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ORIGINAL ARTICLE

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Effect of marination with bioprotective culture-containing marinade on *Salmonella* spp. and *Listeria monocytogenes* in chicken breast meat

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This study investigated the survival of *Pseudomonas* spp., *Salmonella* spp., and *Listeria monocytogenes* in chicken breast meat marinated with a marinade containing bioprotective lactic acid bacteria (*Latilactobacillus curvatus*, *Latilactobacillus sakei*, and *Lactiplantibacillus plantarum*) during storage at 4°C and 8°C. In the first phase, a natural, chemical-free marinade (pH 3.6) was evaluated over 7 days. In this marinade, *Pseudomonas* spp. did not survive, *Salmonella* spp. were inactivated within 7 days, *L. monocytogenes* counts showed negligible reduction, and bioprotective cultures remained stable. In the second phase, chicken breast meat contaminated with *Salmonella* spp. and *L. monocytogenes* was divided into control (non-marinated), marinated control (M-C), and marinated with a marinade containing mixture of bioprotective cultures (M-PC). Initial pH values were 5.99 (control), 5.24 (M-C), and 5.32 (M-PC). At 4°C, *L. monocytogenes* counts in the M-PC group were 4.4 $\log_{10}$ cfu/g lower than the control and 1.4 $\log_{10}$ cfu/g lower than the M-C group on Day 14 ($p < 0.05$). By Day 14, *Pseudomonas* spp. counts were 9.4, 7.3, and 5.7 $\log_{10}$ cfu/g in the control, M-C, and M - PC groups, respectively ($p < 0.05$). At 8°C, *Salmonella* spp. in the M-PC group fell below 1.0 $\log_{10}$ cfu/g by Day 12, and *L. monocytogenes* counts were significantly lower than in the M-C group ($p < 0.05$). Marinating with bioprotective cultures enhanced microbial safety and extended shelf life compared to marinating without them. This approach could offer significant potential for improving the preservation and safety of poultry products.

KEYWORDS
bioprotective culture, chicken meat, *L. monocytogenes*, marination, *Salmonella* spp

Practical Application: Marinated poultry meat, whether prepared domestically by consumers or commercially produced by the poultry meat industry, is widely enjoyed for its flavor and convenience. In this study, bioprotective cultures were incorporated into the marinade as an alternative to chemical preservatives.

The findings demonstrate that marinating chicken breast meat with a marinade composed entirely of natural ingredients and enriched with bioprotective cultures not only extends the product's shelf life but also significantly limits the survival of *Pseudomonas* spp., *Salmonella* spp. and *Listeria monocytogenes*. These results suggest that meat products marinated with bioprotective cultures, or ready-to-use marinades containing such cultures, can be effectively developed and marketed by the meat industry to meet consumer demand for safer, long-lasting, and naturally preserved food products.

1 | INTRODUCTION

The consumption of chicken meat has seen a rapid increase over the past decade, driven by its low-fat content, high-quality protein, affordability, and the absence of significant cultural or religious restrictions limiting its consumption (Dourou et al., 2021). It has been reported that the most consumed meat type in the world is poultry meat, with approximately 140 million tons in 2023 (Statista, 2024).

However, chicken meat provides a suitable environment for microbial growth, which poses significant challenges for food safety. It cannot be stored for extended periods at refrigeration temperatures (4°C) (Serter et al., 2024). In many households, refrigerators often operate inefficiently, with frequent door openings causing temperatures to rise to between 6°C and 8°C or even higher (Ovca et al., 2021). Furthermore, during warmer months or in outdoor settings such as picnics, the temperature of chicken meat may easily exceed the safe 4°C threshold. As temperatures increase, chicken meat and its products may become risky in terms of public health and food safety. *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, and *Clostridium perfringens* are recognized as the most significant pathogens of concern in poultry meat (Karatepe et al., 2025).

In response to growing consumer awareness about healthy eating, there has been a shift toward the use of natural preservatives over chemical alternatives (Gargi & Sengun, 2021; Karatepe et al., 2025). Marinating, an ancient preservation method, has been employed for centuries to extend meat shelf life, enhance its texture, aroma, juiciness, and overall sensory properties (Latoch et al., 2023). In recent years, marinated chicken breast meat, meeting the "ready for thermal treatment" criteria, has gained increasing popularity, particularly in European Union markets (Augustyńska-Prejsnar et al., 2023).

Biopreservation refers to the extension of food shelf life and the enhancement of safety through the use of microorganisms and/or their metabolites. Protective cultures can inhibit the growth of spoilage and pathogenic microorganisms by competing for nutrients or by producing antagonistic compounds such as bacteriocins, organic acids, hydrogen peroxide, and enzymes (Souza et al., 2022). Several studies have demonstrated the antibacterial effects of various lactic acid bacteria (LAB) against foodborne pathogens, contributing to the preservation and shelf life extension of meat products (Ataş et al., 2021; Ibrahim et al., 2021; Pedonese et al., 2020). *Lactiplantibacillus plantarum* is widely used in food preservation due to its ability to produce bacteriocins and organic acids. *Latilactobacillus sakei* synthesizes sakacin bacteriocins and is commonly utilized in food fermentation and industrial production, contributing to the extended shelf life of fermented foods. Additionally, *Latilactobacillus curvatus* has shown the ability to inhibit the growth of spoilage bacteria, such as *Enterobacteriaceae*, *Pseudomonas fragi*, and *Brochothrix thermosphacta*, and produces potent bacteriocins with activity against *Listeria* species (Kingkaew & Tanasupawat, 2023).

Although there are several studies examining the antimicrobial effects of marination and bioprotective cultures in meat products (Mutegi & Patimakorn, 2020; Gargi & Sengun, 2021), to the best of our knowledge, no research has been conducted combining marination with bioprotective LAB in chicken meat. The objective of this study is to evaluate the shelf life of chicken breast meat marinated with a homemade marinade containing bioprotective cultures (*L. curvatus*, *L. sakei*, and *L. plantarum*), and to assess the viability of *Salmonella* spp. and *L. monocytogenes* during storage at both 4°C and 8°C.

2 | MATERIALS AND METHODS

2.1 | Bacterial cultures and inoculum preparation

The bacterial strains used in this study included *L. curvatus* (Bactoferm™ B-LC-48, Chr. Hansen GmbH), *L. sakei* (Bactoferm B-FM, Chr. Hansen GmbH), and *L. plantarum* (Bioferm DSMZ16627). The Bactoferm B-FM starter cul-

TABLE 1 Marinade composition and proportions.

Ingredients	%	Ingredients	%
Water	67	Red chili pepper	1.5
Tomato paste	15	Thyme	1
Lemon juice (fresh)	7	Cumin	0.75
Salt	3	Black pepper powder	0.5
Garlic (crushed)	2	Sugar (sucrose)	0.25
Pepper paste	2		

ture preparation, supplied by Chr. Hansen, comprises a mixture of *L. sakei* and *Staphylococcus xylosus*. Strains were initially activated by incubation in De Man, Rogosa, and Sharpe (MRS) broth at 30°C for 20 h. Subsequently, they were streaked onto MRS agar to isolate single colonies, which were then subjected to Gram staining. Colonies identified as bacilli (*L. sakei*) were purified and utilized in the study.

All bacterial strains were cultivated in sterile polypropylene tubes containing 10 mL of MRS broth and incubated at 30°C for 18–20 h. Following incubation, the cultures were centrifuged, and the resulting bacterial pellets were resuspended in 0.1% peptone water. The final volume in each tube was adjusted to 10 mL. To quantify LAB, 0.1 mL aliquots were serially diluted and plated using the pour plate method on MRS agar. The bacterial counts of each LAB were determined to be approximately 10^9 cfu/mL.

Three different *Salmonella* strains (*S*. Enteritidis ATCC 13076, *S*. Typhimurium ATCC 14028, and NCTC 12416) and *L. monocytogenes* strains (ATCC 13932, ATCC 7644, and N 7144) were separately cultured in sterile polypropylene tubes containing 10 mL of Tryptone Soy Broth at 37°C for 18–20 h. Following incubation, cultures were centrifuged, and the resulting bacterial pellets were resuspended in 2–3 mL of sterile 0.1% peptone water. Subsequently, *L. monocytogenes* and *Salmonella* cells were pooled into separate tubes, and the final volume was adjusted to 10 mL using sterile 0.1% peptone water, yielding a *Salmonella* spp. mix and an *L. monocytogenes* mix. To quantify *Salmonella* and *Listeria* cells, 0.1 mL aliquots were serially diluted and plated using the spread plate method on XLT4 Agar (Merck) and Oxford Listeria Selective Agar (Biokar). The bacterial counts for each pathogen were determined to be approximately 10^8 cfu/mL.

2.2 | Marinade preparation

A modified version of the marinade formulation described by İncili et al. (2020) was employed in this study. The marinade ingredients (Table 1) were carefully selected to ensure they were natural, nonchemical, and readily available in household kitchens.

2.3 | Microbial survival experiment in the marinade

An experimental setup was designed to investigate the viability of *Salmonella* spp., *L. monocytogenes*, and bioprotective cultures in the marinade intended for use in marinating of poultry meat. This experimental setup also allowed for the evaluation of the effects of bioprotective cultures on *L. monocytogenes* and *Salmonella* within the marinade. For this purpose, a 500 g marinade was prepared using the ingredients listed in Table 1, and 5 mL of a pathogen solution containing 10^7 cfu/mL of *Salmonella* spp. and *L. monocytogenes* was added to it. Subsequently, the marinade was divided into five different sterile jars, each containing 100 g. Except for the control group marinade, 1 mL of a solution containing 10^9 cfu/mL of selected bioprotective cultures was added to each of the other four groups. The groups were designed as follows (Figure 1): Group M (control marinade containing *L. monocytogenes* and *Salmonella* spp.); Group M-LP (containing *L. plantarum*, *L. monocytogenes*, and *Salmonella* spp.); Group M-LS (containing *L. sakei*, *L. monocytogenes*, and *Salmonella* spp.); Group M-LC (containing *L. curvatus*, *L. monocytogenes*, and *Salmonella* spp.); and Group M-PC (containing *L. plantarum*, *L. sakei*, *L. curvatus*, *L. monocytogenes*, and *Salmonella* spp.).

Subsequently, each group was further divided into two subgroups and one group of samples was stored at 4°C and the other at 8°C for 7 days. Microbiological and pH analyses were conducted on Days 0, 2, 5, and 7 of storage.

2.4 | Preparation of marinated chicken breast meat groups

Chicken breast meat samples were obtained in their original packaging on the first day of market release and transported to the laboratory under cold chain conditions. The chicken breast meat was then aseptically cut into pieces weighing approximately 25 g using a sterile knife. A total of 500 mL of pathogen inoculation solution was prepared by adding 10 mL of *Salmonella* spp. mix and 10 mL of *L. monocytogenes* mix solutions to 480 mL of sterile 0.1% peptone water. The chicken breast pieces were immersed in the pathogen inoculation solution for 1 min, then removed and placed on sterile grids inside a laminar flow cabinet for 10 min to allow excess liquid to drain and facilitate pathogen adhesion.

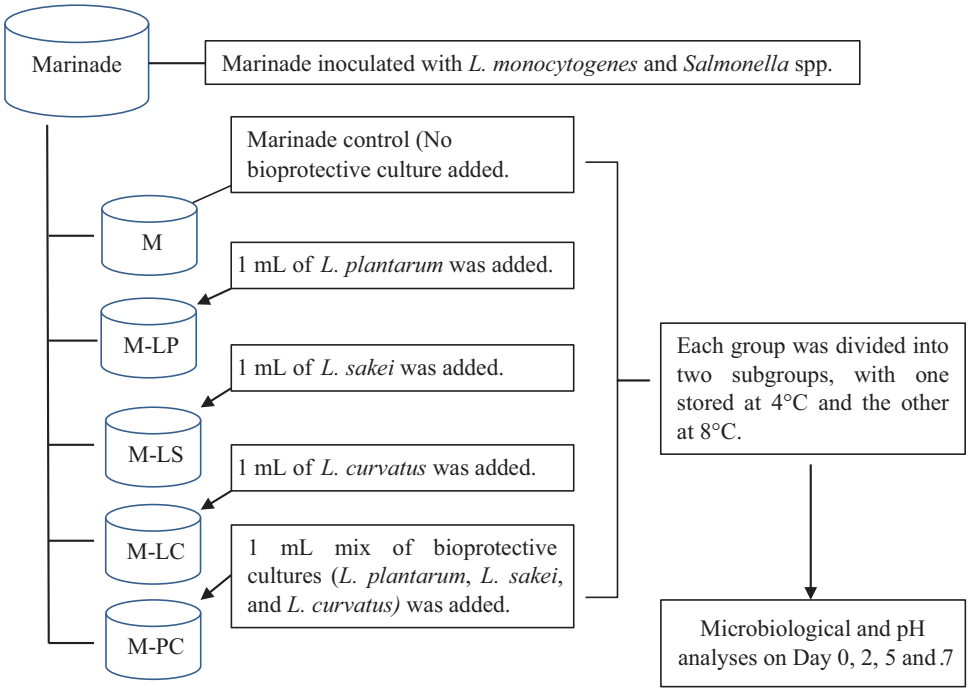

FIGURE 1 Schematic diagram of the preparation of marinade groups and the analyses performed.

TABLE 2 Survival capabilities of bioprotective lactic acid bacteria in marinade stored at 4°C and 8°C (Log_{10} cfu/mL ± SD).

Treatment groups	Storage temperatures (°C)	Days			
		0	2	5	7
M	4	4.2 ± 0.5^{A}	4.6 ± 0.7^{A}	4.7 ± 0.3^{A}	3.9 ± 0.6^{A}
M-LP		7.3 ± 0.8^{B}	7.5 ± 0.4^{B}	7.8 ± 0.5^{B}	7.7 ± 0.2^{B}
M-LS		7.4 ± 0.2^{B}	7.2 ± 0.3^{B}	7.1 ± 0.3^{B}	7.1 ± 0.9^{B}
M-LC		7.4 ± 0.8^{B}	6.9 ± 0.4^{B}	7.4 ± 0.7^{B}	7.2 ± 0.4^{B}
M	8	4.2 ± 0.5^{A}	4.8 ± 0.6^{A}	5.4 ± 0.6^{A}	4.1 ± 0.7^{A}
M-LP		7.3 ± 0.8^{B}	7.7 ± 0.5^{B}	8.1 ± 0.4^{B}	7.2 ± 0.9^{B}
M-LS		7.4 ± 0.2^{B}	7.3 ± 0.7^{B}	7.4 ± 0.5^{B}	6.7 ± 0.4^{B}
M-LC		7.4 ± 0.8^{B}	7.1 ± 0.9^{B}	7.3 ± 0.5^{B}	7.0 ± 0.3^{B}

Note: Numbers in the same column with different superscript are significantly different ($p < 0.05$).
M denotes marinade control (contains no bioprotective culture), M-LP denotes marinade containing *L. plantarum*, M-LS denotes marinade containing *L. sakei*, and M-LC denotes marinade containing *L. curvatus*.

When examined individually, no significant differences were observed in the survival abilities of the three bioprotective LAB in the prepared marinade (Table 2) or in their antibacterial effects against *Salmonella* spp., *L. monocytogenes*, and *Pseudomonas* spp. in the marinade (data not shown). Therefore, rather than testing these bacteria separately during the marination process of chicken breast meat, it was deemed more appropriate to incorporate them as a mixture. Consequently, chicken breast samples inoculated with *Salmonella* spp. and *L. monocytogenes* were divided into three groups (Figure 2):

1. Control (non-marinated).
2. Marinated control (M-C) (contains no bioprotective culture).
3. Marinated with a bioprotective culture-containing marinade (M-PC).

For the control group, chicken breast meat samples were placed in two separate sterile stomacher bags without any further treatment. One bag was stored at 4°C and the other at 8°C for a total of 14 days.

For the marinated control (M-C) group, chicken breast meat samples (total weight approximately 600 g) were placed in a plastic container, and 150 g of marinade was added. The samples were thoroughly mixed by hand using

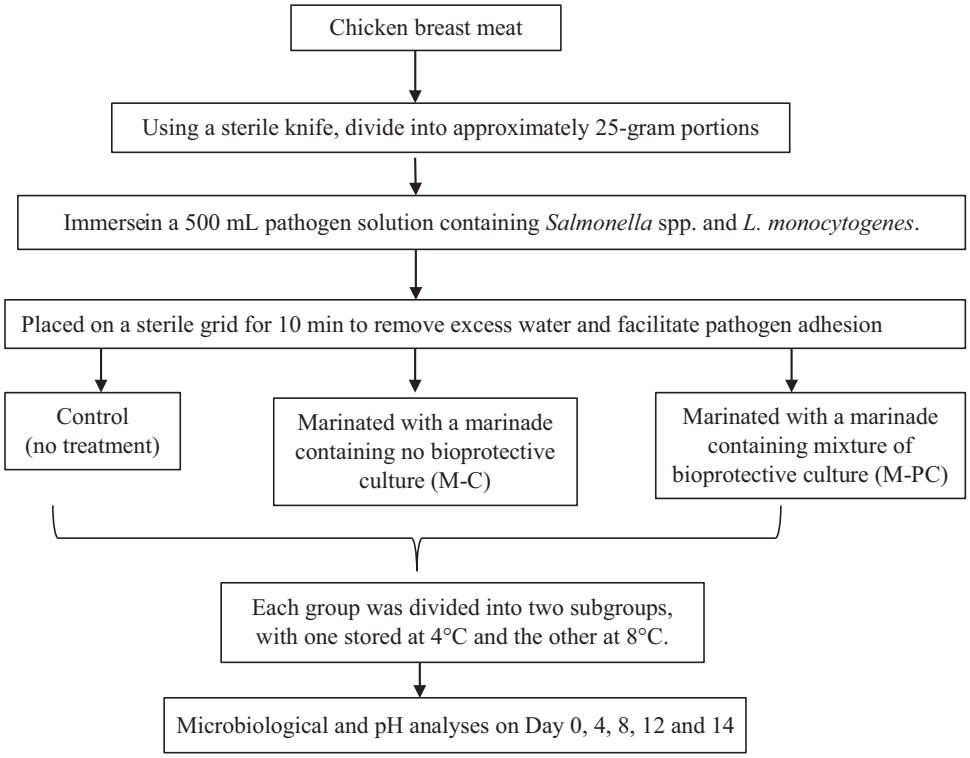

FIGURE 2 Schematic diagram of the preparation of marinated chicken breast meat groups and the analyses performed.

sterile gloves, then transferred into two separate sterile stomacher bags. One bag was stored at 4°C and the other at 8°C for 14 days.

For the M-PC group, approximately 600 g of chicken breast meat samples were placed in a plastic container. A total of 150 g of marinade containing approximately 10^7 cfu/g of each selected bioprotective culture (*L. plantarum*, *L. sakei*, and *L. curvatus*) was added, and the mixture was homogenized. The samples were then divided into two separate sterile stomacher bags and stored for 14 days, with one bag maintained at 4°C and the other at 8°C.

Microbiological and pH analyses were conducted on Days 0, 4, 8, 12, and 14 of storage.

2.5 | Analyses

2.5.1 | pH analysis

pH measurements were conducted immediately following microbiological analyses by immersing the probe of a digital pH meter (HI 2211, Hanna Instruments) directly into the homogenized samples (İncili et al., 2020).

2.5.2 | Microbiological analyses

On each analysis day, two samples were collected from each group for microbiological analysis. For the marinade samples, 1 mL of marinade was taken, serially diluted, and inoculated onto appropriate media. Results were expressed as cfu/mL. For breast meat samples, approximately 25 g of breast meat was placed into sterile stomacher bags, and sterile 0.1% peptone water, at a volume nine times the sample weight, was added. The mixture was then homogenized using a stomacher (IUL 400). Serial dilutions were prepared from 1 mL of the homogenate and inoculated onto appropriate media. Results were reported as cfu/g.

Appropriate dilutions were prepared, and 0.1 mL aliquots were spread-plated onto XLT4 Agar (Merck) for *Salmonella* and Oxford Listeria Selective Agar supplemented with BS003 (Biokar) for *L. monocytogenes*. The plates were incubated at 35°C for 24–48 h, after which characteristic bacterial colonies were counted.

For *Pseudomonas* spp., cultivation was performed on *Pseudomonas* Selective Agar (Merck) containing *Pseudomonas* Selective Supplement, using the spread-plating method. Following incubation at 25°C for 24–48 h, colonies were counted. Five randomly selected colonies were subjected to the oxidase test, and the number of colonies was calculated based on the test results (*Pseudomonas* species are oxidase-positive) (Habeeb et al., 2021).

For LAB, 1 mL of the appropriate dilutions was inoculated on MRS Agar (Biokar) using the pour-plate method. Plates were incubated at 30°C for 48 h before colony enumeration. For total aerobic mesophilic colony (TAMC) counts, 1 mL of the appropriate dilutions was inoculated onto Plate Count Agar (Oxoid) using the pour-plate

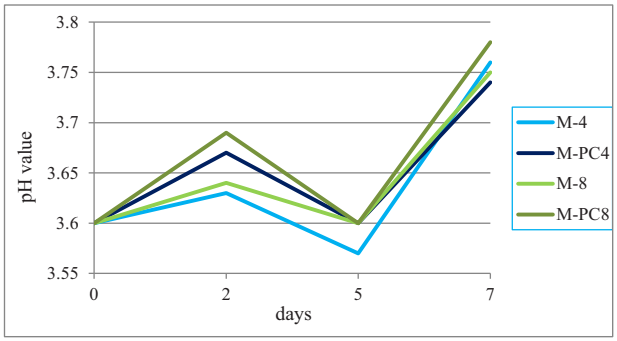

FIGURE 3 pH values of marinade groups with and without bioprotective cultures stored at 4°C and 8°C. (M-4 and M-8: marinade control stored at 4°C and 8°C; M-PC4 and M-PC8: marinade containing mix of bioprotective cultures (*L. plantarum*, *L. sakei*, and *L. curvatus* (in a 1:1:1 ratio), and stored at 4°C and 8°C).

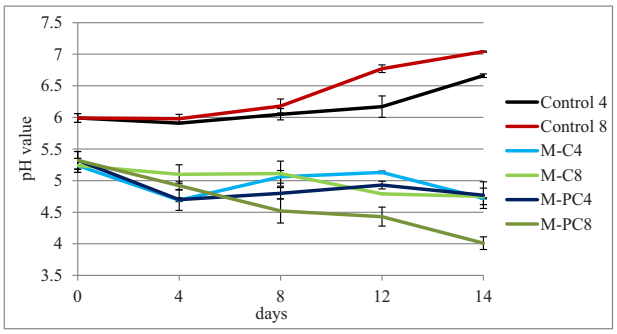

FIGURE 4 The pH values of chicken breast meats marinated with marinades containing and not containing bioprotective cultures, and stored at 4°C and 8°C. (Control 4 and 8, unmarinated chicken breast meat stored at 4°C and 8°C; M-C4 and M-C8, chicken breast meat marinated with a marinade containing no bioprotective cultures, and stored at 4°C and 8°C; M-PC4 and M-PC8, chicken breast meat marinated with a marinade containing a mixture of bioprotective cultures (*L. plantarum*, *L. sakei*, and *L. curvatus* in a 1:1:1 ratio), and stored at 4°C and 8°C.

method. Plates were incubated at 35°C for 48 h before colony enumeration (Güran & İlhak, 2015).

2.5.3 | Statistical analysis

The study was conducted in three replicates. Microbiological data were log-transformed prior to statistical analysis, whereas pH data were analyzed directly. Analysis of variance (ANOVA) was performed to assess the main effects and interactions between treatment groups × storage temperatures × sampling days. All statistical analyses were conducted using SPSS 22 (IBM SPSS), with statistical significance set at $p < 0.05$.

3 | RESULTS AND DISCUSSION

3.1 | pH of marinade

The pH values of the marinades stored at 4°C and 8°C exhibited no significant changes throughout the storage period ($p > 0.05$). Although variations were observed among the different groups, the pH values of the marinades ranged between 3.57 and 3.78 over the 7-day storage period (Figure 3).

In general, homemade marinades tend to be acidic, with pH values typically falling within the range of 2.56 to 3.78, depending on the specific ingredients used (Augustyńska-Prejsnar et al., 2023; İncili et al., 2020; Sengun et al., 2019). Consequently, it can be inferred that the primary antibacterial effect in prepared marinades is largely attributed to their low pH. Lemon juice, a common ingredient in marinades, is primarily composed of citric acid, with minor amounts of malic acid (Augustyńska-Prejsnar et al., 2023; Lytou et al., 2019). Additionally, tomato and pepper pastes, which are naturally acidic due to their content of organic acids such as citric acid, malic acid, and ascorbic acid, further contribute to the overall acidity of the marinade. Typically, the pH values of tomato and pepper pastes are approximately 4.1 and 4.5, respectively (Kaya et al., 2013), which contributes to the low pH of the marinade.

3.2 | pH of marinated chicken breast meat

The low pH of the marinade led to a significant difference ($p < 0.05$) in pH values between marinated and control chicken breast samples stored at 4°C and 8°C (Figure 4). While the control group's initial pH was 5.99, it increased to 6.66 and 7.04 at 4°C and 8°C, respectively, by Day 14 ($p < 0.05$). pH elevation in poultry meat during storage is primarily attributed to biochemical reactions and microbial proteolysis (Lytou et al., 2019). The higher pH increase in the control samples may be linked to the proteolytic activity of psychrotrophic microorganisms, such as *Pseudomonas* spp., with microbial and biochemical activity being more pronounced at 8°C than at 4°C.

The pH values of marinated chicken breast meat decreased to 5.24–5.32 due to the low pH (3.60) of the marinade, showing significant differences compared to the control groups ($p < 0.05$). The higher pH of marinated chicken meat compared to the marinade may be attributed to the buffering effect of proteins in the meat (İncili et al., 2020). Unlike the control group, the pH continued to decrease until the end of storage in both the M-C and M-PC groups as a result of the marinade's effect at

both storage temperatures ($p < 0.05$). In this study, 0.25% sucrose (table sugar) was incorporated into the marinade to serve as a carbon source for bioprotective cultures and to stimulate their growth. Previous studies have shown that sucrose is readily fermented by *L. curvatus* and *L. sakei* (Chen et al., 2020; Zagorec & Champomier-Vergès, 2017), and the addition of 0.3% sucrose to sausage dough has been reported to enhance the growth of *L. plantarum* (Hongthong et al., 2020). This suggests that the 0.25% sucrose added to the marinade was likely metabolized by bacteria. Additionally, the pH values of the M-PC groups stored at 8°C decreased more significantly than those stored at 4°C after the eighth day of storage ($p < 0.05$) (Figure 4). This finding can be attributed to the higher metabolic activity of bioprotective cultures at 8°C compared to 4°C, leading to increased synthesis of organic acids over time.

3.3 | Survival capability of bioprotective cultures, *Salmonella*, *L. monocytogenes*, and background microorganisms in marinade environment

On the first day of storage, the LAB count in the M group marinade was determined to be 4.2 $\log_{10}$ cfu/mL. The initial counts of *L. plantarum*, *L. sakei*, and *L. curvatus* in the M-LP, M-LS, and M-LC groups were 7.3, 7.4, and 7.4 $\log_{10}$ cfu/mL, respectively, and they remained viable in marinades stored at both 4°C and 8°C throughout the storage period without any significant change in their numbers ($p > 0.05$) (Table 2). This finding may be attributed to the suppression of metabolic activity of LAB under the conditions of low pH and low temperature. In general, LAB are known to grow within a temperature range of 2°C–53°C (König & Berkelmann-Löhnertz, 2017). Castellano et al. (2008) reported that *L. curvatus* maintained its metabolic activity and continued to grow, albeit slowly, when incubated in MRS broth at 4°C and 8°C for 14 days. Previous studies have reported that *L. plantarum*, *L. sakei*, and *L. curvatus* can grow at 4°C, albeit slowly (Calasso & Gobbetti, 2011; Castellano et al., 2008; Yu et al., 2024). The findings of this study further indicate that the growth of *L. plantarum*, *L. sakei*, and *L. curvatus* in the marinade environment may be significantly limited due to both the low-storage temperatures (4°C and 8°C) and the acidic pH (3.60–3.78); however, these bacteria were able to survive under these conditions. In light of this information, it can be inferred that while refrigeration at 4°C and 8°C may not be sufficient to completely inhibit the activity of LAB, the inherently low pH of the marinade plays a more critical role in bacterial growth.

Salmonella spp. counts in the M and M-PC groups were 5.2 and 5.1 $\log_{10}$ cfu/mL, respectively, on the first day of storage. However, their numbers decreased significantly over time ($p < 0.05$), dropping below the detectable limit of 1 cfu/mL at both temperatures by the fifth day (Table 3). No significant difference was observed in *Salmonella* inactivation between marinades with and without bioprotective cultures ($p > 0.05$). Previous research has reported that *Salmonella* species cannot survive below pH 4.1 (Kim et al., 2018). Additionally, the antimicrobial properties of spices such as black pepper, garlic, and thyme present in the marinade may have contributed to *Salmonella* inactivation. Volatile oils and peptides found in spices have been shown to exhibit antimicrobial effects (Feknous et al., 2023).

The *L. monocytogenes* counts in the M and M-PC groups, initially ranging from 4.6 to 4.7 $\log_{10}$ cfu/mL on the first day of storage, exhibited an insignificant decrease throughout the storage period ($p > 0.05$), reaching 3.9–4.2 $\log_{10}$ cfu/mL by the seventh day. The findings indicate that while the marinade did not exert a significant bactericidal effect on *L. monocytogenes* at either storage temperature, it demonstrated a strong bacteriostatic effect. These findings are consistent with previous studies reporting that *L. monocytogenes* enters a stationary phase under acidic conditions (Nyhan et al., 2018) and that *L. monocytogenes* counts remained nearly constant in a marinade with a pH of 3.17, suggesting a bacteriostatic effect (İncili et al., 2020).

On the first day of storage, the *Pseudomonas* spp. count was detected at 0.8 $\log_{10}$ cfu/mL in both the M and M-PC groups. However, from the second day onward, *Pseudomonas* counts decreased to below detectable limits (<1 cfu/mL) in marinades stored at both 4°C and 8°C (Table 3), with no statistically significant differences observed between groups or storage temperatures ($p > 0.05$). In general, food spoilage microorganisms proliferate at a pH greater than 4.5 (Alegbeleye et al., 2022). Given that the pH of the marinade in the present study was 3.6 and contained antibacterial spices, it is likely that these factors contributed to the inhibition of *Pseudomonas* spp., which were initially present in low numbers.

The absence of a significant difference in the effects of the marinade on *Salmonella*, *L. monocytogenes*, and *Pseudomonas* spp. between groups with and without bioprotective cultures suggests that the primary inhibitory factor was the low pH (3.6–3.7) of the marinade (Table 3). It is likely that the combination of low storage temperatures (4°C and 8°C) and low pH values (3.6 and 3.78) restricted the metabolic activity of bioprotective cultures despite their survival. Under these conditions, the production of antimicrobial compounds such as organic acids and bacteriocins may have been inhibited. As shown in Table 2, bioprotective culture counts remained relatively stable over the 7-day storage period, further suggesting a significant reduction in their metabolic activity. Bacterial growth and proliferation depend on enzymatic functions,

TABLE 3 Microorganism counts of marinade groups with and without bioprotective culture, and stored at 4°C and 8°C (log_{10} cfu/mL ± SD).

Treatment groups	Storage temperatures (°C)	Days			
		0	2	5	7
		Salmonella spp.			
M	4	5.2 ± 0.4^{x}	2.8 ± 0.6^{y}	0.6 ± 0.5^{z}	nd
M-PC		5.1 ± 0.5^{x}	2.9 ± 0.3^{y}	0.4 ± 0.3^{z}	0.1 ± 0.2^{z}
M	8	5.2 ± 0.4^{x}	2.6 ± 0.6^{y}	0.4 ± 0.4^{z}	nd
M-PC		5.1 ± 0.5^{x}	2.2 ± 0.7^{y}	0.2 ± 0.3^{z}	nd
		Listeria monocytogenes			
M	4	4.6 ± 0.2	4.2 ± 0.4	4.0 ± 0.4	4.0 ± 0.2
M-PC		4.7 ± 0.3	4.2 ± 0.5	4.4 ± 0.5	4.2 ± 0.3
M	8	4.6 ± 0.2	4.5 ± 0.6	4.2 ± 0.3	4.2 ± 0.4
M-PC		4.7 ± 0.3	4.4 ± 0.6	4.4 ± 0.3	3.9 ± 0.3
		Pseudomonas spp.			
M	4	0.8 ± 0.1	nd	nd	nd
M-PC		0.8 ± 0.1	nd	nd	nd
M	8	0.8 ± 0.1	nd	nd	nd
M-PC		0.8 ± 0.1	nd	nd	nd
		Total aerobic colony counts			
M	4	6.7 ± 0.2^{Ax}	5.7 ± 0.6^{Axy}	5.5 ± 0.4^{Axy}	5.0 ± 0.4^{Ay}
M-PC		8.1 ± 0.3^{B}	8.0 ± 1.2^{B}	8.3 ± 0.3^{B}	7.8 ± 0.4^{B}
M	8	6.7 ± 0.2^{Ax}	6.0 ± 0.9^{Axy}	5.6 ± 0.7^{Axy}	4.8 ± 0.7^{Ay}
M-PC		8.1 ± 0.3^{B}	8.1 ± 0.5^{B}	8.0 ± 0.8^{B}	7.7 ± 0.4^{B}

Abbreviation: nd, not defined.

Note: Numbers in the same column with different superscript are significantly different ($p < 0.05$).

Numbers in the same line with different superscript are significantly different ($p < 0.05$).

M denotes marinade control (contains no bioprotective culture) and M-PC denotes marinade containing mix of bioprotective cultures (*L. plantarum, L. sakei, and L. curvatus* [1:1:1]).

which are optimized within specific temperature and pH ranges. When these environmental conditions are unfavorable, enzymatic activity is minimized or may even cease entirely (Rolfe & Daryaei, 2020). Although LAB are generally resistant to acidic environments, the findings of this study indicate that their metabolic activity was significantly suppressed due to the combined effects of low temperature and low pH.

TAMC in the M group was significantly lower compared to the M-PC group ($p < 0.05$), as no bioprotective culture was added to the M group (Table 3). On the first day of storage, TAMC values were 6.7 and 8.1 log_{10} cfu/mL in the M and M-PC groups, respectively. By the seventh day of storage, TAMC decreased to 5.0 and 4.8 log_{10} cfu/mL in the M groups stored at 4°C and 8°C, respectively ($p < 0.05$), but showed nonsignificant decreases in the M-PC groups ($p > 0.05$). This is likely due to the survival of the bioprotective LAB present in the M-PC group within the marinade environment.

3.4 | Microbial survival capability of *Salmonella*, *L. monocytogenes*, *Pseudomonas* spp. bioprotective cultures, and background microorganisms in marinated chicken breast meat

Salmonella spp., which exhibited a 5-log_{10} cfu/mL reduction within 5 days in the marinade with a pH of 3.6 (Table 3), showed a reduction of 2.3 and 2.1 log_{10} cfu/g over 14 days in the M-C and M-PC groups, respectively, when marinated with this solution and stored at 4°C (Table 4). The relatively lower reduction in *Salmonella* counts in marinated chicken meat compared to those in the marinade may be attributed to the buffering capacity of chicken meat proteins, which causes the pH of the marinated meat to range between 5.32 and 4.72. No significant difference in *Salmonella* inactivation was observed between the M-C and M-PC groups ($p > 0.05$), which may be explained by the previously mentioned antimicrobial effect of the

TABLE 4 Microorganism counts of chicken breast meats marinated with marinades containing and not containing bioprotective cultures, and stored at 4°C and 8°C (log_{10} cfu/g ± SD).

Treatment groups	Storage temperature (°C)	Days				
		0	4	8	12	14
		Salmonella spp.				
Control	4	5.2 ± 0.1^{Ax}	3.9 ± 0.5^{Ay}	5.2 ± 0.1^{Ax}	4.4 ± 0.2^{Ay}	4.2 ± 0.1^{Ay}
M-C		4.8 ± 0.1^{Bx}	3.9 ± 0.1^{Ay}	3.9 ± 0.4^{BCy}	3.4 ± 0.3^{ABy}	2.5 ± 0.3^{BCz}
M-PC		4.5 ± 0.1^{Bx}	3.6 ± 0.4^{Ay}	3.0 ± 0.5^{Cyz}	3.3 ± 0.3^{ABy}	2.4 ± 0.4^{Cz}
Control	8	5.2 ± 0.1^{Ax}	4.3 ± 0.1^{Ay}	4.5 ± 0.5^{ABxy}	4.1 ± 0.2^{ABy}	3.2 ± 0.2^{Bz}
M-C		4.8 ± 0.1^{Bx}	3.9 ± 0.2^{Ay}	4.0 ± 0.1^{By}	3.0 ± 0.1^{Bz}	<1.0
M-PC		4.5 ± 0.1^{Bx}	3.7 ± 0.3^{Ax}	3.7 ± 0.4^{BCx}	<1.0	<1.0
		Listeria monocytogenes				
Control	4	4.6 ± 0.1^{Az}	5.3 ± 0.1^{Ay}	6.2 ± 0.3^{Ax}	6.2 ± 0.1^{Bx}	7.1 ± 0.4^{Bw}
M-C		4.5 ± 0.1^{A}	4.2 ± 0.2^{B}	4.2 ± 0.4^{B}	4.2 ± 0.1^{C}	4.1 ± 0.5^{C}
M-PC		4.6 ± 0.1^{Aw}	3.7 ± 0.4^{Bx}	3.4 ± 0.4^{Bxy}	3.3 ± 0.1^{Exy}	2.7 ± 0.4^{Dy}
Control	8	4.6 ± 0.1^{Az}	5.6 ± 0.4^{Ay}	7.1 ± 0.4^{Ax}	7.6 ± 0.1^{Awx}	8.2 ± 0.1^{Aw}
M-C		4.5 ± 0.1^{Awx}	4.1 ± 0.3^{Bwx}	4.0 ± 0.4^{Bx}	4.6 ± 0.1^{Cwx}	4.8 ± 0.2^{Cw}
M-PC		4.6 ± 0.1^{Aw}	4.1 ± 0.3^{Bwx}	3.5 ± 0.2^{By}	3.7 ± 0.2^{Dxy}	3.3 ± 0.2^{Dy}
		Pseudomonas spp.				
Control	4	1.4 ± 0.5^{Bv}	5.6 ± 0.2^{Bw}	7.2 ± 0.3^{Bx}	8.3 ± 0.1^{Ay}	9.4 ± 0.4^{Az}
M-C		<1	1.4 ± 0.3^{Dv}	3.5 ± 0.5^{Dw}	5.8 ± 0.1^{Cx}	7.3 ± 0.2^{Cy}
M-PC		<1	<1	3.5 ± 0.1^{Dv}	4.9 ± 0.1^{Dw}	5.7 ± 0.3^{Dx}
Control	8	2.6 ± 0.1^{Av}	7.4 ± 0.4^{Aw}	8.4 ± 0.4^{Ax}	8.8 ± 0.1^{Axy}	9.4 ± 0.4^{Ay}
M-C		<1	3.3 ± 0.3^{Cw}	5.2 ± 0.1^{Cx}	6.6 ± 0.5^{By}	8.6 ± 0.3^{Bz}
M-PC		<1	2.2 ± 0.4^{Dv}	5.7 ± 0.6^{Cw}	6.4 ± 0.1^{BCw}	7.4 ± 0.1^{Cx}
		Lactic acid bacteria				
Control	4	3.4 ± 0.1^{Av}	3.5 ± 0.3^{ABvw}	4.0 ± 0.1^{ABwx}	4.2 ± 0.2^{Axy}	4.6 ± 0.3^{ABy}
M-C		3.8 ± 0.1^{Avw}	3.8 ± 0.1^{Bvw}	3.3 ± 0.4^{Av}	4.3 ± 0.5^{Aw}	4.5 ± 0.2^{Aw}
M-PC		6.5 ± 0.4^{Bv}	6.9 ± 0.1^{Cvw}	7.4 ± 0.5^{ACw}	7.3 ± 0.2^{Cvw}	7.2 ± 0.1^{Cvw}
Control	8	3.4 ± 0.1^{Av}	3.2 ± 0.2^{Av}	4.6 ± 0.3^{Bw}	4.7 ± 0.2^{ABw}	4.7 ± 0.3^{ABw}
M-C		3.8 ± 0.1^{Av}	3.8 ± 0.1^{Bv}	3.9 ± 0.1^{ABv}	5.4 ± 0.4^{Bw}	5.5 ± 0.4^{Bw}
M-PC		6.5 ± 0.4^{Bv}	7.9 ± 0.2^{Dw}	8.6 ± 0.5^{Dw}	8.8 ± 0.2^{Dw}	8.7 ± 0.6^{Dw}
		Total aerobic mesophilic colony				
Control	4	5.8 ± 0.1^{Av}	5.7 ± 0.6^{Cv}	6.5 ± 0.2^{ABv}	9.2 ± 0.1^{Aw}	9.4 ± 0.6^{ABw}
M-C		3.5 ± 0.4^{Bv}	5.7 ± 0.3^{Cw}	6.4 ± 0.4^{Bwx}	7.3 ± 0.3^{Cxy}	8.1 ± 0.6^{BCy}
M-PC		6.0 ± 0.1^{Av}	7.8 ± 0.1^{Aw}	7.4 ± 0.4^{Aw}	8.4 ± 0.4^{Bw}	7.5 ± 0.9^{Cw}
Control	8	5.8 ± 0.1^{Av}	6.5 ± 0.3^{BCw}	7.4 ± 0.2^{Ax}	9.5 ± 0.2^{Ay}	10.2 ± 0.2^{Az}
M-C		3.5 ± 0.4^{Bv}	6.6 ± 0.3^{Bw}	7.3 ± 0.4^{ABwx}	7.6 ± 0.1^{BCxy}	8.2 ± 0.1^{BCy}
M-PC		6.4 ± 0.6^{Av}	6.8 ± 0.1^{Bv}	7.4 ± 0.3^{Avw}	8.4 ± 0.5^{Bw}	8.4 ± 0.5^{BCw}

Note: Numbers in the same column with different superscript are significantly different ($p < 0.05$).
Numbers in the same line with different superscript are significantly different ($p < 0.05$).
Control denotes unmarinated chicken breast meat, M-C denotes chicken breast meat marinated with marinade without bioprotective cultures, and M-PC denotes chicken breast meat marinated with a marinade containing a mixture of bioprotective cultures (*L. plantarum*, *L. sakei*, and *L. curvatus* in a 1:1:1 ratio).

marinade's low pH rather than the bioprotective cultures at 4°C. On the 12th day of storage, *Salmonella* counts in the M-C group stored at 8°C were 3.0 log_{10} cfu/g, whereas in the M-PC group, they dropped below the detection limit ($p < 0.05$). The pH values of the M and M-PC groups on the eighth day of storage were 5.11 and 4.52, respectively ($p < 0.05$) (Figure 4), and this difference persisted in the following days. This finding suggests that bioprotective cultures were more active at 8°C, exerting an antibacterial effect on *Salmonella* through the production of organic acids and other antimicrobial compounds. Hoyle et al. (2009) reported that LAB strains added to ground beef

did not grow significantly at 5°C, but could still produce substances that inhibit the growth of pathogens such as *Salmonella* spp.

In chicken breast meats stored at 8°C, the *Salmonella* count in the control group decreased by 2.0 $\log_{10}$ cfu/g during storage ($p < 0.05$). This decrease may be attributed to the significant increase in the numbers of TAMC and *Pseudomonas* spp. in the chicken meat, resulting from the effect of elevated storage temperature (Table 4) and the competition between microorganisms. Karatepe et al. (2025) reported that the rapid dominance of microbial groups during the storage of chicken meat negatively affected low-competition bacteria such as *S*. Typhimurium. The rapid proliferation of *Pseudomonas* spp. and TAMC may be responsible for the observed reduction in *Salmonella* counts.

The number of *L. monocytogenes* increased by 2.5 $\log_{10}$ cfu/g in the control group stored at 4°C for 14 days ($p < 0.05$). In contrast, the M-C group showed an insignificant decrease of 0.4 $\log_{10}$ cfu/g ($p > 0.05$), whereas the M-PC group exhibited a significant decrease of 1.9 $\log_{10}$ cfu/g ($p < 0.05$) during the same period (Table 4). At 8°C, by the end of the 14-day storage period, the *L. monocytogenes* count in the control group increased by 3.6 $\log_{10}$ cfu/g ($p < 0.05$), showed insignificant increase in the M-C group ($p > 0.05$), and decreased by 1.3 $\log_{10}$ cfu/g in the M-PC group ($p < 0.05$) (Table 4). Upon evaluation of the results, it was observed that no significant increase or decrease in *L. monocytogenes* counts occurred in the M-C groups stored at both 4°C and 8°C over the 14-day period ($p > 0.05$). In contrast, significant reductions in *L. monocytogenes* numbers were noted in the M-PC groups stored at the same temperatures ($p < 0.05$). Based on these findings, it can be concluded that the marinade exhibited a bacteriostatic effect on *L. monocytogenes* in chicken breast meat, while the addition of a bioprotective culture to the marinade demonstrated a bactericidal effect. LAB and its metabolites have been reported to exert both bactericidal and bacteriostatic effects against *L. monocytogenes* (Webb et al., 2022).

Pseudomonas spp. are psychrotrophic bacteria that play a major role in the spoilage and deterioration of poultry meat stored at refrigeration temperatures (Zhang et al., 2016). In the control group of chicken breast samples stored at 4°C and 8°C, the *Pseudomonas* spp. count increased rapidly, reaching 9.4 $\log_{10}$ cfu/g by Day 14. In the M-C groups stored at the same temperatures, the counts reached 7.3 $\log_{10}$ cfu/g at 4°C and 8.6 $\log_{10}$ cfu/g at 8°C by Day 14 ($p < 0.05$). The observed difference in *Pseudomonas* counts between the control and M-C groups suggests that the low pH of the marinade likely slowed the growth of *Pseudomonas* spp. Furthermore, in the M-PC groups stored at 4°C and 8°C, the *Pseudomonas* counts after 14 days were 5.7 $\log_{10}$ cfu/g and 7.4 $\log_{10}$ cfu/g, respectively, which were significantly lower than those in the M-C groups stored at the same temperatures ($p < 0.05$). This significant reduction can be attributed to the effect of the bioprotective cultures added to the marinade. Pedonese et al. (2020) reported that *Pseudomonadaceae* loads in pork sausages to which bioprotective culture was added had lower values than sausages to which no protective culture was added.

LAB counts showed a gradual increase in all samples stored at 4°C and 8°C throughout the storage period. Due to the addition of bioprotective LAB cultures, the M-PC group exhibited higher LAB counts compared to the control and M-C groups at both storage temperatures ($p < 0.05$). In the M-C and M-PC groups stored at 4°C, LAB counts increased by 0.7 $\log_{10}$ cfu/g after 14 days of storage ($p < 0.05$). Hoyle et al. (2009) reported that LAB strains added to ground beef were active at 5°C, but their numbers did not increase significantly. In contrast, in the M-PC groups stored at 8°C, LAB counts increased 2.2 $\log_{10}$ cfu/g, respectively ($p < 0.05$). These results indicate that the bioprotective cultures remained active and were capable of proliferating in marinated chicken meat at 8°C.

The proliferation of spoilage bacteria can lead to product defects, including undesirable changes in taste, color, odor, texture, and appearance. It has been reported that the upper limit for TAMC is 7 $\log_{10}$ cfu/g (Karatepe et al., 2025). Hoyle et al. (2009) reported in their study that although samples initially inoculated with LAB exhibited higher TAMC and LAB populations, elevated TAMC and LAB numbers did not necessarily indicate spoilage of the product. Specifically, in this study, TAMC values exceeding 7.0 $\log_{10}$ cfu/g on the eighth day of storage at both storage temperatures in the M-PC group containing biopreservative cultures may not reflect product spoilage. The use of low temperatures for poultry meat preservation emphasizes the growth of psychrotrophic microorganisms, which can thrive under such conditions. *Pseudomonas* spp., in particular, are the primary microorganisms responsible for spoilage in poultry meat stored under cold conditions (Zhang et al., 2016). In light of these factors, it can be suggested that monitoring *Pseudomonas* spp. populations, rather than TAMC, would provide a more accurate indicator of the shelf life of poultry meat in this study.

When chicken meat marinated with a marinade containing bioprotective cultures undergoes the cooking process, the majority of the LAB present will be killed. However, it has been reported that viability is not always necessary to derive the beneficial effects of a probiotic. Paraprobiotics, also known as inactive probiotics or ghost probiotics, are defined as nonliving microbial cells that can positively impact the host's immune system when administered in adequate amounts (Manassi et al., 2022). Therefore, it can be argued that even if the bioprotective

cultures in the marinade are killed during cooking, they may still provide beneficial effects to the consumer.

4 | CONCLUSION

This study demonstrated that a marinade, prepared with commonly available spices and auxiliary ingredients, without the inclusion of chemical preservatives, enhances the microbial safety and shelf life of chicken breast meat. It was found that the bioprotective cultures incorporated into this marinade contribute to its antimicrobial properties, with these effects being even more pronounced in instances where the cold chain is compromised.

Future research should focus on identifying LAB with stronger bioprotective properties that can perform effectively in acidic marinating environments, within the cold chain, and in cases where the cold chain is broken. Upon the discovery of bioprotective bacteria that can withstand such harsh conditions, these bacteria could be incorporated into commercial marinade formulations for meat products or freeze-dried and marketed for use in marinades.

AUTHOR CONTRIBUTIONS

Enise Begüm Göçmez: Formal analysis; writing—review and editing; writing—original draft; validation. **Osman İrfan İlhak**: project administration; writing—original draft; writing—review and editing; methodology; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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