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ANTIMICROBIAL EFFICACY OF POSTBIOTICS OF LACTIC ACID BACTERIA AND THEIR EFFECTS ON FOOD SAFETY AND SHELF LIFE OF CHICKEN MEAT*

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Abstract

In this study, the antibacterial effects of postbiotics obtained from *Pediococcus acidilactici*, *Lactiplantibacillus plantarum* and *Latilactobacillus sakei*, which were grown in sterile cow's milk and De Man Rogosa and Sharpe (MRS) broth, against some food pathogens (*Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Brucella melitensis*) were investigated. It was observed that lactic acid bacteria postbiotics produced in MRS broth formed larger inhibition zones than those developed in cow's milk against pathogenic bacteria. In order to investigate the antimicrobial effect of the postbiotics on chicken breast meat and to compare this effect with lactic acid decontamination, samples contaminated with *Salmonella* spp. and *L. monocytogenes* were immersed into the postbiotics of *L. plantarum* and *L. sakei*, 2.1% lactic acid solution, and distilled water for 10 minutes. Microbial changes in the groups were investigated during the storage at 4°C for 17 days. On the 8th day of storage, it was determined that the number of *Salmonella* spp. in the groups treated with postbiotics decreased by 0.9 $\log_{10}$ CFU/g compared to the control and distilled water groups. While the number of *L. monocytogenes* increased during storage in the control and distilled water groups, the postbiotics and 2.1% lactic acid exhibited a bacteriostatic effect on *L. monocytogenes* during storage period. Compared to the postbiotics, 2.1% lactic acid had higher reduction (1.8 $\log_{10}$ CFU/g) rates against *Salmonella* spp. ($P<0.05$), also a significant difference was observed against *L. monocytogenes* in the first and last days of storage ($P<0.05$). While the shelf life of chicken breast meat was determined to be 5 days in the control and distilled water groups, postbiotic treatments extended the shelf life of chicken breast meat by an extra 9 days, and 2.1% lactic acid treatment extended an extra 12 days compared to the control and distilled water treatments.

Key words: postbiotic, *Salmonella*, *L. monocytogenes*, chicken breast meat, shelf life

It is necessary for human beings to consume adequate amounts of nutritious and safe food for their survival. However, rapid population growth, urbanization, natural disasters due to climate change, pandemics, escalating tensions between countries, war and economic crises affect food safety and security. Food loss has become one of the issues that should be emphasized at least as much as food production. Especially, fresh foods with short shelf life constitute the majority of loss and waste (Ishangulyyev et al., 2019). On the other hand, according to the World Health Organization, approximately 600 million people become ill from contaminated food every year, and even 420,000 of them die (WHO, 2015).

Chicken meat is a frequently preferred protein source in the world due to its relatively low production cost, low fat content, high nutritional value, ease of cooking, light sensory characteristics, and culturally and religiously acceptable characteristics compared to red meat. The high nutritional value and high moisture content of chicken meat, as well as the high pH value, cause it to spoil quickly and lose its freshness rapidly. Contamination of chicken meat with pathogens, in addition to its problems in terms of public health, leads to rejection of products during the export and therefore to economic losses. For these reasons, one of the most important focal points of the poultry meat production industry is to extend the shelf life of the product and to maintain its quality during cold storage. To date, a variety of preservation methods have been tried to eliminate or reduce the pathogenic and spoilage microorganisms in poultry meat and meat products (Habeeb et al., 2021; İlhak et al., 2018; Sohaib et al., 2016). However, today's consumers prefer natural or less processed foods instead of foods containing synthetic preservatives (Salanţă and Cropotova, 2022; Balthazar et al., 2022; İncili et al., 2020). When the literature related to decontamination of chicken meat with natural compounds is examined, it is seen that many studies have been carried out on organic acids (Pelyuntha and Vongkamjan, 2022; Habeeb et al., 2021), essential oils of spices (İlhak et al., 2017; Radha et al., 2014), herbal extracts (Rahnemoon et al., 2021),

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natural polymer chitosan (İncili et al., 2021; Bhoir et al., 2019; Petrou et al., 2012) etc. for the decontamination of chicken meat and meat parts.

Recent studies have revealed that non-viable probiotic microorganisms (intact or ruptured) provide benefits to consumers when applied orally or topically in sufficient amounts, and the term "paraprobiotics" (also called inactivated probiotics, ghost probiotics) is used for these microbial cells. Postbiotics (also called metabiotics, biogenics, probiotic cell fragments (PCFs), cell free supernatants (CFS)) are defined as bioactive soluble products or metabolic byproducts produced by live lactic acid bacteria during fermentation (Barros et al., 2020; Cuevas-González et al., 2020). However, although the term "postbiotic" is by far the most commonly used, there is still no universally accepted full definition (Thorakkattu et al., 2022). Cell-free metabolites (postbiotics) from lactic acid bacteria seem to have been relatively less studied in chicken meat (Godoy et al., 2022; İncili et al., 2020, 2021, 2022, 2023; Sabo et al., 2017). In recent years, it has been stated that postbiotics of lactic acid bacteria inhibit many microorganisms including *Salmonella*, *Listeria monocytogenes* (*L. monocytogenes*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, *Yersinia* spp., *Aeromonas* spp., *Bacillus* spp., mold-yeast, and viruses (Mani-López et al., 2022; Moradi et al., 2020; Yolmeh et al., 2017).

In the limited number of studies examining the antimicrobial effects of lactic acid bacteria postbiotics, it is seen that MRS broth is widely used for the growth of lactic acid bacteria and postbiotics production. Cow's milk is a nutrient medium that can be obtained more easily and cheaply than MRS broth. In our literature review, we could not find any study comparing the antimicrobial effect of postbiotics obtained from lactic acid bacteria grown in MRS broth and cow's milk against food pathogens. In addition, we did not find any study comparing the antimicrobial effect of postbiotics with the antimicrobial effect of lactic acid solution, which is widely used in the meat industry.

In line with the above information, in the first stage of this study, it was aimed to determine the titratable acid amounts and pH values of *Pediococcus acidilactici*, *Latilactobacillus sakei* and *Lactiplantibacillus plantarum* postbiotics obtained in MRS broth and cow's milk, and to reveal their antimicrobial effects on some foodborne pathogens (*Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Brucella melitensis*) by the agar well diffusion method. In the second stage of the study, it was aimed to compare the antimicrobial effect obtained by using postbiotics as a decontaminant in chicken breast meat with the antimicrobial effect obtained by using lactic acid solution.

Material and methods

Preparation of postbiotics of lactic acid bacteria

Postbiotics were obtained from lactic acid bacteria with slight modification of a method reported by İncili et al (2021). In the study, *P. acidilactici* (isolated from Bactoferm™ B-LC-78 commercial culture, Chr. Hansen GmbH, Germany), *L. sakei* (Bactoferm™ B-FM isolated from commercial culture, Chr. Hansen GmbH, Germany) and *L. plantarum* (Bioferm DSMZ16627) strains were used. *P. acidilactici*, *L. sakei*, *L. plantarum* strains were propagated separately at 30°C for 20 hours in tubes containing 10 mL of MRS broth. After activation, 0.5 mL of each was taken and placed into falcon tubes containing 10 mL of UHT sterile cow's milk (obtained from a local market) and 10 mL of MRS broth, then incubated at 30°C for 48 hours. In order to measure the effects of these bacteria in the mixed state, 0.5 mL of each was taken (1: 1: 1 v/v) and inoculated into sterile cow's milk and MRS broth in the same way. At the end of the incubation, the tubes were centrifuged at 5,000 rpm for 10 minutes in a refrigerated centrifuge (Hettich, Universal 320 R-Germany) and the supernatants were obtained. Supernatants were sterilized by passing through a 0.22 µm-pore size sterile filter (Millipore, Millex-GP Syringe filter SLGP033RS).

Preparation of pathogenic bacteria inoculum

Salmonella Enteritidis (ATCC 13076), *Salmonella* Typhimurium (ATTC 14028 and NCTC 12416), *Listeria monocytogenes* (ATCC 7644, ATCC 13932 and ATCC 19111), *Escherichia coli* O157:H7 (ATCC 43894, ATCC 43895 and ATCC 35150) and *Brucella melitensis* (provided from Pendik Veterinary Control Research Institute, Istanbul, Turkey) were used as pathogenic bacteria. Each strain of pathogenic bacteria was inoculated in 10 mL of Tryptic Soy Broth (TSB) medium separately and incubated at 37°C for 18−20 hours. At the end of the incubation, the tubes were centrifuged at 5,000 rpm for 10 minutes in a refrigerated centrifuge (Hettich, Universal 320 R-Germany) to separate the bacterial pellet and supernatant, and the pellets were washed with 0.1% sterile peptone water (PW) (Merck, Germany). Each bacterial group (except *B. melitensis*) was collected in separate tubes and the tubes were made up to 10 mL with sterile 0.1% PW and pathogenic bacteria (*Salmonella* spp. mixture, *L. monocytogenes* mixture, *E. coli* O157:H7 mixture and *B. melitensis*) solutions were prepared. These pathogenic bacteria solutions were used for agar well diffusion tests.

Agar-well diffusion challenge, pH and titratable acid analysis

Serial dilutions were made to obtain an inoculation level of approximately 10^6 CFU/mL for each pathogenic bacteria solution. 1 mL of each pathogen solution was taken and placed in separate petri dishes, and 20 mL of Mueller Hinton Agar (MHA) (Biolife, Milano, Italy) was poured over them. After solidification of MHA media, wells with a diameter of 8 mm were punched at certain points on the agar by the help of the blunt part of the sterile pipette tip. After the addition of 100 µl postbiotics obtained from lactic acid bacteria and sterile distilled

water as a control to the wells, the plates were incubated at 37°C for 24 hours. At the end of the incubation, the inhibition zone diameters formed around the wells were measured with the assistance of a digital caliper and the values were recorded.

Then, the pH of the obtained postbiotics was adjusted to 6.0 by using 5 N NaOH solution in order to determine whether the antimicrobial effects of the LAB postbiotics were due to the organic acids or the other metabolites they formed in addition to the organic acids. The antimicrobial effect of postbiotics, whose pH was adjusted to 6.0, were tested with the agar-well diffusion method as described above. The agar-well study was repeated three times and the averages of the inhibition zone diameters were measured.

The pH values of the postbiotics and the homogenates remaining in the stomacher bag after microbiological analysis were measured using a digital pH meter (HI 2211, Hanna Instruments, USA). For the determination of titratable acid that postbiotics contain, a few drops of phenolphthalein indicator solution were added into 10 mL of postbiotic and titrated with 0.25 N NaOH (Merck, Emplura, Darmstadt, Germany) until a light pink color formation was observed. The amount of 0.25 N NaOH spent was multiplied by 10, therefore the amount to be spent for 100 ml of postbiotic was calculated. The result was multiplied by the coefficient of 0.0225 and the values were recorded in g % lactic acid.

Inoculation of chicken breast meat and decontamination experiments

Chicken breast meat was obtained from a local market in their original packaging. Chicken breast meat purchased on the first day of sale was brought to the laboratory by cold chain (≤4°C). By using a sterile forceps and knife, meat was cut into approximately 25 gram pieces and each of them was immersed into a solution containing 10^6–10^7 CFU/mL *Salmonella* spp. and *L. monocytogenes* for 1 minute. After the immersion, breast meat pieces were left on sterile grids for 20 minutes at room temperature to maintain bacterial attachment and remove excess water from the product. Three pieces of the chicken breast meats, contaminated with *Salmonella* spp. and *L. monocytogenes,* were randomly chosen and separated for microbial analyses to determine the initial loads of *Salmonella* spp., *L. monocytogenes*, total psychrotrophic and lactic acid bacteria. The remaining breast meat pieces were divided into 5 groups: control, distilled water (DW), *L. sakei* postbiotic (SP), *L. plantarum* postbiotic (PP), and 2.1% lactic acid group (LA). For the decontamination experiment, *L. sakei* and *L. plantarum* postbiotics were selected, which showed the strongest antimicrobial effect against *Salmonella* spp. and *L. monocytogenes* in agar well diffusion tests. Chicken breast meat pieces in the control group were placed in sterile stomacher bags separately without any treatment and stored at 4°C. Chicken breast meats of DW, SP, PP and LA groups were dipped in beakers containing 250 mL sterile distilled water, *L. sakei* postbiotic, *L. plantarum* postbiotic and 2.1% lactic acid solution for 10 minutes, respectively. At the end of the period, the chicken breast meats removed from the solutions were left at room temperature for 10 minutes to drain the excess liquids, and then they were placed in sterile stomacher bags separately and stored at 4°C. The DW group was used to determine the amount of bacteria removed by the physical effect of water immersion and thus to reveal the actual effects of the decontamination liquids used.

Microbiological analyses

Microbiological analyses were performed on the initial day (day 0), 2, 4, 6, 8, 11, 14 and 17 days of storage. 225 mL of sterile 0.1% PW was added to the sterile stomacher bags containing 25 g chicken breast meat, and a 10^{-1} dilution was prepared by homogenizing in the stomacher for 2 minutes. Serial dilutions up to 10^{-6} were prepared with 0.1% PW by taking 1 mL of homogenate from this dilution. Xylose Lysine Deoxycholate (XLD) Agar and PALCAM agar were used for enumeration of *Salmonella* spp. and *L. monocytogenes*, respectively. Petri dishes were incubated at 35°C for 24−48 hours. De Man, Rogosa and Sharpe (MRS) agar (30°C, 72 h) was used for the determination of lactic acid bacteria, and Plate Count Agar (PCA) (4°C, 7−10 days) was used for psychrotrophic aerobic bacteria.

Statistical analyses

The study was completed by performing three repetitions for each stage. Statistical analyses were performed by using SPSS 24 (IBM SPSS, USA) package program after converting the data to $\log_{10}$ CFU/g value for microbiological analysis, while the pH value and inhibition zones were directly evaluated. Data were subjected to analysis of variance (ANOVA) for main effects and interactions between variables. Statistical significance level was accepted as $P<0.05$.

Results

pH and titratable acid levels of postbiotics

The pH values of postbiotics obtained from *P. acidilactici*, *L. sakei*, *L. plantarum* and LAB mix incubated for 48 hours in MRS broth and in sterile cow's milk were shown in Table 1. There was no difference between LAB postbiotics activated in MRS broth and milk ($P>0.05$). It was determined that the postbiotic obtained from *L. plantarum* grown in MRS broth medium had the lowest pH and the highest % lactic acid. Postbiotics derived from lactic acid bacteria grown in milk were found to have lower lactic acid content than those grown in MRS broth. The pH value of 2.1% lactic acid solution containing almost the same amount of lactic acid as postbiotics was significantly lower than that of postbiotics ($P<0.05$).

Table 1. pH measurements and titratable acidity (g lactic acid) amounts of LAB postbiotics and lactic acid solution (n:3)

	pH	g % lactic acid		
Lactic acid solution	2.23±0.01 y	2.1±0.1 A		
Lactic acid bacteria	MRS broth		UHT cow's milk	
P. acidilactici	3.86±0.03 x	1.9±0.3 A	3.86±0.10	0.9±0.1 B
L. sakei	3.74±0.11 x	2.1±0.1 A	3.90±0.20	0.9±0.0 B
L. plantarum	3.73±0.21 x	2.2±0.1 A	3.90±0.17	0.9±0.0 B
LAB mix	3.76±0.17 x	2.1±0.1 A	3.76±0.16	1.0±0.1 B

A, B: values with different letters in the same line are statistically different (P<0.05).
x, y: values with different letters in the same column are statistically different (P<0.05).

Table 2. Inhibition zones of postbiotics obtained from LAB grown in MRS broth and UHT cow's milk and 2.1% lactic acid solution against pathogenic bacteria (mm±SD) (n:3)

	Inhibition zones (mm±SD)			
	Salmonella spp.	*L. monocytogenes*	*E. coli* O157:H7	*B. melitensis*
2.1% lactic acid	19.0±1.6 Ay	17.9±1.1 Az	17.7±1.0 Az	19.3±0.9 Ay
		MRS broth		
P. acidilactici	18.3±1.5 Ay	17.0±3.0 Az	15.3±1.5 Az	18.0±2.6 Ay
L. sakei	19.7±0.6 ABy	18.3±1.5 Az	21.3±0.6 Bw	19.3±0.6 ABy
L. plantarum	19.3±0.6 Ay	18.7±1.5 Az	20.7±1.2 Aw	18.7±1.2 Ay
LAB mix	19.3±0.6 Ay	18.3±1.2 Az	17.0±2.0 Az	19.0±1.0 Ay
DW (control)*	8.0±0.0 Ax	8.0±0.0 Ax	8.0±0.0 Ax	8.0±0.0 Ax
		UHT milk		
P. acidilactici	8.3±0.6 x	10.7±0.6 y	11.0±1.0 y	10.0±1.7 x
L. sakei	9.0±1.7 x	11.0±0.0 y	11.0±1.0 y	10.0±1.7 x
L. plantarum	9.0±1.7 x	11.3±0.6 y	11.7±1.5 y	9.7±2.1 x
LAB mix	11.0±0.0 x	11.7±0.6 y	11.3±1.2 y	11.0±2.0 x
DW (control)*	8.0±0.0 x	8.0±0.0 x	8.0±0.0 x	8.0±0.0 x

*Well diameter: 8 mm.
A, B: values with different letters in the same line are statistically different (P<0.05).
x y: values with different letters in the same column are statistically different (P<0.05).

Table 3. pH measurement of chicken breast homogenates during cold storage (4°C) (n:3)

Days	Groups				
	Control	Distilled water	*L. sakei*	*L. plantarum*	L.A. (%2.1)
0	5.98±0.04 Ax	6.03±0.07 Ax	5.54±0.11 Bx	5.55±0.09 Bx	5.41±0.05 Bx
2	6.08±0.14 Ax	6.05±0.06 Ax	5.64±0.09 Bxy	5.60±0.14 Bx	5.41±0.18 Bx
4	5.99±0.11 Ax	6.07±0.07 Ax	5.60±0.09 Bxy	5.62±0.07 Bx	5.27±0.15 Cx
6	6.04±0.08 Ax	6.01±0.11 Ax	5.59±0.06 Bxy	5.62±0.12 Bx	5.36±0.07 Cx
8	6.23±0.25 Ax	6.39±0.09 Ay	5.74±0.16 Bxy	5.65±0.19 BCx	5.32±0.09 Cx
11	ND	ND	5.62±0.17 Bxy	5.66±0.34 Bx	5.26±0.12 Bx
14	ND	ND	5.81±0.13 Bxy	5.58±0.02 BCx	5.56±0.10 Cx
17	ND	ND	5.84±0.17 By	5.72±0.10 Bx	5.27±0.20 Cx

ND: Not determined due to spoilage of sample
A, B, C: values with different letters in the same line are statistically different (P<0.05).
x, y: values with different letters in the same column are statistically different (P<0.05).

Agar-well diffusion challenge

It was determined that LAB postbiotics developed in MRS broth formed larger inhibition zones than those obtained from milk (P<0.05) (Table 2). The largest inhibition zone was formed against *E. coli* O157:H7 by the *L. sakei* postbiotic grown in MRS broth (P<0.05). Although no significant difference was observed compared with the postbiotic obtained from *P. acidilactici*, postbiotics of *L. sakei* and *L. plantarum* were found to form a larger inhibition zone against *Salmonella* spp. and *L. monocytogenes*. When the inhibition zone against *Salmonella* spp., *L. monocytogenes* and *B. melitensis* were

compared, there was no difference between postbiotics and 2.1% lactic acid solution (P>0.05). It was observed that *L. sakei* and *L. plantarum* postbiotics were more effective than 2.1% lactic acid solution against *E. coli* O157:H7 (P>0.05). None of the postbiotics adjusted to pH 6.0 with 5 N NaOH formed inhibition zones around the wells (data not shown).

Chicken breast experiments

The initial pH value of chicken breast meat was determined as 5.98 (Table 3). After decontamination with SP, PP and LA solution, a significant decrease was observed in the pH values of the samples (P<0.05). During storage, pH values increased in all groups, except for the LA group. While pH values were above 6.2 for the control and DW groups on the 8th day of storage, pH values were still below 6.0 for the SP, PP and LA groups, even on the 17th day. During storage, the lowest pH values among all groups were detected in the LA group.

On the first day of storage, initial numbers of *Salmonella* spp. were 6.2 and 6.0 $\log_{10}$ CFU/g for the control and DW groups, respectively (Figure 1). It was observed that although decontamination with SP and PP decreased the number of *Salmonella* spp., there was no significant decrease compared to the control and DW groups (P>0.05), while the *Salmonella* count decreased to 4.7 $\log_{10}$ CFU/g in LA group (P<0.05). *Salmonella* count continued to decrease in chicken breast meats decontaminated with LA during storage and was found to be 2.9 $\log_{10}$ CFU/g on the 17th day. Although the number of *Salmonella* spp. was lower for the SP and PP treatments compared to the control and DW groups, no significant difference was observed (P>0.05).

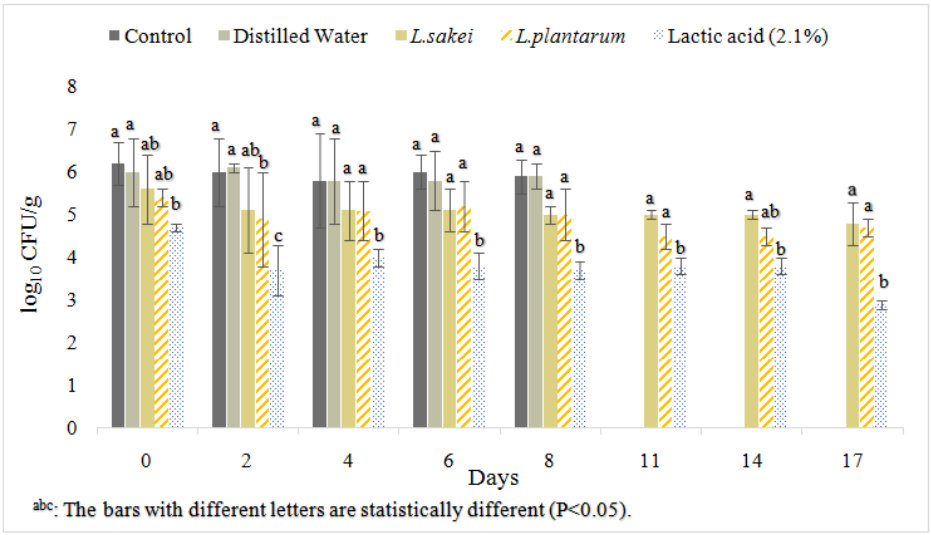

Figure 1. *Salmonella* spp. count of chicken breast meat treated with LAB postbiotics and lactic acid during cold storage (4°C)

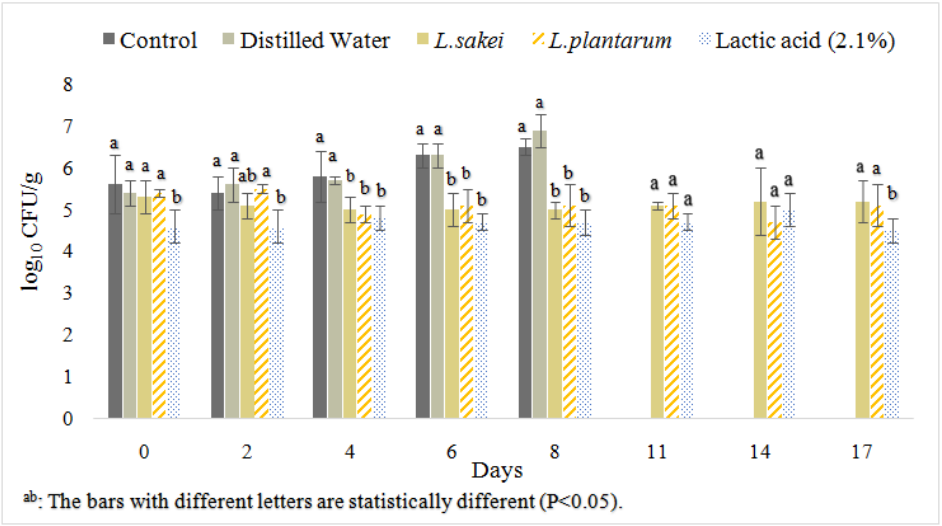

Figure 2. *L. monocytogenes* count of chicken breast meat treated with LAB postbiotics and lactic acid during cold storage (4°C)

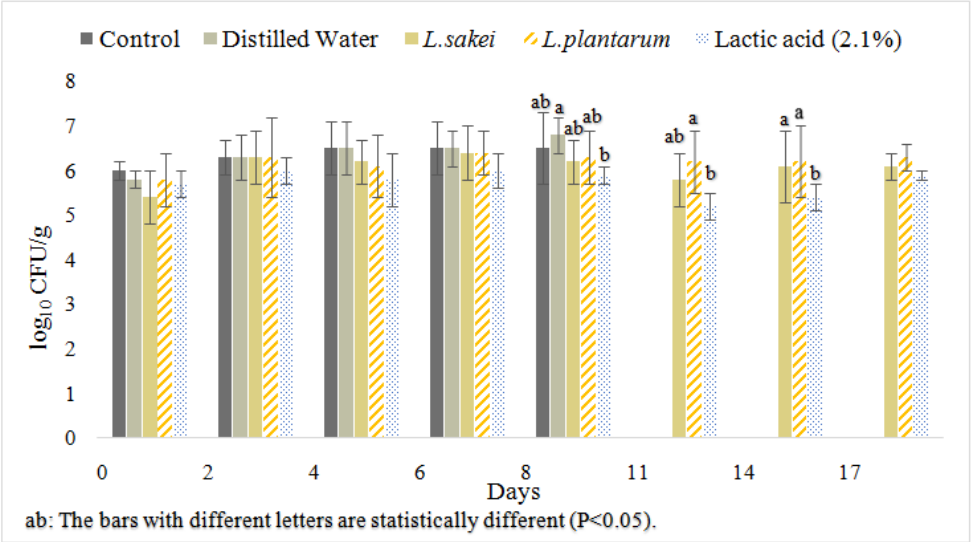

Figure 3. Lactic acid bacteria count of chicken breast meat treated with LAB postbiotics and lactic acid during cold storage (4°C)

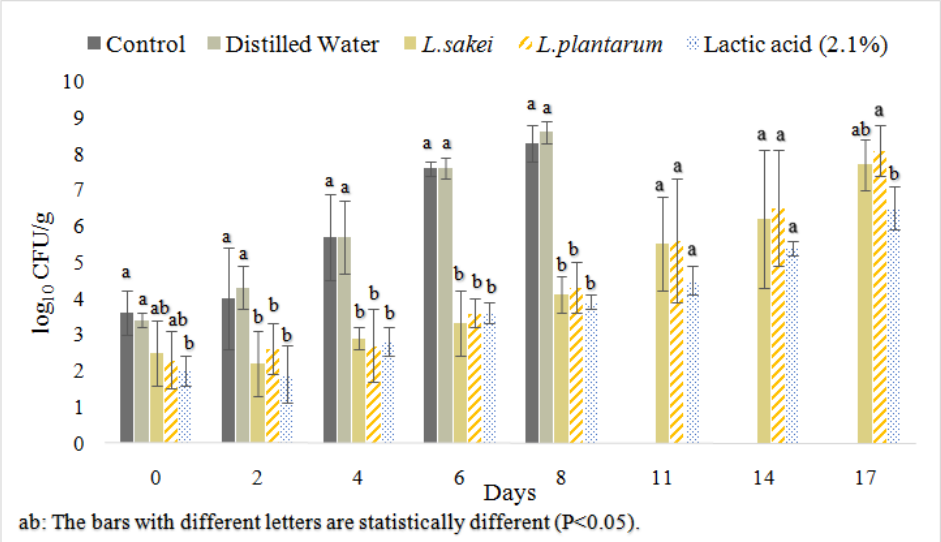

Figure 4. Psychrotrophic bacteria count of chicken breast meat treated with LAB postbiotics and lactic acid during cold storage (4°C)

The initial numbers of *L. monocytogenes* at the beginning of storage in the control and DW groups were determined as 5.6 and 5.4 $\log_{10}$ CFU/g (Figure 2). Decontamination of the chicken breast meat with SP and PP did not cause a significant reduction in the number of *L. monocytogenes* ($P>0.05$), however it dropped to 4.6 $\log_{10}$ CFU/g for the LA group ($P<0.05$). During storage at 4°C, it was determined that the number of *L. monocytogenes* increased continuously for the control and DW groups and there was a difference between the sampling days ($P<0.05$). In SP, PP and LA groups, the number of *L. monocytogenes* remained almost constant throughout the storage period and there was no difference between the days (except for the 2nd and 14th days in the PP group). After the 2nd day of storage, the numbers of *L. monocytogenes* in the SP, PP and LA groups were lower than the control and DW groups ($P<0.05$). On the last day of storage, it was determined that the LA group contained less *L. monocytogenes* than the SP and PP groups ($P<0.05$).

At the beginning of storage, the numbers of lactic acid bacteria for the control and DW groups were determined as 6.0 and 5.8 $\log_{10}$ CFU/g, respectively (Figure 3). Decontamination of the chicken breast meat with SP, PP and LA did not cause a significant decrease in LAB count ($P>0.05$). While there was no difference between the groups in the first 6 days of storage, the LAB count in the DW group (6.8 $\log_{10}$) was found to be significantly

higher than in the LA group (5.9 $\log_{10}$) ($P<0.05$) on the 8th day. While the initial LAB counts in the SP, PP, and LA groups were 5.4, 5.8, and 5.7 $\log_{10}$ CFU/g, respectively, at the end of the 17th day they were found as 6.1, 6.3, and 5.9, respectively, and there was no difference between the days ($P>0.05$).

The numbers of psychrotrophic aerobic bacteria in the control and DW groups were determined as 3.6 and 3.4 $\log_{10}$ CFU/g on day 0, respectively (Figure 4). Although there was no significant difference when compared with the control and DW groups, it was observed that the psychrotrophic bacteria counts were lower for the SP and PP groups (2.5 and 2.3 $\log_{10}$, respectively) ($P>0.05$). In the LA group, the initial psychrotrophic bacteria count (2.0 $\log_{10}$) was significantly lower than in the control and DW groups ($P<0.05$). Psychrotrophic bacteria numbers in the control and DW groups increased rapidly during storage and exceeded 7.0 $\log_{10}$ on the 6th day, and sensory deterioration and putrefaction symptoms were observed in the samples on the 8th day. A slow increase was observed in the SP, PP and lactic acid groups during the storage period, and the numbers of psychrotrophic bacteria in these groups were still below 7.0 $\log_{10}$ CFU/g on the 14th day. On the 17th day of storage, the numbers of psychrotrophic bacteria in the SP and PP groups were 7.7 and 8.1 $\log_{10}$, respectively, while it was 6.5 $\log_{10}$ CFU/g in the group decontaminated with the lactic acid solution ($P<0.05$).

Discussion

In the first stage of the study, the pH and the titratable acidity values of the postbiotics of *P. acidilactici*, *L. sakei*, *L. plantarum* and mixtures of these 3 lactic acid bacteria grown in both MRS broth and sterile milk were determined, and their antimicrobial effect on some food pathogens by agar-well method were detected. As expected, the pH values of the postbiotics decreased as the amount of lactic acid increased (Table 1). Although the titratable acid ratio of the postbiotics of the bacteria grown in MRS broth was found to be higher than those produced in milk ($P<0.05$), no significant difference was observed between the pH values of these two different media ($P>0.05$). As is known, titratable acidity measures the total dissociated and undissociated acids in a solution. The pH is not correlated with the concentration of titratable acids present, but is affected by their ability to dissociate (Tyl and Sadler, 2017). For this reason, there may be no difference between the pH values of the postbiotics of bacteria grown in MRS broth and milk. In addition, this may be due to the different pH buffering capacities of different compounds found in MRS broth and milk. The amount of lactose in the UHT sterile milk purchased from a local market to be used in this study was 4.6% according to the label information. It has been reported that lactic acid bacteria hydrolyze lactose more slowly than glucose, and even the least used sugar by lactic acid bacteria is lactose (Hayek et al., 2019). For these reasons, the amount of lactic acid formed in cow's milk may have been lower than that formed in MRS broth. Similarly, Zalán et al. (2010) compared the titratable organic acids produced by lactic acid bacteria grown in three different media (skimmed milk powder solution, Jerusalem artichoke juice and MRS broth) and found that the amount of lactic acid formed in skimmed milk powder was lower than that in MRS broth.

All the postbiotics obtained from the bacteria grown in MRS broth showed antimicrobial effect on *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7 and *B. melitensis* in the agar-well diffusion method (Table 2). The effect of *P. acidilactici* postbiotic against *L. monocytogenes* by the agar well method is consistent with previous studies (Milillo et al., 2013). Although not statistically significant, the inhibition zones formed by *P. acidilactici* postbiotic were smaller than those formed by *L. sakei* and *L. plantarum* postbiotics. In our study, the titratable acid content of the postbiotic obtained from this bacterium (1.9) was found to be lower than that of *L. sakei* and *L. plantarum* postbiotics, and the pH value (3.86) was higher as expected. On the other hand, homofermentative LAB synthesizes 90−95% lactic acid from glucose, while heterofermentative bacteria are capable of producing 50% of lactic acid together with acetic acid, acetone, CO_2, diacetyl and ethanol (Gunkova et al., 2021). In our study, the amount of % lactic acid in the postbiotics was calculated according to the amount of NaOH spent as a result of acid-base titration of postbiotics. Since the calculation is made by taking into account the dominant organic acid (lactic acid) in the formula, it does not give an idea about the amount of other organic acids produced by lactic acid bacteria. Mun et al. (2019) detected lactic acid (75.2%), acetic acid (17.9%), citric acid (6.5%) and phenyl lactic acid (0.3%) as organic acids in the bacterial filtrate in their study investigating the antimicrobial and antifungal effects of *L. plantarum*. These metabolites may have caused the inhibition zones of heterofermentative *L. sakei* and *L. plantarum* postbiotics to be larger than those of the homofermentative *P. acidilactici* postbiotic. In addition, Habeeb et al. (2021) stated that lactic acid and acetic acid together have a synergistic effect on bacteria. For these reasons, the inhibition zones developed by the postbiotic of *P. acidilactici* against pathogenic bacteria may have been measured smaller than those obtained in *L. sakei* and *L. plantarum*.

In many studies, the acidity of postbiotics has been neutralized by the addition of NaOH to find the metabolite that is the source of the antimicrobial effects of postbiotics (Mani-López et al., 2022; Presti et al., 2015). The absence of a pathogen inhibition in pH-neutralized postbiotics confirms that the metabolite causing antimicrobial activity results from the production of acidic metabolites rather than bacteriocins and other metabolites. In this study, when the pH values of the postbiotics were adjusted to 6.0 with 5 N NaOH, no inhibition zones were detected around the wells. In line with the results ob-

tained, it was concluded that the antimicrobial effects of postbiotics are not caused by bacteriocin or (if any) other metabolites, but by the organic acids they contain. Mani-Lopez et al. (2022) has stated that the antimicrobial effect is mostly due to lactic acid and acetic acid; metabolites such as other organic acids, diacetyl, hydrogen peroxide, bacteriocins, peptides, short and long chain fatty acids also contribute to the antimicrobial effect.

Since there is no study in the literature on the antimicrobial effect of lactic acid bacteria postbiotics against *Brucella* spp., the effect of the LAB postbiotics used in the study against this pathogen was also studied by the agar well method. It was observed that all LAB postbiotics obtained from MRS broth showed antibacterial effect against *B. melitensis* (Table 2). This effect may be due to *Brucella* spp. being easily inhibited in acidic environments. It has been stated that the optimum pH value for the survival and development of *Brucella* spp. is between 6.6 and 7.4 and this bacterium can survive at a minimum pH of 4.1 (Jansen et al., 2019). The pH value of all postbiotics used in our study was below 4.0.

The postbiotics of *L. sakei* and *L. plantarum* grown in MRS broth were selected to apply to chicken breast meat in the second stage of the study, since the postbiotics obtained from lactic acid bacteria grown in milk did not show a strong antimicrobial effect due to the lack of sufficient organic acid. The lactic acid amounts of the postbiotics of *L. sakei* and *L. plantarum* grown in MRS broth were determined as 2.2 and 2.1 g/100 mL, respectively (Table 1). To compare the antimicrobial effects obtained from these postbiotics, 2.1% commercial lactic acid solution was prepared and used.

Compared to the control group, *Salmonella* spp. decreased by 0.6, 0.8, and 1.5 $\log_{10}$ after decontamination of chicken breast meat with SP, PP, and LA, respectively (Figure 1). Anang et al. (2007) found that the number of *S.* Enteritidis decreased by 0.9 $\log_{10}$ in chicken breast meats dipped in 2% lactic acid solution for 10 min and they reported that the number of *Salmonella* in decontaminated samples did not change significantly over 14 days. Edris et al. (2020) found approximately 1.1 and 2.1 $\log_{10}$ reductions in the number of *S.* Enteriditis after decontamination of chicken meat with 1% and 2% lactic acid for 1 min, respectively. In the present study, the number of Salmonella spp. in chicken breast meat immersed in 2.1% lactic acid solution for 10 min decreased by 1.5 $\log_{10}$ compared to the control group on the first day of storage ($P<0.05$). On the 2nd day of storage, the level of reduction was found to be 2.5 $\log_{10}$ compared to the control group. When the results of other researchers who conducted decontamination studies with lactic acid at 1−2% concentrations in chicken meat are examined, it is seen that the antimicrobial effect is generally effective on the first and the following day of decontamination, and there is no significant change in the number of *Salmonella* in the remaining days of storage (Habeeb et al., 2021; İlhak et al., 2017; Cosansu and Ayhan, 2012).

The antimicrobial effects of SP and PP treatments on *L. monocytogenes* were significant ($P<0.05$) after the 4th day of storage compared to the control and DW groups (Figure 2). Although there was an increase in the number of *L. monocytogenes* in the control and DW treated groups during the storage period, no change was observed in the number of this bacterium in the groups decontaminated with SP and PP. Therefore, it can be said that this difference is due to the bacteriostatic effect, not the bactericidal effect of LAB postbiotics against *L. monocytogenes*. After decontamination with 2.1% lactic acid solution, the number of *L. monocytogenes* decreased by 1.0 $\log_{10}$ compared to the control group ($P<0.05$), and similar to the postbiotic decontaminations, the number of this pathogen remained almost constant in the remaining days of storage. It was observed that the pH values of the control and DW groups were close to neutral (≥6.0), while the samples treated with postbiotics and lactic acid were lower. It has been stated that *L. monocytogenes* is highly resistant to low pH values (İncili et al., 2020; Nyhan et al., 2018) and its growth slows down in acidic environments and enters the stationary phase (Buchanan et al., 1993).

LAB, which play a role in the deterioration of raw chicken meat, can multiply under refrigeration conditions (Jay et al., 2008). However, no significant change was observed in the LAB counts of the chicken breast meat in all groups until on day 8 ($P>0.05$) (Figure 3). There was a slight increase in the LAB counts in all groups except the lactic acid treated group, and differences were observed between the 8th and 14th days of storage in the groups due to the fluctuation in the LAB counts ($P<0.05$).

In our study, the initial number of psychrotrophic bacteria in chicken breast meat was found to be 3.6 $\log_{10}$ CFU/g (Figure 4). The number of psychrotrophic bacteria in the control and DW groups increased rapidly in the first 6 days of storage and exceeded 7.0 $\log_{10}$ CFU/g. When the number of aerobic bacteria in raw meat products exceeds 7.0 $\log_{10}$ CFU/g, the product is considered spoiled (Jay et al., 2008). Although the expression "aerobic number" refers to the total viable count (the number of mesophilic aerobic bacteria), Jay et al. (2008) reported that *Pseudomonas* spp. form the dominant flora in spoiled chicken meats stored at refrigerator temperature and that these bacteria are psychrotrophic. In the present study, It was observed that psychrotrophic bacteria exceeded this value on the 6th day in the DW group and the control group, and the product showed clear signs of putrefaction and deterioration on the 8th day. Therefore, analyses were not performed in the control and DW groups after the 8th day of storage. There was a decrease in the number of psychrotrophic bacteria in the postbiotic treated samples compared to the control and DW groups, and these decreases were significant on the 2nd day of storage ($P<0.05$). On the 8th day of storage, psychrotrophic bacteria counts were over 8.0 $\log_{10}$ CFU/g in the control and DW groups, while it was found as 4.1, 4.3 and 3.9 $\log_{10}$ CFU/g in chicken breast meats treated with

SP, PP and LA, respectively. While the numbers of psychrotrophic bacteria in SP and PP treated chicken breast meats were still below 7.0 $\log_{10}$ CFU/g on the 14th day of storage, it showed a value close to 8.0 $\log_{10}$ CFU/g on day 17 of storage. The number of psychrotrophic bacteria in chicken breast meats treated with LA was 6.5 $\log_{10}$ CFU/g on the 17th day of storage.

Jo et al. (2021) reported that cell-free supernatants of lactic acid bacteria (*L. plantarum* and *P. stilesii*) extended the shelf life of fresh fish fillets stored in the refrigerator, and also did not affect the physico-chemical properties and sensory quality of the product. İncili et al. (2021) reported that postbiotics and their combinations with natural preservatives may be an alternative approach to reduce the food-borne pathogens and to extend the shelf-life of poultry meat and meat products, and they (İncili et al., 2023) have also reported that whole cell postbiotic extended the shelf life of chicken breast fillets during storage at 4°C by retarding microbial and chemical deteriorations. Jaspal et al. (2021) were able to extend the shelf life of chicken breast fillets up to 12 days by spraying 1.25% lactic acid solution, and they stated that taste and odor remained within acceptable limits during storage. Considering our study and other studies in the literature, it is seen that decontamination with lactic acid and lactic acid bacteria postbiotics extend the shelf life of chicken meat. The differences in shelf life of the samples in the studies may be due to the differences in lactic acid concentrations, decontamination method and exposure time.

It was observed that postbiotic and lactic acid applications decreased the pH of chicken breast meat ($P<0.05$) (Table 3). Although the pH values of the control, DW, SP and PP groups increased during the storage period, pH values of the SP, PP and LA treated samples remained lower than the control and DW groups ($P<0.05$). The increase in the pH values in chicken breast meat treated with DW, SP, PP and the control groups may be due to the chemical deterioration process, and it may also have resulted from the accumulation of ammonium-containing metabolites as a result of aerobic bacterial growth. Compared to the other groups, the lower pH value in the LA-treated chicken breast meat can be explained by the slower increase in the number of psychrotrophic bacteria in this group and by the fact that the number of psychrotrophic bacteria in this group did not exceed 7 $\log_{10}$ CFU/g during storage.

Appearance is one of the main criteria that determines the final consumer's decision to purchase chicken meat. In our study, as a result of observation made with the naked eye immediately after the decontamination process, it was seen that chicken breast meat treated with lactic acid was lighter in color than control and DW groups, while chicken breast meat treated with postbiotics was darker. Swatland (2008) has reported that low pH chicken breast meat appears pale, while higher pH chicken breast meat appears darker. In the present study, since the pH value of chicken breast meat treated with lactic acid was found to be significantly lower than those of the control and DW groups ($P<0.05$), the color differences between the groups can be attributed to this reason. Although the pH values of the chicken breast meat treated with LAB postbiotics were lower than the control and DW groups, it was noted that the postbiotics treated chicken breast meat was darker than the control and DW groups. The reason of this may be that the postbiotics obtained from MRS broth acquired their original color from this medium and passed this color on to chicken meat. İncili et al. (2021) reported that they did not detect any color change after decontamination in chicken meat that they decontaminated with the postbiotic of *P. acidilactici* grown in tryptic soy broth (TSB) medium. This difference between studies may be due to the use of different microbial media. However, in another study (2023), the same authors reported that they observed a browning in the color of chicken breast meat when they used postbiotics obtained from MRS broth. This finding is consistent with our finding. Mani-López et al. (2022) reported that they observed a dark and brownish color in raw beef pieces to which they applied a 10-fold concentrated cell-free supernatant of *L. plantarum*. In the present study, it was determined that the color difference between the chicken breast meat treated with postbiotics and the control and DW groups diminished after the second day of storage, and they were very similar to each other. It is thought that this may be due to the loss of color properties as a result of oxidation of the colorants originating from MRS broth during storage.

Conclusion

It has been concluded that *L. sakei* and *L. plantarum* postbiotics have antimicrobial effects on *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7 and *B. melitensis* by agar diffusion method, and these antimicrobial effects are due to the organic acids they contain. The types and amounts of organic acids contained in postbiotics should be studied in more detail and their use in foods should be evaluated. In addition, it was concluded that these bacteria could not produce enough organic acids and other antimicrobial substances in cow's milk.

The postbiotics of *L. sakei* and *L. plantarum* reduced the number of *Salmonella* spp. in chicken breast meat by 0.9 $\log_{10}$ CFU/g and showed a bacteriostatic effect on *L. monocytogenes*. Compared to the control groups, it caused a decrease in the number of psychrotrophic bacteria in the chicken breast meat flora at the beginning and a slowdown in the increase of these bacteria in the following days. In our study, it was concluded that *L. sakei* and *L. plantarum* postbiotics applied to chicken breast meat for decontamination extended the shelf life of the product up to 14 days. As a result, it is thought that LAB postbiotics will play an important role in extending the shelf life of foods by using them in appropriate foodstuffs.

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Conflict of interest

The authors have no conflicts of interest to declare.

References

Anang D.M., Rusul G., Bakar J., Ling F.H. (2007). Effects of lactic acid and lauricidin on the survival of *Listeria monocytogenes*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7 in chicken breast stored at 4°C. Food Control, 18: 961–969.

Balthazar C.F., Guimarães J.F., Coutinho N.M., Pimentel T.C., Ranadheera C.S., Santillo A., Albenzio M., Cruz A.G., Sant'Ana A.S. (2022). The future of functional food: Emerging technologies application on prebiotics, probiotics and postbiotics. Compr. Rev. Food Sci. Food Saf., 21: 2560–2586.

Barros C.P., Guimarães J.T., Esmerino E.A., Duarte M.C.K.H., Silva M.C., Silva R., Ferreira B.M., Sant'Ana A.S., Freitas M.Q., Cruz A.G. (2020). Paraprobiotics and postbiotics: Concepts and potential applications in dairy products. Curr. Opin. Food Sci., 32: 1–8.

Bhoir S.A., Jhaveri M., Chawla S.P. (2019). Evaluation and predictive modeling of the effect of chitosan and gamma irradiation on quality of stored chilled chicken meat. J. Food Process. Eng., 42: 13254.

Buchanan R.L., Golden M.H., Whiting R.C. (1993). Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. J. Food Prot., 56: 474–478.

Cosansu S., Ayhan K. (2012). Effects of lactic and acetic acid on survival of *Salmonella* Enteritidis during refrigerated and frozen storage of chicken meats. Food Bioproc. Tech., 5: 372–377.

Cuevas-González P.F., Liceaga A.M., Aguilar-Toalá J.E. (2020). Postbiotics and paraprobiotics: From concepts to applications. Food Res. Int., 136: 109502.

Edris A., Amin R., Eleiwa N., Elnagar R. (2020). Lactic and acetic acids as bacterial decontaminators on chicken carcasses. Benha Med. J., 39: 158–60.

Godoy C.L.A., Costa L.M., Guerra C.A., Oliveira V.S., Paula B.P., Lemos Junior W.J.F., Silva V.D., Luchese R.H., Bautitz I.R., Guerra A.F. (2022). Potentially postbiotic-containing preservative to extend the use-by date of raw chicken sausages and semifinished chicken products. Sustainability, 14: 2646.

Gunkova P.I., Buchilina A.S., Maksimiuk N.N., Bazarnova Y.G., Girel K.S. (2021). Carbohydrate fermentation test of lactic acid starter cultures. IOP Conference Series, Earth Environ. Sci., 852: 012035.

Habeeb G.A., Durmuşoğlu H., İlhak O.İ. (2021). The combined effect of sodium lactate, lactic acid and acetic acid on the survival of *Salmonella* spp. and the microbiota of chicken drumsticks. Slov. Vet. Res., 58: 47–54.

Hayek S.A., Gyawali R., Aljaloud S.O., Krastanov A., Ibrahim S.A. (2019). Cultivation media for lactic acid bacteria used in dairy products. J. Dairy Res., 86: 490–502.

İlhak O.İ., İncili G.K., Durmuşoğlu H. (2017). Evaluation of effect of thymol combined with lactic acid or sodium lactate on psychrophilic bacteria and *Salmonella* spp. on chicken drumstick. Ann. Anim. Sci., 17: 271–280.

İlhak O.İ., İncili G.K., Durmuşoğlu H. (2018). Effect of some chemical decontaminants on the survival of *Listeria monocytogenes* and *Salmonella* Typhimurium with different attachment times on chicken drumstick and breast meat. J. Food Sci. Technol., 55: 3093–3097.

İncili G.K., Akgöl M., Aydemir M.E., Alan S., Mutlu M., İlhak O.İ., Öksüztepe G. (2020). Fate of *Listeria monocytogenes* and *Salmonella* Typhimurium in homemade marinade and on marinated chicken drumsticks, wings and breast meat. LWT – Food Sci. Technol., 134: 110231.

İncili G.K., Karatepe P., Akgöl M., Kaya B., Kanmaz H., Hayaloğlu A.A. (2021). Characterization of *Pediococcus acidilactici* postbiotic and impact of postbiotic-fortified chitosan coating on the microbial and chemical quality of chicken breast fillets. Int. J. Biol. Macromol., 184: 429–437.

İncili G.K., Karatepe P., Akgöl M., Güngören A., Koluman A., İlhak O.İ., Kanmaz H., Kaya B., Hayaloğlu A.A. (2022). Characterization of lactic acid bacteria postbiotics, evaluation in-vitro antibacterial effect, microbial and chemical quality on chicken drumsticks. Food Microbiology, 104: 104001.

İncili G.K., Akgöl M., Karatepe P., Tekin A., Kanmaz H., Kaya B., Hayaloğlu A.A. (2023). Whole-cell postbiotics: An innovative approach for extending the shelf life and controlling major foodborne pathogens in chicken breast fillets. Food Bioproc. Tech. Early Access.

Ishangulyyev R., Kim S., Lee S.H. (2019). Understanding food loss and waste. Why are we losing and wasting food? Foods, 8: 297.

Jansen W., Linard C., Noll M., Nöckler K., Al Dahouk S. (2019). Brucella-positive raw milk cheese sold on the inner European market: A public health threat due to illegal import? Food Control, 100: 130–137.

Jaspal M.H., Ijaz M., ul Haq H.A., Yar M.K., Asghar B., Manzoor A., Badar I.H., Ullah S., Islam M.S., Hussain J. (2021). Effect of oregano essential oil or lactic acid treatments combined with air and modified atmosphere packaging on the quality and storage properties of chicken breast meat. LWT – Food Sci. Technol., 146: 111459.

Jay J.M., Loessner M.J., Golden D.A. (2008). Modern food microbiology: Springer Science & Business Media.

Jo D.M., Park S.K., Khan F., Kang M.G., Lee J.H., Kim Y.M. (2021). An approach to extend the shelf life of ribbonfish fillet using lactic acid bacteria cell-free culture supernatant. Food Control, 123: 107731.

Mani-López E., Arrioja-Bretón D., López-Malo A. (2022). The impacts of antimicrobial and antifungal activity of cell-free supernatants from lactic acid bacteria *in vitro* and foods. Compr. Rev. Food Sci. Food Saf., 21: 604–641.

Milillo S.R., Story R.S., Pak D., O'Bryan C.A., Crandall P.G., Ricke S.C. (2013). Antimicrobial properties of three lactic acid bacterial cultures and their cell free supernatants against *Listeria monocytogenes*. J. Environ. Sci. Health B, 48: 63–68.

Moradi M., Kousheh S., Almasi H., Alizadeh A., Guimarães J., Toy N., Lotfi Javid A. (2020). Postbiotics produced by lactic acid bacteria: The next frontier in food safety. Compr. Rev. Food Sci. Food Saf., 19: 3390–3415.

Mun S.Y., Kim S.K., Woo E.R., Chang H.C. (2019). Purification and characterization of an antimicrobial compound produced by *Lactobacillus plantarum* EM showing both antifungal and antibacterial activities. LWT − Food Sci. Technol., 114: 108403.

Nyhan L., Begley M., Mutel A., Qu Y., Johnson N., Callanan M. (2018). Predicting the combinatorial effects of water activity, pH and organic acids on *Listeria* growth in media and complex food matrices. Food Microbiol., 74: 75–85.

Pelyuntha W., Vongkamjan K. (2022). Combined effects of *Salmonella* phage cocktail and organic acid for controlling *Salmonella* Enteritidis in chicken meat. Food Control, 133: 108653.

Petrou S., Tsiraki M., Giatrakou V., Savvaidis I.N. (2012). Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. Int. J. Food Microbiol., 156: 264–271.

Presti I., D'Orazio G., Labra M., La Ferla B., Mezzasalma V., Bizzaro G., Giardina S., Michelotti A., Tursi F., Vassallo M., Di Gennaro P. (2015). Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their *in vitro* effect. Appl. Microbiol. Biotechnol., 99: 5613–5626.

Radha K.K., Babuskin S., Azhagu S.B.P., Sasikala M., Sabina K., Archana G., Sivarajan M., Sukumar M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. Int. J. Food Microbiol., 171: 32–40.

Rahnemoon P., Sarabi-Jamab M., Bostan A., Mansouri E. (2021). Nano-encapsulation of pomegranate (*Punica granatum* L.) peel extract and evaluation of its antimicrobial properties on coated chicken meat. Food Biosci., 43: 101331.

Sabo S.S., Pérez-Rodríguez N., Domínguez J.M., Oliveira R.P.S. (2017). Inhibitory substances production by *Lactobacillus plantarum* ST16Pa cultured in hydrolyzed cheese whey supplemented with soybean flour and their antimicrobial efficiency as biopreser-

vatives on fresh chicken meat. Food Res. Int., 99: 762–769.
Salanță L.C., Cropotova J. (2022). An update on effectiveness and practicability of plant essential oils in the food industry. Plants, 11: 2488.
Sohaib M., Anjum F.M., Arshad M.S., Rahman U.U. (2016). Postharvest intervention technologies for safety enhancement of meat and meat based products; a critical review. J. Food Sci. Technol., 53: 19–30.
Swatland H.J. (2008). How pH causes paleness or darkness in chicken breast meat. Meat Sci. 80: 396–400.
Thorakkattu P., Khanashyam A.C., Shah K., Babu K.S., Mundanat A.S., Deliephan A., Deokar G.S., Santivarangkna C., Nirmal N.P. (2022). Postbiotics: Current trends in food and pharmaceutical industry. Foods, 11: 3094.
Tyl C., Sadler G.D. (2017) pH and titratable acidity. In: Food Analysis, Nielsen S.S. (ed.). Springer International Publishing. Switzerland, pp. 389–406.
WHO (2015). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007−2015. Geneva: World Health Organization. www.who.int
Yolmeh M., Khomeiri M., Ahmadi Z. (2017). Application of mixture design to introduce an optimum cell-free supernatant of multiple-strain mixture (MSM) for *Lactobacillus* against food-borne pathogens. LWT − Food Sci. Technol., 83: 298–304.
Zalán Z., Hudáček J., Štětina J., Chumchalová J., Halász A. (2010). Production of organic acids by *Lactobacillus* strains in three different media. Eur. Food Res. Technol., 230: 395–404.

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