

THE COMBINED EFFECT OF SODIUM LACTATE, LACTIC ACID AND ACETIC ACID ON THE SURVIVAL OF *Salmonella* spp. AND THE MICROBIOTA OF CHICKEN DRUMSTICKS

Goran Ali Habeeb¹, Halil Durmuşoğlu², Osman İrfan İlhak^{3*}

¹Sulaimani Veterinary Directorate, Veterinary Quarantine, International Border Compound of Bashmakh, Sulaimani, Iraq, ²Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, ³Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Balikesir University, Balikesir, Turkey

*Corresponding author, E-mail: irfan.ilhak@balikesir.edu.tr

Abstract: The poultry processing industry has been investigating the new decontamination applications to prevent foodborne pathogens and extend the shelf life of poultry products. This study investigates the effects of lactic acid, acetic acid and sodium lactate, alone and in combination, on the survival of *Salmonella* spp. and the shelf life of chicken drumsticks. The fresh chicken drumsticks were inoculated with *Salmonella* Typhimurium and *Salmonella* Enteritidis and they were divided into groups as control (sterile tap water), 1% sodium lactate (SL), 1.5% lactic acid (LA), 1.5% acetic acid (AA), and their combinations. The drumstick samples were immersed into the treatment solutions for 5 minutes and stored at 4 °C for eight days, and they were analyzed for aerobic psychrotrophic bacteria (APB), *Pseudomonas* spp., lactic acid bacteria (LAB), *Salmonella* spp. and pH level. On day 5, APB, *Pseudomonas* spp. and LAB numbers exceeded 7.0 log₁₀ CFU ml⁻¹ in the control, SL, LA and LA+ SL groups. The reduction levels of *Salmonella* spp. were 1.2 and 0.9 log₁₀ CFU ml⁻¹ in the LA and AA+LA groups on day 0, and they were significantly different from the control group (P<0.05). The shelf life of the chicken drumsticks that were treated with the solutions containing 1.5% AA (AA, AA+SL, AA+ LA and AA+ LA+ SL) was at least two days longer than the control group. It is concluded that the combinations of 1.5% LA, 1.5% AA and 1% SL can be used to reduce the number of *Salmonella* spp. and to extend the shelf life of chicken drumstick.

Key words: chicken drumstick; lactic acid; acetic acid; sodium lactate; Shelf life; *Salmonella* spp.

Introduction

Chicken meat is one of the important animal protein sources in human diet. However, if raw chicken meat is not properly handled and preserved, it supports the growth of spoilage bacteria and foodborne pathogens such as *Salmonella* spp. (1, 2). Guran et al. (3) found that *Salmonella* spp. prevalence in the skin of chicken drumstick, chicken breast and thighs were 41%, 44.7% and 40.9%, respectively. In Turkey, the data from National *Salmonella* spp. Control Program, which was conducted between 2014 and

2017, revealed that 47% of the carcass samples (n= 691) taken from chicken slaughterhouses were contaminated with *Salmonella* spp. (4). The investigations to find effective applications to control *Salmonella* spp. in poultry carcasses and poultry products have still been continuing. On the other hand, there is increasing concern about the use of chemical preservatives in foodstuff by consumers. More and more people prefer minimally processed foods or foods treated with organic preservatives (5).

Lactic acid (LA) is one of the organic acids naturally occurring in muscles. Sodium lactate (SL) is the sodium salt of lactic acid. Acetic acid (AA), which is commonly known as vinegar, is one of the organic acids that occurs naturally during

the spoilage of fruit and certain other foods by the bacteria of the genus *Acetobacter*. LA, SL and AA are substances affirmed as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA), and they are allowed to be directly added to various foods to inhibit the microbial growth and to extend the shelf life of products (6). The antimicrobial efficacy of SL, LA and AA has been intensely studied by many researchers (7-10). However, there is limited information related to effect of organic acid blends on the shelf life of chicken meat parts and the survival of *Salmonella* spp. on chicken meat. Some researchers have studied the effects of various organic acid blends against the pathogenic microorganisms on poultry carcasses (11-15), and they have noted that the combinations of organic acids may improve the microbiological quality of poultry meat. In meat processing environments, the poultry meat microbiota consists mainly of *Pseudomonas* spp., lactic acid bacteria and many other psychrotrophic bacteria (16). There is limited information regarding the effect of organic acid blends on the microbiota in chicken meat.

The aims of this study were (i) to evaluate the antimicrobial efficacy of the combination of sodium lactate, lactic acid and acetic acid against aerobic psychrotrophic bacteria, *Pseudomonas* spp., lactic acid bacteria and *Salmonella* spp. on chicken drumstick, and (ii) to investigate the shelf life of chicken drumstick that are treated with the blends of sodium lactate, lactic acid and acetic acid.

Materials and methods

Samples and preparation of Salmonella spp. inoculum

In each of the three trials, 36 fresh chicken drumsticks with skin (each one weighed 100 - 150 gram) were used. Their production date was one or two days before the purchase date from a local supermarket. Throughout the experiments, a total of 108 chicken drumsticks were used.

Because of the differences in bacterial strains against antimicrobials, one salmonella cocktail culture, which was composed of one *Salmonella* Enteritidis (RSKK 92 (RSKK is a microorganism culture collection center in Turkish Public Health – Turkey)) and two *Salmonella* Typhimurium (NCTC

12416 and NCTC 74) strains, were used. Each of the *Salmonella* strains was grown in 10 ml of tryptic soy broth (TSB, Acumedia, Maryland) at 37°C for 18 h. Cultures were then centrifuged at 4192 × g for 10 minutes at 5°C, and the supernatant was discarded. The formed pellets were re-suspended in 10 ml of 0.1% sterile peptone water, and then they were centrifuged again to remove the organic residues. The supernatant was removed and the pellets of each strain were re-suspended in 1-2 ml of 0.1% sterile peptone water. These suspensions were combined in a single tube to obtain the salmonella cocktail, and the salmonella cocktail tube was completed to 10 ml with 0.1% sterile peptone water.

Inoculation of chicken drumstick

Before the inoculation procedure, two randomly selected drumstick samples were taken, and tested for the existence of indigenous *Salmonella* spp.. For the inoculation, the *Salmonella* spp. cocktail of 0.25 ml was spread on each of the drumstick samples using a sterile L-shaped spreader. After inoculation, the drumsticks were kept at room temperature for 10 minutes for bacterial attachment. Then, the two samples were taken and analyzed to detect the initial inoculation numbers of *Salmonella* spp.

Decontamination treatments

Sodium lactate solution (60% w/w) (CAS number 72-17-3), L(+) - Lactic acid solution (88-92%) (CAS number: 79-33-4), and Acetic acid (100%) (CAS number: 64-19-7) were used in this study, and they were purchased from Sigma (Sigma-Aldrich, Germany).

The chicken samples in each trial were divided into eight groups. Each group of drumstick samples was dipped into a sterile glass beaker containing 500 ml of one of the following sterile decontamination solutions (v/v) at ambient temperature for 5 minutes. Decontamination solutions (treatment groups) and their pH values were as follows:

- 1- Control (sterile tap water),
- 2- 1.5% Lactic acid (pH 2.3),
- 3- 1.5% Acetic acid (pH 2.75),
- 4- 1% Sodium lactate (pH 6.96),
- 5- 1.5% Lactic acid + 1% Sodium lactate (pH 3.33),

- 6- 1.5% Acetic acid + 1% Sodium lactate (pH 3.72),
- 7- 1.5% Lactic acid + 1.5% Acetic acid (pH 2.20),
- 8- 1.5% Lactic acid + 1.5% Acetic acid + 1% Sodium lactate (pH 3.28)

After the decontamination procedures, the chicken drumsticks were allowed to drain at room temperature for 10 minutes. After draining, each drumstick was individually placed in a separate foam plate, and it was wrapped with cling film and stored at 4°C for eight days.

Microbiological analysis

Microbiological analyses were carried out on days 0 (after the dipping treatment), 3, 5 and 8. Briefly, 100 ml of 0.1% sterile peptone water (PW) was added into a sterile stomacher bag containing a chicken drumstick sample, and the stomacher bag was shaken manually for one minute. Then, 1 ml of the rinse solution was taken from the stomacher bag and serially diluted up to 10^{-6} in sterile tubes containing 9 ml of 0.1% PW, and they were used for the following microbial analyses.

Microbiological analyses were conducted by using the spread plate method and duplicate plates. Since a 1 ml rinse solution was used for microbiological analysis, the numbers of microorganisms were expressed as \log_{10} CFU per ml rinse solution in all the samples.

Plate Count Agar (Merck, Germany) was used to enumerate Aerobic Psychrotrophic Bacteria (APB), and the plates were incubated at 6.5°C for 10 days. *Pseudomonas* Selective Agar (Merck, Germany) supplemented with *Pseudomonas* CFC Selective Supplement (Merck, Germany) was used to detect *Pseudomonas* spp., and the plates were incubated at 25°C for 2 days. After the incubation period, three colonies from each plate were randomly selected and subjected to oxidase test (Bactident Oxidase, Merck, Germany). According to the results obtained in the oxidase test (*Pseudomonas* spp. is oxidase positive), the numbers of *Pseudomonas* spp. were calculated. Lactic acid bacteria (LAB) were enumerated using de Man Rogosa and Sharpe (MRS) Agar (LAB-M, Merck, Germany), and the plates were incubated at 28°C for 2 days. *Salmonella* spp. was enumerated using Xylose Lysine Deoxycholate (XLD) agar (HiMedia, India), and the plates were incubated at 35°C for 24-36 h.

Determination of pH values of the samples

After the microbiological analysis was completed, the pH values of the rinse solution of the samples were measured by using a pH meter (Selecta pH 2001, J.P., Spain).

Statistical analysis

Analyses of the microbiological data and pH values of three independent trials were carried out using SPSS 22 software (IBM, SPSS Statistics, Version 22, USA). The numbers of bacteria were converted to logarithmic values (\log CFU ml^{-1} rinse solution) before calculating means and performing statistical analysis. The data were subjected to analysis of variance (ANOVA) appropriate to replicate \times treatment groups \times sampling times to determine fixed effects and interactions between variables. The Bonferroni test was used for multiple comparisons between the groups. Statistical significant level was expressed as $P < 0.05$.

Results

The decontamination treatment with 1% sodium lactate (SL) did not show bacteriostatic or bactericidal effect on *Salmonella* spp. or microbiota in the chicken drumsticks; however APB, *Pseudomonas* spp. and LAB counts in the groups that were treated with 1.5% lactic acid (LA), 1.5% acetic acid (AA) and their combination were lower than those in the control and SL groups (Table 1).

On day 0, the number of APB and *Pseudomonas* spp. counts in the groups that were treated with AA, AA+LA and AA+LA+SL were between 3.9 and 4.4 \log_{10} CFU ml^{-1} rinse solution, and they were statistically different from the control group (5.4 \log_{10} CFU ml^{-1}) ($P < 0.05$). On day 5, APB, *Pseudomonas* and LAB counts were below 7.0 \log_{10} CFU ml^{-1} rinse solution in the groups that were treated with AA, AA+LA and AA+LA+SL, and they were significantly different from the SL and control groups ($P < 0.05$). During the five days storage, the results showed that *Pseudomonas* numbers of the groups containing AA were numerically lower than the other groups that did not contain AA (Table 1). The drumstick samples in the control, SL and LA+SL groups had a bad odor and a slight slime layer on day 5. Their APB and *Pseudomonas* counts were above 7.2 \log_{10} CFU ml^{-1} rinse solution. The control, SL and LA+SL groups were not analyzed on day 8 because of their apparent sensorial defect.

Table 1: The mean numbers of APB, *Pseudomonas* spp. and LAB of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C (\log_{10} CFU ml⁻¹ rinse solution \pm SD)

Treatment Groups	Storage days			
	0	3	5	8
Aerobic Psychrotrophic Bacteria				
Control	5.4 ^{BCv} \pm 0.2	6.9 ^{Cw} \pm 0.2	7.8 ^{CDx} \pm 0.2	NA
SL	5.7 ^{Cv} \pm 0.1	6.9 ^{Cw} \pm 0.2	8.1 ^{Dx} \pm 0.2	NA
LA	4.9 ^{ABv} \pm 0.3	6.3 ^{BCw} \pm 0.3	7.2 ^{ABCx} \pm 0.1	8.2 ^{Ay} \pm 0.2
AA	4.4 ^{Av} \pm 0.2	5.5 ^{Aw} \pm 0.5	6.8 ^{ABx} \pm 0.1	7.9 ^{Ay} \pm 0.3
LA+ SL	4.9 ^{ABv} \pm 0.1	6.4 ^{BCw} \pm 0.2	7.5 ^{BCDx} \pm 0.2	NA
AA+ SL	4.7 ^{ABv} \pm 0.1	5.8 ^{ABw} \pm 0.2	7.0 ^{ABCx} \pm 0.1	8.3 ^{Ay} \pm 0.4
AA+ LA	4.2 ^{Av} \pm 0.3	5.6 ^{ABw} \pm 0.2	6.5 ^{Ax} \pm 0.1	7.8 ^{Ay} \pm 0.1
AA+LA+ SL	4.2 ^{Av} \pm 0.3	5.6 ^{ABw} \pm 0.1	6.6 ^{Ax} \pm 0.2	7.8 ^{Ay} \pm 0.2
<i>Pseudomonas</i> spp.				
Control	5.4 ^{BCw} \pm 0.4	6.8 ^{Cx} \pm 0.4	7.9 ^{By} \pm 0.4	NA
SL	5.7 ^{Cw} \pm 0.5	6.9 ^{Cx} \pm 0.4	7.9 ^{By} \pm 0.2	NA
LA	4.2 ^{Aw} \pm 0.2	5.6 ^{ABx} \pm 0.3	7.0 ^{ABY} \pm 0.2	7.5 ^{Ay} \pm 0.1
AA	3.9 ^{Aw} \pm 0.1	5.4 ^{ABx} \pm 0.4	6.4 ^{Ay} \pm 0.2	7.6 ^{Az} \pm 0.2
LA+ SL	4.7 ^{ABw} \pm 0.1	5.8 ^{Bx} \pm 0.3	7.2 ^{ABY} \pm 0.3	NA
AA+ SL	4.0 ^{Aw} \pm 0.3	5.2 ^{ABx} \pm 0.2	6.7 ^{Ay} \pm 0.2	7.5 ^{Az} \pm 0.2
AA+ LA	4.1 ^{Av} \pm 0.4	5.2 ^{ABw} \pm 0.2	6.6 ^{Ax} \pm 0.17	7.3 ^{Ax} \pm 0.2
AA+LA+ SL	3.9 ^{Av} \pm 0.2	4.8 ^{Aw} \pm 0.1	6.2 ^{Ax} \pm 0.2	7.2 ^{Ay} \pm 0.4
Lactic Acid Bacteria				
Control	5.1 ^{Ax} \pm 0.1	6.8 ^{CDy} \pm 0.3	7.6 ^{Cz} \pm 0.2	NA
SL	5.2 ^{Ax} \pm 0.2	7.0 ^{Dy} \pm 0.2	7.8 ^{Cz} \pm 0.3	NA
LA	4.7 ^{Ax} \pm 0.2	6.0 ^{ABCy} \pm 0.1	7.3 ^{BCz} \pm 0.3	7.9 ^{Bz} \pm 0.1
AA	4.9 ^{Ax} \pm 0.1	5.5 ^{Ax} \pm 0.4	6.6 ^{ABY} \pm 0.2	7.7 ^{ABz} \pm 0.4
LA+ SL	5.1 ^{Ax} \pm 0.1	6.3 ^{BCDy} \pm 0.4	7.1 ^{ABCz} \pm 0.4	NA
AA+ SL	5.1 ^{Ax} \pm 0.1	5.9 ^{ABxy} \pm 0.4	6.3 ^{Ay} \pm 0.1	7.4 ^{ABz} \pm 0.4
AA+ LA	4.9 ^{Ax} \pm 0.3	5.4 ^{Ax} \pm 0.1	6.5 ^{ABY} \pm 0.1	7.1 ^{AByz} \pm 0.1
AA+LA+ SL	5.0 ^{Aw} \pm 0.1	5.8 ^{ABwx} \pm 0.4	6.5 ^{ABxy} \pm 0.4	7.0 ^{Ayz} \pm 0.5

ABC: Values with different superscripts within the same column are significantly different (P<0.05)

xyz: Values with the different superscript within the same row are significantly different (P<0.05)

NA: Not analyzed SL: Sodium Lactate LA: Lactic acid AA: Acetic acid

The samples which were analyzed for indigenous *Salmonella* spp. showed that the indigenous *Salmonella* spp. in the purchased drumstick samples was below the detection limit (<1 CFU ml⁻¹). The average inoculation level of *Salmonella* spp. colonies in the control samples was 5.4 \log_{10} CFU ml⁻¹ rinse solution (Table 2). After the decontamination treatments (on day 0), the counts of *Salmonella* spp. colonies of the samples reduced up to 1.2 \log_{10} CFU ml⁻¹ depending on the decontamination solutions

when compared with the control sample (P<0.05). After day 0, the numbers of *Salmonella* spp. in the groups were almost stable during the storage time, and no significant differences was observed between the storage days (P>0.05). On day 5, the combination of AA+LA had the best antimicrobial efficacy on *Salmonella* spp. compared to the control group (P<0.05).

Table 2: The mean numbers of *Salmonella* spp. of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C (log₁₀ CFU ml⁻¹ rinse solution ± SD)

Treatment Groups	Storage days			
	0	3	5	8
Control	5.4 ^{Cx} ± 0.1	5.3 ^{ABx} ± 0.2	5.3 ^{Bx} ± 0.1	NA
SL	5.2 ^{BCx} ± 0.1	5.4 ^{Bx} ± 0.2	5.3 ^{Bx} ± 0.1	NA
LA	4.2 ^{Ax} ± 0.2	4.8 ^{ABx} ± 0.2	4.8 ^{ABx} ± 0.3	4.6 ^{ABx} ± 0.1
AA	4.7 ^{ABCx} ± 0.2	4.9 ^{ABx} ± 0.2	4.7 ^{ABx} ± 0.2	4.8 ^{ABx} ± 0.1
LA+ SL	4.9 ^{ABCx} ± 0.2	4.9 ^{ABx} ± 0.1	5.1 ^{ABx} ± 0.1	NA
AA+ SL	5.4 ^{Cx} ± 0.1	5.3 ^{ABx} ± 0.2	5.4 ^{Bx} ± 0.1	5.1 ^{Bx} ± 0.2
AA+ LA	4.5 ^{ABx} ± 0.1	4.6 ^{Ax} ± 0.1	4.5 ^{Ax} ± 0.2	4.4 ^{Ax} ± 0.3
AA+LA+ SL	4.8 ^{ABCx} ± 0.3	4.8 ^{ABx} ± 0.1	4.9 ^{ABx} ± 0.2	4.8 ^{ABx} ± 0.1

^{ABC}: Values with different superscripts within the same column are significantly different (P<0.05)
^{*}: Values with the same superscript within the same row are not significantly different (P>0.05)
 NA: Not analyzed SL: Sodium Lactate LA: Lactic acid AA: Acetic acid

The initial pH of the control sample was 6.78. The pH of the samples that were treated with AA and LA decreased to 5.35 and 5.72 on day 0, respectively (Table 3), and the combination of AA and LA decreased the pH of the chicken drumstick

to 5.02. Those groups were significantly different from the control and SL groups (P<0.05). However, the pH of the samples treated with organic acids, alone and in combination, dramatically increased and approached to the control group on day 3.

Table 3: The mean pH values of the rinse solutions of the drumstick samples immersed into decontamination solutions for 5 min and stored at 4°C (pH ± SD)

Treatment Groups	Storage days			
	0	3	5	8
Control	6.78 ^{Cxy} ± 0.1	6.67 ^{ABx} ± 0.1	7.15 ^{ABy} ± 0.1	NA
SL	7.00 ^{Cxy} ± 0.1	6.87 ^{Bx} ± 0.2	7.33 ^{By} ± 0.2	NA
LA	5.72 ^{Bx} ± 0.1	6.53 ^{ABy} ± 0.2	7.15 ^{ABz} ± 0.1	7.20 ^{Az} ± 0.0
AA	5.35 ^{ABx} ± 0.2	6.39 ^{ABy} ± 0.2	6.90 ^{ABz} ± 0.2	6.91 ^{Az} ± 0.2
LA+ SL	5.76 ^{Bx} ± 0.3	6.68 ^{ABy} ± 0.1	7.07 ^{ABy} ± 0.1	NA
AA+ SL	5.53 ^{ABx} ± 0.2	6.68 ^{ABy} ± 0.2	6.90 ^{ABy} ± 0.1	7.02 ^{Ay} ± 0.1
AA+ LA	5.02 ^{Ax} ± 0.1	6.27 ^{Ay} ± 0.2	6.72 ^{Ayz} ± 0.3	6.85 ^{Az} ± 0.1
AA+LA+SL	5.03 ^{Ax} ± 0.2	6.53 ^{ABy} ± 0.1	6.83 ^{AByz} ± 0.1	6.79 ^{Ayz} ± 0.1

^{ABC}: Values with different superscripts within the same column are significantly different (P<0.05)
^{xyz}: Values with the same superscript within the same row are significantly different (P>0.05)
 NA: Not analyzed SL: Sodium Lactate LA: Lactic acid AA: Acetic acid

Discussion

Eliminating foodborne pathogens and reducing the number of microorganisms causing spoilage in poultry products are among the main goals of the poultry industry. Many researchers have reported that using solutions containing 1% to 3% organic acids have no negative effects on the sensory characteristics in poultry meat or may cause acceptable sensory changes such as insignificant differences in color and taste (7,15,17).

There was no statistical difference between the LA and AA treatments in point of antibacterial effect in the present study; however, the results showed that AA was more effective than LA on the APB and *Pseudomonas* spp. This may be due to the concentrations of undissociated acid molecules of LA and AA. Different acids have various impacts on bacterial survival because of their different dissociation degrees. Undissociated acid molecules penetrate into the bacterial cell and show antimicrobial properties (18). International

Commission on Microbiological Specifications for Foods (ICMSF) reported that un-dissociated proportions of LA and AA at pH 5 were 6.05% and 34.9%, and at pH 6.0 were 0.64% and 5.1%, respectively (19). In this research, the pH of the samples that were treated with AA and LA are 5.35 and 5.72 on day 0, respectively (Table 3). The high efficacy of AA on the APB and *Pseudomonas* spp. can be attributed to the low pH of the samples, because the concentration of undissociated acid molecules of AA is greater than that of LA at the same pH (18).

The drumstick samples in the control, SL and LA+ SL groups had a bad odor and slight slime layer on their skin on day 5 (Table 1). Their APB and *Pseudomonas* spp. counts were above $7.2 \log_{10}$ CFU ml⁻¹ rinse solution. Jay et al. (16) reported that a bad odor in chicken meat can be detected when the surface bacterial number (especially *Pseudomonas* spp.) is between 7.2 and 8.0 \log_{10} CFU cm⁻². As it is known, the initial bacterial load of a product has a great effect on the shelf life of the product. In this study, the initial APB counts of the control and 1% SL groups were 5.4 and 5.7 \log_{10} CFU ml⁻¹, respectively. Therefore, the control and 1% SL groups deteriorated before on day 5. There is limited information regarding the microbiota on chicken meat treated with organic acid blends. Zhu et al. (13) reported that the numbers of total viable bacteria and *Pseudomonas* spp. on the chicken drumsticks that were spray-washed with the combination of 0.5% LA+1% citric acid for 30 s were reduced 1.68 and 1.85 \log_{10} CFU/cm², respectively. Olaimat et al. (15) noted that the count of aerobic mesophilic bacteria on chicken breast meat that were immersed into 0.5% AA+0.5% malic acid (MA) blends for 5 minutes decreased 2 \log_{10} CFU/g.

In this study, none of the treatments showed bactericidal or bacteriostatic effects on LAB during the storage (Table 1). It was observed that LAB is more resistant to LA and AA compared to *Pseudomonas* spp.. In general, the groups treated with AA had a lower number of LAB than the groups treated with LA during the storage. Although there was no significant difference between the groups treated with AA, LA and their combination, it was observed that AA numerically caused more reduction in LAB compared to the LA. Olaimat et al. (15) reported that LAB counts on chicken breast meat treated with 0.5% AA for 5 min remained almost constant (approximately

between 5 and 6 \log_{10} CFU/g), and 0.5%AA+0.5% MA blends solution resulted in approximately 2 \log_{10} CFU/g reduction by the end of 10 days of storage at 4°C.

Nagel et al. (20) and Lee et al. (21) reported that it was not easy to remove *Salmonella* spp. from the folded areas and follicles on chicken skin. İlhak et al. (9) observed that there was no significant reduction in the number of *Salmonella* spp. on chicken drumstick sprayed with 2% LA for 30 sec. Ramirez-Hernandez et al. (14) used the LA+AA blend at the concentration of 2-2.5% (v/v, pH 2.8) and spray method for 15 sec on *Salmonella* spp. in chicken thigh, and they did not find a significant reduction in *Salmonella* spp. counts compared to the control group. In this study, the counts of *Salmonella* spp. in the samples treated with LA and AA+LA combination were reduced 1.2 and 0.9 \log_{10} CFU ml⁻¹ respectively (P<0.05). Mani-López et al. (8) reported that the sensitivity of bacteria to the antimicrobial effect of acetic acid, lactic acid or sodium lactate increases with a decrease in the pH level of food. On the other hand, Tan et al. (22) noted that chicken skin and chicken meat have buffering capacity against changes in pH. This buffering capacity may help the survival of *Salmonella* spp. in chicken meat treated with organic acids.

In this study, the pH of the samples treated with organic acids was above 5 immediately after the treatments. After day 0, the pH of the samples reached above 6.0 (Table 3). The undissociated acid proportions of AA and LA are very few at pH 6.0 and above (19). The physical structure of chicken skin and the high buffering capacity of the chicken meat may have protected *Salmonella* spp. from the effect of organic acids (22). The reductions in the number of *Salmonella* spp. in the treated groups after day 0 were low. This may have been because of the protective effect of the physical structure of chicken skin and the high buffering capacity of the chicken meat and skin. However, Olaimat et al. (15) immersed chicken breast into 0.5 AA%+0.5% malic acid (MA) blends for 5 min, and they found that the acid blend resulted in more than 5.5 \log_{10} CFU/g reduction in viable *Salmonella* spp. at the end of 10 days storage at 4°C. Mikolajczyk (12) noted that immersing breast meat into a solution composed of equal parts of 1% acetic acid, 1% lactic acid, and 1% tartaric acid for 15 min resulted in 2 log Most Probable Number (MPN)/ml reduction in

the number of *Salmonella* spp. after storage at 4 °C for 6 days. When the findings of many studies regarding organic acids applications on poultry carcasses and carcass parts are examined (9, 14, 17, 20, 21), it is seen that the reducing effects of organic acids on *Salmonella* spp. change from insignificant level to about 2.5 log₁₀ CFU ml⁻¹ depending on acid concentration (1-5%), treatment time (15 sec to 20 min) and application methods (spraying, dipping). In our study, the reduction levels in the number of *Salmonella* spp. in the groups treated with organic acids, alone and in combined form, changed between 0 and 1.2 log₁₀ CFU ml⁻¹.

In each experiment, two drumsticks from the chicken drumstick samples, which were purchased from a supermarket, were randomly selected and analyzed for the presence of indigenous *Salmonella* spp.. It is expected that chicken meats purchased from supermarkets should not contain *Salmonella* spp. or should contain at very low concentrations (may be less than 1 log₁₀ CFU/g, at most 2 log₁₀ CFU/g). In this study, the initial inoculation level of *Salmonella* spp. was about 5.4 log₁₀ CFU/ml, which was at least 2000 times higher than the numbers likely to be found on the samples. Because of that, no significant interference was expected from indigenous *Salmonella* spp.

Conclusion

Decontamination of chicken drumstick with 1% SL showed no advantage in extending the shelf life of the samples or in reduction of the number of *Salmonella* spp. The combination of 1% SL with 1.5% LA or 1.5% AA did not have a synergistic or additive antibacterial effect on the spoilage bacteria and *Salmonella* spp. when compared to LA and AA used alone.

In conclusion, the shelf life of chicken drumsticks that were treated with the decontamination solutions containing AA (alone or in combination) was extended at least 2 days when compared to the control group. *Salmonella* spp. was relatively resistant to 1.5% AA and 1.5% LA. This was probably because of the buffering capacity of chicken skin and meat. The buffering capacity of the product should not be neglected when organic acids are used as a decontamination agent. Increasing the acid concentrations would probably give better results in extension of shelf life of the product and elimination of

Salmonella spp. However, the use of organic acid at high concentrations can negatively affect the organoleptic properties of the product. This study contributes to the poultry industry on choosing effective decontamination treatments to improve the shelf life and safety of poultry meats.

Acknowledgement

This study was supported by Scientific Research Projects Coordination Unit of Firat University (FÜBAP) with the Project number of VF.17.11.

References

1. Rouger A, Tresse O, Zagorec M. Bacterial contaminants of poultry meat: sources, species, and dynamics. *Microorganisms* 2017; 5: 50.
2. Silva F, Domingues FC, Nerin C. Trends in microbial control techniques for poultry products. *Crit Rev Food Sci* 2018; 58: 591–609.
3. Guran HS, Mann D, Alali WQ. *Salmonella* prevalence associated with chicken parts with and without skin from retail establishments in Atlanta metropolitan area, Georgia. *Food Control* 2017; 73: 462–7.
4. Republic of Turkey Ministry of Agriculture and Forestry. National *Salmonella* control program, 2018. (In Turkish) <https://www.tarimorman.gov.tr/Duyuru/598/ulusal-salmonella-kontrol-programi-yayinlanmistir-> (March, 2021)
5. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. *Food Control* 2010; 21: 1199–218.
6. FDA (Food and Drug Administration). CFR-Code of Federal Regulations, title 21, part 184. Direct food substances affirmed as generally recognized as safe. Subpart B-listing of specific substances affirmed as gras. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=184>
7. Cosansu S, Ayhan K. Effects of lactic and acetic acid on survival of *Salmonella enteritidis* during refrigerated and frozen storage of chicken meats. *Food Bioprocess Technol* 2012; 5: 372–7.
8. Mani-López E, García HS, López-Malo A. Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Res Int* 2012; 45: 713–21.
9. İlhak Oİ, İncili GK, Durmuşoğlu H. Evaluation of effect of thymol combined with lactic acid or sodium lactate on psychrophilic bacteria and *Sal-*

monella spp. on chicken drumstick. *Ann Anim Sci* 2017; 17: 271–80.

10. Radkowski M, Zdrodowska B, Gomolka-Pawlicka M. Influence of lactic acid on the survival of *Salmonella* on poultry carcass surface. *Med Weter* 2018; 74: 139–42.

11. Rajkovic A, Tomic N, Smigic N, Uyttendaele M, Ragaert P, Devlieghere F. Survival of *Campylobacter jejuni* on raw chicken legs packed in high-oxygen or high-carbon dioxide atmosphere after the decontamination with lactic acid/sodium lactate buffer. *Int J Food Microbiol* 2010; 140: 201–6.

12. Mikołajczyk A. Evaluation of the effects of a mixture of organic acids and duration of storage on the survival of *Salmonella* on turkey carcasses. *J Food Protect* 2015; 78: 585–9.

13. Zhu Y, Xia X, Liu A, et al. Effects of combined organic acid treatments during the cutting process on the natural microflora and quality of chicken drumsticks. *Food Control* 2016; 67: 1–8.

14. Ramirez-Hernandez A, Brashears MM, Sanchez-Plata MX. Efficacy of lactic acid, lactic acid-acetic acid blends, and peracetic acid to reduce *Salmonella* on chicken parts under simulated commercial processing conditions. *J Food Protect* 2018; 81: 17–24.

15. Olaimat AN, Al-Holy MA, Abu Ghoush MH, et al. The use of malic and acetic acids in washing solution to control *Salmonella* spp. on chicken breast. *J Food Sci* 2018; 83: 2197–203.

16. Jay JM, Loessner MJ, Golden DA. Modern

food microbiology. 7th ed. New York : Springer Science+Business Media, 2005.

17. Lecompte JY, Collignan A, Sarter S, Cardinale E, Kondjoyan A. Decontamination of chicken skin surfaces inoculated with *Listeria innocua*, *Salmonella enteritidis* and *Campylobacter jejuni* by contact with a concentrated lactic acid solution. *Br Poultry Sci* 2009; 50: 307–17.

18. Feiner G. Meat products handbook. Practical science and technology. Cambridge : CRC Press, 2006.

19. IFT/FDA. Factors that influence microbial growth. IFT/FDA Report on task order 4. Comprehensive Reviews in Food Science and Food Safety 2003; 2: 21–32.

20. Nagel GM, Bauermeister LJ, Bratcher CL, Singh M, McKee SR. *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. *Int J Food Microbiol* 2013; 165: 281–6.

21. Lee NY, Park SY, Kang IS, Ha SD. The evaluation of combined chemical and physical treatments on the reduction of resident microorganisms and *Salmonella* Typhimurium attached to chicken skin. *Poultry Sci* 2014; 93: 208–15

22. Tan SM, Lee SM, Dykes GA. Fat contributes to the buffering capacity of chicken skin and meat but enhances the vulnerability of attached *Salmonella* cells to acetic acid treatment. *Food Res Int* 2014; 66: 417–23.

KOMBINIRANI UČINEK NATRIJEVEGA LAKTATA, MLEČNE KISLINE IN OČETNE KISLINE NA PREŽIVETJE *Salmonelle* spp. IN OSTALIH MIKROORGANIZMOV NA PIŠČANJH BEDRIH

G. A. Habeeb, H. Durmuşoğlu, O. İ. İlhak

Izveček: Perutninsko predelovalna industrija raziskuje nove prilagoditve za dekontaminacijo mesa, da bi preprečila prenašanje patogenih mikroorganizmov s hrano in podaljšala rok uporabnosti perutninskih izdelkov. V opisani raziskavi so avtorji proučevali učinke mlečne kisline, očetne kisline in natrijevega laktata, samostojno ali v kombinaciji, na preživetje *Salmonelle* spp. in na rok uporabnosti piščančjih beder. Sveža piščančja bedra so inokulirali s *Salmonella typhimurium* in *Salmonella enteritidis* ter jih razdelili v skupine: kontrolna skupina (z dodano sterilno vodo iz vodovoda), skupina z 1% natrijevim laktatom (SL), skupina z 1,5% mlečne kisline (LA), skupina z 1,5% očetne kisline (AA) in njihovih kombinacij. Bedra so za 5 minut potopili v raztopine za obdelavo in jih osem dni hranili pri 4° C ter jih analizirali za prisotnost aerobne psihrotrofne bakterije (APB), *Pseudomonas* spp., mlečnokislinske bakterije (LAB), *Salmonella* spp. in pH-vrednosti. Peti dan so koncentracije APB, *Pseudomonas* spp. in LAB presegale 7,0 log₁₀ CFU ml⁻¹ v kontrolni skupini, skupini SL, LA in LA + SL. Znižanje ravni *Salmonella* spp. so bile 0, 1 in log₁₀ CFU ml⁻¹ v skupinah LA in AA + LA na dan 0 in so se bistveno razlikovale od kontrolne skupine (P < 0,05). Rok uporabnosti piščančjih beder, obdelanih z raztopinami, ki vsebujejo 1,5% AA (AA, AA + SL, AA + LA in AA + LA + SL), je bil vsaj dva dni daljši od kontrolne skupine. Ugotovljeno je bilo, da lahko kombinacije 1,5% LA, 1,5% AA in 1% SL uporabimo za zmanjšanje števila *Salmonella* spp. in podaljšajo rok uporabnosti piščančjih beder.

Ključne besede: piščančja bedra; mlečna kislina; očetna kislina; natrijev laktat; rok uporabnosti; *Salmonella* spp.