LWT - Food Science and Technology 134 (2020) 110231

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

# Fate of *Listeria monocytogenes* and *Salmonella* Typhimurium in homemade marinade and on marinated chicken drumsticks, wings and breast meat

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ARTICLE INFO

*Keywords:*
Chiken meat products
Marination
*Salmonella* typhimurium
*Listeria monocytogenes*

ABSTRACT

In recent years, the increase in consumer demands for the products that are processed with natural additives has highlighted the use of natural substances in food processing and safety. For this purpose, the aim of this study is to determine the effect of homemade marinade that are prepared with the natural substances existing in every kitchens and restaurants on *Salmonella* Typhimurium, *Listeria monocytogenes* and natural microbiota of chicken wings, drumstick and breast meats. The results of this study revealed that the number of *Salmonella* Typhimurium decreased about 4.0 $\log_{10}$ in 24 h in marinade sauce, while no significant change was observed in the number of *Listeria monocytogenes*. The number of *Salmonella* Typhimurium decreased 0.9 and 1.4 $\log_{10}$ in the marinated chicken wing and breast meat. Marinade has a strong bacteriostatic effect on *Listeria monocytogenes*, total mesophilic aerobic bacteria and psycrotrophic bacteria in the wing, drumstick and breast meats. The numbers of *L. monocytogenes*, TMAB and psychrotrophic bacteria in marinated groups were 1.0–3.0 $\log_{10}$ lower than control groups at the end of the storage. The marinades that people can prepare with the natural ingredients existing in their kitchen would enable microbiologically more reliable chicken meats.

## 1. Introduction

Poultry meat and meat products have an important place in human diet. However, pathogenic and spoilage microorganisms likely to be present in these products may cause health problems and economic losses in the poultry industry. In 2018, 94,203 comfirmed human salmonellosis and 2549 listeriosis cases were reported in EU. The percantage of broiler meat and meat products in total salmonellosis was found as 2.4%, 0.6% of the 1206 tested samples were found positive for *L. monocytogenes* (EFSA & ECDC, 2019). 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 (Silva, Domingues & Nerin, 2018).

As a result of globalization and industrialization, consumer behavior has changed and the demand for the ready-to-cook (RTC) and ready-to-eat (RTE) foods, which are easy to prepare and less time-consuming, has increased (Kim et al., 2015). On the other hand, the increasing demand for organic and minimally processed foods that chemical-free or contain less chemicals and accepted as "more healthy" by consumers has led to the use of natural preservation methods to improve the quality and safety of foods (Des Field, Ross, & Hill, 2018). The market of marinated poultry meat and products is increasing in the European Union (Inguglia, Burgess, Kerry, & Tiwari, 2019), and United States (Bowker & Zhuang, 2017).

Marination has been applied to meat and meat products to improve sensory attributes (texture, flavour, juiciness, palatability) and microbial quality (Lytou, Nychas, & Panagou, 2018). A wide variety of ingredients such as vinegar, wine, yogurt, fruit juices, seasonings, salt, sunflower or olive oil, phosphates (acidic or alkaline), organic acids, and different aroma components are being used in marinades (Nisiotou, Chorianopoulos, Gounadaki, Panagou, & Nychas, 2013). Commercial marinated products are prepared by soaking, tumbling, blending, or injection of marination solutions into the product (Thanissery & Smith, 2014). However, due to the difficulty of applying these methods, consumers generally apply immersion type marination. Commercial marinades generally have an alkaline pH, while acidic marinades are also

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https://doi.org/10.1016/j.lwt.2020.110231
Received 5 June 2020; Received in revised form 31 August 2020; Accepted 12 September 2020
Available online 16 September 2020

being used (Karam, Roustom, Abiad, El-Obeid & Savvaidis, 2019; Şengün, Göztepe, & Öztürk, 2019).

Poultry meat is highly perishable food due to having a suitable environment for microbial growth such as high pH and water activity (Silva, Domingues, & Nerín, 2018). Marination has a potential antimicrobial effect to improve microbial quality of these products ( Thanissery & Smith, 2014). However, the antimicrobial effect of marination depends on many factors such as bactericidal or bacteriostatic effects of the components it contains, pH, application method, storage conditions and initial microbial population of the products (Lytou, Tzortzinisa, Skandamis, Nychas, & Panagoua, 2019; Pathania, McKee, Bilgili, & Singh, 2010).

As mentioned above, marinades can be prepared by using many different ingredients, while commercial marinades generally contain polyphosphates and other synthetic chemicals (antimicrobials, antioxidants, etc.) (Baltić et al., 2015). Although consumers purchase commercially marinated RTC and RTE foods from market, a considerable number of people consume marinated poultry meat prepared by the cooks in restaurants, and many people prepare marinated poultry meat at their home. These marinated poultry meat prepared at home and restaurants are sometimes not consumed on the same day they are prepared, and may be stored in refrigerator for few days. Therefore, there is a need to determine the antimicrobial effect of marinade which is prepared by basic ingredients found in kitchens and restaurants on the microbial quality of the poultry meat during storage in refrigerator. For this purpose, the aim of this study was to evaluate the antimicrobial effect of the home-made marinade on the survival of *Salmonella* Typhimurium and *Listeria monocytogenes* on the chicken wing, breast meat, and drumstick, and in the marination sauce, during storage at 4 °C.

## 2. Materials and methods

### 2.1. Preparation and analysis of marinade

The ingredients of the marinade were obtained from a local market. The formulation of the marinade were as follows: 200 g tomato paste, 200 g red pepper paste (Öncü Salça, Turkey) 250 mL sunflower oil (Yudum, Turkey), 15 g red pepper, 10 g black pepper, 10 g cumin (Bağdat Baharat, Turkey), 45 g salt (Billur Tuz, Turkey), 200 mL fresh lemon juice and 70 g garlic rind. The initial total mesophiclic aerobic bacteria (TMAB), yeast-mold, psychrotrophic bacteria, pH (HI 11310, Hanna Instruments, USA) and water activity (aw) (Testo 650, GmbH, Germany) of the marinadee were determined immeadiately after preperation.

### 2.2. Preparation of the chicken meat groups

Fresh chicken wing, drumstick with skin and skinless breast meat samples were purchased from a local market on the day of the experiment. All samples were transported to the laboratory under the cold chain, and experiments were carried out as soon as possible. Before spiking, the breast meat was cut into small pieces (50 ± 5 gr) with a sterile lancet. Then, all samples were spiked with 0.5 mL diluted bacterial cocktail by spreading onto whole surfaces of the samples, and 10 min were given to the bacterial attachment at room temperature. After attachment, samples were randomly divided into two groups were as follows: control (without any marination treatment) and marinated. Marination sauce was covered all surfaces of the samples in the marinated groups. Then all samples packaged with polyethylene film on plastic trays and aerobically stored at 4 °C. All analyses were performed triplicate.

### 2.3. Preparation of inoculum

*Salmonella* Typhimurium (NCTC 12416, 74, and ATCC 14028) and *L. monocytogenes* (N 7144, RSKK 474 and 476 (Refik Saydam National Public Health Agency-Turkey) reference strains were used for the inoculation of the products. Bacteria were incubated in Tyriptic Soy Broth at 37 °C for 18–24 h to obtain fresh culture. After incubation period the supernatnats and pellets were separeted by centrifuging (4000 rpm for 10 min), and then pellets washed in 0.1% pepton water and mixed in a tube. This tube was used for the stock inoculum solution which contains approximately 9.0 $\log_{10}$ CFU/mL for each strains. Decimal dilutions were prepared using the stock solution by using 0.1% pepton water. Approximately 5.0 $\log_{10}$/mL inoculation level was used in the chicken meat products and the survival experiment in the marinade.

### 2.4. Pathogens survival experiment in the marinade

For the evaluation of the antibacterial effect of the marinade, marination sauce was spiked with approximately 5.0 $\log_{10}$ *Salmonella* Typhimurium and *Listeria monocytogenes*. The number of the pathogens were determined just after inoculation, 6 and 24th hours of the incubation period at 4 °C. This experiment was performed triplicate.

### 2.5. Analyses

#### 2.5.1. Microbiological analyses

In the chicken meat products experiment, microbiological analyses were performed on 0, 2, 4, 6, 8, and 10th day of the storage period. Each plastic trays consisted of 2 samples were opened under aseptic conditions, then whole wing, drumstick, and 25 g breast meat samples separately transferred into the sterile sampling bags. One hundred mL 0.1% peptone water (Merck, Darmstadt, Germany) for the wing and drumstick samples, and 225 mL 0.1% peptone water for the breast meat samples were added to the sampling bags. Drumstick and wing samples were shaken manually for 2 min, and breast meat samples were homogenized by using stomacher.

TMAB, psychrotrophic bacteria, yeast-mold, *L. monocytogenes* and *S.* Typhimurium counts were determined in the samples. Plate Count Agar (PCA) (Merck, Darmstadt, Germany) was used for the enumeration of TMAB (35 ± 1 °C for 24–48 h) and psychrotrophic bacteria (5–7 °C for 7–10 days) (USDA, 2011). Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Oxoid, UK) was used for yeast-mold count (25 ± 1 °C for 5 days) (ISO 21527, 2008, p. 95). Oxford agar for *L. monocytogenes*, and Xylose Lysine Tergitol-4 (XLT-4) agar (Merck, Darmsstadt, Germany) for *S.* Typhimurium were used in the study. XLT-4 and Oxford plates were incubated at 35 °C for 24–48 h, and then characteristic colonies were enumerated. The numbers of microorganisms were expressed as $\log_{10}$ CFU/mL rinse fluid for the chicken wing and drumstick samples and $\log_{10}$ CFU/g for the chicken breast meat samples and marinade.

#### 2.5.2. pH analyses

The pH values of the samples (25 ± 1 °C) were determined by using digital pH meter (HI 11310, Hanna Instruments, USA). After microbiological analyzes of the chicken wing and drumstick and breast meat samples, remained liquid (rinse fluid) in the sampling bags was used for the pH analyses.

#### 2.5.3. Statistical analyses

The microbiological data were converted to logarithmic value for the statistical analysis. pH data of the samples were also subjected to the statistical analysis. Indipendent T-Test was used for the comparison of the groups, and ANOVA was used for the comparisons of the sampling days. All statistical analyses were performed by using SPSS 21.0. Statistical significance level was accepted as 0.05.

## 3. Results

### 3.1. Initial values of the samples

The initial TMAB, psychrotrophic bacteria, yeast-mold counts of the

chicken meat products and the marinade, and pH and aw values of the marinade are given in Table 1.

### 3.2. Salmonella Typhimurium

It was found that the number of *Salmonella* Typhimurium decreased 3.6 $\log_{10}$ in 6 h in the marination sauce ($P < 0.05$) (Fig. 1). Although the number of *S.* Typhimurium continued to decrease, the difference between 6 and 24th hours was insignificant ($P > 0.05$). The number of *Salmonella* rapidly decreased in marinade sauce, while a lower reduction rate was obtained in the marinated chicken meat samples.

Compared to the control group, *Salmonella* reduction levels in marinated wing, drumstick and breast meat were 0.9, 0.4 and 1.4 $\log_{10}$ on day 0 (Table 2). The significant reductions were observed in the wing and breast meat ($P < 0.05$). For drumstick samples, significant differences between control and marinated group were observed after on day 4 of the storage ($P < 0.05$). At the end of the storage, *Salmonella* reduction rates were 0.9, 1.4 and 0.7 $\log_{10}$ in marinated wing, drumstick and breast meats, respectively. In marinated breast meat, no significant differences were observed between the storage days ($P > 0.05$).

### 3.3. Listeria monocytogenes

The number of *Listeria monocytogenes* remained almost stable in marinade sauce for 24 h (Fig. 1). The inoculation level was 5.3 $\log_{10}$, and *Listeria monocytogenes* count was found as 4.8 $\log_{10}$ after 24 h at 4 °C ($P > 0.05$). Compared to the control group, the marination sauce did not provide a significant reduction in the number of *Listeria monocytogenes* in the breast meat (0.3 $\log_{10}$) and wing samples (0.3 $\log_{10}$) ($P > 0.05$), while significant reduction was found in the drumstick samples (0.4 $\log_{10}$) ($P < 0.05$) on day 0 (Table 3). The significant differences were observed between the control and marinated groups after the 2, 4 and 6th day of the storage for the breast meat, drumstick and wing, respectively ($P < 0.05$). While *Listeria monocytogenes* counts slowly increased in the control samples of wing, drumstick and breast meat during the storage days ($P < 0.05$), its number in the marinated wing, drumstick and breast meat remained stable, and differences between days were insignificant ($P > 0.05$), except on day 6 for the marinated breast meat.

### 3.4. TMAB

The numbers of TMAB in the marinated and control groups in the wing, drumstick, and breast meat samples were found to be similar on the initial day ($P > 0.05$) (Table 4). It was determined that the number of TMAB did not significantly change during the storage period in all the marinated groups, although significant increases (2–3 $\log_{10}$) were found in the control groups ($P < 0.05$). The counts were reached approximately 7.0 $\log_{10}$ within 4 days in the control groups. After 2nd day of storage, statistical differences were observed between the control and marinated groups of wing and breast meat ($P < 0.05$). It was found that marinade used showed bacteriostatic effect on TMAB, and these counts in the marinated groups reached a maximum of 6.5 $\log_{10}$ during 10 days of the storage period.

**Table 1**
The initial microorganisim counts (Mean $\log_{10}$ CFU/g-mL rinsate±SD), pH and aw values (Mean ± SD) of the chicken drumtsticks, breats meat, wings, and marinade.

| Samples | Total mesophilic aerobic bacteria | Psychrotrophic bacteria | Yeast-mold | pH[a] | Water activity |
|---|---|---|---|---|---|
| **Drumstick** | 4.18 ± 0.24 | 4.49 ± 0.41 | 1.46 ± 0.23 | – | – |
| **Breats meat** | 5.17 ± 0.91 | 4.71 ± 0.98 | 2.01 ± 0.57 | – | – |
| **Wing** | 5.05 ± 0.25 | 5.70 ± 0.17 | 1.21 ± 0.29 | – | – |
| **Marinade** | 5.19 ± 0.3 | 1.52 ± 0.5 | 1.85 ± 0.24 | 3.17 ± 0.2 | 0.876 ± 0.03 |

[a] The initial day pH values of the chicken meat products are initial values of the samples.

### 3.5. Psychrotrophic bacteria

The number of psychrotrophic bacteria was found to be similar between the control and marinated group of the wing and drumstick samples on the initial day ($P > 0.05$), whereas in the breast meat samples the control group was higher than the marinated group ($P < 0.05$) (Table 5). Psychrotrophic bacteria counts rapidly increased to 7.0 $\log_{10}$ within four days in control groups of the wing, drumstick and breast meat, while in the marinated group psychrotrophic bacteria counts did not reach to 7.0 $\log_{10}$ during the storage period. Change in psychrotrophic bacteria in the marinated wing samples was found insignificant between the storage days ($P > 0.05$), whereas significant differences were observed between the storage days for the marinated drumstick and breast meat samples ($P < 0.05$). However, these increases in the drumstick and breast meat samples were found to be lower than the control group samples.

### 3.6. Yeast-mold

The initial yeast-mold counts in control groups of the wing, drumstick and breast meat samples was between 1.2 and 2.3 $\log_{10}$ and lower than those in the marinated products. However, there were no significant differences between the marinated group and the control groups in the wing, drumstick and breast meat samples during the storage period ($P > 0.05$), except for the wing samples on day 8 (Table 6). Yeast-mold numbers in control groups of drumstick, wing and breast meat were found almost stable during the storage period, and no significant differences were observed between the storage days of these products ($P > 0.05$). Although the yeast-mold numbers were found to be fluctuating in marinated wing and drumstick samples, the differences between the initial and 10th days of the storage were not significant ($P > 0.05$). Similar fluctuating was observed in the marinated breast meat samples fluctuated, while differences among the sampling days were significant ($P < 0.05$).

### 3.7. pH

The initial pH of the control samples of wing, drumstick and breast meat were 6.59, 6.53 and 5.58, while initial pH of marinated wing, drumstick and breast meat were 3.58, 3.72 and 4.73, respectively. During the storage period, pH values of the wing, drumstick, and breast meat samples of the control group were found to be higher than those of the marinated groups ($P < 0.05$) (Table 7). The pH level of the control group of wing and drumstick were found to be above 7.0 on day 2. None of the pH level of the marinated samples reached to 7.0 during the storage period.

## 4. Discussion

The number of *S.* Typhimurium in the marination sauce significantly decreased in 6 h. Similar to this finding, Şengün et al. (2019) reported that *S.* Typhimurium count decreased 3.47 $\log_{10}$ in marination sauce made from koruk (*Vitis vinifera* L.) juice (pH 2.56–2.91) in 18 h. Pathania et al. (2010) noted that *S.* Typhimurium count decreased from 5.65 $\log_{10}$ CFU to 0.9 $\log_{10}$ in the sauce containing teriyaki marinade (pH 3.71–3.78) at 4 °C for 24 h. Although the less decrease was obtained in the number of *S.* Typhimurium compared to the marinade, its number significantly decreased also in marinated chicken meat parts. Thanissery and Smith (2014) noted that the reduction levels in the number of *S.*

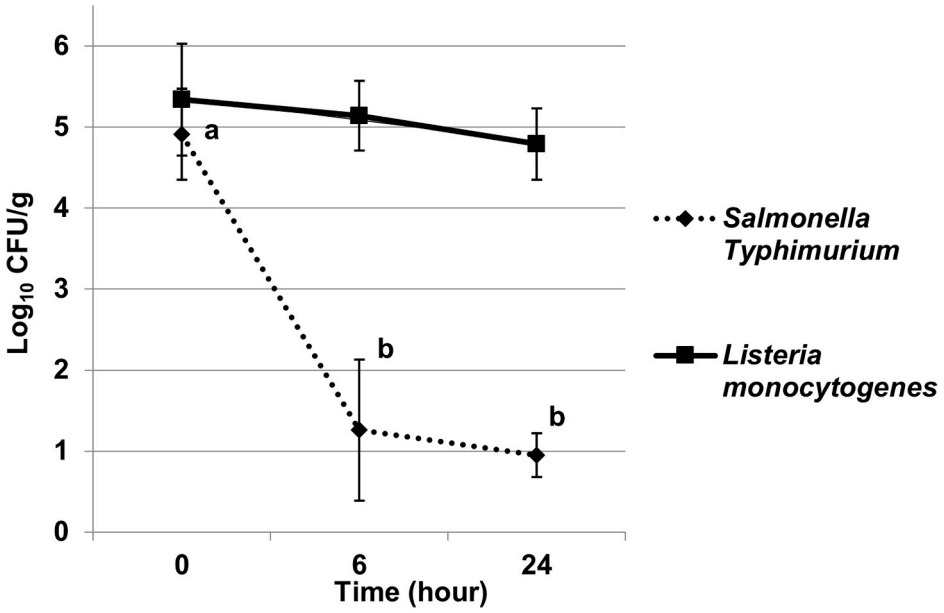


**Fig. 1.** The survival of *Salmonella* Typhimurium and *Listeria monocytogenes* in the homemade marinade during 24 h at 4 °C.

**Table 2**
The mean number of *Salmonella* Typhimurium in chicken meat products during storage period at 4 °C ($log_{10}$ CFU/g-mL rinsate±SD).

| Day | Wing | | Drumstick | | Breast Meat | |
|---|---|---|---|---|---|---|
| | Control | Marinated | Control | Marinated | Control | Marinated |
| **0** | $4.50 \pm 0.15^{Ax}$ | $3.62 \pm 0.14^{Bx}$ | $4.46 \pm 0.05^{Ax}$ | $4.05 \pm 0.52^{Ax}$ | $5.75 \pm 0.13^{Ax}$ | $4.37 \pm 0.38^{Bx}$ |
| **2** | $3.95 \pm 0.12^{Axy}$ | $2.91 \pm 0.38^{Bxy}$ | $4.29 \pm 0.73^{Ax}$ | $3.16 \pm 0.56^{Axy}$ | $5.01 \pm 0.67^{Ax}$ | $4.21 \pm 0.21^{Ax}$ |
| **4** | $3.81 \pm 0.29^{Axy}$ | $3.34 \pm 0.5^{Axy}$ | $3.82 \pm 0.31^{Ax}$ | $2.96 \pm 0.32^{Bxy}$ | $4.84 \pm 0.78^{Ax}$ | $4.33 \pm 0.34^{Ax}$ |
| **6** | $3.18 \pm 0.59^{Ay}$ | $2.34 \pm 0.64^{xy}$ | $4.33 \pm 0.26^{Ax}$ | $2.63 \pm 0.57^{By}$ | $4.68 \pm 0.37^{Ax}$ | $3.80 \pm 0.92^{Ax}$ |
| **8** | $3.74 \pm 0.37^{Axy}$ | $2.46 \pm 0.15^{By}$ | $3.87 \pm 0.23^{Ax}$ | $1.97 \pm 0.55^{By}$ | $4.74 \pm 0.77^{Ax}$ | $3.68 \pm 0.15^{Ax}$ |
| **10** | $3.27 \pm 0.1^{Ay}$ | $2.73 \pm 0.1^{Bxy}$ | $4.23 \pm 0.10^{Ax}$ | $2.69 \pm 0.19^{By}$ | $4.75 \pm 0.12^{Ax}$ | $3.66 \pm 0.14^{Bx}$ |

**AB:** The mean values with different letters in the same line are significantly different ($P < 0.05$).
**xy:** The mean values with different letters in the same column are significantly different ($P < 0.05$).

**Table 3**
The mean number of *Listeria monocytogenes* in chicken meat products during storage period at 4 °C ($log_{10}$ CFU/g-mL rinsate±SD).

| Day | Wing | | Drumstick | | Breast Meat | |
|---|---|---|---|---|---|---|
| | Control | Marinated | Control | Marinated | Control | Marinated |
| **0** | $4.80 \pm 0.05^{Ayz}$ | $4.46 \pm 0.25^{Ax}$ | $4.82 \pm 0.13^{Ayz}$ | $4.39 \pm 0.16^{Bx}$ | $5.23 \pm 0.3^{Ay}$ | $4.93 \pm 0.02^{Ax}$ |
| **2** | $4.56 \pm 0.31^{Az}$ | $3.94 \pm 0.6^{Ax}$ | $4.72 \pm 0.11^{Az}$ | $4.04 \pm 0.66^{Ax}$ | $5.22 \pm 0.16^{Ay}$ | $4.62 \pm 0.21^{Bxy}$ |
| **4** | $5.32 \pm 0.58^{Axyz}$ | $4.27 \pm 0.6^{Ax}$ | $4.58 \pm 0.17^{Az}$ | $4.22 \pm 0.46^{Bx}$ | $5.56 \pm 0.29^{Ay}$ | $4.73 \pm 0.26^{Bxy}$ |
| **6** | $5.50 \pm 0.40^{Axy}$ | $4.18 \pm 0.29^{Bx}$ | $4.91 \pm 0.12^{Ayz}$ | $4.46 \pm 0.16^{Bx}$ | $5.84 \pm 0.16^{Axy}$ | $4.19 \pm 0.19^{By}$ |
| **8** | $5.37 \pm 0.14^{Axyz}$ | $4.44 \pm 0.22^{Bx}$ | $5.14 \pm 0.12^{Axy}$ | $4.42 \pm 0.49^{Bx}$ | $6.41 \pm 0.48^{Ax}$ | $4.47 \pm 0.32^{Bxy}$ |
| **10** | $5.78 \pm 0.11^{Ax}$ | $4.62 \pm 0.12^{Bx}$ | $5.42 \pm 0.13^{Ax}$ | $4.33 \pm 0.08^{Bx}$ | $6.42 \pm 0.28^{Ax}$ | $4.79 \pm 0.24^{Bxy}$ |

**AB:** The mean values with different letters in the same line are significantly different ($P < 0.05$).
**xyz:** The mean values with different letters in the same column are significantly different ($P < 0.05$).

Enteritidis in chicken breast meat and wing samples marinated with 6% NaCl and 3% sodium tripolyphosphate were 1.2 and 1.0 $log_{10}$, respectively. In another study, it was reported that the marination sauce containing 50% and 100% lemon juice provided 2.0 and 3.0 $log_{10}$, reduction in the number of *Salmonella* in chicken fillets stored at 4 °C for 6 days (Eldin, Talaat, Elbaba, & Ibrahim, 2020).

As it is known, the optimal pH range for *Salmonella* is 6.5–7.5, and it can survive between pH 4.5 and 9.0 when the water activity is $\geq 0.94$ (Lawley, Curtis, & Davis, 2008). In this regard, the pH (3.17) and $a_w$ (0.876) values of the marinade used in this study can explain the reason of the reduction in the number of *S.* Typhimurium. In addition, garlic existed in the marination sauce contain substances such as cysteine sulfoxides, and these compounds are converted to thiosulfinate compounds exhibiting antimicrobial effect when the vegetable is damaged (Benkeblia, 2004). Besides, citric acid, allicin, piperine and piperic acids from lemon juice, garlic and black pepper, respectively, have antibacterial effects against bacteria (Eldin et al., 2020; Kim et al., 2015; Zarai, Boujelbene, Salem, Gargouri, & Sayari, 2013). The results showed that *Salmonella* was less inactivated in marinated chicken meat parts compared to those inactivated in the marinade. The reason of this result may be that the bacteria that are firmly attached to the product can be protected from the effects of the antibacterial agents (İlhak, İncili, & Durmuşoğlu, 2018).

In the present study, it was detected that the number of *S.* Typhimurium in the control groups decreased during the storage. This may be because of the rapid increase in the number of TMAB and psychotrophic bacteria in the control groups, since *Salmonella* is not a good competitive bacteria and the background microflora affect the survival and growth rate, they may also cause sublethal stress and injuries (Oscar, 2007). In addition, decrease in *Salmonella* count in the control groups may be caused by the decrease in the ability to grow in specific agars due to the sublehtal injury (Rhoades, Kargiotou, Katsanidis, & Koutsoumanis, 2013).

It was determined that the marinade used in this study had a strong bacteriostatic effect on *L. monocytogenes*. Similar to these findings, Carroll, Alvarado, Brashears, Thompson & Boyse (2007) noted that the marination sauce combination of sodium diacetate, sodium citrate and lactate/diacetate significantly slowed down the growth rate of

**Table 4**
The mean number of total mesophilic aerobic bacteria in chicken meat products during storage period at 4 °C ($log_{10}$ CFU/g-mL rinsate±SD).

| Day | Wing | | Drumstick | | Breast Meat | |
|---|---|---|---|---|---|---|
| | Control | Marinated | Control | Marinated | Control | Marinated |
| **0** | 5.91 ± 0.49$^{Az}$ | 5.27 ± 0.04$^{Axy}$ | 5.36 ± 0.04$^{Az}$ | 5.25 ± 0.39$^{Ax}$ | 5.86 ± 0.68$^{Az}$ | 6.39 ± 0.11$^{Ax}$ |
| **2** | 6.56 ± 0.55$^{Ayz}$ | 5.40 ± 0.09$^{Bxy}$ | 6.04 ± 0.40$^{Ayz}$ | 5.07 ± 0.51$^{Ax}$ | 6.71 ± 0.12$^{Ayz}$ | 6.04 ± 0.13$^{Bx}$ |
| **4** | 7.28 ± 0.5$^{Axy}$ | 5.28 ± 0.3$^{Bxy}$ | 6.91 ± 0.04$^{Ay}$ | 5.18 ± 0.09$^{Bx}$ | 7.77 ± 0.40$^{Axy}$ | 6.28 ± 0.48$^{Bx}$ |
| **6** | 7.32 ± 0.4$^{Axy}$ | 5.16 ± 0.49$^{By}$ | 7.12 ± 0.16$^{Ay}$ | 5.36 ± 0.39$^{Bx}$ | 7.99 ± 0.76$^{Axy}$ | 6.32 ± 0.09$^{Bx}$ |
| **8** | 7.92 ± 0.33$^{Ax}$ | 5.72 ± 0.21$^{Bxy}$ | 8.81 ± 1.21$^{Ax}$ | 6.51 ± 1.13$^{Ax}$ | 8.40 ± 0.28$^{Ax}$ | 6.52 ± 0.24$^{Bx}$ |
| **10** | 8.04 ± 0.12$^{Ax}$ | 5.93 ± 0.15$^{Bx}$ | 8.43 ± 0.14$^{Axy}$ | 5.88 ± 0.15$^{Bx}$ | 8.69 ± 0.17$^{Ax}$ | 6.31 ± 0.46$^{Bx}$ |

**AB:** The mean values with different letters in the same line are significantly different ($P < 0.05$).
**xyz:** The mean values with different letters in the same column are significantly different ($P < 0.05$).

**Table 5**
The mean number of psychrotrophic bacteria in chicken meat products during storage period at 4 °C ($log_{10}$ CFU/g-mL rinsate±SD).

| Day | Wing | | Drumstick | | Breast Meat | |
|---|---|---|---|---|---|---|
| | Control | Marinated | Control | Marinated | Control | Marinated |
| **0** | 4.71 ± 0.71$^{Az}$ | 4.47 ± 0.57$^{Aw}$ | 4.19 ± 0.59$^{Az}$ | 4.43 ± 0.51$^{Axy}$ | 5.90 ± 0.77$^{Ay}$ | 4.00 ± 0.63$^{Bxy}$ |
| **2** | 6.01 ± 1.03$^{Ayz}$ | 4.40 ± 1.21$^{Aw}$ | 5.91 ± 0.34$^{Ay}$ | 4.16 ± 0.14$^{By}$ | 5.72 ± 0.65$^{Ay}$ | 3.69 ± 1.40$^{Ay}$ |
| **4** | 7.33 ± 0.14$^{Axy}$ | 4.52 ± 1.05$^{Bw}$ | 7.51 ± 0.63$^{Ax}$ | 4.27 ± 0.95$^{Bxy}$ | 7.68 ± 0.93$^{Ax}$ | 5.75 ± 0.66$^{Bwx}$ |
| **6** | 8.26 ± 0.59$^{Awx}$ | 4.98 ± 1.00$^{Bw}$ | 8.36 ± 0.52$^{Awx}$ | 5.18 ± 0.61$^{Bwx}$ | 8.73 ± 0.12$^{Awx}$ | 5.98 ± 0.11$^{Bw}$ |
| **8** | 9.14 ± 0.65$^{Aw}$ | 5.05 ± 1.26$^{Bw}$ | 9.28 ± 0.63$^{Aw}$ | 6.51 ± 0.39$^{Bw}$ | 9.33 ± 0.08$^{Aw}$ | 5.89 ± 0.36$^{Bwx}$ |
| **10** | 8.18 ± 0.09$^{Awx}$ | 4.01 ± 0.29$^{Bw}$ | 8.53 ± 0.11$^{Awx}$ | 6.19 ± 0.06$^{Bw}$ | 9.44 ± 0.31$^{Aw}$ | 6.52 ± 0.1$^{Bw}$ |

**AB:** The mean values with different letters in the same line are significantly different ($P < 0.05$).
**wxyz:** The mean values with different letters in the same column are significantly different ($P < 0.05$).

**Table 6**
The mean number of yeast-mold in chicken meat products during storage period at 4 °C ($log_{10}$ CFU/g-mL rinsate±SD).

| Day | Wing | | Drumstick | | Breast Meat | |
|---|---|---|---|---|---|---|
| | Control | Marinated | Control | Marinated | Control | Marinated |
| **0** | 1.23 ± 0.48$^{Av}$ | 1.73 ± 0.21$^{Avw}$ | 1.08 ± 0.45$^{Av}$ | 2.02 ± 0.21$^{Av}$ | 2.26 ± 0.24$^{Av}$ | 2.42 ± 0.2$^{Ay}$ |
| **2** | 1.78 ± 0.93$^{Av}$ | 2.31 ± 0.19$^{Avw}$ | 1.18 ± 0.36$^{Av}$ | 1.81 ± 0.22$^{Av}$ | 2.32 ± 0.15$^{Av}$ | 1.93 ± 0.31$^{Az}$ |
| **4** | 1.61 ± 0.72$^{Av}$ | 2.48 ± 0.47$^{Av}$ | 1.08 ± 0.78$^{Av}$ | 1.94 ± 0.82$^{Av}$ | 3.09 ± 0.59$^{Av}$ | 3.17 ± 0.08$^{Axy}$ |
| **6** | 1.47 ± 0.34$^{Av}$ | 1.95 ± 0.6$^{Avw}$ | 1.13 ± 0.30$^{Av}$ | 2.90 ± 0.95$^{Av}$ | 3.19 ± 0.6$^{Av}$ | 4.22 ± 0.27$^{Avw}$ |
| **8** | 1.80 ± 0.63$^{Av}$ | 2.68 ± 0.34$^{Bv}$ | 1.38 ± 0.48$^{Av}$ | 3.09 ± 1.14$^{Av}$ | 3.11 ± 0.61$^{Av}$ | 4.39 ± 0.38$^{Av}$ |
| **10** | 1.39 ± 0.18$^{Av}$ | 1.39 ± 0.36$^{Avw}$ | 1.71 ± 0.24$^{Av}$ | 1.84 ± 0.09$^{Av}$ | 3.74 ± 0.72$^{Av}$ | 3.42 ± 0.56$^{Awx}$ |

**AB:** The mean values with different letters in the same line are significantly different ($P < 0.05$).
**v-z:** The mean values with different letters in the same column are significantly different ($P < 0.05$).

**Table 7**
pH values of the chicken meat products during storage period at 4 °C (Mean ± SD).

| Day | Wing | | Drumstick | | Breast Meat | |
|---|---|---|---|---|---|---|
| | Control | Marinated | Control | Marinated | Control | Marinated |
| **0** | 6.59 ± 0.39$^{Ay}$ | 3.58 ± 0.32$^{By}$ | 6.53 ± 0.38$^{Ay}$ | 3.72 ± 0.07$^{By}$ | 5.58 ± 0.25$^{Az}$ | 4.73 ± 0.15$^{By}$ |
| **2** | 7.25 ± 0.37$^{Ax}$ | 4.18 ± 0.33$^{Bxy}$ | 7.19 ± 0.29$^{Ax}$ | 4.32 ± 0.06$^{Bx}$ | 6.51 ± 0.06$^{Ay}$ | 5.54 ± 0.32$^{Bxy}$ |
| **4** | 7.14 ± 0.18$^{Axy}$ | 4.27 ± 0.12$^{Bx}$ | 7.07 ± 0.16$^{Axy}$ | 4.49 ± 0.03$^{Bx}$ | 6.35 ± 0.45$^{Ay}$ | 5.60 ± 0.08$^{Bxy}$ |
| **6** | 7.27 ± 0.1$^{Ax}$ | 4.40 ± 0.08$^{Bx}$ | 7.33 ± 0.05$^{Ax}$ | 4.59 ± 0.26$^{Bx}$ | 6.43 ± 0.16$^{Ay}$ | 5.55 ± 0.41$^{Bxy}$ |
| **8** | 7.25 ± 0.05$^{Ax}$ | 4.60 ± 0.27$^{Bx}$ | 7.18 ± 0.08$^{Ax}$ | 4.77 ± 0.36$^{Bx}$ | 7.28 ± 0.09$^{Ax}$ | 5.66 ± 0.67$^{Bxy}$ |
| **10** | 7.61 ± 0.09$^{Ax}$ | 4.68 ± 0.13$^{Bx}$ | 7.20 ± 0.03$^{Ax}$ | 4.76 ± 0.05$^{Bx}$ | 7.37 ± 0.12$^{Ax}$ | 6.37 ± 0.12$^{Bx}$ |

**AB:** The mean values with different letters in the same line are significantly different ($P < 0.05$).
**xyz:** The mean values with different letters in the same column are significantly different ($P < 0.05$).

*L. monocytogenes* in turkey deli loaves. Fouladkhah, Geornaras, Nychas, and Sofos (2013) made a study with chicken breast meat and reported that the number of *L. monocytogenes* significantly increased in control groups, and its count was 2 $log_{10}$ low in the groups marinated with lemon juice at the ends of 7 days compared to the control group.

It is stated that *L. monocytogenes* is highly resistance to low temperature, pH and $a_w$ (Nyhan et al., 2018). These characteristics of *L. monocytogenes* may explain the survival of this bacterium at low pH and aw of the marinade. It is also stated that growth of *L. monocytogenes* in acidic conditions has slowed down and it enters the stationary phase (Buchanan, Golden, & Whiting, 1993). In the current study, the pH values in the marinated groups partly raised during the storage. These increases may have enabled *L. monocytogenes* to survive. It is also noted that the pH alone is not sufficient to inactivate bacteria (Rhoades et al., 2013), and dissociation (dissociated-undissociated) rate of the substances that provide acidity is also important for the inactivation (Alvarado & McKee, 2007). It is reported that citric acid in the lemon juice has a very low amount of undissociated acid rate and exhibits bacteriostatic effect against *L. monocytogenes* (Conner, Scott, & Bernard, 1990). In addition, it has been noted that marinades containing different ingredients have different antimicrobial effects on the microbiota of meat, and gram negative bacteria are more sensitive to acidic condition than gram positive bacteria (Choi, Bae, Kim, Kim, & Rhee, 2009). This situation may explain the reason why *Salmonella* is inactivated in the acidic marinade used in the present study while *L. monocytogenes* survives.

The number of 7.0 $log_{10}$ TMAB is accepted as the upper limit for microbiological quality of raw meats (Karam, Roustom, Abiad, El-Obeidd, & Savvaidis, 2019). In the present study, while the control groups exceeded this upper limit in a short time (4th day), none of the marinated groups reached 7.0 $log_{10}$ during the storage of 10 days. In some studies in the literature, significant reductions in TMAB numbers were achieved (Doğu-Baykut & Güneş, 2014; Janjic et al., 2019; Lytou, Panagou, & Nychas, 2017; Şengün et al., 2019), while in some other studies (Karam et al., 2019; Lytou et al., 2019; Lytou, Panagou, & Nychas, 2016) marinade delayed or slowed down growth of TMAB. Possible reasons of those results may arise from the factors such as the content of the marinade, the pH value, application methods, meat type, initial microbial load of the products and storage temperature.

It is stated that bacteria that can grow at low temperatures such as *Pseudomonas* spp., *Brochothrix thermosphacta* and psycrophilic LAB are play an important role in spoilage of poultry meats (Björkroth, 2005; Karam et al., 2019). In addition, the growth of psychrotrophic bacteria and *Pseudomonas* spp. varies depending on the content of marinades used. Especially, the presence of organic acids in marinade may

significantly slowed down the growth of those bacteria (Smaoui, Hlima, Salah, & Ghorbel, 2011). This information may explain why the marination sauce used in the present study delayed or slowed down the growth of psychrotrophic bacteria in the marinated groups.

In the present study, yeast-mold counts in the marinated chicken breast meat significantly increased during the storage. Similar to this finding, Lytou et al. (2019) found that yeast number in acidic marination sauce became dominant during storage. Karam et al. (2019) noted that the numbers of yeast-molds were the lowest amount of microorganism in the initial microbiota, but their numbers increased during the aerobic storage. In addition, Doğu-Baykut and Güneş (2014) reported that yeast-mold counts increased from 3.0 to 5.0 $\log_{10}$ in 9 days in aerobic packaging. In this study, yeast-mold counts showed unexpectedly fluctuation in the marinated samples. The reason of these results may possibly be due to the yeast-mold cells in microbiota of the tomato and pepper paste used in the marinade may not show a uniform distribution.

The pH levels in both the control and marinated groups increased during the storage. Similar to this finding, Baltić et al. (2015) noted pH increases in all marinated chicken breast meat. The pH increases in the marinated groups were less than those in the control groups. The reason of the rapid increase in pH levels of the control groups may be due to the proteolytic activity of the microorganisms such as *Pseudomonas* spp. which are capable of growth at low temperatures (Lytou et al., 2019). It is also stated that this increase in the pH levels may be caused by the high buffering capacity of the proteins existing in breast meat (Björkroth, 2005).

## 5. Conclusion

In conclusion, the home-made marinate used in this study showed bactericidal effect against *S. Typimurium* and TMAB. It was detected a bacteriostatic effect against *L. monocytogenes* and psychrotrophic bacteria. It can be recomended that the marination sauce can be used to decrase the microbial risks and to extend the shelf-life of poultry meat products. Further studies investigating the effects of home-made marinates on the food-borne pathogens and the shelf-life of meat and meat products may provide useful information for all people who dealing with food.

## CRediT authorship contribution statement

**Gökhan Kürşad İncili:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Müzeyyen Akgöl:** Formal analysis, Investigation. **Mehmet Emin Aydemir:** Formal analysis, Investigation. **Selçuk Alan:** Formal analysis, Investigation. **Muhsin Mutlu:** Formal analysis, Investigation. **Osman İrfan İlhak:** Formal analysis, Investigation, Writing - original draft. **Gülsüm Öksüztepe:** Methodology, Formal analysis, Investigation, Writing - original draft.

## Declaration of competing interest

The authors declare that no conflict of interests in this study. The manuscript has not been published previously and not under consideration for publication in any other journal. Manuscript is read and approved by all authors.

## References


Alvarado, C., & McKee, S. (2007). Marination to improve functional properties and safety of poultry meat. *The Journal of Applied Poultry Research, 16*, 113–120.

Baltić, T., Baltić, Ž. M., Mišić, D., Ivanović, J., Janjić, J., Bošković, M., et al. (2015). Influence of marination on Salmonella spp. growth in broiler breast fillets. *Acta Veterinaria Beograd, 65*, 417–428.

Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). *Lebensmittelwissenschaft und Technologie, 37*, 263–268.

Björkroth, J. (2005). Microbiological ecology of marinated meat products. *Meat Science, 70*, 477–480.

Bowker, B., & Zhuang, H. (2017). Freezing-thawing and sub-sampling influence the marination performance of chicken breast meat. *Poultry Science, 96*(9), 3482–3488.

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. *Journal of Food Protection, 56*, 474–478.

Carroll, C. D., Alvarado, C. Z., Brashears, M. M., Thompson, L. D., & Boyce, J. (2007). Marination of Turkey breast fillets to control the growth of Listeria monocytogenes and improve meat quality in deli loaves. *Poultry Science, 86*, 150–155.

Choi, Y. M., Bae, Y. Y., Kim, K. H., Kim, B. C., & Rhee, M. S. (2009). Effects of supercritical carbon dioxide treatment against generic Escherichia coli, Listeria monocytogenes, Salmonella Typhimurium, and E. coli O157:H7 in marinades and marinated pork. *Meat Science, 82*, 419–424.

Conner, D. E., Scott, V. N., & Bernard, D. T. (1990). Growth, inhibition, and survival of Listeria monocytogenes as affected by acidic conditions. *Journal of Food Protection, 53*(8), 652–655.

Des Field, D., Ross, R. P., & Hill, C. (2018). Developing bacteriocins of lactic acid bacteria into next generation biopreservatives. *Current Opinion in Food Science, 20*, 1–6.

Doğu-Baykut, E., & Güneş, G. (2014). Quality of ready-to-cook marinated chicken drumsticks as affected by modified atmosphere packaging during refrigerated storage. *Journal of Food Processing and Preservation, 38*, 615–621.

Efsa & Ecdc-(European Food Safety Authority and European Centre for Disease Prevention and Control). (2019). The European Union one health 2018 zoonoes report. *EFSA Journal, 17*(12), Article e05926.

Eldin, R. M. B., Talaat, D., Elbaba, A. H., & Ibrahim, M. S. (2020). Antibacterial activity of some plant extracts on different bacteria in chicken fillet. *European Journal of Pharmaceutical and Medical Research, 7*(1), 84–95.

Fouladkhah, A., Geornaras, I., Nychas, G.-J. E., & Sofos, J. N. (2013). Antilisterial properties of marinades during refrigerated storage and microwave oven reheating against post-cooking inoculated chicken breast meat. *Journal of Food Science, 78*(2), 285–289.

Inguglia, E. S., Burgess, C. M., Kerry, J. P., & Tiwari, B. K. (2019). Ultrasound-assisted marination: Role of frequencies and treatment time on the quality of sodium-reduced poultry meat. *Foods, 8*, 473.

Iso 21527 1. (2008). *Microbiology of food and animal feeding stuffs -horizontal method for the enumeration of yeasts and moulds-Part 1: Colony count technique in products with water activity greater than 0*.

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. *Journal of Food Science & Technology, 55*(8), 3093–3097.

Janjic, J., Ciric, J., Grbic, S., Boskovic, M., Glisic, M., Mitrovic, R., et al. (2019). Reduction of microbiota in marinated vacuum-packaged poultry breast fillets. *Meat Technology, 60*(1), 1–7.

Karam, L., Roustom, R., Abiad, M. G., El-Obeidd, T., & Savvaidis, I. N. (2019). Combined effects of thymol, carvacrol and packaging on the shelf-life of marinated chicken. *International Journal of Food Microbiology, 291*, 42–47.

Kim, H.-Y., Kim, K.-J., Lee, J.-W., Kim, G.-W., Choe, J.-H., Kim, H.-W., et al. (2015). Quality characteristics of marinated chicken breast as influenced by the methods of mechanical processing. *Korean Journal of Food Science of Animal Resources, 35*(1), 101–107.

Lawley, R., Curtis, L., & Davis, J. (2008). Salmonella. In *The food safety hazard guidebook* (pp. 60–66). Cambridge, UK: RCS Publishing.

Lytou, A. E., Nychas, G.-J. E., & Panagou, E. Z. (2018). Effect of pomegranate based marinades on the microbiological, chemicaland sensory quality of chicken meat: A metabolomics approach. *International Journal of Food Microbiology, 267*, 42–53.

Lytou, A., Panagou, E. Z., & Nychas, G.-J. E. (2016). Development of a predictive model for the growth kinetics of aerobic microbial population on pomegranate marinated chicken breast fillets under isothermal and dynamic temperature conditions. *Food Microbiology, 55*, 25–31.

Lytou, A. E., Panagou, E. Z., & Nychas, G.-J. E. (2017). Effect of different marinating conditions on the evolution of spoilage microbiota and metabolomic profile of chicken breast fillets. *Food Microbiology, 66*, 141–149.

Lytou, A. E., Tzortzinisa, K., Skandamis, P. N., Nychas, G.-J. E., & Panagoua, E. Z. (2019). Investigating the influence of organic acid marinades, storage temperature and time on the survival/inactivation interface of Salmonella on chicken breast fillets. *International Journal of Food Microbiology, 299*, 47–57.

Nisiotou, A., Chorianopoulos, N. G., Gounadaki, A., Panagou, E. Z., & Nychas, G.-J. E. (2013). Effect of wine-based marinades on the behavior of Salmonella Typhimurium and background flora in beef fillets. *International Journal of Food Microbiology, 164*, 119–127.

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 Microbiology, 74*, 75–85.

Oscar, T. P. (2007). Predictive model for growth of Salmonella Typhimurium DT104 from low and high initial density on ground chicken with a natural microflora. *Food Microbiology, 24*, 640–651.

Pathania, A., McKee, S. R., Bilgili, S. F., & Singh, M. (2010). Antimicrobial activity of commercial marinades against multiple strains of Salmonella spp. *International Journal of Food Microbiology, 139*, 214–217.

Rhoades, J., Kargiotou, C., Katsanidis, E., & Koutsoumanis, K. P. (2013). Use of marination for controlling Salmonella enterica and Listeria monocytogenes in raw beef. *Food Microbiology, 36*, 248–253.

Şengün, İ. Y., Göztepe, E., & Öztürk, B. (2019). Efficiency of marination liquids prepared with koruk (Vitis vinifera L.) on safety and some quality attributes of poultry meat. *LWT - Food Science and Technology, 113*, 108317.

Silva, F., Domingues, F. C., & Nerín, C. (2018). Trends in microbial control techniques for poultry products. *Critical Reviews in Food Science and Nutrition, 58*(4), 591–609.

Smaoui, S., Hlima, H. B., Salah, R. B., & Ghorbel, R. (2011). Effects of sodium lactate and lactic acid on chemical, microbiological and sensory characteristics of marinated chicken. *African Journal of Biotechnology, 10*(54), 11317–11326.

Thanissery, R., & Smith, D. P. (2014). Marinade with thyme and orange oils reduces Salmonella Enteritidis and Campylobacter coli on inoculated broiler breast fillets and whole wings. *Poultry Science, 93*, 1258–1262.

USDA/FSIS. (2011). *Microbiology Laboratory Guidebook. Metot 3.01. Quantitative analysis of bacteria in foods as sanitary indicators*.

Zarai, Z., Boujelbene, E., Salem, N. B., Gargouri, Y., & Sayari, A. (2013). Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from Piper nigrum. *LWT-Food Science and Technology, 50*, 634–641.