

Effect of in-bag carcass decontamination method on shelf life of whole chicken carcasses packaged in plastic bags

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Abstract: This study was undertaken to investigate the changes in the microbiological and sensorial attributes of fresh chicken carcasses immersed in decontamination solutions, followed by packaging in plastic bags in which decontamination solution was added during storage at 4 °C. The samples were taken on days 0, 3, 7, and 10 for microbiological and sensory attributes. Results showed that there was a limited antimicrobial effect on microbial flora in the treatment groups within the first 3 days of storage compared to the control group. However, the difference between the groups completely disappeared on days 7 and 10. The changes in the numbers of the microflora between days 0 and 10 varied between 4.5–7.9 log₁₀ CFU/mL for total aerobic mesophiles, 3.6–8.58 log₁₀ CFU/mL for psychrotrophic bacteria, 2.7–6.7 log₁₀ CFU/mL for coliforms, and 2.2–5.9 log₁₀ CFU/mL for yeast and molds. Sensory evaluation of the cooked breast meat of the carcasses indicated that there was no appreciable difference between the groups between days 0 and 7. It was concluded that the in-pack decontamination method described here should be improved for antimicrobial effect and has potential to be used without affecting sensory attributes of the carcasses in poultry.

Key words: Chicken, coliform, decontamination, *Salmonella* spp., shelf life

1. Introduction

Poultry meat and poultry meat products account for 30% of total meat consumption in the world. Due to its high consumption rate, reliability, lower degradation rate, color, taste, and appearance, poultry meat is an important food staple. However, in addition to the general appearance and quality problems of contaminated poultry meat by microorganisms, it plays an important role in the transmission of food-borne pathogens to humans [1–3].

In order to produce healthy and extended shelf-life poultry meat, microbial contamination should be minimized. Although slaughtering hygiene is taken into consideration, contamination of poultry meat cannot be completely prevented during slaughtering processes. It was reported that the initial microflora of poultry carcasses is generally between 10²–10⁶ CFU/g [4]. Broiler growing conditions, transportation methods, slaughterhouse processes such as chilling, cutting, type of packaging, storage, distribution, and many other factors affect the microbial quality of carcasses [5]. In addition, especially in the slaughterhouses, scalding, defeathering, evisceration, and the cooling steps involved play an important role in crosscontamination and proliferation of microflora in

poultry meat [6]. The initial microflora of broiler meat may include microorganisms that are not only caused by spoilage such as *Moraxella* spp., *Acinetobacter* spp., *Flavobacterium* spp., and *Pseudomonas* spp. but also due to pathogenic bacteria such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Bacillus cereus*, *E. coli*, and *Clostridium* spp. It was reported that *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, and pathogenic *E. coli* are major pathogens responsible for poultry meat-borne infections and poisonings [7–10].

Many studies have been carried out on decontamination practices at different stages of slaughterhouse processes to extend the shelf life of poultry meat and to inactivate pathogenic microorganisms such as *Salmonella* spp. and *C. jejuni* [1, 7–11]. Numerous studies have also been conducted to investigate the antimicrobial activity of chemicals [8,9,12–14]. The most commonly used chemicals are organic acids [10], trisodium phosphate [15], chlorinated disinfectants [9], acidified sodium chloride [7], peracetic acid [11], and cetylpyridinium chloride [16]. The antimicrobial effect of these chemicals varies depending on concentration, exposure time, administration method, chemical substance, and pH [1].

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The results of these studies reveal that *Salmonella* spp. counts can be reduced 2–3 log by using these chemicals.

The literature presented above shows that studies carried out with the aim to increase food safety and extend the shelf life of chicken carcasses are mostly focused on the slaughterhouse and prepackaging stages. However, with the exception of cold storage, there is a need for applications that can be effective on microorganisms during shelf life. Whole chicken carcasses are usually packaged in a plastic bag, which is low-cost and easy to handle. Since poultry carcasses come into contact with water or decontamination liquids at many stages during the slaughtering process, some liquid can still be found on the carcass surface in the process leading up to packaging, and it can remain in the packaging during storage. Because this occurs, this study aimed to investigate whether adding a small amount of decontaminant fluid in the bag may cover the whole external surface of the carcass due to tight packaging and provide protection against the growth of pathogenic and spoilage bacteria during refrigerated storage.

2. Materials and methods

This study was carried out in a commercial poultry slaughterhouse with an air chilling system. Whole chicken carcasses chilled and sorted/graded prior to packaging were used. Experimental groups were as follows: the control group, 1% lactic acid (LA) group, 0.1% cetylpyridinium chloride group (CPC), and 8% trisodium phosphate (TSP) group. The groups were prepared at the point of sorting and packaging in the slaughter processing line. Eight randomly selected carcasses weighing 1.5–1.8 kg were used for each group. Three liters of decontamination solutions were prepared in separate plastic containers allowing complete immersion of the chicken carcass. Selected carcasses were first immersed into the decontamination solution without holding and handed to the slaughterhouse workers for stuffing into the plastic bag used for the company's own products. Approximately 15 mL of the decontamination solution was then taken from the container and added to the stuffed plastic bag; following this, the bags were clipped as usual by using the slaughterhouse's clippers. No immersion or fluid addition in the bags was carried out in the control group. All of the chicken carcasses were kept in cold storage (4 ± 1 °C) and microbiological, chemical, and sensory analyses were performed on days 0, 3, 7, and 10 of the storage period. The study was carried out in 3 independent repetitions, 8 carcasses for each group, and 32 for each repetition; 96 carcasses were used in total.

On the days of analysis, broiler carcasses were sampled by using the carcass-rinse method for *Salmonella* spp. detection [17]. The chicken carcasses were briefly removed from cold storage and opened under aseptic conditions,

then taken into sterile homogenization bags of 380 × 580 mm size. Approximately 400 mL of sterile buffered peptone water (BPW) (Merck, Darmstadt, Germany) was then added to the homogenization bag and manually shaken for 2 min. Although this sampling method was mainly developed for *Salmonella* analysis, it was used in this study to determine the number of total mesophilic aerobic colony, psychrotrophic bacteria, coliform, yeast-mold, and for the presence of *Salmonella* spp.

2.1. Microbiological analysis

Decimal dilutions of the rinse solution were made using 0.1% peptone water, and plate count agar (PCA) (Merck, Darmstadt, Germany) (48 h at 35 °C) was used for the total number of mesophilic aerobic colonies; psychrotrophic bacteria PCA (7–10 days at 7 °C) [18], violet red bile agar (Merck, Darmstadt, Germany) (24 h at 35 °C) for coliform bacteria [19]; and dichloran rose Bengal chloramphenicol agar (Merck, Darmstadt, Germany) (4–5 days at 25 °C) for the number of yeast-molds [20]. In order to determine the presence of *Salmonella* spp., rinsing liquid obtained with BPW was incubated for 24 h at 37 °C for preenrichment. After preenrichment, 0.1 mL and 1 mL of preenrichment culture were inoculated in 10 mL rappaport vassiliadis (RV) and 10 mL tetrathionate (TT) broth, respectively. RV medium was incubated at 42 °C for 24 h, and TT medium was incubated at 37 °C for 24 h. Then, xylose lysine deoxycholate agar (Merck, Darmstadt, Germany) and xylose lysine tergitol-4 (Merck, Darmstadt, Germany) agar were used for both selective enrichment fluids, and the plates were incubated at 37 °C for 24 h. At least 2 of the black-centered colonies surrounded by black or yellow zones were taken and added to triple sugar iron agar and lysine iron agar (Merck, Darmstadt, Germany). Following 24 h of incubation at 37 °C, typical *Salmonella* reaction cultures were confirmed by biochemical (API 20E) (Biomerieux, France) and serological agglutination with polyvalent *Salmonella* antisera (Oxoid, UK) [21].

2.2. Chemical and sensory analyses

The pH values of the samples (25 ± 1 °C) were determined by pH meter (P selecta pH 2001). After opening the chicken sampling bags, the liquid in the packaging bag (decontaminant added) was collected aseptically and used for pH measurement.

Sensory analysis was carried out by 9 panelists using hedonic scales with a range of 0–9 points. Five points were selected as the lowest acceptable level. The bags were opened, the decontamination fluids inside the bags were drained, and the color/appearance of the raw carcass was evaluated first; then, the breast meat was isolated from the carcass and cooked in the oven for 45 min at 180 °C. The panelists evaluated the samples for appearance, texture, flavor, and general acceptance [22].

2.3. Statistical analyses

Microbiological findings were converted to log₁₀ CFU/mL and statistical analyses were performed by using the variance analysis in accordance with 4 (×) 4 (test group (×) sampling times) factorial design. Mean counts were separated using Fisher's Least Square Differences method. Statistical analysis was performed using the statistical analysis system package program [23]. The statistical significance level was accepted as P < 0.05.

3. Results

Microbiological findings are shown in Table 1. Although there were limited antimicrobial effects in the first 3 days in the treatment groups compared to the control group on the microbial flora (P > 0.05), these differences disappeared on the 7th and 10th days (P > 0.05). No significant differences were found among the groups in any of the microbial analyses on sampling days. On the other hand, the number of total mesophilic aerobic colony, psychrotrophic colony numbers, and coliforms showed significant changes in all experimental groups during storage. The amount of yeast and mold increased in all groups, as well, but the change within 10 days was not significant (P > 0.05). All carcasses

sampled were found to be *Salmonella* spp. positive in all groups throughout the storage period.

3.1. pH results

The pH values of the fluids collected from the bags are shown in Figure 1. The pH of the CPC groups remained relatively constant at approximately 6.70 between days 0 and 10. The pH values in the TSP and LA groups exhibited significant changes. The pH of the TSP group decreased from 11.29 to 7.04, while that of LA increased from 3.97 to 6.16 during storage. pH values were not measured in the control group since no liquid was added to the packaging bags.

3.2. Sensory analyses results

As a result of sensory evaluation of cooked chicken breast meat, no significant differences were found among groups between days 0 and 7 (P > 0.05). On the 10th day, the sensory panel was not performed because the sensory qualities of all groups were considered unacceptable. The results of sensory analyses are shown in Table 2.

4. Discussion

The reason for immersing the chilled-chicken carcasses into decontamination solution and adding 15 mL of the

Table 1. Changes in the number of microorganisms during storage at 4 °C in whole broiler carcasses in plastic packaging bags containing antimicrobial solution (log₁₀ CFU/mL ± SD).

Analysis	Groups	Storage period (day)			
		0	3	7	10
Total aerobic mesophilic colony	control	4.71 ± 0.26 ^b	5.6 ± 0.3 ^b	6.43 ± 1.06 ^{ab}	7.88 ± 0.58 ^a
	CPC 0.1%	4.48 ± 0.6 ^b	5.08 ± 0.43 ^b	5.94 ± 0.77 ^{ab}	7.6 ± 0.33 ^a
	TSP 8%	4.6 ± 0.83 ^b	5.03 ± 0.27 ^b	5.6 ± 0.96 ^{ab}	7.7 ± 0.3 ^a
	LA 1%	4.81 ± 1.06 ^b	4.95 ± 0.88 ^b	6.16 ± 0.44 ^{ab}	7.66 ± 0.5 ^a
Psychrotrophic bacteria	Control	4.48 ± 0.98 ^c	5.97 ± 0.36 ^b	7.19 ± 0.7 ^b	8.58 ± 0.17 ^a
	CPC 0.1%	4.14 ± 0.2 ^b	5.56 ± 0.6 ^b	7.06 ± 0.34 ^a	8.15 ± 0.06 ^a
	TSP 8%	3.61 ± 0.21 ^b	5.13 ± 0.78 ^b	7.13 ± 0.34 ^a	8.12 ± 0.38 ^a
	LA 1%	4.23 ± 0.25 ^b	5.0 ± 0.28 ^b	7.07 ± 0.7 ^a	7.86 ± 0.06 ^a
Coliform	Control	3.14 ± 0.28 ^b	4.06 ± 0.13 ^b	5.4 ± 0.16 ^{ab}	6.14 ± 0.26 ^a
	CPC 0.1%	2.73 ± 1.05 ^b	3.83 ± 0.4 ^b	5.61 ± 0.25 ^a	6.71 ± 0.58 ^a
	TSP 8%	3.13 ± 0.24 ^b	3.21 ± 0.5 ^b	4.76 ± 1.03 ^{ab}	5.9 ± 0.3 ^a
	LA 1%	3.95 ± 1.0 ^b	3.07 ± 0.39 ^b	4.22 ± 0.1 ^{ab}	5.65 ± 0.15 ^a
Mold-yeast	Control	2.85 ± 0.1	3.26 ± 2.49	5.07 ± 0.84	5.9 ± 1.28
	CPC 0.1%	2.4 ± 0.34	4.48 ± 2.17	4.92 ± 1.27	5.54 ± 1.78
	TSP 8%	2.24 ± 0.34	3.33 ± 1.89	3.98 ± 0.29	5.51 ± 0.53
	LA 1%	2.81 ± 0.61	4.2 ± 1.56	4.94 ± 0.65	5.78 ± 0.57

CPC: cetylpyridinium chloride; TSP: trisodium phosphate; LA: lactic acid.
 abc: values containing different letters in the same line are statistically different (P < 0.05).

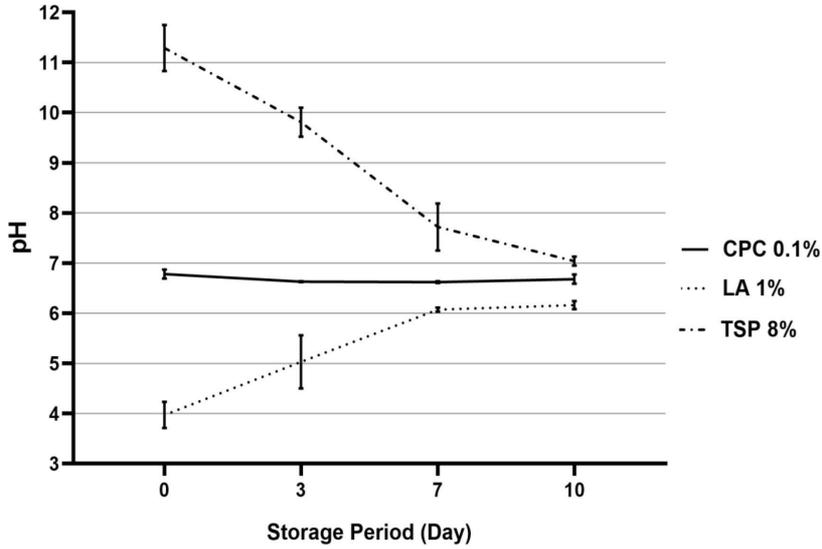


Figure 1. pH values of the remaining liquids in the chicken packaging bags. CPC: cetylpyridinium chloride; TSP: trisodium phosphate; LA: lactic acid.

Table 2. Changes in sensory attributes of whole broiler carcasses in plastic bags with an antimicrobial solution during storage at 4 °C (mean value ± SD).

Sensory analysis	Day	Group			
		Control	CPC 0.1%	TSP 8%	LA 1%
Color	0	7.9 ± 0.3	7.7 ± 0.5	7.5 ± 0.5	7.5 ± 0.5
	3	7.7 ± 0.8	8.2 ± 0.6	8.1 ± 0.7	7.9 ± 0.7
	7	7.3 ± 0.5	7.5 ± 0.5	7.3 ± 0.8	7.0 ± 0.9
Appearance	0	8.1 ± 0.3	7.9 ± 0.5	7.9 ± 0.5	7.9 ± 0.5
	3	7.9 ± 0.7	8.2 ± 0.8	8.2 ± 0.7	8.0 ± 0.7
	7	7.5 ± 0.5	7.3 ± 0.5	7.0 ± 0.9	7.0 ± 0.9
Odor	0	8.0 ± 0.1	8.1 ± 0.3	7.9 ± 0.5	7.6 ± 0.8
	3	7.8 ± 0.5	8.0 ± 0.4	7.9 ± 0.5	7.8 ± 0.6
	7	7.0 ± 0.6	6.8 ± 0.8	7.0 ± 1.1	6.3 ± 1.2
Firmness	0	7.9 ± 0.7	8.0 ± 0	7.9 ± 0.3	7.9 ± 0.7
	3	7.6 ± 1.0	7.8 ± 0.6	7.7 ± 0.9	7.7 ± 0.7
	7	7.0 ± 0.9	7.0 ± 0.9	7.2 ± 1.0	6.8 ± 1.0
Flavor	0	8.0 ± 0	8.0 ± 0	8.0 ± 0	8.0 ± 0
	3	7.6 ± 1.0	7.8 ± 1.1	7.7 ± 1.2	7.5 ± 1.1
	7	7.0 ± 1.1	7.3 ± 0.5	6.8 ± 1.6	6.2 ± 1.3
General acceptance	0	8.2 ± 0.2	8.2 ± 0.4	7.8 ± 0.4	7.8 ± 0.4
	3	7.6 ± 1.1	7.8 ± 1.1	7.7 ± 1.3	7.6 ± 1.1
	7	7.0 ± 0.6	7.3 ± 0.5	7.2 ± 1.0	6.3 ± 1.0

CPC: cetylpyridinium chloride; TSP: trisodium phosphate; LA: lactic acid.

solution into the bag was to take advantage of the residual antimicrobial effect of the solutions that could remain effective during storage. Therefore, it could be expected that by the time consumers purchased the product, the activity of the spoilage microorganism had decreased and *Salmonella* had become inactive if at all present. However, this did not occur. Even if the antimicrobial effects of these compounds have been proven by a number of studies [24–29] and commonly used by the broiler industry in the US and in other countries, their effects are influenced by many factors including time and concentration, microbial load, mode of application, and temperature. The majority of broiler carcass decontamination solutions focused on the inactivation of *Salmonella* spp. before packaging. In general, the results of these studies showed that immersing carcasses in TSP (8–10% w/v) for various times (10–30 min) at different temperatures (25–35 °C) can yield 1.0–2.0 log reduction in *Salmonella* spp. numbers on the carcasses [24–29]. As for lactic acid, Izat et al. [30] sprayed *Salmonella*-contaminated carcasses after the precooling stage with 2%–5% lactic acid solution, and they did not find any reduction in *Salmonella* numbers, similar to our findings. Kanellos and Burriel [31] reported that *Salmonella* spp. counts decreased 3.0 log₁₀ CFU/mL by immersing the carcasses in a 1.5% LA solution for 30 min. Hwang and Beuchat [26] obtained a 2.0 log₁₀ CFU/cm² reduction in the number of *Salmonella* spp. by immersing the chicken breast meat in 1% LA solution at 25 °C for 30 min. Kim and Slavik [16] reported that 0.1% cetylpyridinium chloride resulted in 0.9–1.7 log₁₀ CFU/cm² by spray method and 1.0–1.6 log₁₀ CFU/cm² with the immersing method. One factor should be underlined in the current study and that is all of the carcasses were found positive for *Salmonella* spp., indicating that the slaughter hygiene of the factory was very poor. *Salmonella* spp. was not expected to be found in the carcasses. However, it turned out that the carcasses were carrying *Salmonella* spp. as if they had been inoculated for experimental purposes. Nevertheless, this gave us a better opportunity to evaluate the effects of the treatments against *Salmonella* spp.. The main reason why there were no decreases in microbial numbers or presence of *Salmonella* spp. in our treatment groups could be due to an insufficient volume of antimicrobial solutions added to the packaging bags. A volume of 15 mL was chosen to avoid any undesired reaction by the consumers that could be related to excessive fluid in the bag. In addition, the solutions probably did not uniformly spread to the entire surface area, which resulted in differences in the molarity of the antimicrobial substance per unit area. It was assumed that the tight packaging would allow the liquid to stay in the space between the carcass surface and the packaging material and spread to the whole carcass. However, this did not effectively occur due to the irregular

shape of the carcass (folded regions: the tail and the wing, etc.) and because of gravity.

The effects of the tested antimicrobial solutions on bacterial and fungal flora of the chicken carcasses were studied to a lesser extent compared to *Salmonella* spp.. The reductions were between 1.0–2.5 log CFU depending on the application method [13,26,32–34]. For example, Xiong et al. [34] reported a 2.2–2.5 log₁₀ CFU/g reduction in the total mesophilic aerobic colony count in chicken breast meat by spraying with a 5–10% TSP solution for 30 s at the slaughtering stage. Sinhamahapatra et al. [13] found a 1.4 log₁₀ CFU/cm² reduction in the total mesophilic aerobic colony count by immersing the carcasses in a 2% LA solution for 30 s at the slaughtering stage. In another study, Hwang and Beuchat [26] found 1.8 log₁₀ CFU/cm² reduction in the number of psychrotrophic colonies by immersing chicken wing parts with skin decontaminated in 1% TSP solution at 25 °C for 30 min. Sinhamahapatra et al. [13] reported that 1.1 log₁₀ CFU/cm² reduction in the number of coliform bacteria was found at the slaughterhouse stage on the chicken carcasses by spraying 2% lactic acid solution for 30 s. Sakhare et al. [35] found a 3.0 log₁₀ CFU/cm² reduction in the number of coliform bacteria on chicken carcasses at the slaughtering stage by immersion for 60 s with 0.25% LA solution. As for the yeast and mold in chicken carcasses, there is very limited data. Kanellos and Burriel [31] reported that 0.25% lactic acid significantly reduced the number of yeasts and molds. As these studies indicate, TSP and LA usually yield reductions varying between 1.0 to 3.0 log depending on the microflora tested. However, in the current study no differences occurred among the control and treatment groups in mesophilic, psychrotrophic colonies, and coliform bacteria numbers. The reasons explained for *Salmonella* spp. are also valid for normal flora. In addition, an interesting finding of this study was a trend in the effect of flora on the pH values of decontaminant solutions. As seen in Figure 1, the pH of TSP solution collected from the packages, which was very alkaline, decreased from 11.3 to 7.04, while the pH of the lactic acid increased from 3.97 to 6.16. This indicated that the changes in the microbial flora were able to drive changes toward neutral pH values, which promote microbial growth.

In addition to having proper slaughter hygiene and decontamination applications during the slaughterhouse process, innovative applications are required to maintain the antimicrobial effect during storage of chicken meat. In-pack antimicrobial applications have been developed and applied to cooked meat products and have become successful against *Listeria monocytogenes* [36]. However, the results of this study indicated that the concentration and volume of the solutions need to be greatly increased to overcome the survival strategies of microbial flora.

In conclusion; poultry meat is a nutritious but quite perishable food. Although modern slaughterhouses where food safety systems such as Good Manufacturing Practices, Good Hygiene Practices, and Hazard Analysis and Critical Control Point applied are essential for assuring the microbial safety and shelf life of the product, maintaining the cold chain still plays a key role during shelf life. In regions or countries where the cold chain can not be assured during transportation or at retail points, innovative approaches may be required to prevent microbial growth. In this regard, the use of the in-bag decontamination solutions described in this study can be considered as a first step in providing relevant initial

data. However, further studies are required to achieve acceptable prevention of microbial growth with the use of safe antimicrobials.

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Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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