Spraying Chicken Skin with Selected Chemicals to Reduce Attached Salmonella typhimurium

HUA XIONG,1 YANBIN LI,1,* MICHAEL F. SLAVIK,2 AND JOEL T. WALKER1

1Department of Biological & Agricultural Engineering and 2Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, USA

MS 97-19: Received 31 January 1997/Accepted 4 February 1997

Abstract

Aqueous solutions of 5% and 10% trisodium phosphate (TSP), 0.1% and 0.5% cetylpyridinium chloride (CPC), 1% and 2% lactic acid (LA), and 0.1% and 0.5% grapefruit seed extract (DF-100) were evaluated in prechill spraying for reducing Salmonella typhimurium attached on chicken skins. Chicken skins were inoculated with S. typhimurium and then sprayed with the selected chemical solutions for 30 sec at 206 kPa and 20°C. After chemical spraying, the skins were rinsed by spraying tap water for 30 sec. Each skin was stomached in buffered peptone water (BPW) for 1 min. The stomaching water was then diluted serially, inoculated onto both xylose lysine tergitol (XLT4) agar and Aerobic Plate Count (APC) Petrifilm®, and incubated for 24 hr at 37°C. The results showed that the numbers of Salmonella on the chicken skins after the chemical spraying were significantly lower than those without spray (P < 0.05). The CPC reduced Salmonella by 1.5 to 1.9 log10, TSP resulted in a 2.1 to 2.2 log10 reduction of Salmonella, and DF-100 produced a 1.6 to 1.8 log10 reduction of Salmonella. The LA had a number of Salmonella with a 2.2 log10 reduction. The 0.5% CPC resulted a significantly greater reduction in Salmonella than 0.1% CPC. There were no significant differences in Salmonella reduction between different concentrations of the other three chemicals.

Contamination of poultry products with Salmonella has been and continues to be of concern to consumers and the poultry industry (3, 6). Research is needed to find effective and safe methods to reduce incidences of Salmonella on poultry carcasses. Various chemicals have been tested with varying degrees of success for reducing bacterial contamination on chicken carcasses. Trisodium phosphate (TSP), cetylpyridinium chloride (CPC), and lactic acid (LA) have shown their bactericidal ability on chicken carcasses and chicken skins in both immersion and spray treatments (1, 11–15, 18, 19, 21, 26). Since its approval for use in poultry processing, TSP treatments have received much attention as a method to control bacterial contamination (1, 7, 8, 19). TSP not only has high antimicrobial activity on chicken carcasses but it also reduces bacteria by removing a thin layer of lipids from the chicken skin surfaces (7, 8). CPC is approved for use as a mouthwash and has a neutral pH (17, 20). CPC has potential applications in poultry processing because it has strong germicidal activity (2, 17, 20). CPC also causes no carcass bloating or skin discoloration and is noncorrosive on equipment (2, 20). LA has been investigated as an antibacterial agent (10, 26) and is widely used in the food industry because of its generally regarded status as safe (26). Grapefruit seed extract (DF-100) has been evaluated for potential use as an antimicrobial agent in poultry processing and in the storage of various fruits and vegetables (4, 15). Sawaya et al. (21) studied the effects of a pretreatment with LA buffer on the shelf life of broiler carcasses stored at simulated market conditions of 4°C and 7°C. The treated group was dipped for 1 min in a 2% LA solution and then drained for 15 min before packaging. The shelf life of the treated broiler carcasses was extended by 6 to 7 days and 5 to 6 days for the 4°C and 7°C storage temperatures, respectively.

Seven chemicals, including TSP, sodium tripolyphosphate, monosodium phosphate, sodium acid pyrophosphate, sodium hexametaphosphate, LA, and NaOH, were investigated by Hwang and Beuchat (9) for their effectiveness in reducing pathogenic populations on chicken skins. They found that the Salmonella on chicken skins was reduced by 1.0 to 1.6 log10 using a 1% TSP washing treatment for 30 min. Kim et al. (12) investigated a 15-sec immersion of chicken skins in a 10% TSP solution. A 1.7 and 2.2 log10 reduction of Salmonella was found on chicken skins treated with TSP solution at temperatures of 10°C and 50°C, respectively. Kim et al. (13) also showed that the incidence of Salmonella on chicken skins would be reduced from 12 to 24% to 0 to 8% using a 10% TSP dipping treatment for 15 sec in comparison with water dipping. Lillard (19) treated inoculated chicken carcasses by immersing them in a 10% TSP solution for 15 min, and a 2 log10 reduction of Salmonella was subsequently observed. Slavik et al. (22) conducted trials in which postchill chicken carcasses were dipped in a 10% TSP solution for 15 sec at 10°C and 50°C. They found that the levels of Campylobacter on chicken carcasses were reduced by an average of 1.5 and 1.2 log10 in storage at 40°C for 1 and 6 days, respectively. Rathgeber and Waldroup (23) used Brifisol K® (BK Ladenburg Corp., Ladenburg, Germany) (a commercial blend of SAPP) solution in chill water to evaluate its bactericidal activity. There were significant reductions in Escherichia coli and aerobic

* Author for correspondence. Tel: (501) 575-2424; Fax: (501) 575-7139; E-mail: yli@saturm.uark.edu
plate count (APC) when chicken carcass halves were chilled with 1.5% TSP at 1°C for 60 min.

Lattin et al. (17) conducted a series of trials to investigate the efficacy of CPC in reducing Salmonella and Campylobacter on chicken carcasses. Immersing prechill poultry carcasses in 0.1% CPC solution for 10 min reduced Salmonella by 2.3 log10 and Campylobacter by 1.7 log10, and the APC by 1.7 to 2.2 log10 per carcass.

Cho et al. (4) investigated the antibacterial and antifungal effects of DF-100 for its use as a sanitizer, disinfectant, and preservative. Their results showed that DF-100 destroys microorganisms by disrupting the function of the microbial cell wall membrane and microbial spores. Kim et al. (15) dipped chicken skins in DF-100 solution for 1 and 3 min, and the number of Salmonella on chicken skins was reduced by 0.8 to 1.2 log10, respectively, at a concentration of 0.1% and 1.6 to 1.7 log10, respectively, at a concentration of 0.5%.

Chemical spraying treatments have been evaluated for reducing or eliminating pathogenic bacteria on chicken carcasses. CPC spraying was investigated for its effectiveness in reducing attached Salmonella on chicken skins (20). The results showed that a 1- to 3-min 0.5% CPC spraying on chicken skins resulted in a 0.9 to 2 log10 reduction of Salmonella. Li et al. (18) sprayed chicken carcasses at two pressures with either CPC, TSP, sodium chloride (NaCl), or sodium bisulfate (SBS) to reduce attached Salmonella. At both 207 and 345 kPa spray pressures, 5% TSP and 5% SBS reduced S. typhimurium by 1.4 to 1.6 log10, and the 10% TSP and 10% SBS treatments resulted in 3.7 and 2.4 log10 reductions, respectively. The objective of this study was to compare selected chemicals, including water, for their effectiveness as spraying treatments in reducing Salmonella attached to chicken skin.

**MATERIALS AND METHODS**

**Bacterial culture.** Salmonella typhimurium (ATCC 14028) maintained in a brain-heart infusion (BHI) agar slant at 4°C was cultured in BHI broth at 37°C for 18 to 20 h. Then the culture was loop-transferred to 4.5 ml of BHI broth and incubated at 37°C for 18 to 20 h. The titer of the final culture was approximately 10⁹ colony-forming units (CFU)/ml.

**Chemical spraying system.** A chemical spraying system was designed and constructed for the tests on chicken skins. The system consisted of a spray solution storage tank, a pressure pump, a pressure regulator, three chicken skin holders, and a spraying chamber with three spray nozzles. The chemical solutions were prepared in the storage tank and pumped to the nozzles through the pressure regulator. Inoculated chicken skins were mounted on the skin holders for exposure to the chemical spraying treatments. Another chamber was used for water rinse after the chemical spray.

The chamber had dimensions of 80 × 50 × 40 cm (width × depth × height) and was made of clear Plexiglas® plastic (Cope Plastics Inc., Little Rock, AR), allowing viewing of the spray patterns. The lid of the chamber could be opened to insert the skin holders on a mounted, adjustable stainless steel rail. The spray nozzles were installed in front, and the distance between the nozzles and skin holders could be adjusted from 15 to 45 cm by moving the holder rail.

Two sizes of Standard FullJet nozzles (Spraying Systems Co., Wheaton, IL) were used. Nozzles with a diameter of 0.08 cm (1/8GG-SS1) were selected for the chemical spraying and nozzles with a diameter of 0.12 cm (1/8GG-SS2) for the water rinses. The FullJet nozzles gave a solid cone-shaped spray pattern with a circular impact area and produced a uniform spray over a wide range of pressures. GG series FullJet nozzles with removable caps and vanes made it possible to remove the working end for cleaning without having to remove the nozzle bodies from the header. A booster pump was used to provide high-pressure spray. The spray pressure could be adjusted from 0 to 1,104 kPa by the pressure regulator (Grainger®, W.W. Grainger, Inc., Springfield, AR). The chicken skin holder consisted of two plastic plates, each with a dimension of 10 × 10 cm. One plate had a 0.1-cm-deep, 7-cm-diameter circular recess for holding the skin. The other plate served as a cover with a 1.5-cm-deep edges and a 7-cm-diameter hole for exposing the skin to the spray.

**Chicken skin samples.** Prechill chicken carcasses were obtained from the end of an eviscerating line at a processing plant, stored in a carbon box with a plastic bag, and then transported to our poultry-processing pilot plant in a half hour. The chicken skin samples, approximately 9 to 10 cm in diameter, were aseptically cut from the breast area. Each sample was placed in a skin holder for spraying. Each sample had a 38-cm² exposure area, which was inoculated with S. typhimurium by gently spreading 0.1 ml of inoculum on the skin surface and leaving it for 20 min to allow for bacterial attachment. Loosely attached Salmonella were washed off by dipping the skins three times in a bucket filled with 15 liters of tap water. The three skins in their holders were then placed on the holder rail in the spray test chamber for the spraying treatments.

**Spraying treatments.** Four chemicals were evaluated in the spraying treatments, including 5% or 10% trisodium phosphate (TSP, Rhone-Poulenc, Cranbury, NJ), 0.1% or 0.5% cetlypyridinium chloride (CPC, Sigma, St. Louis, MO), 1% or 2% lactic acid (LA, Fisher Scientific, Fairlawn, NJ) and 0.1% or 0.5% grapefruit seed extract (DF-100, Chemie Research & Manufacturing Co., Casselberry, FL). The skin samples were treated by spraying the selected chemical solution for 30 sec at 207 kPa at room temperature. The distance between the skin and nozzle was 15 cm. After the chemical spraying, the skin samples in the holders were rinsed in a rinse chamber to remove chemical residues by spraying tap water for 30 sec at 207 kPa at room temperature. A group of three skin samples without spray treatment was dipped five times each in each of two buckets with 15 liters of tap water. The spray treatment was performed for each of the selected chemicals and tap water. All trials were duplicated once.

**Microbial enumeration.** Each treated skin sample was put into a Whirl-pak™ plastic bag (Nasco, Fort Atkinson, WI) containing 50 ml of 0.1% buffered peptone water (Difco Laboratories, Detroit, MI) and automatically stomached for 1 min using a Stomacher 400 Lab Blender (Seward Medical, London, UK). One milliliter of the stomaching solution in each bag was transferred into 9 ml of buffered peptone water, which was then serially diluted to 10⁻³.

Enumeration of Salmonella was performed by serially plating 0.1 ml of diluted solution on xylose lysine tergitol 4 agar (Difco), incubating at 37°C for 18 to 20 h, and then counting black colonies of Salmonella. APC were performed to determine the injured cells by serially plating 1-ml dilutions of the stomaching water on Aerobic Plate Count Petrifilm® (3M, Brookings, SD), incubating at 37°C for 18 to 20 h, and then counting pink colonies on Petrifilm. The APC and number of Salmonella on chicken skins in each treatment were converted to log10 CFU/ml for statistical analysis.

**Statistical analysis.** The values of means, standard deviations, and significant differences (P < 0.05) were analyzed using
the general linear model with Tukey’s tests (5, 16) to determine any statistical differences among different treatments.

RESULTS AND DISCUSSION

The effectiveness of the selected chemical and water spray treatments in reducing bacterial populations on the chicken skins was determined by evaluating the differences in numbers of S. typhimurium between the treatment and the no-spray control specimens (Table 1). All selected chemicals have been found to be effective against microorganisms when sprayed on chicken skins. As shown in Table 1, there was no significant difference between the APC and the numbers of Salmonella in each treatment, indicating that no Salmonella cells were injured by the chemical spraying treatments.

The results in Table 1 show that the water spraying caused a significant reduction of Salmonella on chicken skins in comparison with the water-immersion treatments. The numbers of Salmonella in water spray treatments were reduced by 1.0 to 1.5 log_{10} compared with the immersion treatment. This study supports the finding that water spray washing used as a practical method in poultry processing for improving hygiene should be somewhat successful in reducing bacterial contamination (24, 25).

The chicken skins sprayed with 0.1% CPC showed a 1.5 log_{10} reduction in Salmonella, whereas 0.5% CPC resulted in a 1.9 log_{10} reduction, compared with without-spray treatment. A 2.1 log_{10} and 2.2 log_{10} reduction resulted when TSP was sprayed at 5% and 10%, respectively. The application of 1% and 2% LA spraying reduced Salmonella by 2.2 log_{10}. The solutions of 0.1% and 0.5% DF-100 reduced Salmonella by 1.6 log_{10} and 1.8 log_{10}, respectively. Comparing the reductions in Salmonella, the 0.5% CPC gave a significantly greater value than 0.1% CPC. There were no significant differences in Salmonella reductions between different concentrations of the other three chemicals. No changes in color or texture of skin were apparent after any of the chemical treatments under the conditions of this study.

Of the selected chemical treatments, DF-100 and CPC showed strong bactericidal abilities at low concentrations. These solutions have neutral pH values, do not cause discoloration of the chicken skin, and have no deleterious effects on equipment, which is a major advantage over some other chemicals. To obtain stronger bactericidal effects, CPC and DF-100 may be used at higher concentrations. TSP and LA have been approved for use in food processing, but extremely alkaline or acidic pH values were needed for strong bactericidal ability. The disposal of used TSP solution at a pH of 12 to 14 or LA solution at a pH of 1 to 2 would cause serious environmental pollution: this is the main reason that neither has been adopted for routine treatment in poultry processing. Further research on TSP or LA should include investigating methods of recycling the chemicals. Parameters such as spray temperature, pressure, spraying times, and setting times also need to be evaluated for applications of chemical spraying methods in poultry processing.

ACKNOWLEDGMENTS

This research was funded in part by the Food Safety Consortium and the Southeastern Poultry & Egg Association. The authors thank Jinping Huang and George Dwyer for their help in the experiment.

REFERENCES


### TABLE 1. Reduction of aerobic plate count and number of Salmonella on chicken skins sprayed with chemicals at different concentrations for 30 sec, at room temperature and 207-kPa spray pressure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APC(^a)</th>
<th>Reduction</th>
<th>Salmonella(^b)</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>No spray</td>
<td>6.7 ± 0.24(^a)</td>
<td>—</td>
<td>6.5 ± 0.25(^a)</td>
<td>—</td>
</tr>
<tr>
<td>Water spray</td>
<td>5.6 ± 0.45(^b)</td>
<td>1.1</td>
<td>5.5 ± 0.25(^b)</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1% CPC</td>
<td>5.1 ± 0.39(^c)</td>
<td>1.4</td>
<td>5.0 ± 0.24(^c)</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5% CPC</td>
<td>4.7 ± 0.28(^d)</td>
<td>1.9</td>
<td>4.6 ± 0.39(^b)</td>
<td>1.9</td>
</tr>
<tr>
<td>No spray</td>
<td>7.1 ± 0.16(^a)</td>
<td>—</td>
<td>6.9 ± 0.17(^a)</td>
<td>—</td>
</tr>
<tr>
<td>Water spray</td>
<td>5.6 ± 0.23(^b)</td>
<td>1.5</td>
<td>5.6 ± 0.19(^b)</td>
<td>1.3</td>
</tr>
<tr>
<td>5% TSP</td>
<td>4.9 ± 0.39(^c)</td>
<td>2.2</td>
<td>4.8 ± 0.36(^c)</td>
<td>2.1</td>
</tr>
<tr>
<td>10% TSP</td>
<td>4.6 ± 0.31(^c)</td>
<td>2.5</td>
<td>4.7 ± 0.19(^b)</td>
<td>2.2</td>
</tr>
<tr>
<td>No spray</td>
<td>7.2 ± 0.13(^a)</td>
<td>—</td>
<td>7.0 ± 0.20(^a)</td>
<td>—</td>
</tr>
<tr>
<td>Water spray</td>
<td>5.9 ± 0.08(^b)</td>
<td>1.3</td>
<td>5.9 ± 0.14(^b)</td>
<td>1.1</td>
</tr>
<tr>
<td>1% Lactic acid</td>
<td>4.9 ± 0.20(^c)</td>
<td>2.3</td>
<td>4.8 ± 0.23(^c)</td>
<td>2.2</td>
</tr>
<tr>
<td>2% Lactic acid</td>
<td>5.0 ± 0.16(^c)</td>
<td>2.2</td>
<td>4.8 ± 0.19(^c)</td>
<td>2.2</td>
</tr>
<tr>
<td>No spray</td>
<td>7.2 ± 0.31(^c)</td>
<td>—</td>
<td>6.9 ± 0.21(^a)</td>
<td>—</td>
</tr>
<tr>
<td>Water spray</td>
<td>6.2 ± 0.54(^b)</td>
<td>1.0</td>
<td>5.9 ± 0.25(^b)</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1% DF-100</td>
<td>5.5 ± 0.53(^c)</td>
<td>1.7</td>
<td>5.3 ± 0.44(^c)</td>
<td>1.6</td>
</tr>
<tr>
<td>0.5% DF-100</td>
<td>5.1 ± 0.13(^c)</td>
<td>2.1</td>
<td>5.1 ± 0.41(^c)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

\(^a\) Means ± standard deviations of two tests or a total of six chicken skins.

\(^b\) Values within one column or one row with different superscripts (A, B, C, D) are significantly different (P < 0.05).