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Reduction of *Salmonella* Typhimurium on Poultry Skin Utilizing Sodium Metasilicate in Prechill and Postchill Applications

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Abstract: The comparison of antimicrobial properties of sodium metasilicate (SMS) to conventional chlorine applications in pre and postchill processing of poultry was evaluated. Fresh broiler chicken breast skin was cut aseptically into 5×5 cm portions and treated with either water only (negative control), inoculum+water (positive control, 10^7 cfu/mL of *Salmonella* Typhimurium), inoculum +50 ppm chlorine solution, inoculum +2% SMS solution, inoculum +2% SMS solution followed by 50 ppm chlorine solution (prechill), or inoculum +50 ppm chlorine solution followed by 2% SMS solution (postchill) and analyzed after 0 and 24 h storage at 4°C for *Salmonella* spp., total plate count and pH. Sodium metasilicate postchill treatment reduced aerobic plate counts at 0 and 24 h by 2.97 and 5.31 logs, respectively and *Salmonella* spp. counts at 0 and 24 h by 5.82 and 6.47 logs, respectively. The data revealed that SMS was most effective as postchill treatment. This research revealed the possibility of sodium metasilicate being used as an antimicrobial intervention in poultry chill tanks to control *Salmonella*.

Key words: Sodium metasilicate, antimicrobials, carcass chilling, microbiology, *Salmonella* Typhimurium

INTRODUCTION

Losses attributed to foodborne pathogens have resulted in 9.4 million foodborne illnesses, 55,961 hospitalizations and 1,351 deaths in the United States with nontyphoidal *Salmonella* spp. (11%) and *Campylobacter* spp. (9%) being the leading bacterial causes (Scallan *et al*., 2011). Poultry meat has been cited as the most common cause of illnesses among the 235 outbreaks attributed to a single food commodity (Center for Disease Control and Prevention, 2010). The most recent *Salmonella* outbreak which originated with Foster Farms in central California has resulted in 278 individuals becoming ill in 17 states (Andrews, 2013). Although antimicrobial interventions are currently used in the poultry industry, the continued presence of *Salmonella* in the food supply suggests the need for additional anti-Salmonella interventions. Approved antimicrobials currently employed in the poultry industry include acidified sodium chlorite (Oyarzabal *et al*., 2004; Kemp *et al*., 2001; USDA FSIS, 2013a), organic acids (Shafit and Williams, 2010; Zhao *et al*., 2009; Bauermeister *et al*., 2008), trisodium phosphate (Bourassa *et al*., 2004; Capita *et al*., 2002), cetylpyridium chloride (USFDA, 2013; Kim and Slavik, 1996), peracetic acid (Bauermeister *et al*., 2008) and natural antimicrobials (Friedman *et al*., 2002; Fisher and Phillips, 2006; Piskernik *et al*., 2011; Skandamis *et al*., 2002; Burt, 2004).

An additional antimicrobial that has been proven to reduce *Salmonella* (Sharma *et al*., 2012) and psychrotrophic bacteria (Huang *et al*., 2011) on fresh broiler breast meat is sodium metasilicate (SMS). Sodium metasilicate is approved as an antimicrobial component of marinades for raw meat and poultry products at levels not to exceed 2% by weight of the marinade and up to a 6% solution for ready-to-eat poultry products applied to the surface of the poultry at a rate not to exceed 300 ppm of the finished product (USDA FSIS, 2013b). In addition, a blend of sodium metasilicate and sodium carbonate can be applied to the surface of ready to eat poultry at up to 15 percent of a solution of sodium metasilicate and sodium carbonate (sodium metasilicate not to exceed 6 percent) at a rate not to exceed 700 ppm by weight of the finished poultry product. SMS is highly alkaline and results in pH 12.5 in a 1% SMS solution.

In regards to mode of action of SMS, flow cytometry analysis of *Salmonella* Typhimurium cells suspended in SMS solutions revealed that the cell membrane integrity was compromised and propidium iodide was able to stain the cells. Transmission electron microscopy of *Salmonella* Typhimurium cells treated with SMS revealed changes in cell morphology, cytoplasmic contents, cell wall invaginations and cell lysis (Sharma *et al*., 2013). Research studies have also revealed that the antimicrobial effect of SMS may be due to its high pH as seen when SMS solutions were neutralized (unpublished data). To date, no research was available concerning antimicrobial properties of sodium metasilicate in poultry chillers.

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The objectives of this study were to determine the efficacy of SMS at USDA FSIS approved levels in the reduction of *Salmonella* Typhimurium on broiler carcasses and compare antimicrobial properties of SMS to conventional chlorine applications in processing of broiler carcasses.

MATERIALS AND METHODS

Culture preparation: A frozen *Salmonella* Typhimurium (ATCC 14028) culture was obtained from ABC Research Corporation in Gainesville, Florida and used as the inoculum in this study. Frozen *S.* Typhimurium culture was thawed at room temperature for 10 min before being streaked onto tryptic soy agar (TSA, DF 02-676-22, Fisher Scientific, Pittsburgh, PA). The TSA plates were incubated for 24 h at 37°C. One colony of the culture was isolated and transferred into 10 mL of sterile trypticase soy broth (TSB, DF 0369-17-6, Difco Laboratories, Detroit, MI) and incubated at 37ºC for 24 h. Following 24 h incubation, the culture was transferred to 10 mL of sterile 0.1% peptone water (DF O1897-17-4, BD Diagnostics, Sparks, MD) and centrifuged (Dupont Instruments Sorball RC-5 Super Speed Refridgerated Centrifuge, Newton, CT) at 5000 rpm for 10 min at 16°C. The supernatant was decanted and the pellet was rinsed with 10 mL of sterile 0.1% peptone water and recentrifuged. This step was repeated three times. The cells were resuspended into 10 mL of sterile 0.1% peptone water and used to prepare the serial dilutions. The inoculum contained approximately 10^6-10^7 cfu/mL.

Sample preparation and microbiological analyses: Chicken broiler breast skins were obtained from a local supermarket that markets boneless and skinless chicken breast meat. The skins were transferred on ice to the Meat Research Microbiology laboratory and stored at 4°C for a maximum of 4 h prior to treatment. The skins were cut aseptically into 5×5 cm pieces and utilized in the study as described by Zhao *et al.* (2009). Equal quantities of the skin were divided into six groups and treated with water only (negative control), inoculum+ water (positive control, 10^7 cfu/ml of *Salmonella* Typhimurium), inoculum +50 ppm chlorine (Austin's® A-1 Bleach 6% sodium hypochlorite, AUS61-CS, Deland, FL) solution, inoculum +2% SMS (w/w, Avgard®XP, Danisco USA Inc., New Century, KS) solution, inoculum +2% SMS solution followed by 50 ppm chlorine solution (pre-chill), or inoculum +50 ppm chlorine solution followed by 2% SMS solution (post chill). Except for the negative control, all skin samples were inoculated with 10^7 cfu/mL of *Salmonella* Typhimurium and stored at 22°C (room temperature) for 20 min to allow sufficient bacterial attachment. For each treatment, the inoculated skin samples were placed into a stomacher bag containing the antimicrobial solutions (200 mL solution for each skin sample) at 4ºC for a contact time of 5 min with intermittent hand massage (every 30 sec). The chlorine concentration and 2% SMS was based on current USDA guidelines for a poultry processing facility. Each treated sample was removed from the antimicrobial solution, placed in a stomacher bag which contained 9 mL of sterile 0.1% peptone water and massaged for one minute. One mL of the rinsate was placed into 9 ml of peptone water to prepare the appropriate serial dilutons. A 0.1 mL aliquot of each serial dilution was plated on prehardened duplicate Xylose Lactose Tergitol™ 4 agar (XLT-4, DF0225-17-0, BD Diagnostics) for *Salmonella* and TSA for aerobic plate counts (APCs). Plates were incubated at 37°C for 48 h. After incubation the plates were counted, averaged and the results reported as colony forming units per cm^2 (cfu/cm^2).

pH analysis: The pH analyses were conducted immediately after the microbiological analyses were completed using the same sample rinsates. Two pH measurements were recorded for each sample using an Accumet pH meter (model AB15, Fisher Scientific, Pittsburgh, PA). The probe was placed into the 10^{-1} sample rinsate and allowed to reach equilibrium for 1 min before the readings were recorded.

Statistical analysis: The experiment was constructed in a complete randomized design. A total of 72 samples were analyzed (6 treatments, two storage periods, 3 trials and 2 samples per treatment). Data were analyzed using SAS Proc GLM procedure (SAS, 2008). Significant differences among means were determined using Duncan Multiple Range Test with level of significance being alpha p = 0.05.

RESULTS AND DISCUSSION

pH analyses: The pH of the SMS solutions was higher ($p<0.05$) than the distilled water only and the 50 ppm Cl solution (Table 1). The data demonstrated a 5.61 unit increase in pH for the SMS solution when compared to the chlorine solution.

Table 1: pH and chlorine concentration for treatment solutions

Treatment solutions	pH	Chlorine Conc. (ppm)
Distilled water	6.97	-
Chlorine solution	6.87	50.0
Sodium Metasilicate solution (Prechill)	12.58	-
Sodium Metasilicate solution (Postchill)	12.58	-

Microbiological analyses: Aerobic Plate Counts. Except for the prechill SMS treatment, APCs were lower ($p<0.05$) for all skin treated with SMS when compared to the controls and skin treated with chlorine solution only at 0 and 24 h (Table 2). APCs for the skin treated with SMS prechill and Cl treatment had similar ($p>0.05$) APCs after 24 h storage. APCs were similar ($p>0.05$) for control skin

Table 2: Aerobic plate count for commercial broiler breast skin inoculated with *Salmonella* Typhimurium, treated with sodium metasilicate and stored for 0 and 24 h at 4±1°C

	Aerobic Plate Count (cfu/cm^2) Storage time (h)	
Treatment[1]	0	24
Negative Control	7.38^{a,x}	7.38^{a,x}
Control (S. Typhimurium inoculum)	7.62^{a,x}	7.61^{a,x}
Chlorine (Cl)	6.94^{a,x}	6.68^{ab,x}
Sodium metasilicate (SMS)	4.93^{b,x}	3.30^{c,y}
SMS, followed by CL, Prechill	4.82^{b,x}	5.36^{b,x}
Cl, followed by SMS, Postchill	4.65^{b,x}	2.31^{c,y}

[a-c]Means in the same column bearing different superscripts differ significantly (p<0.05)
[x-y]Means in the same row bearing different superscripts differ significantly (p<0.05)

Table 3: Salmonella spp. counts for commercial broiler breast skin inoculated with *Salmonella* Typhimurium, treated with sodium metasilicate and stored for 0 and 24 h at 4±1°C

	Salmonella Count (cfu/cm^2) Storage time (h)	
Treatment[1]	0	24
Negative Control	2.15^{c,x}	2.15^{c,x}
Control (S. Typhimurium inoculum)	7.77^{a,x}	7.77^{a,x}
Chlorine (Cl)	5.55^{b,y}	7.08^{a,x}
Sodium metasilicate (SMS)	2.81^{c,x}	2.09^{c,x}
SMS, followed by CL, Prechill	5.13^{b,x}	4.47^{b,x}
Cl, followed by SMS, Postchill	1.95^{c,x}	1.30^{d,x}

[a-c]Means in the same column bearing different superscripts differ significantly (p<0.05)
[x-y]Means in the same row bearing different superscripts differ significantly (p<0.05)

and skin treated with chlorine after 0 and 24 h. All skin treated with SMS had similar (p>0.05) APCs on day 0. After 24 h storage, the SMS only and SMS post chill treatments had lower APCs than skin treated with SMS as prechill. The data revealed that the Cl only, SMS only, SMS prechill and SMS postchill treatments resulted in 0.68, 2.69, 2.80 and 2.97 log reductions in APCs, respectively, after 0 h storage and 0.93, 4.31, 2.25 and 5.30 log reductions, respectively, after 24 h storage when compared to the inoculated control.

***Salmonella* spp**: On day 0, all treatments had lower (p<0.05) *Salmonella* counts than the negative and inoculated controls (Table 3). Except for the negative control, skin treated with SMS alone and SMS in post chill had lower (p<0.05) *Salmonella* than all other treatments. The data also revealed that *Salmonella* present on the untreated skin was 2.15 logs cfu/mL initially (0 h) and remained at the same level after 24 h storage. After 24 h storage, all skin treated with SMS only and SMS in prechill and post chill had lower (p<0.05) *Salmonella* than the inoculated control and the CL only treatment. A 1.53 log increase (p<0.05) in *Salmonella* was revealed for the Cl only treatment after 24 h. The data revealed that the Cl only, SMS only, SMS prechill and SMS postchill treatments resulted in 2.32, 4.96, 2.64 and 5.82 log reductions in *Salmonella*, respectively, after 0 h storage and 0.69, 5.68, 3.30 and 6.47 log reductions in *Salmonella*, respectively, after 24 h storage when compared to the inoculated control. The log reductions in *Salmonella* in this study surpassed those reported by Nagel *et al*. (2013) for broiler carcasses inoculated with *S.* Typhimurium and submerged in postchill treatment solutions containing 0.04 or 0.1% peracetic acid. Nagel *et al*. (2013) reported approximately 3 log reductions in S. Typhimurium for both treatments when compared to the positive *S.* Typhimurium control.

In summary, SMS was effective in reducing APCs and *Salmonella* at 0 and 24 h storage. The data demonstrated that the SMS alone and SMS postchill methods resulted in 4.96 and 5.82 log reductions in *Salmonella*, respectively after 0 h storage and 5.68 and 6.47 log reductions, respectively, after 24 h. All SMS treatments resulted in 2.61 to 2.97 log reductions in APCs initially (0 h) and 4.31 and 5.30 log reductions after 24 h for SMS only and SMS postchill treatments, respectively. The Cl treatment resulted in less than 1 log reduction in APCs and *Salmonella* initially and after 24 h. The *Salmonella* counts increased by 1.53 logs after 24 h storage, which suggested limited antimicrobial activity of the CL solution. This research revealed the possibility of sodium metasilicate being used as an antimicrobial intervention in poultry chill tanks to control *Salmonella*. Additional studies will be conducted to evaluate SMS on whole broiler carcasses under commercial plant conditions.

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