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Research Note

Comparison of the Efficacy of a Sulfuric Acid–Sodium Sulfate Blend and Lactic Acid for the Reduction of *Salmonella* on Prerigor Beef Carcass Surface Tissue

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ABSTRACT

A study was conducted to compare the efficacy of a commercially available sulfuric acid–sodium sulfate blend (SSS) and lactic acid (LA) in reducing inoculated *Salmonella* populations on beef. Sixty pieces of prerigor beef carcass surface brisket tissue, collected directly from the processing line of a commercial beef processing plant, were cut into two sections (10 by 10 cm each) and spot inoculated (6 to 7 log CFU/cm^2) on the adipose side with a six-strain mixture of *Salmonella*. One section per piece of brisket tissue was left untreated (control), while the second section was spray treated (5 s, 15 lb/in^2, and 33 mL/s flow rate) with unheated (21°C) or heated (52°C) solutions of SSS (pH 1.1) or LA (4%). Unheated and heated SSS lowered ($P < 0.05$) total bacterial counts from 6.3 to 4.6 and 4.3 log CFU/cm^2, respectively. Likewise, unheated and heated LA reduced ($P < 0.05$) total bacterial counts from 6.3 to 4.7 and 4.4 log CFU/cm^2, respectively. Initial counts of inoculated *Salmonella* populations (6.1 to 6.2 log CFU/cm^2) were reduced ($P < 0.05$) to 4.2 and 3.9 log CFU/cm^2 following treatment with unheated and heated SSS, respectively, and to 3.7 and 3.8 log CFU/cm^2 after treatment with unheated and heated LA, respectively. Overall, the temperature of the chemical solutions had a small (0.3 log CFU/cm^2), but significant ($P < 0.05$), effect on total bacterial counts but not ($P > 0.05$) on *Salmonella* counts. Regardless of solution temperature, *Salmonella* counts for LA-treated samples were 0.3 log CFU/cm^2 lower ($P < 0.05$) than those of samples treated with SSS. These results indicate that both unheated and heated solutions of SSS and LA are effective interventions for reducing *Salmonella* contamination on prerigor beef carcass surface tissue.

Key words: Decontamination; Lactic acid; Prerigor beef; *Salmonella*; Sulfuric acid and sodium sulfate blend

More than 1 million illnesses occur each year in the United States due to ingestion of food contaminated with nontyphoidal *Salmonella (19)*. Moreover, this bacterial pathogen is estimated to be the leading cause of foodborne illness–associated hospitalizations and deaths *(19)*. According to latest surveillance data compiled by the Centers for Disease Control and Prevention, *Salmonella* was the confirmed etiologic agent of 140 foodborne outbreaks in 2014 *(7)*.

Cattle are one of the known reservoirs of *Salmonella,* and numerous studies *(2, 4, 5, 14, 18, 20)* have reported on the prevalence of this pathogen in or on cattle hide and fecal samples, carcass surfaces before and after exposure to various decontamination treatments, beef trimmings, lymph nodes, and ground beef. In 2015, *Salmonella* was recovered from 1.32% of 1,290 bench trim samples, 1.74% of 3,218 manufacturing trim samples, and 2.91% of 11,047 ground beef samples collected from establishments regulated by the U.S. Department of Agriculture, Food Safety and Inspection Service *(24)*. Over 38 years (1973 to 2011), consumption of beef was associated with 96 salmonellosis outbreaks, with ground beef as the implicated vehicle in 22 (23%) of these outbreaks *(15)*. Of particular concern are foodborne-related infections with *Salmonella* strains that are multidrug resistant. Infection with multidrug-resistant *Salmonella* may be associated with an increased risk of hospitalization and treatment failure of infected individuals *(6, 23)*. In 2011, consumption of ground beef contaminated with a *Salmonella* Typhimurium strain that was resistant to eight antibiotics resulted in 20 cases of salmonellosis across seven states *(6)*. The hospitalization rate in this outbreak was 47% *(6)*.

Various chemical decontamination interventions are used by beef processing facilities in North America for pathogen control on surfaces of carcasses, cuts, and beef trimmings. The efficacy of these antimicrobial chemicals in reducing *Salmonella* contamination levels, as well as contamination levels of *Escherichia coli* O157:H7 and non-O157 Shiga toxin–producing *E. coli,* on beef tissue surfaces has been extensively evaluated *(1, 8–13, 17, 21, 26)*. Among these chemicals, lactic acid (LA) is one of the most commonly used organic acids in commercial beef processing plants, with numerous studies demonstrating its

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antimicrobial effect *(1, 8–10, 12, 17, 21, 26, 27)*. Nevertheless, the beef industry continues to look for novel chemical treatments for use as part of a multiple hurdle system to effectively control pathogen contamination on carcasses and resulting beef products.

A commercially available blend of sulfuric acid and sodium sulfate (SSS), which was recently shown to effectively reduce inoculated *Salmonella* populations on chicken wings *(22),* is approved for use as an antimicrobial treatment of meat and poultry products *(25)*. Published data on the efficacy of SSS against *Salmonella* on beef carcass surface tissue are limited. Therefore, the objective of this study was to evaluate the efficacy of SSS, applied at two temperatures, against *Salmonella* inoculated onto prerigor beef carcass surface tissue and to compare its efficacy to LA.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. The inoculum used in this study consisted of a mixture of six *Salmonella* serotype strains of bovine origin, namely, *Salmonella* Agona, *Salmonella* Anatum, *Salmonella* Newport FSL S5-436, *Salmonella* Reading, *Salmonella* Saintpaul, and *Salmonella* Typhimurium DT104 var. Copenhagen. Three of the *Salmonella* serotype strains were multidrug resistant (i.e., *Salmonella* Newport, Reading, and Typhimurium DT104 var. Copenhagen), and three were antibiotic susceptible (i.e., *Salmonella* Agona, Anatum, and Saintpaul) *(3, 11)*. The *Salmonella* Newport strain was kindly provided by Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY), while the five remaining strains were previously isolated by Bacon et al. *(2, 3)* and were available in our laboratory's culture collection. All six strains were hydrogen sulfide producers, as indicated by the formation of black-centered colonies on xylose lysine deoxycholate agar (XLD; Acumedia, Neogen Corporation, Lansing, MI).

Working cultures of the inoculum strains were maintained on XLD agar. Two days prior to the start of each trial, a single colony of each strain was separately inoculated into 10 mL of tryptic soy broth (TSB; Difco, BD, Sparks, MD) and incubated at 35°C for 22 to 24 h. Afterwards, a 0.1-mL aliquot of the original broth culture was transferred to 10 mL of fresh TSB and again incubated at 35°C (22 to 24 h). On the day of the experiment, broth cultures of all six *Salmonella* strains were combined, and cells were harvested by centrifugation (3,220 × *g,* 20 min, 4°C; Eppendorf, model 5810 R, Brinkmann Instruments Inc., Hamburg, Germany). Cell pellets were washed with 10 mL of phosphate-buffered saline (pH 7.4; Sigma-Aldrich, St. Louis, MO), centrifuged again, and the final cell pellets resuspended in 60 mL of phosphate-buffered saline. The concentration of the inoculum mixture was approximately 8 to 9 log CFU/ml.

Collection and inoculation of beef samples. On three separate days, 20 pieces of prerigor (warm) beef carcass surface brisket tissue were collected directly from the processing line of a commercial beef processing plant in northern Colorado. The beef tissue samples were obtained from carcasses that had been subjected to a hot water treatment but prior to treatment with LA. Samples were placed in insulated containers and transported to the laboratory of the Center for Meat Safety & Quality (Department of Animal Sciences, Colorado State University, Fort Collins) within 1 h of collection. Two sections (10 by 10 cm) were cut from each warm (25 ± 5°C) beef brisket tissue sample. One section per piece of brisket tissue was left untreated (control), while the second section was spray treated with one of four antimicrobial treatment groups (described in the next section). Only the external adipose side of each sample was inoculated, and this was done by randomly distributing 0.2 mL (approximately 10 drops) of the inoculum over the surface. The target inoculation level was 6 to 7 log CFU/cm^2. Inoculated samples were left undisturbed for 15 min to allow for cell attachment and were then either subjected to one of the antimicrobial treatments or, in the case of untreated control samples, were microbially analyzed for initial bacterial counts.

Antimicrobial treatment of beef samples. The LA (L-isomer; Purac America, Lenexa, KS) and SSS (Centron [formerly, AFTEC 3000], Zoetis Inc., Florham Park, NJ) solutions were prepared at approved concentrations or pH level *(25)* and were applied per manufacturer recommendations. The four antimicrobial treatments evaluated in the study were (i) unheated SSS (pH 1.1, 21°C), (ii) heated SSS (pH 1.1, 52°C), (iii) unheated LA (4%, 21°C), and (iv) heated LA (4%, 52°C). Treatments were applied by using a custom-built spray cabinet (Chad Co., Olathe, KS) designed to simulate a commercial beef carcass spray cabinet. Individual beef tissue samples were suspended on a hook and spray treated for 5 s (15 lb/in^2, 33 mL/s flow rate). Following treatment, excess liquid was allowed to drip off of samples for 10 min before being processed for microbial analysis.

Microbiological and pH analyses. Untreated (control) and treated beef tissue samples were transferred to Whirl-Pak filter bags (Nasco, Fort Atkinson, WI) to which 100 mL of Dey-Engley neutralizing broth (Difco, BD) was added. Samples were mechanically agitated (2 min; Masticator, IUL Instruments, Barcelona, Spain) and then serially diluted in 0.1% buffered peptone water (Difco, BD). Appropriate dilutions were surface plated in duplicate onto tryptic soy agar (TSA; Acumedia, Neogen Corporation), for determination of total bacterial counts, and onto XLD agar, for determination of inoculated *Salmonella* counts. Colonies were manually counted after incubation of plates at 25°C for 72 h (TSA) or 35°C for 24 h (XLD agar). Counts were recorded and converted to log CFU per square centimeter. On each experiment day, five uninoculated beef samples were also analyzed for any naturally present hydrogen sulfide–producing microflora.

Uninoculated beef tissue samples treated with the designated antimicrobial treatments were analyzed for pH. These samples were diluted with deionized water (1:5 sample-to-water ratio) and mechanically agitated (Masticator) for 2 min. The pH of samples was measured with a calibrated pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO).

Statistical analysis. Three repetitions of the study were conducted on three separate days. The study was designed as a randomized complete block with a 2 × 2 factorial arrangement (n = 15 per treatment). Each repetition (test day) was considered as a blocking factor. Separate analyses were performed for each treatment (unheated SSS, heated SSS, unheated LA, and heated LA) to evaluate the antimicrobial efficacy against the total bacterial populations and inoculated *Salmonella* populations. This was done by comparing the counts of untreated and treated samples using a pairwise t test (mixed procedure of SAS, Version 9.3; SAS Institute Inc., Cary, NC). Bacterial populations were expressed as least-squares means. To compare the antimicrobial efficacy between treatments, counts for untreated samples served as a covariate, and counts for treated samples were adjusted and then compared to determine the main effect of chemical (SSS or LA), solution temperature (unheated or heated) and their interaction. Data were analyzed by using the mixed procedure of SAS, with chemical,

TABLE 1. *Least-squares mean bacterial counts, recovered with TSA and XLD agar, for* Salmonella*-inoculated prerigor beef carcass surface tissue that was left untreated or was spray treated with unheated (21°C) or heated (52°C) solutions of SSS or LA*[a]

		Mean (SE) counts (log CFU/cm^2)			
		TSA		XLD	
Chemical	Unheated/heated	Untreated	Treated	Untreated	Treated
SSS	Unheated	6.3 (0.1) A[b]	4.6 (0.1) B	6.2 (0.1) A	4.2 (0.1) B
	Heated	6.3 (0.1) A	4.3 (0.1) B	6.2 (0.1) A	3.9 (0.1) B
LA	Unheated	6.3 (0.1) A	4.7 (0.1) B	6.1 (0.2) A	3.7 (0.2) B
	Heated	6.3 (0.1) A	4.4 (0.1) B	6.2 (0.1) A	3.8 (0.1) B

[a] TSA, tryptic soy agar; XLD, xylose lysine deoxycholate; SSS, surfuric acid and sodium sulfate blend (pH 1.1); LA, lactic acid (4%).
[b] Within each row and culture medium (i.e., TSA or XLD), least-squares means with different letters are different ($P < 0.05$).

solution temperature, and their interaction as independent variables. The pH data from treated samples were analyzed to determine treatment effects on the final pH of treated beef samples. All differences were reported by using a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Antimicrobial effect of treatments. Naturally occurring hydrogen sulfide–producing microflora were not recovered (1 CFU/cm^2 detection limit) from the uninoculated samples analyzed (data not shown); therefore, colony counts obtained from the XLD agar were those of the *Salmonella* inoculum used in this study. Bacterial counts of the inoculated untreated (control) and treated prerigor beef carcass surface tissue samples are shown in Table 1. Counts from the nonselective (TSA) and selective (XLD) agars were similar for the control samples, which indicated that total bacterial counts recovered with TSA consisted largely of the inoculated *Salmonella* strains. Spray treatment of beef tissue samples with unheated (21°C) or heated (52°C) solutions of SSS or LA effectively ($P < 0.05$) reduced microbial populations. More specifically, initial total bacterial counts (6.3 log CFU/cm^2) were reduced by 1.7 and 2.0 log CFU/cm^2 by the unheated and heated SSS treatments, respectively, and by 1.6 and 1.9 log CFU/cm^2 following treatment with the unheated and heated LA solutions, respectively (Table 1). In comparison, numerically greater reductions (by 0.3 to 0.8 log CFU/cm^2) were observed based on counts recovered with the selective agar, and were likely owing to the inability of sublethally injured cells to recover and grow in the presence of the selective ingredients included in the XLD agar formulation. As seen in Table 1, spray treatment of beef samples with the unheated and heated solutions of SSS reduced ($P < 0.05$) initial *Salmonella* counts (6.2 log CFU/cm^2) by 2.0 and 2.3 log CFU/cm^2, respectively, and for the unheated and heated LA treatments, initial pathogen counts (6.1 to 6.2 log CFU/cm^2) were reduced ($P < 0.05$) by 2.4 log CFU/cm^2, irrespective of solution temperature.

Comparison of treatments. Inoculation level of the test organism may affect the antimicrobial efficacy of treatments in vitro, as a higher inoculation level may result in proportionately higher reductions and a lower inoculation level may lead to relatively lower proportional reductions in bacterial counts. Because the inoculation level was slightly different for each treatment in the present study (Table 1), counts of initial inoculation levels on untreated control samples served as a covariate, and the least-squares means of counts for treated samples were adjusted. Therefore, only the adjusted counts were used to compare the main effects of chemical, solution temperature, and their interaction.

As indicated by our data, the interaction of chemical (SSS or LA) and solution temperature (unheated or heated) was not significant ($P > 0.05$); therefore, only the main effects of chemical and solution temperature are presented (Table 2). The chemical solution temperature had a significant ($P < 0.05$) effect on adjusted least-squares mean total bacterial counts but not ($P > 0.05$) on adjusted least-squares mean *Salmonella* counts (Table 2). Spray treatment of beef tissue samples with heated (52°C) chemical solutions resulted in a 0.3 log CFU/cm^2 greater ($P < 0.05$) reduction in total bacterial counts as compared with reductions obtained with unheated (21°C) solutions. Note, however, that a 0.3-log difference is generally not considered biologically significant *(16)*. The adjusted least-squares mean total bacterial counts of samples treated with SSS did not differ ($P > 0.05$) from those of LA-treated beef samples, indicating that use of either compound would be expected to be equally effective against total bacterial populations. In contrast, chemical type had a significant ($P < 0.05$) effect on adjusted least-squares mean *Salmonella* counts. More specifically, *Salmonella* counts from LA-treated samples were 0.3 log CFU/cm^2 lower ($P < 0.05$) than those of SSS-treated samples.

Comparison of the pH data of treated beef samples showed that solution temperature did not ($P > 0.05$) have an effect on the final pH of treated samples, whereas chemical type did ($P < 0.05$; data not shown). Beef carcass tissue samples spray treated with LA had a pH (pH 4.18) that was 0.53 pH units lower ($P < 0.05$) than the pH of SSS-treated samples (pH 4.71).

Use of LA for reducing bacterial pathogen contamination on beef carcasses and derived cuts has been extensively investigated *(1, 8–10, 12, 17, 21, 26)*. Factors that may affect the decontamination efficacy of LA in inoculated challenge studies include the type and temperature of the beef tissue used, inoculation method, inoculation level, attachment time of the tested pathogen, solution concentration and temperature, and treatment application method and parameters *(26)*.

TABLE 2. *Effect of chemical solution temperature and chemical type on least-squares mean bacterial counts, recovered with TSA and XLD agar, for* Salmonella*-inoculated prerigor beef carcass surface tissue*[a]

		Mean (SE) counts (log CFU/cm^2)			
		Chemical solution temperature[b]		Chemical[c]	
Culture medium	Untreated/treated	Unheated	Heated	SSS	LA
TSA	Untreated	6.3	6.3	6.3	6.3
	Treated[d]	4.6 (0.1) A[e]	4.3 (0.1) B	4.4 (0.1)	4.6 (0.1)
XLD	Untreated	6.2	6.2	6.2	6.2
	Treated	3.9 (0.1)	3.8 (0.1)	4.0 (0.1) A	3.7 (0.1) B

[a] TSA, tryptic soy agar; XLD, xylose lysine deoxycholate.
[b] Unheated solution temperature was 21°C; heated solution temperature was 52°C.
[c] SSS, sulfuric acid and sodium sulfate blend (pH 1.1); LA, lactic acid (4%).
[d] Counts for treated samples were adjusted by using the counts of untreated samples as a covariate.
[e] Within each row and main effect (i.e., chemical solution temperature or chemical), adjusted least-squares means with different letters are different ($P < 0.05$).

Cutter and Rivera-Betancourt *(8)* reported 3.2-log CFU/cm^2 reductions of *Salmonella* on the surface of beef flanks that were sprayed (15 s, 125 ± 5 lb/in^2, and 4.8 L/min flow rate) with 2% LA (35 ± 2°C). In another study *(21),* immersion of inoculated (approximately 4 log CFU/cm^2) adipose or lean muscle surfaces of beef cheek meat in 5% LA for 1 min reduced *Salmonella* levels by 2.0 and 1.4 log CFU/cm^2, respectively. When lean beef trimmings were immersed for 30 s in 25°C or 55°C LA (5%), inoculated *Salmonella* counts (3.0 to 3.3 log CFU/cm^2) were reduced by 1.3 to 1.5 log CFU/cm^2 (25°C solution) and 1.6 to 1.9 log CFU/cm^2 (55°C solution) *(10)*.

A limited number of studies have evaluated the antimicrobial efficacy of SSS. A 30-s immersion treatment of beef trimmings in SSS (pH 1.2) resulted in 0.5- to 0.7-log CFU/cm^2 reductions of *Salmonella* Typhimurium and *Salmonella* Newport *(11). Salmonella* levels on beef cheek meat samples were reduced by 1.0 to 1.5 log CFU/cm^2 following an immersion treatment in 1% SSS for 1, 2.5, or 5 min *(21)*. In another study *(22),* chicken wings immersed for 10 or 20 s in SSS (pH 1.1) lowered inoculated *Salmonella* populations by 0.8 to 0.9 and 1.1 to 1.2 log CFU/mL of rinse solution, respectively.

In conclusion, the results of the study indicate that spray treatment of prerigor beef carcass surface tissue with SSS at pH 1.1 or 4% LA effectively reduces *Salmonella* contamination. In addition, under the experimental conditions of our study, heating of the chemical solutions to 52°C was found not to have a practical effect on their antimicrobial effectiveness against *Salmonella*.

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