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Research Note

Efficacy of Antimicrobial Spray Treatments in Reducing *Salmonella enterica* Populations on Chilled Pork

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ABSTRACT

Studies reporting on alternative antimicrobial interventions for pathogen control on chilled pork carcasses and cuts are limited. In this study, the antimicrobial effects of various spray treatments against *Salmonella enterica* inoculated on skin-on pork samples were evaluated. Chilled pork jowls were portioned (10 by 5 by 1 cm) and inoculated, on the skin side, with a mixture of six *S. enterica* serotype strains to target levels of 6 to 7 log CFU/cm^2 (high inoculation level) or 3 to 4 log CFU/cm^2 (low inoculation level). Samples were then left nontreated (control) or were treated (10 s) using a laboratory-scale spray cabinet with water, formic acid (1.5%), a proprietary blend of sulfuric acid and sodium sulfate (SSS, pH 1.2), peroxyacetic acid (PAA, 400 ppm), or PAA (400 ppm) that was pH-adjusted (acidified) with acetic acid (1.5%), formic acid (1.5%), or SSS (pH 1.2). Samples (n = 6) were analyzed for *Salmonella* populations after treatment application (0 h) and after 24 h of refrigerated (4°C) storage. Irrespective of inoculation level, all spray treatments effectively reduced ($P < 0.05$) *Salmonella* levels immediately following their application. Overall, pathogen reductions for the chemical treatments, compared to the respective high and low inoculation level nontreated controls, ranged from 1.2 to 1.9 log CFU/cm^2 (high inoculation level) and 1.0 to 1.7 log CFU/cm^2 (low inoculation level). Acidification of PAA with acetic acid, formic acid, or SSS did not ($P \geq 0.05$) enhance the initial bactericidal effects of the nonacidified PAA treatment. *Salmonella* populations recovered from all treated samples following 24 h of storage were, in general, similar ($P \geq 0.05$) or up to 0.6 log CFU/cm^2 lower ($P < 0.05$) than those recovered from samples analyzed immediately after treatment application. The results of the study may be used by processing establishments to help identify effective decontamination interventions for reducing *Salmonella* contamination on pork.

The Centers for Disease Control and Prevention (CDC) estimates that nontyphoidal *Salmonella enterica* subspecies *enterica* (hereafter *Salmonella*) is responsible for more than 1.3 million infections (includes foodborne and nonfoodborne) in the United States every year and is the leading cause of foodborne illness-related hospitalizations and deaths (Batz, Hoffmann, & Morris, 2012; Scallan et al., 2011; Centers for Disease Control and Prevention (2022a)). According to surveillance data compiled by the CDC, *Salmonella* was the confirmed etiologic agent of 172 food-related outbreaks in 2018 (Centers for Disease Control and Prevention, 2022b). In the United States, pork is considered the third most common vehicle of salmonellosis outbreaks, with eggs and chicken constituting the first and second most common causes, respectively (Snyder et al., 2019). From 1998 to 2020, 144 pork-related *Salmonella* outbreaks were reported, resulting in a total of 4486 illnesses, 552 hospitalizations, and four deaths (Centers for Disease Control and Prevention, 2022b). One of the largest salmonellosis outbreaks linked to pork products occurred in 2015 and was associated with 192 cases and 30 hospitalizations across five U.S. states. A single pork slaughter facility identified as the source of the contaminated products issued a recall for more than 500,000 lbs (>226,000 kg) of pork that was potentially contaminated with *Salmonella* I 4,[5],12:i:- and *Salmonella* Infantis (Centers for Disease Control and Prevention, 2015; U.S. Department of Agriculture, Food Safety and Inspection Service, 2015).

Swine are usually asymptomatic carriers (primarily in their intestinal tract) and shedders of *Salmonella* (Baer et al., 2013; Bonardi, 2017; Dickson et al., 2019). Reports indicate that stresses (e.g., handling, comingling, adverse weather conditions, noise) encountered by the animals during transport to the slaughter facility and subsequent lairage can lead to increased shedding of the pathogen (Larsen et al.,

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2003). As a consequence, transmission of *Salmonella* from animal to animal increases, as does the risk of contamination of carcasses during the slaughter process (Baer et al., 2013; Mannion et al., 2012). High levels of *Salmonella* contamination have been reported on carcasses postexsanguination (i.e., before scalding) (Sanchez-Maldonado et al., 2017; Schmidt et al., 2012). In a study conducted by Schmidt et al. (2012) at two large U.S. processing establishments, 1386 of 1520 (91.2%) pork carcasses sampled at the prescalding stage of the processing line were positive for the pathogen. Subsequent processing steps, such as scalding and singeing, have been shown to decrease *Salmonella* contamination levels on carcasses, but do not completely eliminate its presence (Dickson et al., 2019). In the Schmidt et al. (2012) study, *Salmonella* was isolated from 19.1% of carcasses sampled after scalding, singeing, and polishing but before evisceration, and from 3.7% of samples collected after carcass chilling. Excessive handling involved during the fabrication of chilled carcasses into cuts and trim can potentially lead to the recontamination of products. In a 12-month baseline study of raw pork products from federally inspected slaughter and processing establishments in the United States, *Salmonella* was detected in 5.3% of intact cuts, 3.9% of nonintact cuts, and 28.9% of comminuted products (Scott et al., 2020).

Physical processes and sprays, rinses, or washes with approved antimicrobial products (U.S. Department of Agriculture, Food Safety and Inspection Service, 2021) are used in food-animal processing establishments in the United States as part of a multihurdle approach to reduce the prevalence of enteric pathogens, such as *Salmonella* and Shiga toxin-producing *Escherichia coli*, on carcasses and final products. Such pathogen control strategies, and especially those related to the use of chemical decontamination treatments, have been extensively researched in relation to beef and poultry processing (Britton et al., 2018; Cano, Meneses, & Chaves, 2021; Geornaras et al., 2012a, 2012b; Gonzalez, Geornaras, Nair, & Belk, 2021; Kim, Park, Lee, Owens, & Ricke, 2017; Kocharunchitt, Mellefont, Bowman, & Ross, 2020; Muriana et al., 2019; Nagel, Bauermeister, Bratcher, Singh, & McKee, 2013; Ramirez-Hernandez, Brashears, & Sanchez-Plata, 2018; Scott et al., 2015; Sohaib, Anjum, Arshad, & Rahman, 2016). In comparison, however, published studies on the effectiveness of antimicrobial interventions for reducing pathogen contamination on pork carcasses and cuts are limited (Eastwood et al., 2021; Loretz et al., 2011; Totton et al., 2016). Some examples of decontamination treatments that have been evaluated for their efficacy in reducing *Salmonella* populations on pork include hot or warm water, steam, lactic acid, acetic acid, and peroxyacetic acid (PAA) (Baer et al., 2013; Eastwood et al., 2021; Loretz et al., 2011; Totton et al., 2016; Young et al., 2016). Additional research on antimicrobial interventions that are specific to pork tissue would greatly benefit the industry. Moreover, studies evaluating decontamination treatments applied onto chilled pork surface tissue would help identify pathogen control interventions that can be applied during or after carcass chilling and/or subsequent fabrication processes. Therefore, the objectives of this study were to (i) evaluate the antimicrobial effects of formic acid, a proprietary blend of sulfuric acid and sodium sulfate (SSS), PAA, and PAA that was pH-adjusted (hereafter acidified PAA) with acetic acid, formic acid, or SSS, when applied to chilled pork surface tissue inoculated with *Salmonella* and, (ii) determine the antimicrobial efficacy of the test solutions against two target inoculation levels (6–7 log CFU/cm^2 and 3–4 log CFU/cm^2) of *Salmonella*.

Materials and methods

Bacterial strains and inoculum preparation. The inoculum comprised a mixture of six *S. enterica* serotype strains of swine origin. The strains were provided by Dr. Thomas Edrington (previously at U.S. Department of Agriculture, Agricultural Research Service, Food and Feed Research Unit, College Station, TX, USA) and included *Salmonella* Agona B E1-09, *Salmonella* Anatum E B1-03, *Salmonella* Derby B E1-13, *Salmonella* Montevideo C1 B2-51, *Salmonella* Schwartzengrund B B1-10, and *Salmonella* Tennessee C1 E3-10. The strains were stored at -80°C in tryptic soy broth (TSB, Difco, Becton Dickinson and Company [BD]) containing 15% glycerol. Working cultures of the strains were maintained at 4°C on xylose lysine deoxycholate (XLD) agar (Acumedia-Neogen) plates. All six strains were hydrogen sulfide producers, indicated by the formation of black-centered colonies on the XLD agar.

Before each experiment, the *Salmonella* strains were individually cultured and subcultured in 10 mL TSB (35°C, 22 ± 1 h). After incubation, cultures of the six strains were combined and centrifuged at 6000 × *g* for 15 min at 4°C (Sorvall Legend X1R centrifuge, Thermo Scientific). The cell pellet was resuspended and washed once with 10 mL of phosphate-buffered saline (PBS, pH 7.4; Sigma-Aldrich), and the final washed cell pellet comprised of the six strains was resuspended in 60 mL of PBS. This cell suspension was either left undiluted (ca. 9 log CFU/mL concentration) or was diluted 1000-fold (ca. 6 log CFU/mL concentration) in PBS and used to inoculate pork tissue samples to the high (6–7 log CFU/cm^2) or low (3–4 log CFU/cm^2) target inoculation levels, respectively. Actual concentrations of the undiluted and diluted 6-strain mixtures were determined to be 9.0 and 5.9 log CFU/mL, respectively. These were determined by serial dilution (1:10) of inoculum aliquots in 0.1% buffered peptone water (Difco, BD) followed by plating of appropriate dilutions onto tryptic soy agar (TSA; Acumedia-Neogen). Colonies were manually counted after incubation of plates at 35°C for 24 h.

Inoculation of pork samples. For this study, the skin side of pork jowls was used to represent the surface of pork carcasses and skin-on pork primal and subprimal cuts. Pork jowls were collected from carcasses on the harvest floor of a major commercial pork processor and were shipped overnight, on dry ice, to the Department of Animal Sciences, Colorado State University (Fort Collins, CO, USA). On arrival, jowls were held at 3°C and were used within one or two days. Two trials (repetitions) of the study, conducted on two separate days and with different production lots of jowls, were performed for each *Salmonella* inoculation level. The day before inoculation and treatment application, pork jowls were cut into 10 by 5 cm portions with an approximate thickness of 1 cm. Portioned samples were placed in a bag and refrigerated (3°C) until the next day.

On the day of the experiment, pork tissue samples were randomly assigned to one of eight treatments. For inoculation, pork samples were placed on trays lined with alcohol-sterilized aluminum foil, with the outer (skin side) surface facing up, and were inoculated under a biological safety cabinet. A 0.2-mL (200 μL) aliquot of the low or high concentration of the *Salmonella* inoculum was deposited, with a micropipette, on the skin side of each portion and then spread over the entire surface (50 cm^2) with a sterile disposable spreader. Samples remained undisturbed for 15 min to allow for bacterial cell attachment. The target inoculation level of samples inoculated with the ca. 6 log CFU/mL or ca. 9 log CFU/mL concentration of the inoculum mixture was 3–4 log CFU/cm^2 and 6–7 log CFU/cm^2, respectively.

Antimicrobial treatment of pork samples. Inoculated pork tissue samples were left nontreated, to serve as controls, or were subjected to one of the following treatments: water, formic acid (1.5%; BASF Corporation), SSS (pH 1.2; Titon, Zoetis), PAA (400 ppm; Kroff), PAA (400 ppm) acidified with acetic acid (1.5%; Fisher Scientific; AA-aPAA), PAA (400 ppm) acidified with formic acid (1.5%; FA-aPAA), or PAA (400 ppm) acidified with SSS (pH 1.2; SSS-aPAA). The water treatment was included to determine the rinsing effect of the spray application method utilized in this study. Antimicrobial treatment solutions were prepared according to the manufacturers' instructions, and the pH of solutions was measured (Orion Star A200 Series pH meter and Orion Ross Ultra pH electrode; Thermo Scientific). The average pH values of the formic acid, SSS, and PAA solutions were 2.9, 1.2, and 3.4, respectively. For the AA-aPAA, FA-aPAA, and SSS-

aPAA solutions, average pH values were 2.6, 2.9, and 1.2, respectively. A hydrogen peroxide and peracetic acid test kit (LaMotte Company) was used to verify the PAA concentration.

The treatment solutions (room temperature; 20-25°C) were applied using a custom-built laboratory-scale spray cabinet (Birko/Chad Equipment) fitted with six 0.38 L/min FloodJet spray nozzles (Spraying Systems Co.) positioned above the product belt. Treatments were sprayed onto the inoculated surface of the pork sections at a flow rate of approximately 3.8 L/min at 124–131 kPa, and a product contact time of 10 s per sample. Following the spray treatment, samples were placed on a sterile wire rack for 5 min to allow the excess solution to drip off samples before microbial analysis or storage. In each of the two trials conducted for each *Salmonella* inoculation level, three samples per treatment were analyzed for *Salmonella* population counts following treatment application (0-h analysis) or, in the case of nontreated control samples, immediately following the inoculation procedure. An additional three samples per treatment were placed in sterile plastic containers, covered with aluminum foil (without it touching the product), and analyzed after a 24 ± 1 h storage period at 4°C.

Microbiological analysis. At each sampling time (0 h and 24 h), individual nontreated and treated pork samples were analyzed for *Salmonella* levels using previously described procedures (Geornaras et al., 2012b; Scott-Bullard et al., 2017). Briefly, samples were placed into separate Whirl-Pak filter bags (710-mL, Nasco) containing 75 mL of Dey/Engley neutralizing broth (Difco, BD). Samples were mechanically pummeled for 2 min (Masticator, IUL Instruments), serially diluted (1:10) in 0.1% buffered peptone water, and appropriate dilutions were surface-plated, in duplicate, onto XLD agar. Colonies were counted after 24 h of incubation of plates at 35°C. Three noninoculated and nontreated samples were also analyzed on each of the inoculation and treatment application days, for levels of the natural microflora (on TSA; 25°C for 72 h), and any naturally present hydrogen sulfide-producing *Salmonella* populations (on XLD agar). The detection limit of the microbiological analysis was 0.2 log CFU/cm^2 (1.5 CFU/cm^2).

Statistical analysis. The study was designed as an 8 (treatments) × 2 (sampling times) factorial for each inoculation level (low, high), blocked by trial day. It was repeated on two separate days for each inoculation level, and three samples were analyzed per treatment and sampling time (0 h and 24 h) in each trial (i.e., a total of six samples per treatment and sampling time). For each inoculation level, recovered *Salmonella* populations for each treatment were analyzed within and across the two sampling times. Bacterial populations were expressed as least squares means for log CFU/cm^2 under the assumption of a log-normal distribution of plate counts. Data were analyzed using the emmeans package (Lenth, 2020) in R (version 3.5.1; R Core Team, 2022). Means were separated with Tukey adjustment using a significance level of $\alpha = 0.05$.

Results

Bacterial contamination level of noninoculated and nontreated pork samples. Naturally occurring hydrogen sulfide-producing *Salmonella* populations were not detected (0.2 log CFU/cm^2 detection limit) in any of the noninoculated and nontreated pork samples analyzed. Therefore, *Salmonella* populations recovered with the XLD agar from the inoculated nontreated and treated samples (Tables 1 and 2) were those of the inoculum strains used in this study. Aerobic plate counts of the noninoculated pork tissue ranged from 2.4 to 4.6 log CFU/cm^2, with a mean of 3.2 ± 0.5 log CFU/cm^2 (data not shown in tables).

Decontamination effects against high *Salmonella* contamination level. The pathogen inoculation level on the nontreated (control) pork tissue samples was 6.2 ± 0.1 log CFU/cm^2, and subsequent aerobic storage at 4°C for 24 h did not ($P \geq 0.05$) have an effect on this initial *Salmonella* population level (Table 1). All seven spray treatments effectively ($P < 0.05$) reduced the initial inoculated contamination level. Overall, at the 0-h sampling time, counts of all treated samples were 0.8 (water) to 1.9 (SSS-aPAA) log CFU/cm^2 lower ($P < 0.05$) than counts obtained for the nontreated samples (Table 1, Supplemental Table 1). Furthermore, apart from the formic acid treatment, all of the chemical spray treatments resulted in greater ($P < 0.05$) *Salmonella* reductions than those obtained by spraying the samples with water. Compared to the control treatment, formic acid, SSS, and PAA lowered ($P < 0.05$) *Salmonella* populations by 1.2, 1.6, and 1.5 log CFU/cm^2, respectively. No ($P \geq 0.05$) differences in efficacy were obtained between SSS, PAA, and any of the acidified

Table 1
Mean ($n = 6$) *Salmonella* populations (log CFU/cm^2 ± standard deviation [SD]) for inoculated (6 to 7 log CFU/cm^2) pork samples that were left nontreated (control) or were spray-treated (10 s, 124 to 131 kPa, 3.8 L/min flow rate) with various treatment solutions. Samples were analyzed for surviving *Salmonella* populations after treatment (0 h) and after a 24-h storage period at 4°C

Treatment	Mean *Salmonella* populations (log CFU/cm^2 ± SD)	
	0 h	24 h
Control	6.2 ± 0.1^{a,z}	6.0 ± 0.1^{a,z}
Water	5.4 ± 0.1^{b,z}	4.9 ± 0.2^{b,y}
Formic acid (1.5%)	5.0 ± 0.3^{bc,z}	4.7 ± 0.3^{bc,z}
SSS (pH 1.2)	4.6 ± 0.2^{cd,z}	4.8 ± 0.2^{bc,z}
PAA (400 ppm)	4.7 ± 0.4^{cd,z}	4.5 ± 0.3^{bcd,z}
AA-aPAA	4.5 ± 0.3^{cd,z}	4.5 ± 0.2^{bcd,z}
FA-aPAA	4.5 ± 0.4^{d,z}	4.3 ± 0.3^{cd,z}
SSS-aPAA	4.3 ± 0.2^{d,z}	4.1 ± 0.1^{d,z}

SSS: sulfuric acid and sodium sulfate blend; PAA: peroxyacetic acid; AA-aPAA: PAA (400 ppm) acidified with 1.5% acetic acid; FA-aPAA: PAA (400 ppm) acidified with formic acid (1.5%); SSS-aPAA: PAA (400 ppm) acidified with SSS (pH 1.2).
[a-d]Least squares means in the same column without a common superscript letter are different ($P < 0.05$).
[y-z]Least squares means in the same row without a common superscript letter are different ($P < 0.05$).

Table 2
Mean ($n = 6$) *Salmonella* populations (log CFU/cm^2 ± standard deviation [SD]) for inoculated (3 to 4 log CFU/cm^2) pork samples that were left nontreated (control) or were spray-treated (10 s, 124 to 131 kPa, 3.8 L/min flow rate) with various treatment solutions. Samples were analyzed for surviving *Salmonella* populations after treatment (0 h) and after a 24-h storage period at 4°C.

Treatment	Mean *Salmonella* populations (log CFU/cm^2 ± SD)	
	0 h	24 h
Control	3.5 ± 0.0^{a,z}	3.3 ± 0.1^{a,z}
Water	2.8 ± 0.1^{b,z}	2.5 ± 0.4^{b,y}
Formic acid (1.5%)	2.5 ± 0.2^{bc,z}	1.9 ± 0.3^{c,y}
SSS (pH 1.2)	2.2 ± 0.2^{cd,z}	1.6 ± 0.2^{c,y}
PAA (400 ppm)	2.1 ± 0.2^{cde,z}	1.9 ± 0.2^{c,z}
AA-aPAA	1.9 ± 0.1^{de,z}	1.7 ± 0.3^{c,z}
FA-aPAA	2.1 ± 0.2^{cde,z}	1.5 ± 0.5^{c,y}
SSS-aPAA	1.8 ± 0.3^{e,z}	1.5 ± 0.2^{c,z}

SSS: sulfuric acid and sodium sulfate blend; PAA: peroxyacetic acid; AA-aPAA: PAA (400 ppm) acidified with 1.5% acetic acid; FA-aPAA: PAA (400 ppm) acidified with formic acid (1.5%); SSS-aPAA: PAA (400 ppm) acidified with SSS (pH 1.2).
[a-e]Least squares means in the same column without a common superscript letter are different ($P < 0.05$).
[y-z]Least squares means in the same row without a common superscript letter are different ($P < 0.05$).

PAA treatments (i.e., AA-aPAA, FA-aPAA, or SSS-aPAA). For pork samples treated with FA-aPAA, *Salmonella* counts were 0.5 log CFU/cm^2 lower ($P < 0.05$) than those of samples that had been treated with the formic acid solution.

Within each treatment, pathogen counts for samples analyzed after the refrigerated storage period were, in general, similar ($P \geq 0.05$) to counts of the corresponding treatment at 0 h (Table 1). The only exception for this was the water treatment, where counts of samples analyzed after 24 h were 0.5 log CFU/cm^2 lower ($P < 0.05$) than those of the corresponding 0-h samples. Overall, after 24 h of cold storage, it was noted that the only treatments with lower ($P < 0.05$) *Salmonella* populations than those of the water treatment were FA-aPAA and SSS-aPAA.

Decontamination effects against low *Salmonella* contamination level. The initial level of inoculated *Salmonella* on control samples was 3.5 ± 0.0 log CFU/cm^2 (Table 2). Pathogen reductions obtained immediately after application of the spray treatments were, in general, similar to those obtained for the samples inoculated with the high concentration of the pathogen. Specifically, all spray treatments resulted in significant ($P < 0.05$) reductions of *Salmonella* that ranged from 0.7 (water) to 1.7 (SSS-aPAA) log CFU/cm^2 (Table 2, Supplemental Table 1). Recovered *Salmonella* populations from samples treated with formic acid were not ($P \geq 0.05$) different from those of the water-treated samples. Surviving *Salmonella* populations on pork samples treated with the formic acid, SSS, or PAA were 1.0, 1.3, and 1.4 log CFU/cm^2 lower ($P < 0.05$) than counts of the control treatment. No ($P \geq 0.05$) differences in antimicrobial effects were noted between formic acid and the FA-aPAA treatment. Similarly, no ($P \geq 0.05$) differences in decontamination efficacy were obtained between PAA and any of the three acidified PAA treatments. On the other hand, spray treatment of samples with SSS-aPAA was found to be more ($P < 0.05$) effective than treating the samples with SSS on its own.

Salmonella counts of the control samples and those treated with PAA, AA-aPAA, or SSS-aPAA and subsequently stored at 4°C for 24 h were similar ($P \geq 0.05$) to the counts obtained at the 0-h sampling time for each treatment (Table 2). For samples treated with SSS, formic acid, or FA-aPAA, pathogen counts obtained after the refrigerated storage period were 0.6 log CFU/cm^2 lower ($P < 0.05$) than the corresponding 0-h counts. For the 24-h sampling time, contrary to the results obtained for samples that had been inoculated at the high contamination level, populations recovered from refrigerated samples that had been treated with any of the six chemical treatments were all lower ($P < 0.05$) than those recovered from the refrigerated water-treated samples. Also, statistical comparison of *Salmonella* counts obtained at the 24-h sampling time indicated no ($P \geq 0.05$) differences between any of the chemical spray treatments.

Discussion

Foodborne outbreak data collected for 12 food commodity groups over a 20-year period (i.e., 1998–2017) show an increasing trend in the proportion of pork-associated *Salmonella* outbreaks (Williams and Ebel, 2022). These data, in part, have led to the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) proposing pathogen reduction performance standards for *Salmonella* in raw intact and nonintact pork cuts, and comminuted pork products (U.S. Department of Agriculture, Food Safety and Inspection Service (2022)). Therefore, establishments subject to these performance standards may have to implement additional or new pathogen reduction practices or processes to ensure that they meet these performance standards once they come into effect. Antimicrobial washes, rinses, and/or sprays are a cost-effective postharvest pathogen control strategy widely used in the meat and poultry industry (Jensen et al., 2015). In the current study, formic acid, SSS, PAA, and three acidified PAA treatments were evaluated for their antimicrobial effects against two levels of *Salmonella* contamination on chilled pork surface tissue. Overall, the results showed that compared to the *Salmonella* populations of the nontreated controls, all chemical spray treatments were effective in reducing the initial pathogen load, regardless of the inoculation level. Furthermore, all except the formic acid treatment had greater ($P < 0.05$) initial *Salmonella* reductions than the water treatment. As indicated previously, the water treatment was included in the study to serve as a control for the physical removal of bacterial cells from the sample surface by the spray application parameters used in this study. Therefore, under the experimental conditions of our study, compared to the water treatment, 1.5% formic acid was not an effective antimicrobial intervention for reducing initial (0 h) *Salmonella* populations on pork. For the pork samples inoculated at the low contamination level, *Salmonella* populations recovered from stored samples that had been treated with any of the six chemical solutions were all lower (by 0.5–1.0 log CFU/cm^2; $P < 0.05$) than the recovered populations of the water-treated samples. For stored samples originally inoculated at the ca. 6 log CFU/cm^2 contamination level, only FA-aPAA- and SSS-aPAA-treated samples had lower (by 0.6 and 0.8 log CFU/cm^2, respectively; $P < 0.05$) pathogen populations than those of the samples sprayed with water.

The SSS blend (sometimes referred to as AFTEC 3000 or Titon in the literature; Geornaras et al., 2012a; Pozuelo et al., 2021; Scott-Bullard et al., 2017) is approved for use as a spray, wash, or dip of meat and poultry products at concentrations sufficient to reach a pH range of 1.0–2.2 (U.S. Department of Agriculture, Food Safety and Inspection Service, 2021). The antimicrobial effects of SSS have been evaluated mostly on beef and poultry products (Acuff, 2017; Geornaras et al., 2012a, 2012b; Gonzalez, Geornaras, Nair, & Belk, 2021; Kim, Park, Lee, Owens, & Ricke, 2017; Muriana et al., 2019; Olson, Wythe, Dittoe, Feye, & Ricke, 2020; Scott et al., 2015; Scott-Bullard et al., 2017). Initial *Salmonella* reductions of 1.6 and 1.3 log CFU/cm^2 were obtained in the current study when pH 1.2 SSS was sprayed onto skin-on chilled pork tissue samples inoculated with the high or low concentrations of the pathogen, respectively. McCullough (2016) also evaluated SSS, but at pH levels of 1.0 and 1.5, for its decontamination effects against *Salmonella* on pork. Briefly, McCullough (2016) inoculated the surface of bone-in pork shoulder portions with the same mixture of *S. enterica* serotype strains used in the current study and used a spray cabinet to apply the SSS treatments for 11 s at 138 kPa. Initial *Salmonella* levels of 6.5 log CFU/g were reduced by 0.9 and 0.8 log CFU/g following treatment with pH 1.0 and 1.5 SSS, respectively (McCullough, 2016). Additionally, McCullough (2016) conducted an in-plant study in a commercial pork harvest facility to evaluate the decontamination effects of SSS (pH 1.0 and 1.3) followed by a snap-chill cycle against inoculated (6–7 log CFU/cm^2) nonpathogenic *E. coli* surrogates for enteric pathogens, on the surface of prerigor carcasses. The pH 1.0 and 1.3 SSS treatments reduced ($P < 0.05$) surrogate populations by 1.31 and 0.89 log CFU/cm^2, respectively, and surviving populations were reduced ($P < 0.05$) by another 1.46 and 1.54 log CFU/cm^2, respectively, after the rapid chill cycle (McCullough, 2016). Low pH antimicrobial compounds, such as SSS, kill or retard bacterial growth by passing through the cell membrane in their undissociated form and dissociate upon encounter with the neutral intracellular pH, generating an excess of protons that the cell then needs to efflux to maintain its pH (Mani-López et al., 2012; Stratford et al., 2009). This process consumes the cell's energy and leads to the disruption of cellular metabolic functions.

Peroxyacetic acid is a commercially available oxidizing agent, and its use as an antimicrobial treatment, applied at different application parameters, has been extensively evaluated against pathogens, including *Salmonella*, on beef and poultry products (Britton et al., 2018; Ellebracht et al., 2005; Geornaras et al., 2012b; Kim, Park, Lee, Owens, & Ricke, 2017; King et al., 2005; Kocharunchitt, Mellefont, Bowman, & Ross, 2020; Mohan & Pohlman, 2016; Nagel,

Bauermeister, Bratcher, Singh, & McKee, 2013; Penney et al., 2007; Ramirez-Hernandez, Brashears, & Sanchez-Plata, 2018; Scott et al., 2015). In comparison, published data on the decontamination effects of PAA against *Salmonella* on pork products are limited (Totton et al., 2016). One recently published study (Eastwood et al., 2021) reported 0.8 and 1.1 log CFU/cm^2 reductions of rifampicin-resistant *Salmonella* Typhimurium inoculated (ca. 6 log CFU/cm^2) on skin-on and skinless chilled pork, respectively, following a 3–5 s spray application of 400 ppm PAA using a garden sprayer. In the present study, use of a spray cabinet for the application of 400 ppm PAA onto skin-on chilled pork portions reduced ($P < 0.05$) high (6.2 log CFU/cm^2) and low (3.5 log CFU/cm^2) *Salmonella* contamination levels by 1.5 and 1.4 log CFU/cm^2, respectively. The reductions reported in our study and those by Eastwood et al. (2021) cannot be directly compared due to differences in testing parameters, such as the inoculum strains, inoculation method, pork tissue temperature at the time of inoculation, bacterial cell attachment time before treatment exposure, and spray application methodologies used. Pozuelo et al. (2021) also evaluated 400 ppm PAA for its antimicrobial effects on pork, but in this case, the target microorganism was Shiga toxin-producing *E. coli* (STEC). The PAA was applied onto prerigor pork carcass sides using a commercial-grade wash cabinet that was operated under simulated industrial-scale wash or spray parameters. Inoculated STEC populations of 5 log CFU/cm^2 were reduced by 3.3 and 1.6 log CFU/cm^2 following a high-volume wash (12 s, 345 kPa, 303 L/min) or low-volume spray (16 s, 207 kPa, 9 L/min) application of the antimicrobial (Pozuelo et al., 2021). Subsequent chilling of PAA-treated carcasses for 18 h at 2°C did not have an effect on the surviving STEC populations (Pozuelo et al., 2021).

Acidified PAA treatments combine two mechanisms of action, oxidation from PAA and cytoplasmic acidification, and this combination could result in a synergistic antimicrobial effect. A few recently published studies have investigated the decontamination effects of acidified PAA on beef, poultry, and pork (Britton et al., 2018; Gonzalez et al., 2021; Olson et al., 2020; Pozuelo et al., 2021). In the current study, regardless of sampling time, antimicrobial effects against both contamination levels of *Salmonella* were similar ($P \geq 0.05$) for PAA and all three evaluated pH-adjusted PAA solutions. Similar findings were reported in a study by Britton et al. (2018). The investigators used a spray cabinet application to evaluate the antimicrobial effects of acidified and nonacidified PAA solutions against nonpathogenic *E. coli* surrogates for enteric pathogens on prerigor beef carcass surface tissue samples. Reductions of 1.9, 1.7, and 1.9 log CFU/cm^2 were obtained immediately following a 10 s spray application at 103 kPa of 400 ppm PAA and PAA (400 ppm) acidified with 2% acetic acid or pH 1.2 SSS, respectively (Britton et al., 2018). Moreover, the previously mentioned Pozuelo et al. (2021) study also evaluated an acidified PAA treatment, and similar antimicrobial effects for PAA and acidified PAA were found. More specifically, a low-volume spray application of 400 ppm PAA acidified to pH 1.2 using SSS reduced STEC populations on prerigor pork carcasses by 2.3 log CFU/cm^2. This 2.3-log reduction was numerically, but not statistically ($P > 0.05$), greater than the 1.6-log reduction the investigators reported for the nonacidified PAA treatment (Pozuelo et al., 2021).

In conclusion, the results of the current study showed that compared to the nontreated samples, all evaluated chemical spray treatments effectively ($P < 0.05$) reduced *Salmonella* contamination levels on the surface of chilled pork tissue, regardless of the inoculum level. Pathogen reductions for the chemical treatments ranged from 1.2 to 1.9 log CFU/cm^2 (6.2 log CFU/cm^2 inoculation level) and 1.0 to 1.7 log CFU/cm^2 (3.5 log CFU/cm^2 inoculation level). Recovered *Salmonella* populations from samples treated with 1.5% formic acid were not ($P \geq 0.05$) different from those of water-treated samples. Regardless of contamination level and sampling time (0 h or 24 h), no ($P \geq 0.05$) differences in efficacy were obtained between PAA and any of the acidified PAA treatments evaluated. Under the conditions of this study, pH 1.2 SSS, 400 ppm PAA, and 400 ppm PAA acidified with acetic acid (1.5%), formic acid (1.5%), or pH 1.2 SSS were effective interventions for reducing high and low contamination levels of *Salmonella* on skin-on chilled pork surfaces.

CRediT authorship contribution statement

Sara V. Gonzalez: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Mahesh N. Nair:** Writing – review & editing, Supervision, Resources. **Keith E. Belk:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Ifigenia Geornaras:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfp.2023.100068.

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