Effect of caprylic acid alone or in combination with peracetic acid against multidrug-resistant *Salmonella* Heidelberg on chicken drumsticks in a soft scalding temperature-time setup[1]

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ABSTRACT The antimicrobial efficacy of caprylic acid (**CA**), a medium-chain fatty acid, against multidrug-resistant *Salmonella* Heidelberg (**MDR SH**) on chicken drumsticks in a soft-scalding temperature-time setup was investigated. Based on the standardization experiments in nutrient media and on chicken breast fillet portions, intact chicken drumsticks were spot inoculated with MDR SH and immersed in water with or without antimicrobial treatments at 54°C for 2 min. The treatments included 0.5% CA, 1% CA, 0.05% peracetic acid (**PAA**), 0.5% CA + 0.05% PAA, and 1.0% CA + 0.05% PAA. Additionally, the efficacy of the potential scald treatments against MDR SH survival on drumsticks for a storage period of 48 h at 4°C was determined. Furthermore, the effect of these treatments on the surface color of the drumsticks was also evaluated. Appropriate controls were included for statistical comparisons. The antimicrobial treatments resulted in a significant reduction of MDR SH on drumsticks. For the lower inoculum (~2.5 $\log_{10}$ CFU/g) experiments, 0.5% CA, 1% CA, 0.05% PAA, 0.5% CA + 0.05% PAA, and 1.0% CA + 0.05% PAA resulted in 0.7-, 1.0-, 2.5-, 1.4-, and 1.5- $\log_{10}$ CFU/g reduction of MDR SH on drumsticks, respectively ($P < 0.05$). The same treatments resulted in 0.9-, 1.3-, 2.5-, 2.2-, and 2.6- $\log_{10}$ CFU/g reduction of MDR SH when the drumsticks were contaminated with a higher inoculum (~4.5 $\log_{10}$ CFU/g) level ($P < 0.05$). Moreover, the antimicrobial treatments inactivated MDR SH in the treatment water to undetectable levels, whereas 2.0- to 4.0- $\log_{10}$ CFU/mL MDR SH survived in the positive controls ($P < 0.05$). Also, the treatments were effective in inhibiting MDR SH on the drumsticks compared to the respective controls during a storage period of 48 h at 4°C; however, the magnitude of reduction remained the same as observed during the treatment ($P < 0.05$). Additionally, none of the treatments affected the color of the drumsticks ($P > 0.05$). Results indicate that CA could be an effective natural processing aid against MDR SH on chicken products.

Key words: *Salmonella* Heidelberg, caprylic acid, scalding, peracetic acid

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INTRODUCTION

Nontyphoidal *Salmonella* is a leading cause of human illnesses, hospitalizations, and deaths caused by foodborne pathogens (Scallan et al., 2011). Foodborne salmonellosis results in an estimated 1.2 million infections, 23,000 hospitalizations, and 450 deaths in the USA every year. The direct medical cost of salmonellosis is estimated to be $3.6 billion annually (USDA, 2014). *Salmonella* outbreaks are associated with poultry foods, although foods produced from other food animals, vegetables, and fruits are also implicated. In a previous study, *Salmonella* was recovered from 9.1% retail chicken samples, and multidrug-resistant (**MDR**) strains were high in poultry meat ranging from 20 to 36% (2014).

Salmonella Heidelberg (**SH**) is a highly invasive serotype and one among the major *Salmonella* isolated from poultry and poultry products. Resistance to critically

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important antibiotics used as first-line treatment for human salmonellosis has been reported markedly higher in serotypes associated with poultry (Hoffmann et al., 2012). A recent European study showed that SH isolated from imported poultry meat had MDR profiles, including resistance to extended-spectrum cephalosporins and fluoroquinolones (Campos et al., 2018).

To reduce the incidence of foodborne outbreaks through poultry, interventions should be employed in processing steps, in addition to the application of preharvest control strategies. In the processing facility, scalding is the first step where stunned and bled birds are exposed to a common water bath, where a high potential for cross-contamination exists. Scalding is the process in which carcasses are dipped in hot water to ease de-feathering (removal of feathers). The USDA-approved maximum temperature and time combination for soft-scalding conditions in broiler processing is 54°C and 120 s (USDA, 2021). Studies have shown that reduction of *Salmonella* at 55°C and 60°C were similar (Yang et al., 2001), but at a higher temperature, the oily layer on the chicken skin surface prevented chlorine from reaching the bacteria. Additionally, the presence of high organic matter in scalding water makes the antimicrobials less effective. The optimum pH for *Salmonella* growth is 6.5 to 7.5; creating an acidic pH in the scalding water could result in *Salmonella* reduction (Okrend et al., 1986). Studies have been conducted with several organic acids as antimicrobials in the scalding tank to provide an acidic environment to control *Salmonella*.

Peracetic acid (**PAA**), a mixture of hydrogen peroxide and acetic acid, is an organic oxidizer commonly used in the chilling steps of poultry processing. It is found that PAA is affected by organic materials to a lesser degree than chlorine (Bauermeister et al., 2008b). PAA was tested alone or in combination with other antimicrobials in postscalding steps (Río et al., 2007; Chen et al., 2014). However, studies on the application of PAA in scalding water are not available in the literature. Also, there are recent concerns over PAA use in processing facilities due to its capacity to cause pulmonary issues in processing facility personnel (Hawley et al., 2018).

Caprylic acid (**CA**) is an eight-carbon, medium-chain fatty acid (**MCFA**) naturally present in coconut oil and bovine milk (Jensen et al., 1990; Marina et al., 2009). It is a Generally Recognized as Safe (**GRAS**) − status food additive by the Food and Drug Administration (**FDA**). Caprylic acid has antimicrobial activity against Gram-positive and Gram-negative bacterial pathogens in various food systems. It has been tested alone or in combination with essential oils and antimicrobials in meat and meat products (Hulankova et al., 2013; Moschonas et al., 2012). However, its potential as a natural processing aid has not been explored, especially for scalding purpose. Additionally, the effect of the combination of CA and PAA that produces active peroxyoctanoic acid has not been investigated in scalding steps. Hence, this study's objectives were to determine the potential of CA alone or in combination with PAA against MDR SH on intact chicken drumsticks in a soft-scalding temperature-time setup in water.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Two strains of MDR SH from the 2013 Tennessee correctional facility outbreak (SH 1904 [N13X001904] and SH 466 [N13F0000466]; Division of Laboratory Services, Tennessee Department of Health) were used in this study (Dewi et al., 2021). Each strain was cultured separately from the glycerol stocks stored at -80°C. Working cultures of SH 1904 and 466 were prepared by adding 100 μL of stock culture to 10 mL tryptic soy broth (**TSB**; catalog no. C7141, Criterion, Hardy Diagnostics, Santa Maria, CA) containing nalidixic acid sodium salt (**NA**; CAS no. 3374-05-8, Alfa Aesar, Haverhill, MA) at 50 μg/mL and incubated at 37°C for 24 h. The SH strains were preinduced for resistance to 50 μg/mL of NA to facilitate selective enumeration of the pathogen. Growth of bacteria was determined by the presence of black colonies after plating an appropriate dilution of overnight culture on xylose lysine desoxycholate agar (**XLD**; catalog no. C7322, Criterion, Hardy Diagnostics, Santa Maria, CA) plates containing 50 μg/mL NA and incubating at 37°C for 24 h. While SH 1904 was used for screening experiments in nutrient broth and fillet portions, a cocktail of SH 1904 and 466 was used in all drumstick experiments. Bacterial inoculum was prepared from overnight broth cultures (approximately 9 $\log_{10}$ CFU/mL) after centrifugation (3600 Xg, 15 min, 4°C) and resuspending the pellet in sterile PBS (Kollanoor-Johny et al., 2012) to obtain appropriate inoculum levels in the study.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The bacterial time-kill assay and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination of CA (Product no. W279927, >98%, natural, food-grade, kosher, Sigma Aldrich, St. Louis, MO) against SH were conducted (Kollanoor et al., 2007) to determine the effective concentrations to be applied in the drumstick experiments. Seven different concentrations of CA (0.03%, 0.06%, 0.12%, 0.25%, 0.5%, 1%, and 2%) were prepared in 10 mL TSB, separately. A 100 μL bacterial inoculum of 5 $\log_{10}$ CFU/mL prepared from the overnight culture of SH 1904 was added into the treatment tubes and incubated at 37°C for 24 h. Appropriate samples of TSB inoculated with or without MDR SH were kept as positive and negative controls. Surviving SH populations were enumerated at 0 and 24 h of incubation after plating appropriate dilutions of bacteria on XLD + NA agar. Samples were also tested for surviving bacteria by

enriching 1 mL of sample in 10 mL selenite cystine broth (**SCB**; Criterion, Hardy Diagnostics, Santa Maria, CA) at 37°C for 24 h and streaked on XLD + NA plates. Black colonies on XLD plates after 24 h of incubation were considered enrichment positive.

Preparation of Treatments

Caprylic acid treatments (0.5, 1, and 2%) were prepared by mixing the appropriate amount of the compound in autoclaved tap water in Whirlpak bags (Whirl-Pak, Nasco, Madison, WI) and vortexing for 30 s. Two concentrations (0.05% and 0.12%) of PAA (Perasan MP 2; Envirotech, Modesto, CA) and different combinations of the 2 compounds (0.05% CA + 0.05% PAA, 0.1% CA + 0.05% PAA, 2% CA + 0.12% PAA, 1% CA + 0.12% PAA) were also prepared in autoclaved tap water in Whirlpak bags and mixed thoroughly using a stomacher (100/125V, 50/60Hz; Neutec Group Inc., 200 Central Ave, Farmingdale, NY) for 30 s. The temperature of treatment water was set at 54°C in a water bath to simulate the USDA-recommended scalding temperature. The treatment water temperature was confirmed using a thermocouple dipped in water in Whirlpak bags with no added treatments.

Determination of the Effect of CA and PAA Against MDR SH on Breast Fillet Portions

An experiment was conducted with retail fresh, natural chicken breast fillets (boneless, skinless, 99% fat-free, no-antibiotic ever [**NAE**] commercial brand) purchased from a Minnesota grocery store to determine the effect of adding CA and PAA in the water against SH 1904. This step using a small portion of meat was necessary to fine-tune the concentrations of treatment for the drumstick experiments. Meat samples were cut into 25 g pieces and the upper inoculating surface exposed to UV light for 15 min in a biosafety cabinet to kill surface bacteria before SH inoculation for better attachment (Nair and Kollanoor Johny, 2017; Dewi et al., 2021). Different concentrations of CA (1 and 2%), PAA (0.12%) and their combinations (1% CA + 0.12% PAA and 2% CA + 0.12% PAA) were prepared in 250 mL autoclaved tap water in Whirlpak bags maintained at 54°C and vortexed for 30 s. The PAA at 0.12% was chosen because this concentration is the recommended maximum dose for meat application purposes by the manufacturer as approved by the FDA (Food Contact Substance Notification# FCN-001738).

Fillet samples were spot inoculated with a diluted culture of SH at a dose of ~3 $\log_{10}$ CFU/g and spread all over the meat samples with a glass rod. After providing 20 min of attachment time for SH at room temperature, each meat sample was separately dipped in appropriate treatment maintained at 54°C for 2 min. Meat samples were homogenized in 250 mL PBS for 1 min immediately after the dip treatment. The survival rate of the pathogen was determined in both treatment water and meat samples. Autoclaved tap water in Whirlpak bag without any treatments maintained at 54°C and 20°C inoculated with the pathogen were kept as positive controls (PC54, and PC20, respectively). Uninoculated fillet samples dipped in autoclaved tap water were used as negative control (**NC**).

Determining the Antibacterial Effect of CA and PAA Against MDR SH Cocktail on Chicken Drumsticks in a Soft-Scalding Temperature-Time Set-Up

All-natural, fresh retail chicken drumsticks (commercial brand) obtained from a retail store were used for this study. Drumsticks will better represent a whole carcass since the portion has meat covered by loose skin and some exposed flesh on the top (except that there are no feathers). Drumsticks were weighed and the upper inoculating surface exposed to UV light in a biosafety cabinet for 15 min to kill the microflora before inoculating with SH for better attachment. A lower (2.5 $\log_{10}$ CFU/g) and a higher inoculum (~4.5 $\log_{10}$ CFU/g) of SH were tested in 2 sets of experiments. Treatments were prepared in 350 mL sterile tap water, kept in a Whirlpak bag at 54°C, and vortexed for 30 s for uniform mixing. After providing 20 min of pathogen attachment time, the drumsticks were dipped in the treatment solution for 2 min. Treated drumsticks were transferred to 350 mL PBS in whirl pack bags and mixed well by vigorous shaking by hand for 60 s. Uninoculated drumsticks dipped in tap water served as NC, and SH-inoculated drumsticks dipped in sterile tap water without any treatments and maintained at 54°C and 20°C served as the positive controls.

Effect of CA and PAA Applied in Treatment Water Against MDR SH Cocktail After Chilling and Storage

The selected effective concentrations (1% CA, 0.05% PAA, and 1% CA + 0.05% PAA) were used to prepare treatments for this set of experiment. PAA at 0.05% was selected after a preliminary experiment testing different concentrations of PAA (0.005, 0.01, 0.02, and 0.05%) and selecting the effective concentration that would not affect sensory attributes (Nair et al., 2015). A cocktail of 2 strains of SH at a final concentration of 4.5 $\log_{10}$ CFU/g was inoculated on the drumsticks. The experiment was repeated as in the previous experiment until the dipping step. After 2 min of dipping the drumsticks in the treatment solutions, they were transferred to 350 mL autoclaved tap water kept in Whirlpak bags maintained at 4°C for 30 min to simulate chilling conditions. After 30 min of chilling, samples were transferred to 350 mL PBS and mixed well for 60 s, and SH survival was determined (and Kollanoor JohnyNair and Kollanoor Johny, 2017) in both chilling water and on drumsticks. After chilling treatment, additional sets of

samples were transferred to whirl pack bags and were kept at 4°C for 48 h (storage time). The surviving SH populations were determined after 2 d of storage by surface plating and enrichment.

Microbiological Analysis

Surviving SH populations on drumsticks and dipping water were determined by the broth dilution assays. After homogenizing the samples, 1 mL of rinsate was serially diluted 10-fold, and 200 μL from appropriate dilutions was surface plated on XLD + NA plates. SH colonies were enumerated after 24 h of incubation at 37°C. One mL each of the dipping solution and rinsate was enriched in 10 mL SCB, incubated for 8 h, and streaked on XLD + NA plates to detect bacteria when not identified by direct plating (Kollanoor-Johny et al., 2012).

Determination of Treatment Water pH

The pH of the treated water used for the experiments was measured by placing the pH meter probe (Symphony B10P, VWR, Radnor, PA) in the water taken in Whirlpak bags. Readings were taken immediately after the dip experiment.

Effect of CA and PAA on the Surface Color of Drumsticks

After 2 min of dipping in treatments at scalding temperature, color differences between the drumsticks applied with treatments were measured using a hunter handheld colorimeter (Hunter Lab MiniScan EZ 4500S Spectrophotometer, Reston, VA). The L* (lightness), a* (redness), and b* (yellowness) values were recorded from 3 different points (forming an inverted triangle with base on the top and vertex on the bottom) on both sides of a single drumstick after calibrating the chroma meter (Nair et al., 2015).

Statistical Analysis

A completely randomized design was used to determine the effect of CA and PAA against SH in all experiments. Each sample (culture tube, fillet, drumstick, treatment water) served as an experimental unit, with every experiment repeated at least 3 times in duplicates. The number of bacterial colonies was logarithmically transformed before analysis to achieve homogeneity of variance. The samples from which no bacteria were detected after direct plating but positive after enrichment were assumed a value of 0.90 for analysis (Seo et al., 2000; Young et al., 2007). Data were analyzed using the PROC-MIXED procedure of SAS. Separate analyses were conducted at each time point to understand the effects of treatments compared to the controls (Tukey test). A P value of <0.05 was considered statistically significant.

Table 1. Effect of caprylic acid against *Salmonella* Heidelberg 1904 in broth culture at 0 h and 24 h of incubation in broth (Mean ± SE; n = 6).

	Salmonella counts ($\log_{10}$ CFU/mL)	
Treatments	0 h	24 h
PC	5.97 ± 0.04^{a}	8.93 ± 0.09^{a}
0.03% CA	5.86 ± 0.05^{a}	8.10 ± 0.07^{b}
0.06% CA	5.91 ± 0.07^{a}	7.73 ± 0.15^{b}
0.12% CA	5.94 ± 0.12^{a}	7.84 ± 0.11^{b}
0.25% CA	4.95 ± 0.30^{b}	5.11 ± 0.21^{c}
0.5% CA	NG^{c}	NG^{d}
1% CA	NG^{c}	NG^{d}
2% CA	NG^{c}	NG^{d}

[a-d] Values with different superscripts in a column are significantly different from each other at $P < 0.05$.Abbreviations: CA, caprylic acid; NG, no growth detected; PC, positive control.

RESULTS

Effect of CA Against MDR SH in Nutrient Broth

As expected, MDR SH 1904 in the control group grew from 6 to 9 $\log_{10}$ CFU/mL over 24 h of incubation at 37°C (Table 1). Concentrations of 0.03%, 0.06%, and 0.12% CA resulted in 1 $\log_{10}$ CFU/mL reduction in SH growth compared to the PC over time ($P < 0.05$). Whereas 0.25% resulted in a 4-log reduction of SH compared to PC after 24 h of incubation, all concentrations above 0.25% resulted in a rapid reduction of MDR SH to undetectable levels even after 30 s of treatment. The MIC and MBC of CA against MDR SH were 0.25% and 0.5%, respectively (Table 1).

Effect of CA, PAA, and Their Combinations Against MDR SH on Breast Fillet Portions in Treatment Water

All treatments resulted in a significant reduction ($P <$ 0.05) in MDR SH 1904 on breast fillets compared to the PC (1.25−1.57 $\log_{10}$ CFU/g; Table 2). Populations of SH in PC at both temperatures remained at ~2.5 $\log_{10}$ CFU/g (Table 2). There was no significant difference between MDR SH populations on the breast fillets

Table 2. Effect of caprylic acid against *Salmonella* Heidelberg 1904 on chicken breast fillet and treatment water at 54°C for 2 min (Mean ± SE; n = 6).

	Salmonella counts	
Treatments	Meat ($\log_{10}$ CFU/g)	Water ($\log_{10}$CFU/mL)
PC (20°C)	2.69 ± 0.13^{a}	1.43 ± 0.21^{a}
PC (54°C)	2.47 ± 0.11^{a}	1.40 ± 0.21^{a}
1% CA	1.28 ± 0.19^{b}	NG^{b}
2% CA	1.06 ± 0.29^{b}	NG^{b}
0.12% PAA	1.22 ± 0.32^{b}	NG^{b}
1% CA + 0.12% PAA	0.90 ± 0.23^{b}	NG^{b}
2% CA + 0.12% PAA	1.22 ± 0.28^{b}	NG^{b}

[a,b]Values with different superscripts in a column are significantly different from each other at $P < 0.05$.Abbreviations: CA, caprylic acid; NG, no growth detected; PAA, peracetic acid; PC (20°C), positive control at 20°C; PC (54°C), positive control at 54°C.

dipped in water maintained at 54°C and 20°C among the controls. Among the treatments, 0.12% PAA and the combination of 2% CA and 0.12% PAA caused a similar numerical reduction, which was 1.25 $\log_{10}$ CFU/g lower compared to PC. The combination of 1% CA and 0.12% PAA resulted in maximum reduction (1.57 $\log_{10}$ CFU/g; $P < 0.05$) compared to PC ($P < 0.05$), although these treatments were not significantly different from 0.12% PAA or the combination of 2% CA and 0.12% PAA groups ($P > 0.05$). All treatments reduced MDR SH populations in the treatment water to undetectable levels, while the PC group had 1.4 $\log_{10}$ CFU/mL of SH (Table 2).

Effect of CA, PAA, and Their Combinations Against MDR SH cocktail on Drumsticks in Treatment Water

Based on the experiments using a single strain inoculation (SH 1904) on breast fillet portions, the lower (2.5 $\log_{10}$ CFU/g) and higher inoculum (4.5 $\log_{10}$ CFU/g) experiments using a cocktail of MDR SH 1904 and 466 were conducted (Tables 3 and 4, respectively). There was no effect of temperature (54°C and 20°C) on MDR SH survival on drumsticks. Both PCs had 2.5 $\log_{10}$ CFU/g of bacteria attached to them (Table 3). With the lower inoculum, 0.05% PAA resulted in 2.5 $\log_{10}$ CFU/g reduction of MDR SH followed by the combination of CA and PAA treatments. The addition of CA at 0.5% and 1% caused a reduction of 0.69 and 0.98 $\log_{10}$ CFU/g, respectively, compared to PC (Table 3), and the reduction of MDR SH was slightly higher when PAA was combined with CA at these concentrations (1.36 and 1.52 $\log_{10}$ CFU/g, respectively) (Table 3).

When a higher inoculum was used (Table 4), 0.5 and 1% CA resulted in the reduction of ~0.93 and 1.33 $\log_{10}$ CFU/g SH, respectively ($P < 0.05$). The addition of 0.05% PAA could still result in ~2.5 $\log_{10}$ CFU/g reduction of MDR SH compared to PCs. Similar to the lower inoculum experiment, no additive effect was observed in bacterial reduction when combinations were used. The combination of 0.5% CA and 0.05% PAA, and 1% CA and 0.05% PAA resulted in 2.21- and 2.6- $\log_{10}$ CFU/g reduction of MDR SH, compared to PC (Table 4).

The PC had 2- and 4- $\log_{10}$ CFU/mL SH in the water after dip treatment in the lower and higher inoculum experiments, respectively. However, in both studies, no SH was recovered from the treatment water even after enrichment (Tables 3 and 4).

Table 3. Effect of caprylic acid against *Salmonella* Heidelberg 1904 and 466 (low inoculum) on chicken drumsticks and treatment water at 54°C and 2 min (Mean ± SE; n = 6).

	Salmonella counts	
Treatments	Drumsticks ($\log_{10}$ CFU/g)	Water ($\log_{10}$CFU/mL)
PC (20°C)	2.54 ± 0.11^{a}	1.96 ± 0.15^{a}
PC (54°C)	2.51 ± 0.17^{a}	2.04 ± 0.18^{a}
0.5% CA	1.81 ± 0.17^{b}	NG[b]
1% CA	1.53 ± 0.12^{bc}	NG[b]
0.05% PAA	NG[d]	NG[b]
0.5% CA + 0.05% PAA	0.99 ± 0.07^{c}	NG[b]
1% CA + 0.05% PAA	0.17 ± 0.07^{c}	NG[b]

[a-d]Values with different superscripts in a column are significantly different from each other at $P < 0.05$.Abbreviations: CA, caprylic acid; NG, no growth detected; PAA, peracetic acid; PC (20°C), positive control at 20°C; PC (54°C), positive control at 54°C.

Table 4. Effect of caprylic acid against *Salmonella* Heidelberg 1904 and 466 (high inoculum) on chicken drumsticks and treatment water at 54°C for 2 min (Mean ± SE; n = 6).

	Salmonella counts	
Treatments	Drumsticks ($\log_{10}$ CFU/g)	Water ($\log_{10}$CFU/mL)
PC (20°C)	4.82 ± 0.09^{a}	4.16 ± 0.22^{a}
PC (54°C)	4.57 ± 0.13^{a}	4.25 ± 0.23^{a}
0.5% CA	3.64 ± 0.09^{b}	NG[b]
1% CA	3.25 ± 0.11^{b}	NG[b]
0.05% PAA	2.13 ± 0.31^{c}	NG[b]
0.5% CA + 0.05% PAA	2.36 ± 0.33^{c}	NG[b]
1% CA + 0.05% PAA	1.98 ± 0.28^{c}	NG[b]

[a,b,c]Values with different superscripts in a column are significantly different from each other at $P < 0.05$.Abbreviations: CA, caprylic acid; NG, no growth detected; PAA, peracetic acid; PC (20°C), positive control at 20°C; PC (54°C), positive control at 54°C.

Effect of CA, PAA, and Their Combinations on MDR SH Cocktail Survival on Drumsticks After Chilling and Storage

The PC had approximately 4.5 $\log_{10}$ CFU/g MDR SH on the drumsticks. The addition of 1% CA resulted in ~1 $\log_{10}$ CFU/g reduction of SH on drumsticks after 30 min of chilling ($P < 0.05$; Table 5), which was similar to the reduction obtained immediately after the soft-scalding in the low inoculum experiment. This reduction was maintained even after 48 h of storage (Table 5). On the other hand, PAA applied at 0.05% resulted in a reduction of 1.25 $\log_{10}$ CFU/g of MDR SH, significantly different from the 1% CA group ($P < 0.05$; Table 5). However, this reduction increased to 1.6 $\log_{10}$ CFU/g

Table 5. Effect of caprylic acid against *Salmonella* Heidelberg 1904 and 466 survival (high inoculum) on chicken drumsticks after 30 min of chilling and 48 h of storage and in chilling water at 4°C (Mean ± SE; n = 6).

	Salmonella counts		
	Drumsticks ($\log_{10}$ CFU/g)		
Treatments	D 0	D 2	Water ($\log_{10}$CFU/mL)
PC (54°C)	4.32 ± 0.08^{a}	4.47 ± 0.13^{a}	3.60 ± 0.56^{a}
1% CA	3.47 ± 0.13^{b}	3.68 ± 0.07^{b}	2.08 ± 0.25^{b}
0.05% PAA	3.03 ± 0.08^{c}	2.83 ± 0.09^{c}	1.16 ± 0.38^{c}
1% CA + 0.05% PAA	2.95 ± 0.09^{c}	2.65 ± 0.26^{c}	1.34 ± 0.25^{c}

[a,b,c]Values with different superscripts within a column are significantly different from each other at $P < 0.05$.Abbreviations: CA, caprylic acid; PAA, peracetic acid; PC, positive control.

Table 6. Effect of treatments on pH of treatment water.

Treatments	pH
NC	6.58 ± 1.06^{a}
PC 54°C	6.15 ± 0.93^{a}
PC 20°C	6.64 ± 0.75^{a}
0.5% CA	4.22 ± 0.05^{b}
1% CA	4.19 ± 0.05^{b}
0.05% PAA	3.39 ± 0.10^{b}
0.5% CA + 0.05% PAA	3.33 ± 0.11^{b}
1% CA + 0.05% PAA	3.29 ± 0.12^{b}

[a,b]Values with different superscripts within a column are significantly different from each other at $P < 0.05$.Abbreviations: CA, caprylic acid; NC, negative control; PAA, peracetic acid; PC, positive control.

after 48 h of storage (Table 5). The combination of PAA at 0.05% and 1% CA resulted in a significant reduction of ~2 $\log_{10}$ CFU/g after 48 h of storage (Table 5).

All 3 treatments significantly reduced SH in chilling water compared to PC ($P < 0.05$). Reductions of 1.5-, 2.4- and 2.25- $\log_{10}$ CFU/mL in chilling water were obtained with treatments of 1% CA, 0.05% PAA, and 1% CA + 0.05% PAA, respectively (Table 5).

Effect of CA and PAA on Water pH

The treatments were significantly different from the controls ($P < 0.05$). The addition of CA resulted in a pH close to 4 (range: 4.19−4.22), and PAA alone (3.39) or combination with CA resulted in pH close to 3 (range: 3.29−3.33) in treatment water, compared to the controls (pH range = 6.15−6.64) (Table 6).

Effect of CA, PAA, and the Combinations on the Surface Color of Drumsticks After Dip Treatment at 54°C for 2 Minutes

There were no significant differences in L*, a*, and b* values of drumsticks dipped in treatment water maintained at 54°C for 2 min ($P > 0.05$; Table 7).

DISCUSSION

Scalding is the first critical control point in poultry processing since the step could result in *Salmonella*'s initial attachment to the skin. Such *Salmonella*-contaminated carcasses could result in cross-contamination of fresh incoming carcasses through water and carryover of contamination throughout the subsequent stages of processing (Mcbride et al., 1980). Hard scalding (59−64°C for 30−75 s) and soft scalding (51−54°C for 90−120 s) are 2 scalding methods commonly employed in broiler processing. A temperature above 47°C is enough to control *Salmonella* growth since the organisms cannot grow at and above that temperature (USDA, 2021). Yang et al. (2001) found that *Salmonella* counts could be reduced with higher water temperatures (55−60°C) and a long duration of dip. In this study, the temperature alone could not significantly reduce MDR SH on the skin and in treatment water. A possible reason is that the skin temperature cannot reach up to scalding water temperature within the recommended short dip time (Yang et al., 2001). Although not investigated in depth in this study, a better thermal tolerance for these strains could be a reason. The other approach to reduce *Salmonella* in scalding water is maintaining a pH below or above the optimum pH for *Salmonella* growth (6.5 −7.5). The pH above 8.5 and below 4 profoundly affects *Salmonella* growth (Humphrey et al., 1981). We observed that CA and PAA resulted in reducing the pH of the water (Table 6). The mechanism by which the acidic environments are thought to inactivate *Salmonella* is by altering the activity of functional enzymes (Sun et al., 1998).

In this study, 2 compounds, CA and PAA, were tested, either alone or in combination at the soft scalding temperature-time combination setup in water. Both compounds resulted in a significant reduction in SH survival on drumsticks after the soft-scalding, chilling, and 48 h of storage without adversely affecting the surface color of chicken drumsticks. PAA was the most effective of all treatments in our study (Tables 3, 4, and 5). Previously, Bauermeister et al. (2008a, b) had tested PAA as an intervention strategy in poultry chiller to decrease *Salmonella* and *Campylobacter*. Peracetic acid hydrogen peroxide (**PAHP**) at 85 ppm (0.0085%) reduced *Salmonella* positive carcasses by 92%, whereas 30 ppm chlorine (0.003%) reduced *Salmonella* by 57%. Additionally, PAHP reduced *Campylobacter* on carcasses exiting the chiller by 43%, while chlorine resulted in only a 13% reduction (Bauermeister et al., 2008b). The group also reported that 0.02% (200 ppm) PAA decreased *S.* Typhimurium by >1.5 logs CFU/ sample compared to a chlorine treatment and extended shelf-life of products without compromising organoleptic properties (Bauermeister et al., 2008b).

Mechanisms of action of PAA could be due to acidification and oxidation effects. The substantial oxidizing property of PAA could disrupt the cell membrane's permeability and alter protein synthesis through reaction with sulfhydryl groups, sulfides, and nucleotides. Indirect antimicrobial action could occur by acidifying the carcass surface and penetrating undissociated acids into bacterial cells (Oyarzabal, 2005; Table 6). The reduction of bacteria due to PAA addition is generally higher with a temperature rise and decreases with the biochemical oxygen demand (Stampi et al., 2001). Although PAA is

Table 7. Color values of drumsticks (n = 6) dipped in different treatment water at 54°C for 2 min (Mean ± SE).

	Color designators		
Treatments	L*	a*	b*
NC	67.4 ± 3.2	0.69 ± 0.6	6.2 ± 0.6
0.5% CA	71.9 ± 2.7	0.75 ± 0.7	7.7 ± 0.8
1% CA	69.5 ± 3.2	0.94 ± 1.0	5.3 ± 1.0
0.05% PAA	70.1 ± 0.8	0.88 ± 0.6	4.5 ± 1.1
0.5% CA + 0.05% PAA	71.9 ± 1.2	1.00 ± 0.7	6.3 ± 1.5
1% CA + 0.05%PAA	72.9 ± 1.4	1.00 ± 0.3	4.9 ± 1.0

Color values among the treatments do not differ significantly ($p > 0.05$).
Abbreviations: CA, caprylic acid; NC, negative control; PAA, peracetic acid.

a good option, it has limitations for use in processing facilities, including its pulmonary-irritant nature, neutralization in the presence of organic matter, and evaporation at scalding temperatures (Kitis, 2004; Hawley et al., 2018).

A comparable reduction of the pathogen with CA was observed in treatment water (Tables 2, 3, and 4). Besides, CA was better than the PC in all experiments resulting in significant reductions of SH populations on fillets and drumsticks, indicating its potential as a natural antibacterial aid in scalding steps of the broiler processing operations. Caprylic acid could cause intracellular acidification after entering through the lipophilic cell membrane and result in the dissociation of the compound into H+ and caprylate ions. The decrease in the pH would result in the reduced or diminished activity of enzymes resulting in bacterial death (Sun et al., 1998).

Other scald additives containing sodium hydroxide have been reported to reduce *S.* Typhimurium on carcasses compared to soft scald and hard scald dip without antimicrobials (McKee et al., 2008). Chlorine will immediately get deactivated by the organic load in the scalder and can gas off due to a higher scalder temperature (USDA, 2021). Organic acids were also tested against *Salmonella* at 50°C and found that a combination of formic and propionic acids was more effective, followed by lactic acid and acetic acid (Cherrington et al., 1992). In another study, trisodium phosphate (8% or 80,000 ppm) and acetic acid (5% or 50,000 ppm) caused approximately 1.8 to 2 $\log_{10}$ CFU reduction on the skin surface. However, acetic acid at 5% caused discoloration on the skin (Tamblyn et al., 1997). Lactic acid at 0.25% and potassium sorbate at 2.5% were also found to be effective under scalding conditions against *S.* Typhimurium and *S.* Sofia*;* however, lactic acid treatment under high temperature resulted in undesirable color and texture of the chicken carcasses (Morrison and Fleet, 1985). Succinic acid, along with hot water, was effective in reducing *S.* Montevideo (Juven et al., 1974), but heat treatment resulted in changes in odor and gross appearance (Juven et al., 1974; Cox et al., 1974). As in our study, carcasses that are chilled or stored after 6% phosphate (sodium tripolyphosphate and sodium hexametaphosphate) treatment during scalding caused a reduction of *S.* Typhimurium in the chill water and storage (Thomson et al., 1978). In one of our previous studies, the use of pimenta essential oil was found to reduce *Salmonella* on turkey skin by >2 $\log_{10}$ CFU/sq. in. under scalding and chilling conditions (Nair and Kollanoor Johny, 2017). It is promising that comparable reductions of SH were obtained with CA and PAA in the current study without affecting the surface color of drumsticks.

Caprylic acid was also tested in combination with other antimicrobials. Moschonas et al. (2012) evaluated the antimicrobial efficacy of CA with carvacrol and polylysine to reduce *Salmonella* contamination in non-RTE, surface-browned, frozen, and breaded chicken products. They found a dose-dependent reduction with all 3 individual antimicrobials and 1% CA reducing the initial pathogen populations (4.8−4.9 $\log_{10}$CFU/g) below the detectable limit. A lower concentration (0.0625%) of CA combined with either of the other 2 antimicrobials was found to be effective in reducing 1.8 $\log_{10}$ CFU/g *Salmonella* in the final product after storage. Similarly, a combination of the 3 antimicrobials at a very low concentration (0.0625%) of CA resulted in 2.4 $\log_{10}$ CFU/g reduced *Salmonella* in the final product after frozen storage (Moschonas et al., 2012). In another study, (Hulankova et al. 2013) tested the additive effect of essential oregano oil, citric acid, and CA in vacuum packaged minced beef. A 2.5 $\log_{10}$ reduction of *Listeria* and 1.5 log reduction of lactic acid bacteria were noticed in combination treatments after 10 d of storage at 3°C. However, a negative impact on color and sensory properties was observed with CA treatment. Studies conducted by Burnett et al. (2007) using CA to reduce *L. monocytogenes* in RTE poultry products observed reduction in the pathogen populations and scoring equivalently in organoleptic evaluation with the controls (Burnett et al., 2007)

The results of this study indicate that CA could be a natural alternative antimicrobial against SH on chicken parts in water without affecting the color attributes, indicating its potential as a scalding antimicrobial. The next step will be to validate the efficacy of CA on whole chicken carcasses at different levels of organic content in the scalding water. Since the addition of CA did not result in significant changes to the color, a complete sensory evaluation of the poultry parts, carcasses, and ground meat exposed to CA at effective antimicrobial concentrations will be conducted.

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DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. Mckee. 2008a. Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. J. Food Prot. 71:1119–1122.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008b. The Microbial and quality properties of poultry carcasses treated with peracetic acid as an antimicrobial treatment. Poult. Sci. 87:2390–2398.

Burnett, S. L., J. H. Chopskie, T. C. Podtburg, T. A. Gutzmann, S. E. Gilbreth, and P. W. Bodnaruk. 2007. Use of octanoic acid as a postlethality treatment to reduce *Listeria monocytogenes* on ready-to-eat meat and poultry products. J. Food. Prot. 70:392–398.

Campos, J., J. Mourao, L. Silveira, M. Saraiva, C. B. Correia, A. P. Macas, L. Peixe, and P. Antunes. 2018. Imported poultry meat as a source of extended-spectrum cephalosporin-resistant CMY-2-producing *Salmonella* Heidelberg and *Salmonella* Minnesota in the European Union, 2014−2015. Int. J. Antimicrob. Agents. 51:151–154.

CDC. 2014. National antimicrobial resistance monitoring system 2014 human surveillance report. Accessed Sept. 2021. https://www.cdc.gov/narms/pdf/2014-annual-report-narms-508c.pdf.

Chen, X., J. Bauermeister, G. N. Hill, M. Singh, S. F. Bilgili, and S. R. Mckee. 2014. Efficacy of various antimicrobials on reduction of *Salmonella* and *Campylobacter* and quality attributes of ground chicken obtained from poultry parts treated in a postchill decontamination tank. J. Food Prot. 77:1882–1888.

Cherrington, C. A., V. Allen, and M. Hinton. 1992. The influence of temperature and organic matter on the bactericidal activity of short-chain organic acids on *Salmonella*. J. Appl. Bacteriol. 72:500–503.

Cox, N. A., A. J. Mercuri, B. J. Juven, J. E. Thomson, and V. Chew. 1974. Evaluation of succinic acid and heat to improve the microbiological quality of poultry meat. J. Food Sci. 39:985–987.

Dewi, G., D. V. T. Nair, C. Peichel, T. J. Johnson, S. Noll, and A. Kollanoor Johny. 2021. Effect of lemongrass essential oil against multidrug-resistant *Salmonella* Heidelberg and its attachment to chicken skin and meat. Poult. Sci. 100:101116.

Hawley, B., M. Casey, M. A. Virji, K. J. Cummings, A. Johnson, and J. Cox-Ganser. 2018. Respiratory symptoms in hospital cleaning staff exposed to a product containing hydrogen peroxide, peracetic acid, and acetic acid. Ann. Work Expo. Heal. 62:28–40.

Hoffmann, M., S. Zhao, Y. Luo, C. Li, J. P. Folster, J. Whichard, M. W. Allard, E. W. Brown, and P. F. Mcdermott. 2012. Genome sequences of five *Salmonella enterica* serovar Heidelberg isolates associated with a 2011 multistate outbreak in the United States. J. Bacteriol. 194:3274–3275.

Hulankova, R., G. Borilova, and I. Steinhauserova. 2013. Combined antimicrobial effect of oregano essential oil and caprylic acid in minced beef. Meat Sci. 95:190–194.

Humphrey, T. J., D. G. Lanning, and D. Beresford. 1981. The effect of pH adjustment on the microbiology of chicken scald-tank water with particular reference to the death rate of *Salmonellas*. J. Appl. Bacteriol. 51:517–527.

Jensen, R. G., A. M. Ferris, C. J. Lammi-Keefe, and R. A. Henderson. 1990. Lipids of bovine and human milks: a comparison. J. Dairy Sci. 73:223–240.

Juven, B. J., N. A. Cox, A. J. Mercuri, and J. E. Thompson. 1974. A hot acid treatment for eliminating *Salmonella* from chicken meat. J. Milk Food Technol. 37:237–239.

Kitis, M. 2004. Disinfection of wastewater with peracetic acid: a review. Environ. Int. 30:47–55.

Kollanoor, A., P. Vasudevan, M. Kumar, M. Nair, T. Hoagland, and K. Venkitanarayanan. 2007. Inactivation of bacterial fish pathogens by medium-chain lipid molecules (caprylic acid, monocaprylin and sodium caprylate). Aquacult. Res. 38:1293–1300.

Kollanoor-Johny, A., A. Upadhyay, S. A. Baskaran, I. Upadhyaya, S. Mooyottu, N. Mishra, M. J. Darre, M. I. Khan, A. M. Donoghue, D. J. Donoghue, and K. Venkitanarayanan. 2012. Effect of therapeutic supplementation of the plant compounds *trans*-cinnamaldehyde and eugenol on *Salmonella enterica* serovar Enteritidis colonization in market-age broiler chickens. J. Appl. Poult. Res. 21:816–822.

Marina, A. M., Y. B. Che Man, S. A. H. Nazimah, and I. Amin. 2009. Chemical properties of virgin coconut oil. J. Am. Oil Chem. Soc. 86:301–307.

Mcbride, G. B., B. J. Skura, I. R. Y. Yadal, and E. J. Bowmer. 1980. Relationship between incidence of *Salmonella* contamination among pre-scalded, eviscerated and post-chilled chickens in a poultry processing plant. J. Food Prot. 43:538–543.

McKee, S. R., J. C. Townsend, and S. F. Bilgili. 2008. Use of a scald additive to reduce levels of *Salmonella* Typhimurium during poultry processing. Poult. Sci. 87:1672–1677.

Morrison, G. J., and G. H. Fleet. 1985. Reduction of *Salmonella* on chicken carcasses by immersion treatments. J. Food Prot. 48:939–943.

Moschonas, G., I. Geornaras, J. D. Stopforth, D. Wach, D. R. Woerner, K. E. Belk, G. C. Smith, and J. N. Sofos. 2012. Activity of caprylic Acid, carvacrol, ε-polylysine and their combinations against *Salmonella* in not-ready-to-eat surface-browned, frozen, breaded chicken products. J. Food Sci. 77:405–411.

Nair, D. V. T., and A. Kollanoor Johny. 2017. Food grade pimenta leaf essential oil reduces the attachment of *Salmonella enterica* Heidelberg (2011 Ground Turkey Outbreak Isolate) on to turkey skin. Front. Microbiol. 8:2328.

Nair, D., A. Kiess, W. Schilling, R. Nannapaneni, and S. Sharma. 2015. The combined efficacy of carvacrol and modified atmosphere packaging on the survival of *Salmonella, Campylobacter jejuni* and Lactic acid bacteria on turkey breast cutlets. Food Microbiol. 49:134–141.

Okrend, A. J., R. W. Johnston, and A. B. Moran. 1986. Effect of acetic acid on the death rates at 52 C of *Salmonella newport, Salmonella typhimurium* and *Campylobacter jejuni* in poultry scald water. J. Food Prot. 49:500–503.

Oyarzabal, O. A. 2005. Reduction of Campylobacter spp. by commercial antimicrobials applied during the processing of broiler chickens: a review from the United States perspective. J. Food Prot. 68:1752–1760.

Ríő, E. D., R. Ríő, R. Muriente, M. Prieto, C. Alonso-Calleja, and R. Capita. 2007. Effectiveness of trisodium phosphate, acidified sodium chlorite, citric acid, and peroxyacids against pathogenic bacteria on poultry during refrigerated storage. J. Food Prot. 70:2063–2071.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States−major pathogens. Emerg. Infect. Dis. 17:7–15.

Stampi, S., G. De Luca, and F. Zanetti. 2001. Evaluation of the efficiency of peracetic acid in the disinfection of sewage effluents. J. Appl. Microbiol. 91:833–838.

Seo, K. H., P. S. Holt, R. K. Gast, and C. L. Hofacre. 2000. Combined effect of antibiotic and competitive exclusion treatment on *Salmonella* Enteritidis fecal shedding in molted laying hens. J. Food Prot. 63:545–548.

Sun, C. Q., C. J. O'Connor, S. J. Turner, G. D. Lewis, R. A. Stanley, and A. M. Roberton. 1998. The effect of pH on the inhibition of bacterial growth by physiological concentrations of butyric acid: Implications for neonates fed on suckled milk. Chem. Biol. Interact. 113:117–131.

Tamblyn, K. C., D. E. Conner, and S. F. Bilgili. 1997. Utilization of the skin attachment model to determine the antibacterial efficacy of potential carcass treatments. Poult. Sci. 76:1318–1321.

Thomson, J. E., J. S. Bailey, and N. A. Cox. 1978. Phosphate and heat treatments to control *Salmonella* and reduce spoilage and rancidity on broiler carcasses. Poult. Sci. 58:139–143.

USDA ERS. 2014. Cost estimates of foodborne illness. Accessed Sept. 2021. https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses/

USDA FSIS. 2021. FSIS guideline for controlling *Salmonella* in raw poultry. Accessed Sept. 2021. https://www.fsis.usda.gov/sites/default/files/media_file/2021-07/FSIS-GD-2021-0005.pdf

Yang, H., Y. Li, and M. G. Johnson. 2001. Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. J. Food Prot. 64:770–776.

Young, S. D., O. Olusanya, K. H. Jones, T. Liu, K. A. Liljebjelke, and C. L. Hofacre. 2007. *Salmonella* incidence in broilers from breeders vaccinated with live and killed *Salmonella*. J. Appl. Poult. Res. 16:521–528.