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Immersion in Antimicrobial Solutions Reduces *Salmonella enterica* and Shiga Toxin–Producing *Escherichia coli* on Beef Cheek Meat†

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ABSTRACT

The objective of this study was to determine the effect of immersing beef cheek meat in antimicrobial solutions on the reduction of O157:H7 Shiga toxin–producing *Escherichia coli* (STEC), non-O157:H7 STEC, and *Salmonella enterica*. Beef cheek meat was inoculated with O157:H7 STEC, non-O157:H7 STEC, and *S. enterica* on both the adipose and muscle surfaces. The inoculated cheek meat was then immersed in one of seven antimicrobial solutions for 1, 2.5, or 5 min: (i) 1% Aftec 3000 (AFTEC), (ii) 2.5% Beefxide (BX), (iii) 300 ppm of hypobromous acid (HOBR), (iv) 2.5% lactic acid (LA2.5), (v) 5% lactic acid (LA5), (vi) 0.5% levulinic acid and 0.05% sodium dodecyl sulfate (LEV-SDS), or (vii) 220 ppm of peroxyacetic acid (POA). Inoculated cheek meat was also immersed in 80°C tap water (HW) for 10 s. In general, increasing immersion duration in antimicrobial solutions did not significantly ($P \geq 0.05$) increase effectiveness. Immersion in HW for 10 s was the most effective intervention, reducing STEC and *S. enterica* by 2.2 to 2.3 log CFU/cm^2 on the adipose surface and by 1.7 to 1.8 log CFU/cm^2 on the muscle surface. Immersion for 1 min in AFTEC, BX, LA2.5, LA5, or POA was also effective as an intervention, reducing STEC and *S. enterica* by 0.8 to 2.0 log CFU/cm^2 on the adipose surface and by 0.6 to 1.4 log CFU/cm^2 on the muscle surface. Immersion for 1 min in HOBR or LEV-SDS was not an effective intervention because STEC and *S. enterica* reductions ranged from 0.1 to 0.4 log CFU/cm^2, which were not significantly different ($P \geq 0.05$) from the reductions obtained when cheek meat was immersed in room temperature tap water. We conclude that immersion of cheek meat in HW for 10 s and immersion for 1 min in AFTEC, BX, LA2.5, LA5, or POA effectively reduced levels of STEC and *S. enterica*.

Cheek meat recovered from bovine heads during processing may be sold without further processing or may be incorporated in several downstream products, including ground beef, chopped beef, fabricated beef steaks, corned beef, and chili con carne *(32)*. Higher levels of bacteria have been observed on cheek meat compared to other muscle tissues, and Shiga toxin–producing *Escherichia coli* (STEC) serotype O157:H7 has been detected in cheek meat *(9)*. The head is in the lowest position on the carcass during processing, and it has been theorized that this position contributes to cheek meat contamination since washes employed after hide removal flow over the head as they drain off the carcass *(17)*. We are unaware of any published study that has examined cheek meat for non-O157 STEC or *Salmonella enterica* contamination. However, it can reasonably be assumed that cheek meat may be sporadically contaminated by non-O157 STEC and *S. enterica* since cattle hides are the primary source of carcass contamination by STEC O157:H7, as well as by non-O157 STEC and *S. enterica (1, 3, 4, 7, 27)*.

Because heads, typically removed from the carcass prior to the application of final carcass antimicrobial interventions, are not subjected to the same antimicrobial interventions as the rest of the carcass, further intervention after head removal may be warranted. Spray treatment of bovine heads with 2.0% lactic acid, hot water (74°C), or FreshFx has been demonstrated to reduce STEC O157:H7 during processing *(21)*. However, STEC O157:H7 has been detected in samples obtained from the bovine oral cavity during processing, indicating a source of contamination that may be unaffected by antimicrobial spray treatments of the external beef head surface *(23, 25)*. Identification and adoption of novel, effective antimicrobial interventions for cheek meat following removal from the head would improve public health by lowering exposure to foodborne pathogens.

Processing of cheek meat after removal from the head may include a centrifugation step to remove liquids gained

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TABLE 1. *Schedule of pathogen inoculum mixtures and antimicrobial solutions evaluated*

Day	Antimicrobial solutions evaluated	Pathogen inoculum				
		STEC O157:H7 strain[a]	STEC non-O157		*Salmonella enterica*	
			Serotype	Strain	Serotype	Strain
1	LA5, LA2.5, POA, HOBR, HW, TW	FSIS4	O26:H11	3392	Typhimurium	DT104
2	LA5, LA2.5, POA, HOBR, HW, TW	ATCC 43895	O121:H19	O1E-2074	Newport	3-1055
3	LA5, LA2.5, POA, HOBR, HW, TW	FSIS4	O111:NM	1665	Anatum	6-3230
4	LA5, LA2.5, POA, HOBR, HW, TW	ATCC 43895	O145:NM	GS5578620	Montevideo	5-1170
5	LA5, LA2.5, POA, HOBR, HW, TW	FSIS4	O103:H2	MDR0089	Agona	4-1093
6	LA5, LA2.5, POA, HOBR, HW, TW	ATCC 43895	O45:H2	O1E-1269	Dublin	SD2793
7	BX, AFTEC, LEV-SDS	FSIS4	O26:H11	3392	Typhimurium	DT104
8	BX, AFTEC, LEV-SDS	ATCC 43895	O121:H19	O1E-2074	Newport	3-1055
9	BX, AFTEC, LEV-SDS	FSIS4	O111:NM	1665	Anatum	6-3230
10	BX, AFTEC, LEV-SDS	ATCC 43895	O145:NM	GS5578620	Montevideo	5-1170
11	BX, AFTEC, LEV-SDS	FSIS4	O103:H2	MDR0089	Agona	4-1093
12	BX, AFTEC, LEV-SDS	ATCC 43895	O45:H2	O1E-1269	Dublin	SD2793

[a] STEC, Shiga toxin–producing *Escherichia coli*.

during the harvest process. Beef producers have indicated that normal routines would be minimally disturbed by the immersion of cheek meat in an antimicrobial solution for a period of up to 5 min following removal from the head but before centrifugation. The goal of this study was to determine the effectiveness of the following six antimicrobial solutions, which are approved for use in beef processing, for reducing STEC and *S. enterica* in cheek meat: 1% (vol/vol) Aftec 3000 (AFTEC); 2.5% (vol/vol) Beefxide (BX); 300 ppm of hypobromous acid prepared from 24% (wt/vol) hydrogen bromide in aqueous solution (HOBR); 2.5% (wt/vol) lactic acid (LA2.5); 5% (wt/vol) lactic acid (LA5); and 220 ppm of peroxyacetic acid (POA) *(31)*. In addition, we determined the efficacy of a mixture of 0.5% (vol/vol) levulinic acid and 0.05% (wt/vol) sodium dodecyl sulfate (LEV-SDS), which is currently under investigation for use as an antimicrobial intervention in cattle processing *(35, 36)*. At the request of beef processing companies, we also determined the efficacy of immersion in water at 80°C (HW) for 10 s. Because it has been demonstrated that spray treatment of beef carcasses with organic acids is more effective for reducing STEC O157:H7 on adipose surfaces than on lean muscle surfaces *(12)*, we determined the effectiveness of immersion in each antimicrobial solution for both the adipose and lean muscle surfaces of cheek meat.

MATERIALS AND METHODS

Antimicrobial solutions. Antimicrobial solutions were evaluated over 12 days according to the schedule in Table 1. Room-temperature antimicrobial solutions AFTEC (Advanced Food Technologies, LLC, Shreveport, LA), BX (Birko Corp., Henderson, CO), HOBR (Enviro Tech Chemical Services Inc., Modesto, CA), LA2.5 (Purac, Chicago, IL), LA5 (Purac), and POA (Ecolab, St. Paul, MN) were prepared according to the manufacturers' recommendations. AFTEC is a proprietary formulation of buffered sulfuric acid. BX is a proprietary formulation of buffered lactic and citric acids. Levulinic acid and sodium dodecyl sulfate were obtained from Sigma Co. (St. Louis, MO). LEV-SDS was prepared in deionized water. All antimicrobial solutions were prepared within 16 h of use and were protected from light exposure until use. HW was prepared by placing tap water in a sanitized stainless steel tray, which was then heated on a hot plate until the water reached a temperature of 80.0 ± 3.0°C. Room-temperature water (TW) was prepared by dispensing tap water into a sterilized beaker 3 h prior to use to permit equilibration to room temperature.

Preparation of cheek meat and inoculation of cheek meat. Each day cheek meat was collected from a local beef cattle processing plant immediately following removal from the head and was transported within 2 h to the U.S. Meat Animal Research Center (USMARC) laboratory in insulated containers. Cheek meat consists of surfaces that are primarily lean muscle (hereafter referred to as the ''muscle surface'') or primarily adipose and connective tissue (hereafter referred to as the ''adipose surface''). Edible ink was used to mark 50-cm^2 surface areas on the cheek meat, which was then trimmed with a sanitized knife to generate individual pieces with a marked 50-cm^2 area on either the adipose surface or the muscle surface. Each piece of cheek meat was inoculated on the marked surface with the inoculum mixture used on that day (Table 1).

The inoculum mixture contained a *S. enterica* strain, a non-O157 STEC strain, and an O157:H7 STEC strain (Table 1). Six different inoculum mixtures were used to represent each of the six non-O157 STEC serogroups (O26, O45, O103, O111, O121, and O145) declared to be adulterants in nonintact beef by the U.S. Food Safety Inspection Service (Table 1). Each inoculum mixture also contained a different serotype of *S. enterica* (Table 1). All bacterial strains used in this study were obtained from the USMARC culture collection and were grown for 16 to 18 h at 37°C in nutrient broth (BD, Sparks, MD). Each strain was adjusted in nutrient broth to an approximate concentration of 1.5×10^8 CFU/ml using a spectrophotometer set at a wavelength of 600 nm. Equal volumes of each strain were then mixed to prepare an inoculum mixture containing approximately 5.0×10^7 CFU/ml of each strain. Cheek meat pieces were inoculated by pipetting 50 μl of inoculum mixture onto the 50-cm^2 surface area marked with edible ink and spread over the 50-cm^2 area with a sterile cell spreader. Inoculated cheek meat pieces were incubated undisturbed for 15 min at 37°C to permit bacterial cell attachment. On each day for each evaluated permutation of antimicrobial solution and immersion duration, four pieces of cheek meat were inoculated, two on the muscle surface and two on the adipose surface. To

determine the preimmersion populations on each surface of cheek meat, 12 ''untreated control'' pieces of cheek meat were inoculated each day, six on the muscle surface and six on the adipose surface. On each day, the untreated control pieces of cheek meat were placed into filtered bags (Whirl-Pak, Nasco, Fort Atkinson, WI) immediately after the 15-min attachment period.

Immersion in antimicrobial solutions and bacterial enumeration. For each of the antimicrobial solutions evaluated, except HW, 12 pieces of inoculated cheek meat were placed into a sterile beaker using sanitized tongs. Then, 1.5 liters of antimicrobial solution was added to the beaker, ensuring that all 12 pieces were fully immersed. At 1, 2.5, and 5 min after the addition of the antimicrobial solution, sterile tongs were used to remove two cheek meat pieces inoculated on the adipose side and two cheek meat pieces inoculated on the muscle side from the antimicrobial solution to a sanitized consumer salad spinner (model SALA-5, Progressive International, Kent, WA), which was spun manually for 30 s to remove excess liquid. Sterile tongs were then used to place the individual cheek meat pieces into filtered bags.

Similarly, when HW was evaluated, sterile tongs were used to fully immerse a cheek meat piece inoculated on the muscle side in HW. After 10 s, sterile tongs were used to remove the piece from HW to a sanitized consumer salad spinner. The salad spinner was then spun manually for 30 s to remove excess liquid. The cheek meat piece was then placed into a filtered bag using sterile tongs. This process was repeated for a cheek meat piece inoculated on the adipose side, followed by a cheek meat piece inoculated on the muscle side, followed by a cheek meat piece inoculated on the adipose side.

Immediately following the placement of the cheek meat in a filtered bag, 100 ml of Dey-Engley broth (BD) supplemented with 0.3% soytone and 0.25% sodium chloride was added to neutralize the sample. The samples were homogenized for 1 min using a stomacher (Bag Mixer 400, Interscience, Weymouth, MA). A 1-ml aliquot was then removed from each sample and was 10-fold serial diluted in maximum recovery diluent (BD). Appropriate dilutions were plated on USMARC chromogenic agar (UCA) plates *(22)* and Petrifilm AC plates (3M Microbiology, St. Paul, MN). Petrifilm AC plates were incubated at 37°C for 48 h, and aerobic plate counts (APC) were determined according to the manufacturer's directions. The lower limit of detection for Petrifilm AC was 27 CFU/cm^2. UCA plates were incubated at 37°C for 24 h and then at room temperature for 30 min to allow full color development. Colonies on UCA plates were enumerated as follows: turquoise blue, O26 STEC; blue-green, O45 STEC; light green, O103 STEC; dark blue-green, O111 STEC; light blue-gray, O121 STEC; purple, O145 STEC; green, O157 STEC; colorless with pink halo, *S. enterica*. For each sample, up to three presumptive colonies of each inoculated strain on UCA plates were confirmed by PCR *(5, 19, 28, 29)*. The lower limit of detection on UCA plates was 40 CFU/cm^2. For inoculated samples that were below the detection level, an arbitrary value of 20 CFU/cm^2 was assigned.

Measurement of surface pH. For each antimicrobial solution evaluated, edible ink was used to mark 50 cm^2 of adipose surface and 50 cm^2 of muscle surface on three uninoculated pieces of cheek meat. The three pieces were immersed in the antimicrobial solution; at 1, 2.5, or 5 min, one piece of cheek meat was removed from the solution and spun in a consumer salad spinner for 30 s. pH readings were taken at three locations on the adipose surface and three locations on the muscle surface of each cheek meat piece using a PH100 pH meter and PH105 electrode (Extech Instruments Corp., Nashua, NH) before immersion and after spinning.

Statistical analysis. Colony counts were transformed to values expressed as log CFU per square centimeter. For each cheek meat piece, reductions were determined by subtracting the log CFU per square centimeter on each cheek meat piece after exposure to antimicrobial solution from the mean initial inoculated log CFU per square centimeter determined from the six untreated control pieces inoculated on the same surface (adipose or muscle) for that day. Mean reductions for each antimicrobial solution, immersion duration, and surface permutation were determined by pooling the reduction values obtained from 12 pieces, which comprised two pieces for each of the six inoculation mixtures. Mean log reduction and postimmersion pH values were compared between antimicrobial solutions or immersion durations by one-way statistical analysis of variance using Tukey's multiple comparison test performed with the Prism 5.0 program (GraphPad Software, La Jolla, CA). P values <0.05 were considered significant.

RESULTS

Effect of immersion in antimicrobial solutions on surface pH. The pH of the adipose surface of cheek meat prior to immersion in the antimicrobial solutions ranged from 6.4 to 7.0. The muscle surface pH measured prior to immersion in the antimicrobial solutions ranged from 6.3 to 6.8. Immersion in LA5, LA2.5, BX, AFTEC, POA, or LEV-SDS for 1-, 2.5-, or 5-min durations resulted in adipose surface and muscle surface pH values that were significantly ($P < 0.05$) lower than the pH values measured prior to immersion (data not shown). Increasing the immersion duration in LEV-SDS from 1 to 2.5 min significantly ($P < 0.05$) decreased the pH from 5.0 to 4.5 on the adipose surface and from 5.1 to 4.5 on the muscle surface (Table 2). Increasing the immersion duration in LA5, LA2.5, BX, AFTEC, POA, or HOBR from 1 to 2.5 min did not significantly alter ($P \geq 0.05$) adipose surface or muscle surface pH values. For all antimicrobial solutions examined, neither the adipose surface pH values nor the muscle surface pH values following 5 min of immersion were significantly different from their respective values following immersion for 2.5 min. For LA2.5, BX, POA, and LEV-SDS, the adipose surface pH following 5 min of immersion was significantly different ($P < 0.05$) from the pH following 1 min of immersion in the same antimicrobial solution. For LA2.5, AFTEC, and LEV-SDS, the muscle surface pH following immersion for 5 min was significantly different ($P < 0.05$) from the pH following immersion for 1 min (Table 2).

Reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the adipose surface of cheek meat following immersion in antimicrobial solutions. The levels of STEC O157:H7, non-O157 STEC, and *S. enterica* on the adipose surface of untreated control pieces ranged from 3.8 to 4.3 log CFU/cm^2, with respective means of 3.9, 4.0, and 4.1 log CFU/cm^2 (data not shown). Immersion of cheek meat in LA5, LA2.5, BX, AFTEC, or POA for 1 min resulted in reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the adipose surface that ranged from 0.8 to 2.0 log CFU/cm^2 (Tables 3 through 5). These reductions differed significantly ($P < 0.05$) from the reductions of 0.1 to 0.2 log CFU/cm^2 observed following 1 min of immersion

TABLE 2. *pH of antimicrobial solutions and cheek meat surfaces following immersion in antimicrobial solution*

Antimicrobial solution	Antimicrobial solution abbreviation	pH of antimicrobial solution	Mean surface pH following immersion in antimicrobial solution for indicated duration[a]					
			Adipose surface			Muscle surface		
			1 min	2.5 min	5 min	1 min	2.5 min	5 min
5% lactic acid	LA5	2.2	3.0 A	2.8 A	2.7 A	3.0 A	2.8 A	2.7 A
2.5% lactic acid	LA2.5	2.3	3.3 A	3.0 AB	2.9 B	3.3 A	3.1 AB	3.0 B
2.5% Beefxide	BX	2.5	3.7 A	3.5 AB	3.2 B	3.6 A	3.3 A	3.3 A
1% Aftec 3000	AFTEC	1.8	3.1 A	2.7 A	2.6 A	3.1 A	3.0 AB	2.6 B
220 ppm of peroxyacetic acid	POA	2.6	5.2 A	4.8 AB	4.5 B	5.0 A	4.9 A	4.8 A
300 ppm of hypobromous acid	HOBR	6.7	6.6 A	6.5 A	6.4 A	6.3 A	6.5 A	6.4 A
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	2.8	5.0 A	4.5 B	4.5 B	5.1 A	4.5 B	4.6 B
Tap water	TW	6.8	7.0 A	6.9 A	6.8 A	6.7 A	6.8 A	6.8 A

[a] Within a surface type, means in the same row with no common letter are significantly different ($P < 0.05$). For each value, $n = 6$.

in TW (Tables 3 through 5). Immersion of cheek meat in HOBR or LEV-SDS for 1 min resulted in reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the adipose surface that ranged from 0.1 to 0.4 log CFU/cm^2, but these reductions did not differ significantly ($P \geq 0.05$) from the reductions obtained by immersion in TW for 1 min (Tables 3 through 5).

The reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the adipose surface of cheek meat following immersion in LA5, LA2.5, BX, AFTEC, or POA for durations of 2.5 or 5 min did not differ significantly ($P \geq 0.05$) from the reductions obtained following 1 min of immersion in the same antimicrobial solutions (Tables 3 through 5). Immersion in HOBR for 5 min resulted in reductions of STEC O157:H7 and non-O157 STEC on the adipose surface that were significantly different ($P < 0.05$) from the reductions obtained by immersion for 1 min in HOBR, but these reductions did not differ significantly ($P \geq 0.05$) from the reductions obtained by immersion in TW for 5 min (Tables 3 and 4). Immersion in LEV-SDS for 5 min resulted in reductions of non-O157 STEC and *S. enterica* on the adipose surface that were significantly different ($P < 0.05$) from the reductions obtained after immersion for 1 min in LEV-SDS, but these reductions did not differ significantly ($P \geq 0.05$) from the reductions obtained by immersion in TW for 5 min (Tables 4 and 5).

Reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the muscle surface of cheek meat following immersion in antimicrobial solutions. The levels of STEC O157:H7, non-O157 STEC, and *S. enterica* on the muscle surface of untreated control pieces ranged from 3.8 to 4.6 log CFU/cm^2, with respective means of 4.2, 4.3, and 4.2 log CFU/cm^2 (data not shown). Immersion of cheek meat in LA5, LA2.5, BX, AFTEC, or POA for 1 min resulted in reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the muscle surface that ranged from 0.6 to 1.4 log CFU/cm^2 (Tables 3 through 5). These reductions differed significantly ($P < 0.05$) from the reductions of 0.0 to 0.1 log CFU/cm^2 obtained by immersion for 1 min in TW (Tables 3 through 5). Immersion of cheek meat in HOBR or LEV-SDS for 1 min resulted in reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the muscle surface that ranged from 0.2 to 0.3 log CFU/cm^2; however, these reductions did not differ significantly ($P \geq 0.05$) from the reductions obtained by immersion in TW for 1 min (Tables 3 through 5).

The reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the muscle surface of cheek meat following immersion in LA5, LA2.5, HOBR, or LEV-SDS for durations of 2.5 or 5 min did not differ significantly ($P \geq 0.05$) from the reductions obtained following 1 min of immersion in the same antimicrobial solutions (Tables 3 through 5). Immersion in BX for 5 min resulted in reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* on the muscle surface that ranged from 1.4 to 1.6 log CFU/cm^2, significantly different ($P < 0.05$) from the reductions obtained by immersion for 1 min in BX, which ranged from 0.8 to 1.1 log CFU/cm^2 (Tables 3 through 5).

TABLE 3. *Reductions of STEC O157:H7 on cheek meat surfaces following immersion in antimicrobial solution*[a]

Antimicrobial solution	Antimicrobial solution abbreviation	Mean reduction (log CFU/cm^2) following immersion for the indicated duration[b]					
		Adipose surface			Muscle surface		
		1 min	2.5 min	5 min	1 min	2.5 min	5 min
5% lactic acid	LA5	1.9 A a	1.9 A a	2.1 A a	1.2 A a	1.6 A a	1.8 A a
2.5% lactic acid	LA2.5	1.6 AB a	1.5 AB a	1.8 AB a	1.1 AB a	1.2 AB a	1.3 AB a
2.5% Beefxide	BX	1.3 AB a	1.5 AB a	1.6 AB a	0.8 AB b	1.2 AB ab	1.4 AB a
1% Aftec 3000	AFTEC	1.3 AB a	1.4 AB a	1.4 AB a	0.8 ABC b	0.9 BC ab	1.2 AB a
220 ppm of peroxyacetic acid	POA	1.1 B a	1.1 B a	1.3 BC a	0.7 BCD a	0.8 BC a	1.0 BC a
300 ppm of hypobromous acid	HOBR	0.2 C b	0.3 C ab	0.7 CD a	0.3 CDE a	0.4 CD a	0.5 CD a
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	0.1 C a	0.2 C a	0.3 D a	0.2 DE a	0.0 D a	0.1 D a
Tap water	TW	0.2 C a	0.1 C a	0.2 D a	0.0 E a	0.2 D a	0.2 D a

[a] STEC, Shiga toxin–producing *Escherichia coli.*

[b] Means in the same column with no common uppercase letter are significantly different ($P < 0.05$). Within a surface type, means in the same row with no common lowercase letter are significantly different ($P < 0.05$). For each value, $n = 12$.

TABLE 4. *Reductions of non-O157 STEC on cheek meat surfaces following immersion in antimicrobial solution*[a]

Antimicrobial solution	Antimicrobial solution abbreviation	Mean reduction (log CFU/cm^2) following immersion for the indicated duration[b]					
		Adipose surface			Muscle surface		
		1 min	2.5 min	5 min	1 min	2.5 min	5 min
5% lactic acid	LA5	2.0 A a	1.7 A a	2.0 A a	1.4 A a	1.8 A a	1.8 A a
2.5% lactic acid	LA2.5	1.4 B a	1.6 A a	1.9 AB a	1.1 AB a	1.2 B a	1.5 AB a
2.5% Beefxide	BX	1.3 B a	1.5 AB a	1.6 AB a	1.1 AB b	1.2 B ab	1.5 A a
1% Aftec 3000	AFTEC	1.1 B a	1.2 AB a	1.4 B a	1.0 AB b	1.0 B ab	1.3 AB a
220 ppm of peroxyacetic acid	POA	1.0 B a	1.0 BC a	1.3 B a	0.7 BC a	0.8 BC a	1.0 BC a
300 ppm of hypobromous acid	HOBR	0.3 C b	0.5 CD ab	0.7 C a	0.3 CD a	0.4 CD a	0.5 CD a
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	0.1 C b	0.2 D ab	0.4 C a	0.3 CD a	0.2 D a	0.3 D a
Tap water	TW	0.1 C a	0.1 D a	0.1 C a	0.1 D a	0.2 D a	0.2 D a

[a] STEC, Shiga toxin–producing *Escherichia coli.*

[b] Means in the same column with no common uppercase letter are significantly different ($P < 0.05$). Within a surface type, means in the same row with no common lowercase letter are significantly different ($P < 0.05$). For each value, $n = 12$.

TABLE 5. *Reductions of* Salmonella enterica *on cheek meat surfaces following immersion in antimicrobial solution*

Antimicrobial solution	Antimicrobial solution abbreviation	Mean reduction (log CFU/cm^2) following immersion for the indicated duration[a]					
		Adipose surface			Muscle surface		
		1 min	2.5 min	5 min	1 min	2.5 min	5 min
5% lactic acid	LA5	2.0 A a	1.8 A a	2.0 A a	1.4 A a	1.8 A a	1.9 A a
2.5% lactic acid	LA2.5	1.5 AB a	1.5 AB a	1.9 A a	1.2 A a	1.2 BCD a	1.5 ABC a
2.5% Beefxide	BX	1.2 BC a	1.5 AB a	1.7 AB a	1.0 AB b	1.3 ABC ab	1.6 AB a
1% Aftec 3000	AFTEC	1.1 BC a	1.5 AB a	1.5 AB a	1.0 AB a	1.1 CD a	1.3 BC a
220 ppm of peroxyacetic acid	POA	0.8 CD a	0.9 BC a	1.1 BC a	0.6 BC b	0.7 DE ab	1.0 CD a
300 ppm of hypobromous acid	HOBR	0.4 DE a	0.4 CD a	0.5 CD a	0.3 CD a	0.4 EF a	0.4 DE a
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	0.1 E b	0.2 D ab	0.4 D a	0.2 CD a	0.1 F a	0.2 E a
Tap water	TW	0.1 E a	0.1 D a	0.1 D a	0.1 D a	0.2 F a	0.2 E a

[a] Means in the same column with no common uppercase letter are significantly different ($P < 0.05$). Within a surface type, means in the same row with no common lowercase letter are significantly different ($P < 0.05$). For each value, $n = 12$.

Immersion in AFTEC for 5 min resulted in reductions of STEC O157:H7 and non-O157 STEC on the muscle surface of 1.2 and 1.3 log CFU/cm^2, respectively, significantly different ($P < 0.05$) from the respective reductions of 0.8 and 1.0 log CFU/cm^2 obtained following immersion for 1 min in AFTEC (Tables 3 and 4). Immersion in POA for 5 min resulted in a 1.0-log CFU/cm^2 reduction of *S. enterica* on the muscle surface that was significantly different ($P < 0.05$) from the 0.6-log CFU/cm^2 reduction obtained by immersion for 1 min in POA (Table 5).

Reductions of APC on cheek meat surfaces following immersion in antimicrobial solutions. The exposure of a population of bacteria to antimicrobial treatments lethally injures a portion of the bacterial population, whereas the remainder are sublethally injured or uninjured *(21)*. A disadvantage of the use of selective medium to enumerate specific bacteria following antimicrobial treatment is that selective media may inhibit the growth of injured bacteria, leading to an overestimation of reductions. APC reductions following treatment were measured using nonselective media that permitted the recovery and growth of sublethally injured bacteria for the sole purpose of ensuring that the reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* determined using selective media were not exaggerated. Thus, the inclusion of APC counts in this study should not be construed as an endorsement of the use of APC reductions as an indicator of pathogen reduction in validation studies. It is important to emphasize that the cheek meat pieces were inoculated with a concentrated mixture of pathogens; and, thus, the observed APC reductions largely represent the reduction of the inoculated bacterial population.

For each solution, immersion duration, and meat surface permutation tested, the difference between the APC reduction and the STEC O157:H7, non-O157 STEC, or *S. enterica* reduction was ≤0.4 log CFU/cm^2 (data not shown), demonstrating that the observed reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* were likely caused by lethal injury. The APC concentrations on the adipose surface of the untreated control pieces ranged from 4.8 to 5.4 log CFU/cm^2, with an overall mean of 5.0 log CFU/cm^2 (data not shown). Immersion of cheek meat in LA5, LA2.5, BX, AFTEC, or POA for 1 min resulted in APC reductions on the adipose surface that ranged from 0.7 to 1.7 log CFU/cm^2, and each of these reductions differed significantly ($P < 0.05$) from the APC reduction of 0.2 log CFU/cm^2 on the adipose surface obtained by immersion for 1 min in TW (Table 6). The APC reductions of 0.3 and 0.0 log CFU/cm^2 obtained on the adipose surface following immersion for 1 min in HOBR and LEV-SDS, respectively, were not significantly different ($P \geq 0.05$) from the APC reduction obtained by immersion for 1 min in TW (Table 6). Significant differences ($P < 0.05$) between the APC reductions on the adipose surface were observed for the 1- and 5-min immersions in LA2.5 and AFTEC. In addition, the 1.2-log CFU/cm^2 reductions of APC on the adipose surface for the 2.5- and 5-min immersions in POA were significantly different ($P < 0.05$) from the 0.7-log

TABLE 6. *Reductions of APC on cheek meat surfaces following immersion in antimicrobial solution*[a]

Antimicrobial solution	Antimicrobial solution abbreviation	Mean reduction (log CFU/cm^2) following immersion for the indicated duration[b]					
		Adipose surface			Muscle surface		
		1 min	2.5 min	5 min	1 min	2.5 min	5 min
5% lactic acid	LA5	1.7 A a	1.6 A a	2.0 A a	1.2 A b	1.5 A ab	1.7 A a
2.5% lactic acid	LA2.5	1.3 AB b	1.5 A ab	1.7 AB a	1.1 A a	1.1 AB a	1.3 AB a
2.5% Beefxide	BX	1.1 BC a	1.2 A a	1.4 B a	1.0 A a	1.1 AB a	1.2 B a
1% Aftec 3000	AFTEC	1.0 BC b	1.3 A ab	1.5 AB a	1.0 A b	1.1 AB ab	1.3 AB a
220 ppm of peroxyacetic acid	POA	0.7 CD b	1.2 A a	1.2 B a	0.5 B b	0.6 BC ab	0.9 B a
300 ppm of hypobromous acid	HOBR	0.3 DE a	0.3 B a	0.5 C a	0.2 BC a	0.3 CD a	0.4 C a
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	0.0 E a	0.2 B a	0.2 C a	0.1 C a	0.1 D a	0.1 C a
Tap water	TW	0.2 E a	0.0 B a	0.2 C a	0.0 C a	0.1 D a	0.2 C a

[a] APC, aerobic plate count.

[b] Means in the same column with no common uppercase letter are significantly different ($P < 0.05$). Within a surface type, means in the same row with no common lowercase letter are significantly different ($P < 0.05$). For each value, $n = 12$.

CFU/cm^2 reduction of APC obtained by immersion for 1 min in POA (Table 6).

The APC concentrations on the muscle surface of the untreated control pieces ranged from 4.9 to 5.3 log CFU/cm^2, with an overall mean of 5.1 log CFU/cm^2 (data not shown). Immersion of cheek meat in LA5, LA2.5, BX, AFTEC, or POA for 1 min resulted in APC reductions on the muscle surface that ranged from 0.5 to 1.2 log CFU/cm^2, and each of these reductions differed significantly ($P < 0.05$) from the APC reduction of 0.0 log CFU/cm^2 on the adipose surface obtained by immersion for 1 min in TW (Table 6). The APC reductions of 0.2 and 0.1 log CFU/cm^2 on the muscle surface following 1 min of immersion in HOBR or LEV-SDS, respectively, were not significantly different ($P \geq 0.05$) from the APC reduction obtained by immersion for 1 min in TW. Significant differences ($P < 0.05$) between the APC reductions on the muscle surface were observed for immersion for 1 and 5 min in LA5, AFTEC, and POA (Table 6).

Reductions of STEC O157:H7, non-O157 STEC, *S. enterica,* and APC on cheek meat surfaces following immersion in HW for 10 s. Immersion of cheek meat in HW for 10 s reduced STEC O157:H7, non-O157 STEC, *S. enterica,* and APC on the adipose surface by 2.2, 2.3, 2.2, and 2.5 log CFU/cm^2, respectively (data not shown). Immersion of cheek meat in HW for 10 s reduced STEC O157:H7, non-O157 STEC, *S. enterica,* and APC on the muscle surface by 1.8, 1.8, 1.7, and 1.8 log CFU/cm^2, respectively (data not shown).

DISCUSSION

Because cheek meat may be contaminated on both the adipose and muscle surfaces, the effectiveness of each permutation of treatment solution and immersion duration for the control of STEC O157:H7, non-O157 STEC, *S. enterica,* and APC on both surfaces was determined. The differences in the reductions of STEC O157:H7, non-O157 STEC, *S. enterica,* and APC between the adipose and muscle surfaces ranged from a 0.7-log CFU/cm^2 greater reduction on the adipose surface to a 0.2-log CFU/cm^2 greater reduction on the muscle surface (Table 7). Because the log CFU per square centimeter reductions between adipose and muscle surfaces were significantly different ($P < 0.05$) for only 14 of the 100 tested permutations, we concluded that, in general, immersion was equally effective for each surface. Cutter and Siragusa *(12)* found that reductions of STEC O157:H7 were 1 log CFU/cm^2 greater on the adipose surface of beef carcass tissue than on the lean surface of beef carcass tissue when sprayed with lactic, acetic, or citric acid. Cutter and Siragusa *(12)* also found that the postspray surface pH on adipose beef carcass tissue was 0.2 to 0.9 units lower than that on lean beef carcass tissue treated with the same antimicrobial solution, and they concluded that the lower postspray surface pH was correlated with a greater reduction of STEC O157:H7. However, we found that, for all treatments, the differences in the postimmersion pH values on the adipose and muscle surfaces were <0.4 pH units. The similar postimmersion

TABLE 7. *Difference in reductions of bacteria on adipose and muscle surfaces of cheek meat*

Antimicrobial solution	Intervention solution abbreviation	Immersion duration	Mean log CFU/cm^2 reduction on adipose surface − mean log CFU/cm^2 reduction on muscle surface[a]							
			O157:H7 STEC[b]	*P* value	non-O157 STEC	*P* value	*Salmonella enterica*	*P* value	APC[c]	*P* value
80°C water	HW	10 s	0.4	0.11	0.0	0.88	0.5	0.15	0.7	0.01
5% lactic acid	LA5	1 min	0.7	0.01	0.6	0.02	0.6	0.02	0.5	0.01
5% lactic acid	LA5	2.5 min	0.3	0.23	−0.1	0.66	0.0	1.00	0.1	0.47
5% lactic acid	LA5	5 min	0.3	0.33	0.2	0.30	0.1	0.63	0.3	0.20
2.5% lactic acid	LA2.5	1 min	0.5	0.47	0.3	0.16	0.3	0.17	0.2	0.16
2.5% lactic acid	LA2.5	2.5 min	0.3	0.18	0.4	0.04	0.3	0.14	0.4	0.04
2.5% lactic acid	LA2.5	5 min	0.5	0.05	0.4	0.09	0.4	0.10	0.4	0.03
2.5% Beefxide	BX	1 min	0.5	0.05	0.2	0.15	0.2	0.14	0.1	0.15
2.5% Beefxide	BX	2.5 min	0.3	0.23	0.3	0.16	0.2	0.39	0.1	0.67
2.5% Beefxide	BX	5 min	0.2	0.32	0.1	0.65	0.1	0.63	0.2	0.34
1% Aftec 3000	AFTEC	1 min	0.5	0.01	0.1	0.12	0.1	0.57	0.0	0.96
1% Aftec 3000	AFTEC	2.5 min	0.5	0.00	0.2	0.13	0.4	0.02	0.2	0.14
1% Aftec 3000	AFTEC	5 min	0.2	0.40	0.1	0.63	0.2	0.20	0.2	0.41
220 ppm of peroxyacetic acid	POA	1 min	0.4	0.03	0.3	0.05	0.2	0.10	0.2	0.41
220 ppm of peroxyacetic acid	POA	2.5 min	0.3	0.23	0.2	0.28	0.2	0.32	0.6	0.01
220 ppm of peroxyacetic acid	POA	5 min	0.3	0.10	0.3	0.06	0.1	0.34	0.3	0.08
300 ppm of hypobromous acid	HOBR	1 min	−0.1	0.53	0.0	0.46	0.1	0.32	0.1	0.32
300 ppm of hypobromous acid	HOBR	2.5 min	−0.1	0.66	0.1	0.60	0.0	0.80	0.0	0.56
300 ppm of hypobromous acid	HOBR	5 min	0.2	0.25	0.2	0.27	0.1	0.57	0.1	0.43
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	1 min	−0.1	0.58	−0.2	0.22	−0.1	0.21	−0.1	0.42
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	2.5 min	0.2	0.06	0.0	0.47	0.1	0.12	0.1	0.23
0.5% levulinic acid–0.05% sodium dodecyl sulfate	LEV-SDS	5 min	0.2	0.02	0.1	0.18	0.2	0.02	0.1	0.60
Tap water	TW	1 min	0.2	0.09	0.0	0.88	0.0	0.36	0.2	0.24
Tap water	TW	2.5 min	−0.1	0.33	−0.1	0.30	−0.1	0.06	−0.1	0.38
Tap water	TW	5 min	0.0	0.96	−0.1	0.33	−0.1	0.36	0.0	0.47

[a] Values are means of differences in reduction values on each surface, $n = 12$.
[b] STEC, Shiga toxin–producing *Escherichia coli.*
[c] APC, aerobic plate count.

surface pH values and log reductions of bacteria on each cheek meat surface can be attributed to more even exposure of the cheek meat to the antimicrobial solution during immersion compared to spray application of antimicrobial solutions. Wolf et al. *(33)* demonstrated that immersion in 4.4% lactic acid reduced levels of STEC O157:H7, non-O157 STEC, and *S. enterica* on beef trim more effectively than spray application of 4.4% lactic acid.

For each antimicrobial solution, immersion duration, and cheek meat surface permutation tested, the differences among the reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* were $\leq$0.3 log CFU/cm^2. We concluded that, when used to immerse cheek meat, the tested antimicrobial solutions were equally effective for the reduction of STEC O157:H7, non-O157 STEC, and *S. enterica*. Similarly, Kalchayanand et al. *(22)* determined that there was no significant difference among the reductions of STEC serogroups O26, O45, O103, O111, O121, O145, and O157 on the surfaces of beef flanks when sprayed with acidified sodium chlorite, peroxyacetic acid, lactic acid, or hot water (85°C). Likewise, Arthur et al. *(2)* determined that reductions of *Salmonella* serotypes Newport, multidrug-resistant Newport, Typhimurium, and multidrug-resistant Typhimurium; human-disease–associated STEC O157:H7; and non-human-disease–associated STEC O157:H7 were generally similar when beef flanks were sprayed with 2% acetic acid, electrolyzed oxidizing water, FreshFx, hot water (74°C), 2% lactic acid, or ozonated water. Geornaras et al. *(16)* obtained similar reductions of STEC O157:H7 and of *Salmonella* serotypes Newport, multidrug-resistant Newport, Typhimurium, and multidrug-resistant Typhimurium when beef trimmings were immersed in acidified sodium chlorite, sodium metasilicate, Bromitize Plus, or Aftec 3000. Fouladkhah et al. *(15)* determined that reductions of STEC O157:H7; non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145; and *Salmonella* serotypes Newport, multidrug-resistant Newport, Typhimurium, and multidrug-resistant Typhimurium were generally similar when beef trimmings were immersed in 5% lactic acid.

In general, a longer immersion in an antimicrobial solution increased the numerical reduction of STEC O157:H7, non-O157 STEC, and *S. enterica,* but most of these increases were not statistically significant. This result is not unexpected, because increasing the immersion duration in these solutions from 1 to 5 min resulted in small additional reductions in pH of 0.3 to 0.5 pH units; the antimicrobial actions of LA5, LA2.5, BX, AFTEC, and LEV-SDS rely on the reduction of surface pH. Thus, we concluded that increasing the immersion duration beyond 1 min does not significantly improve antimicrobial efficacy.

Immersion in HW for 10 s was the most effective intervention for reducing STEC O157:H7, non-O157 STEC, and *S. enterica* on beef cheek meat surfaces, in agreement with several other studies that have determined that ''hot water'' (tap water heated to 74 to 85°C) is an effective beef processing antimicrobial intervention *(6, 13, 21, 22, 34)*. Immersion of cheek meat in LA5, LA2.5, BX, AFTEC, and POA for $\geq$1 min effectively reduced STEC O157:H7, non-O157 STEC, and *S. enterica* levels. Excluding immersion in HW for 10 s, immersion in LA5 resulted in the greatest reductions of STEC O157:H7, non-O157 STEC, and *S. enterica,* but these reductions frequently did not differ significantly ($P > 0.05$) from the reductions of STEC O157:H7, non-O157 STEC, and *S. enterica* obtained following the same immersion duration in LA2.5, BX, or AFTEC.

Immersion in either LA5 or LA2.5 effectively reduced STEC O157:H7, non-O157 STEC, and *S. enterica* on beef cheek meat surfaces, analogous to numerous studies that have demonstrated lactic acid to be an effective beef processing antimicrobial intervention *(10, 12, 13, 15, 18, 22, 24, 33)*. We concluded that immersing cheek meat in a higher concentration of lactic acid did not result in increased efficacy because the log reductions were not significantly different ($P \geq 0.05$) between immersions in LA5 and LA2.5 for the same duration for 21 of the 24 permutations evaluated in this study. Similarly, Harris et al. *(18)* found that reductions of *E. coli* O157:H7 and *S. enterica* were not different between spray applications of beef trim with 2 and 4% lactic acid. Conversely, Cutter and Siragusa *(12)* determined that increasing the spray-applied lactic acid concentration from 1 to 5% increased reductions of *E. coli* O157:H7 on beef carcass tissue. Immersion of beef trim in AFTEC for 30 s reduced STEC O157:H7 and *S. enterica* by 0.4 to 0.7 log CFU/cm^2 *(16),* less than the 0.8- to 1.5-log CFU/cm^2 reductions of STEC O157:H7 and *S. enterica* we observed on cheek meat following immersion in AFTEC for 1 to 5 min. The lower surface pH on cheek meat following AFTEC immersion may explain the increased reductions we observed; surface pH values for cheek meat following AFTEC immersion ranged from 2.6 to 3.1, whereas the postimmersion surface pH for beef trim was 4.7 *(16)*. The reductions of STEC O157:H7 and *S. enterica* on cheek meat following immersion in BX were similar to the reductions on beef tips following spray application of BX *(26)*. Immersion of cheek meat in POA reduced STEC and *S. enterica* by levels similar to the reductions obtained following spray application of POA on beef flanks *(21)* and immersion of beef trim in POA *(14, 16)*.

It is unclear why HOBR did not effectively reduce bacterial populations on the surfaces of cheek meat, as our laboratory previously demonstrated that spray application of 1,3-dibromo-5,5-dimethylhydantoin, which is hydrolyzed to the active hypobromous acid form in aqueous solution, reduced STEC O157:H7 levels on beef cutaneous trunci sections and beef hearts by 1.6 to 2.1 log CFU/cm^2 *(20)* and determined that spray application of hypobromous acid on cattle hides reduced *E. coli* by 2.2 to 3.8 log CFU/cm^2 *(30)*.

Zhao et al. *(36)* demonstrated that immersion of chicken skin in LEV-SDS for 1 to 5 min reduced *S. enterica* by 2.9 to 5.3 log CFU/cm^2. Neither 0.5% levulinic acid nor 0.05% sodium dodecyl sulfate alone has an antimicrobial effect on *S. enterica,* but sodium dodecyl sulfate has bactericidal activity at pH values between 1.5 and 3.0 *(8, 11, 36)*. We observed that the surface pH of cheek meat following immersion in LEV-SDS ranged from 4.5 to 5.1. Thus, the lack of LEV-SDS antimicrobial activity on cheek meat is likely explained by the failure of levulinic acid to lower the pH on the cheek meat surface to the level required for sodium dodecyl sulfate bactericidal activity.

In summary, we demonstrated that immersion in HW for 10 s is the most effective intervention of the methods examined herein to reduce levels of the foodborne pathogens STEC O157:H7, non-O157 STEC, and *S. enterica* present on both adipose and muscle surfaces of cheek meat. We also demonstrated that immersion of cheek meat in LA5, LA2.5, BX, AFTEC, or POA for 1 min effectively reduced levels of the foodborne pathogens STEC O157:H7, non-O157 STEC, and *S. enterica*. In general, increasing the duration of immersion did not significantly increase antimicrobial effectiveness. We demonstrated that levels of STEC O157:H7, non-O157 STEC, and *S. enterica* were equally reduced on adipose and muscle surfaces when cheek meat was immersed in antimicrobial solutions. Because currently used head washing systems may not be effective against pathogens present in the oral cavity *(21),* incorporation of an antimicrobial intervention following the removal of cheek meat from the head will have a positive impact on the food safety of ground beef by reducing the levels of pathogenic *E. coli* and *S. enterica* on cheek meat.

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