Validation of Lactic Acid Bacteria, Lactic Acid, and Acidified Sodium Chlorite as Decontaminating Interventions To Control Escherichia coli O157:H7 and Salmonella Typhimurium DT 104 in Mechanically Tenderized and Brine-Enhanced (Nonintact) Beef at the Purveyor

ALEJANDRO ECHEVERRY, J. CHANCE BROOKS, MARKUS F. MILLER, JESSE A. COLLINS, GUY H. LONERAGAN, and MINDY M. BRASHEARS*

Department of Animal and Food Sciences, Texas Tech University, Box 42141, Lubbock, Texas 79409, USA

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ABSTRACT

After three different outbreaks were linked to the consumption of nonintact meat products contaminated with Escherichia coli O157:H7, the U.S. Food Safety and Inspection Service published notice requiring establishments producing mechanically tenderized and moisture-enhanced beef products to reassess their respective hazard analysis and critical control point systems, due to potential risk to the consumers. The objective of this study was to validate the use of lactic acid bacteria (LAB), acidified sodium chlorite (ASC), and lactic acid (LA) sprays when applied under a simulated purveyor setting as effective interventions to control and reduce E. coli O157:H7 and Salmonella Typhimurium DT 104 in inoculated U.S. Department of Agriculture (USDA) Choice strip loins (longissimus lumborum muscles) pieces intended for either mechanical blade tenderization or injection enhancement with a brine solution after an aging period of 14 or 21 days at 4.4°C under vacuum. After the mechanical process, translocation of E. coli O157:H7 and Salmonella Typhimurium DT 104 from the surface into the internal muscles occurred at levels between 1.00 and 5.72 log CFU/g, compared with controls. LAB and LA reduced internal E. coli O157:H7 loads up to 3.0 log, while ASC reduced the pathogen 1.4 to 2.3 log more than the control (P < 0.05), respectively. Salmonella Typhimurium DT 104 was also reduced internally 1.3 to 2.8, 1.0 to 2.3, and 1.4 to 1.8 log after application of LAB, LA, and ASC, respectively. The application of antimicrobials by purveyors prior to mechanical tenderization or enhancement of steaks should increase the safety of these types of products.

Escherichia coli O157:H7 and Salmonella are pathogens capable of causing high morbidity and mortality among humans, with the majority of infections linked to the consumption of contaminated foods, including meat and meat products (32, 36). These two pathogens can have a large socioeconomic impact due to illness, medical costs, lost of productivity, disability, deaths, litigation, and recalls due to contaminated products (7).

In October 1994 under the Federal Meat Inspection Act, the U.S. Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) declared E. coli O157:H7 to be an adulterant in raw ground beef (39, 42). This decision occurred in response to a multistate outbreak due to consumption of contaminated beef patties, which resulted in approximately 700 illnesses and four deaths (3). Afterward, the FSIS established new provisions for all meat and poultry plants, requiring the mandatory implementation of a hazard analysis and critical control point (HACCP) system in order to identify risky and potentially hazardous practices that account for microbial contamination. Adoption of the HACCP system gives plants a tool to identify the hazards and control measures (also known as interventions in the North American meat industry) that can be implemented in each step of their process.

In May 2005, the FSIS published notice that establishments producing mechanically tenderized beef, enhanced beef, or any other type of nonintact beef products were required to reassess their HACCP plans, because recent outbreaks indicated that E. coli O157:H7 was a hazard reasonably likely to occur (29, 40) due to microbial transfer from the external surfaces into the internal muscle (19, 21, 35, 37). If these products are undercooked, then there will be a risk that consumers may become ill due to the surviving bacteria in these tissues.

In the United States, physical, chemical, or biological interventions for the carcasses and their subprimals are implemented by meat processors, and with the new regulation, processors are required to demonstrate that the control and decontamination measures in place are effective

* Author for correspondence. Tel: 806-742-2805, Ext 235; Fax: 806-742-4003; E-mail: mindy.brashears@ttu.edu.
in reducing the presence of pathogens in their products. Previous studies have shown the effectiveness of chemical interventions in reducing microbial contamination in beef hides (1, 2), carcasses (8–11, 14, 17, 24, 28), beef trim–variety meats and ground beef (25, 34), and mechanically tenderized or injected beef products (15); however, the previous studies have focused on the use of preventive control steps at the slaughter and the processing plant. There is lack of scientific data on the use of some of these interventions once the meat has left the processing facility and is distributed, as is the case of meat that is purchased by purveyors, retailers, supermarkets, and restaurants, and beef that is intended for nonintact beef products.

Even though the outbreaks in which the FSIS took the decision to mandate reassessment of the HACCP plans for nonintact beef products did not involve Salmonella, control measures taken to reduce this pathogen at all stages of the meat production system might prove to be beneficial in decreasing the high number of illnesses caused by other enteric bacteria of public health concern (41). In the United States, the mechanical tenderization and/or the injection process can occur at different points during the meat supply chain continuum (Fig. 1). Usually, the slaughter plant handles the harvesting of the cattle (where the majority of the antimicrobial interventions are applied) and it can be involved with further processing, mechanical processes, and distribution; however, once the meat is shipped, no further interventions are expected by purveyors. In the case of those cuts that are considered tough, they can be subjected to either needle–blade tenderization, moisture–brine enhancement, or their combination to increase the tenderness and juiciness of the final product before it reaches consumers. In these processes, the meat is penetrated by very sharp blades, without stretching or tearing apart the muscle fibers of the meat, or injected with solutions that increase the juiciness and palatability of the final product. The product is usually placed on a conveyor belt system, and as the product moves down the line, it is penetrated with the blades. The use of this technique is a common practice to improve the quality of whole-muscle cuts, such as chucks, ribs, tenderloins, and

FIGURE 1. Generic slaughter and fabrication flow diagram. Different interventions or decontamination strategies can be applied along the process. In the United States, many of these interventions are mandatory for meat processors, as they are required by the FSIS to implement them in order to reduce pathogen loads.
strip loins. Tenderness can also be increased by aging the carcass under refrigerated temperatures for up to 21 days; however, this process can be costly to the processors due to carcass weight loss, microbial spoilage, and energy and space requirements.

The objective of this study was to validate the use of lactic acid bacteria (LAB), acidified sodium chloride (ASC), and lactic acid (LA) sprays, when applied under a simulated purveyor setting, as interventions to control and reduce E. coli O157:H7 and Salmonella Typhimurium DT 104 in USDA Choice strip loins (longissimus lumborum muscle) pieces intended for either mechanical–blade tenderization (MT) or injection–enhancement (EN) with a brine solution in a simulated purveyor environment.

MATERIALS AND METHODS

Experimental design and statistical analysis. The experiment was a randomized complete split-plot design, with individual pieces defined as experimental units. Experiments were carried out in triplicate. Four different sampling sites within each individual piece ("surface swab," "top," "A," and "B"), as described by Echeverry et al. (15), were analyzed individually. The two processes, MT and EN, although similar because they pierce the meat, were analyzed separately. For each set of treatments, duplicate plates were obtained for each dilution at each sampling time and averages obtained. Average surface swab counts were transformed into log CFU per square centimeter, while top, A, and B core counts were transformed into log CFU per gram of meat, respectively, allowing control and stabilization of statistical variance and fulfillment of the requirements for normality prior to the analysis. Log counts were considered a dependent variable of interest. All data were imported into commercially available software package and analyzed with the MIXED procedure in the Statistical Analysis System 9.1.3 software (33). Comparisons of least square means were obtained. In all tests, the significance level was set at α = 0.05.

Bacterial cultures. Experiments involving the two different pathogens were conducted on separate occasions in triplicate. For the E. coli O157:H7 study, a three-strain cocktail of E. coli O157:H7 (strains 922, 944, and 966) originally isolated from cattle, were used to inoculate the pieces used in this study. Frozen stock cultures were grown individually in 200 ml of Trypticase soy broth (TSB) at 37°C for 24 h and passed three times prior to experimental use. For the Salmonella study, a three-strain cocktail of Salmonella enterica serotype Typhimurium definitive phage type 104 (strains 205, 206, and 214) originally isolated from humans, was used to inoculate the pieces, and grown in a similar procedure as described above.

Preparation of cocktail cultures. Three 200-ml portions of TSB were prepared for each strain, after which cells were centrifuged (20 min at 4,000 rpm; Marathon 21000R, Fisher Scientific, Waltham, MA) under refrigerated conditions (4°C) and resuspended into 30 ml of TSB to create a concentrated culture. On the day of the experiment, the concentrated cultures were transported to the pathogen processing facility, where they were combined with 1,000 ml of buffered peptone water to allow for enough volume of the cocktail to inoculate the meat, as described below. The final mean E. coli O157:H7 cocktail concentration used to inoculate the pieces was $5.76 \times 10^8$ CFU/ml (standard deviation [SD] = 1.16). The final mean Salmonella cocktail concentration used to inoculate the pieces was $1.70 \times 10^9$ CFU/ml (SD = 1.16). All cultures were obtained from the Texas Tech University Food Microbiology Laboratory Stock Collection (Lubbock).

Meat preparation. All the experiments were conducted in the Biosafety Level 2 pathogen processing facility in the Food Technology Building at Texas Tech University. This 750-ft² (~70-m²) facility allows validation and processing studies of food inoculated with pathogens under simulated industrial conditions.

USDA Choice grade, boneless beef strip loins, obtained from a commercial processor, were transported to the pathogen laboratory under refrigeration. The subprimal were trimmed and fabricated for uniformity into pieces measuring approximately 20.3 by 12.7 by 7.6 cm, as shown in our previous study (15).

The concentrated cocktail cultures were prepared to allow dilution to the targeted concentration and to facilitate inoculation of the large number of pieces. The pieces were inoculated by dipping each one into a sanitized container containing the diluted microbial cocktail (either E. coli O157:H7 or Salmonella serotype Typhimurium DT 104, to be referred only as Salmonella in this article) for 1 min, transferred to sterile, stainless steel mesh racks, and held at refrigerated temperatures for 1 h to allow dripping of excessive moisture and facilitate attachment of bacteria inocula before processing. After attachment, pieces were randomly assigned to one of the treatments, individually vacuum packed, and held under refrigeration (<4.4°C) for either 14 or 21 days.

Equipment sanitation. Prior to experimentation, the pathogen processing laboratory was subjected to a full 3-day cleaning and sanitation process with a quaternary ammonium sanitizer (Bi-Quat, Birko Corp., Henderson, CO) of all walls, ceilings, processing equipments, racks, and other utensils and instruments to guarantee absence of any pathogens and background flora with the potential of misleading results. For quality assurance and once the pieces were processed, the conveyor belt system and all equipment were cleaned with a commercial detergent and sanitized with a three-way quaternary ammonium disinfectant (Ala-Quat, Birko Corp.) between interventions, within a replication that was followed by a rinse with hot water (65.5 to 82.2°C) prior to the exposure of the pieces to each of the interventions. Environmental and swab samples of the equipment were obtained between interventions to validate the cleaning and sanitation process.

To guarantee pureness of the sprayed solutions, the conveyor’s tank was emptied between treatments and the system was operated with hot water (>80°C) for 2 min, followed by sterile distilled water for 1 min in order to rinse any impurities before refilling with the next intervention. Similarly, the multineedle injector as well as the manual mechanical blade tenderizer were cleaned and sanitized after the previous procedure (after processing of each of the pieces). The brine used in the enhancement process was not recirculated (as occurs in the industry) in order to prevent cross-contamination and unintentional introduction of pathogens inside the subsamples via injection of the runoff, with the potential of misleading microbial results.

Microbial challenge and processing of pieces. After the aging period under refrigerated conditions (14 or 21 days to simulate the time that the cuts can be held before they are subjected to further processing or cutting into steaks by the purveyor or end user), the inoculated samples were transported to the pathogen laboratory for further processing. For each treatment at any given aging period, two pieces were analyzed. Pieces were fed to a six-nozzle, trim-sanitizing spray cabinet (Chad Co., Olathe, KS) with a...
conveyor belt system (series 800, Intralox, Inc., Harahan, LA), similar to those used in the meat industry, and treated by spraying one of the antimicrobial interventions at a flow rate of 0.11 gal (0.42 liter)/min per nozzle with a pressure of 20 lb/in² (~138 kPa) onto the surface of the product as it moved down the belt. The following interventions were evaluated: (i) sterile distilled water (CTRL; pH = 6.85); (ii) lactic acid bacteria (LAB; pH = 6.07, ~ 4.70 × 10⁶ CFU/ml; Lactiguard, Nutrition Physiology Corp., Amarillo, TX); (iii) acidified sodium chlorite (ASC; pH = 3.36, 1,000 to 1,200 ppm; Keeper, Bio-Cide International, Inc., Norman, OK); and (iv) 3% lactic acid (LA; pH = 2.21; Birko Corp.). The pH for each of the spray solutions was obtained on-site with a pH meter (Orion model 550A, Thermo Fisher Scientific, Waltham, MA; the ASC solution was acidified and activated with powdered citric acid and its concentration tested with the BCI’s Keeper Test Kit, following the manufacturer’s directions. The spraying process was performed at room temperature (20°C). Sprayed pieces were collected at the end of the line on sanitized stainless mesh racks, transferred to the refrigerated room, allowed to drip for 5 min, and randomly assigned to one of the following process: (i) mechanical tenderization (MT) with a manual tenderizer (Manual Tenderizer Model H, Jaccard, Orchard Park, NY) consisting of 544 stainless steel surgical knives with two directional sharpness, providing approximately an area of 192 cm² of tenderization per cycle, or (ii) enhancement with a chilled (2 to 4°C) brine solution (EN), formulated to provide 0.3% sodium chloride and 0.3% sodium tripolyphosphate in the final product by using a multineedle injector (Injectamatic Pi21 Automatic Brine Injector, Koch Equipment, L.L.C., Kansas City, MO) consisting of 21 needles (4-mm diameter) operating at 41 strokes per min at a pressure of approximately 9.0 lb/in² (~62 kPa). EN samples were pumped to approximately 110% of their original weight. All equipment was sanitized as described earlier between each of the treatments. After the mechanical process, all pieces were individually sealed without vacuum in high-barrier Cryovac bags under refrigerated condition, stored in a plastic cooler with ice packs, and transported directly to the Food Microbiology Laboratory in the Experimental Sciences Building at Texas Tech University and examined within 30 to 60 min after collection. Pieces were analyzed for microbial counts on days 0 (surface only), and 14 and 21 (surface, top, A and B).

**Microbial analysis of samples.** Samples held for 0 days were transported to the Food Microbiology Lab immediately after being vacuum packaged. For each sample, a surface area 50 cm² was swabbed by using a sterile cotton tip and a sterile template (USDA-050, Biotrace International, Muncie, IN). The cotton tip was placed into a tube containing 9 ml of buffered peptone water, vortexed, and serial dilutions were performed to allow bacterial enumeration. For each dilution, 100 μl was plated into duplicate plates by using the thin agar layer method, which allows injured cells (due to the spray interventions or the refrigeration conditions) to resuscitate and grow on the media, while inhibiting other microorganisms and native background flora (5, 44, 45). **E. coli** O157:H7 dilutions were plated onto petri dishes containing an overlay of MacConkey agar (approximately 7 ml) with two successive 7-ml layers of tryptic soy agar (TSA; total of 14 ml) and incubated for 24 h at 37°C. Similarly, *Salmonella* Typhimurium DT 104 dilutions were plated onto an overlay of xylose lysine decarboxylase (XLD) medium (approximately 7 ml) with two successive 7-ml layers of tryptic soy agar (TSA; total of 14 ml) and incubated for 24 h at 37°C. TSA overlays were used to facilitate recovery of injured cells (5, 44, 45). After incubation, plates were counted with the Spiral Biotech Q Count software (version 2.0, Spiral Biotech, Norwood, MA). For both pathogens, negative plates or plates with counts under the detection limit (<0 CFU/g) were assigned an arbitrary small value of “1.0” prior to the statistical analysis in order to normalize the data distribution.

Samples that were treated with the interventions and processed with either MT or EN were analyzed after the aging period, with an identical methodology as described before on day 0. In addition to the surface counts, for each of the processed pieces, another three sections (top surface, approximately 0.6-cm deep, and subsections A and B, obtained from core samples about 1- and 1.5-cm deep, respectively) were obtained aseptically and analyzed as previously described by Echeverry et al. (15). These subsections were analyzed to determine pathogen loads in the interior of the product. For each of these subsections, 11 g was collected, placed in stomacher bags, and 99 ml of buffered peptone water was added. Samples were then stomached for 2 min, and serial dilutions were performed. Plating and other microbiological analysis were performed similarly as explained previously.

**RESULTS.**

**E. coli** O157:H7 surface counts: MT samples. Microbial surface counts were taken on days 0, 14, and 21 from inoculated, nontreated samples to confirm inoculation levels, which were 5.73, 5.23, and 5.40 log CFU/cm², respectively (data not shown). On day 14, samples treated with LAB presented a statistically significant (P < 0.05) reduction on the numbers of **E. coli** O157:H7 (0.66 log CFU/cm²) when compared with the water treatment. On day 21, there was a significant (P < 0.05) reduction on the numbers of **E. coli** O157:H7 (>1.3 log CFU/cm²) after application of LAB; however, no significant reduction was observed with ASC or LA (Fig. 2).

**Salmonella** surface counts: MT samples. Microbial surface counts were taken on days 0, 14, and 21 from inoculated, nontreated samples to confirm inoculation levels, which were 5.52, 4.80, and 4.49 log CFU/cm² (data not shown). On day 14, there was a statistically significant (P < 0.05) difference among treatments, with the most
significant reduction (>0.66 log CFU/cm²) on surface counts observed after application of LA (Fig. 3). On day 21, no significant reductions were observed between the treatments and the CTRL (Fig. 3).

**E. coli O157:H7 surface counts: EN samples.** Microbial surface counts were taken on day(s) 0, 14, and 21 from inoculated, nontreated samples to confirm inoculation levels, which were 5.73, 5.60, and 5.63 log CFU/cm², respectively (data not shown). Microbial surface counts on day 14 revealed that there was a significant (P < 0.05) reduction in the numbers of *E. coli* O157:H7 (>0.8 log CFU/cm²) after the application of LA when compared with the CTRL (Fig. 4). After 21 days of aging, the total amount of *E. coli* O157:H7 recovered was also significantly reduced (0.89 log CFU/cm²) by the application of LAB (P < 0.05) when compared with treatment with water. No differences were observed after application of ASC or LA (Fig. 4).

**Salmonella surface counts: EN samples.** Microbial surface counts were taken on days 0, 14, and 21 from inoculated, nontreated samples to confirm inoculation levels, which were 5.52, 4.63, and 4.86 log CFU/cm², respectively (data not shown). On day 14, there was a statistically significant (P < 0.05) reduction on *Salmonella* surface counts after treatment with the LAB spray (0.7 log CFU/cm²); however, there were no differences among the interventions (Fig. 5). On day 21, no differences were observed among treatments when compared with CTRL (Fig. 5).

**E. coli O157:H7 internal counts: MT samples.** No internal sections were enumerated on day 0, as the samples were not mechanically tenderized until after the end of the aging period. On day 14, there was a significant difference (P < 0.05) between treatments on the top subsection, with the greatest reduction (>0.7 log CFU/g) obtained with the application of LAB (Table 1). In subsection A, all the samples subjected to the interventions were significantly (P < 0.05) different from the CTRL, with counts ranging between 1.9 and 3.00 log CFU/g lower than those counts of the CTRL. Subsection B samples had similar differences for all the interventions when compared with the CTRL, with the lowest transfer (3.00 log CFU/g less) observed in those samples treated with LAB (Table 1). On day 21, the top
On day 14, there were no significant differences in the top subsection between the interventions and the CTRL (Table 2). Subsections A and B samples treated with ASC and LAB, respectively, presented the lowest pathogen counts (up to 2.16 log CFU/g) compared with the recovery levels obtained from the CTRL samples ($P < 0.05$) (Table 2). On day 21, the top subsection of mechanically tenderized samples did not present significant differences between interventions and CTRL ($P > 0.05$) (Table 2). On day 21, subsection A had lower total numbers of *Salmonella* isolated (~2.4 log CFU/g) on those samples subjected to LA when compared with the CTRL samples ($P < 0.05$), although significant reductions were also observed with the use of LAB and ASC. On day 21, subsection B samples treated with LA presented the only significant reduction (~2.0 log CFU/g) when compared with CTRL.

**Salmonella internal counts: MT samples.** On day 14, there were no significant differences in the top subsection between the interventions and the CTRL (Table 2). Subsections A and B samples treated with ASC and LAB, respectively, presented the lowest pathogen counts (up to 2.16 log CFU/g) when compared with the recovery levels obtained from the CTRL samples ($P < 0.05$) (Table 2). On day 21, the top subsection of mechanically tenderized samples did not present significant differences between interventions and CTRL ($P > 0.05$) (Table 2). On day 21, subsection A had lower total numbers of *Salmonella* isolated (~2.4 log CFU/g) on those samples subjected to LA when compared with the CTRL samples ($P < 0.05$), although significant reductions were also observed with the use of LAB and ASC. On day 21, subsection B samples treated with LA presented the only significant reduction (~2.0 log CFU/g) when compared with CTRL.

**E. coli O157:H7 internal counts: EN samples.** On day 14, there were no significant differences in the top subsections between interventions and CTRL. The total *E. coli* O157:H7 isolated from subsections A and B had the greatest reduction (~1.5 and ~2.3 log CFU/g) when the pieces were sprayed with LAB and ASC, respectively ($P < 0.05$) (Table 3). On day 21, the top subsection on EN samples contained significantly ($P < 0.05$) fewer *E. coli* O157:H7 when treated with LAB and ASC, with the greatest reduction (~1.0 log CFU/g) observed when sprayed with the latter solution (Table 3). For subsection A, significant ($P < 0.05$) reductions were observed after application of ASC (2.2 log CFU/g), while on subsection B all the interventions presented significant decreases in the numbers of cells when compared with the CTRL (1.0 to ~1.8 log CFU/g) (Table 3).

**Salmonella internal counts: EN samples.** On day 14, there were no significant differences in the top subsections between the interventions and the CTRL (Table 4). On day 14, all the interventions were effective ($P < 0.05$) in reducing the translocation of *Salmonella* into subsections A (between 1.9 and ~2.9 log CFU/g) and subsection B (~2.9 log CFU/g) (Table 4). After 21 days of aging, no differences were observed in the number of *Salmonella* recovered from the top subsection after application of the interventions; however, all the interventions were effective ($P < 0.05$) on both subsections A and B when compared with the CTRL. Subsection A presented the highest reduction (~2.8 log CFU/g) after application of ASC, and subsection B presented the highest reduction (~1.5 log CFU/g) after application of LA (Table 4).

### TABLE 1. Effects of different interventions applied after an aging period of 14 or 21 days in beef striploins inoculated with *Escherichia coli* O157:H7 and subjected to mechanical tenderization

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Top</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td>4.66 (0.3536)</td>
<td>3.93 (0.3536)</td>
<td>4.00 (0.3536)</td>
</tr>
<tr>
<td>LAB</td>
<td>4.20 (0.3536)</td>
<td>2.36 (0.3536)</td>
<td>3.94 (0.3536)</td>
</tr>
<tr>
<td>ASC</td>
<td>4.34 (0.3536)</td>
<td>2.36 (0.3536)</td>
<td>3.94 (0.3536)</td>
</tr>
<tr>
<td>LA</td>
<td>3.52 (0.3536)</td>
<td>1.85 (0.3536)</td>
<td>1.85 (0.3536)</td>
</tr>
<tr>
<td>CTRL</td>
<td>4.08 (0.3536)</td>
<td>1.85 (0.3536)</td>
<td>1.85 (0.3536)</td>
</tr>
</tbody>
</table>

*Means with different letters within a row for each aging period differ significantly ($P < 0.05$).*

*CFU per gram of meat. ± standard errors of the mean.*

*CTRL, sterile distilled water; LAB, ~10¹⁰ CFU/ml lactic acid bacteria; ASC, 1,000 to 1,200 ppm of acidified sodium chloride; LA, 3% lactic acid. Values are expressed in least-square means log CFU/ml.*
### TABLE 2. Effects of different interventions applied after an aging period of 14 or 21 days in beef strip loins inoculated with *Salmonella Typhimurium DT 104* and subjected to mechanical tenderization

<table>
<thead>
<tr>
<th>Subsection within the meat</th>
<th>14 days of aging&lt;sup&gt;b&lt;/sup&gt;</th>
<th>21 days of aging&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTRL</td>
<td>LAB</td>
</tr>
<tr>
<td>Top</td>
<td>4.91</td>
<td>5.36</td>
</tr>
<tr>
<td></td>
<td>(0.2690)</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>4.41</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>(0.8274)</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>3.30</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>(0.8614)</td>
<td>A</td>
</tr>
</tbody>
</table>

<sup>a</sup> CTRL, sterile distilled water; LAB, \(\sim10^7\) CFU/ml lactic acid bacteria; ASC, 1,000 to 1,200 ppm of acidified sodium chlorite; LA, 3% lactic acid. Values are expressed in least-square means log CFU per gram ± standard errors of the mean.

<sup>b</sup> Data within days of aging are separate and distinct studies (not the same steak).

<sup>c</sup> Means with different letters within a row for each aging period differ significantly (\(P < 0.05\)).

### TABLE 3. Effects of different interventions applied after an aging period of 14 or 21 days in beef strip loins inoculated with *Escherichia coli O157:H7* and subjected to brine enhancement

<table>
<thead>
<tr>
<th>Subsection within the meat</th>
<th>14 days of aging&lt;sup&gt;b&lt;/sup&gt;</th>
<th>21 days of aging&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>CTRL</td>
<td>LAB</td>
</tr>
<tr>
<td>Top</td>
<td>4.80</td>
<td>3.53</td>
</tr>
<tr>
<td></td>
<td>(0.3536)</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>3.12</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>(0.7635)</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>3.31</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>(0.8614)</td>
<td>AB</td>
</tr>
</tbody>
</table>

<sup>a</sup> CTRL, sterile distilled water; LAB, \(\sim10^7\) CFU/ml lactic acid bacteria; ASC, 1,000 to 1,200 ppm of acidified sodium chlorite; LA, 3% lactic acid. Values are expressed in least-square means log CFU per gram ± standard errors of the mean.

<sup>b</sup> Data within days of aging are separate and distinct studies (not the same steak).

<sup>c</sup> Means with different letters within a row for each aging period differ significantly (\(P < 0.05\)).


**DISCUSSION**

Under current U.S. regulations, any processor or establishment that produces beef products must reassess their written HACCP plan on an annual basis (38). Establishments that produce any type of raw or cooked nonintact beef products as defined by the National Advisory Committee on Microbiological Criteria for Foods and the FSIS “[a] cut of whole muscle(s) that has not been injected, mechanically tenderized, or reconstructed” must take into account in their next reassessment the different *E. coli* O157:H7 outbreaks that have been epidemiologically linked to the consumption of mechanically tenderized meat (12, 40, 43). However, processors lack scientific information on the effectiveness of the current interventions to control this pathogen in this type of products.

Sensory and quality attributes of tenderized meat have been studied extensively by many authors before; however, the microbiological aspects of this process have not received much attention until very recently. It is generally accepted that bacteria associated with meat are derived from the ingesta, the environment, and the instruments used in the fabrication of the carcass, occurring only on the surface of the meat (16, 23). The internal muscles and deep tissues of the carcass are sterile unless they are subjected to a considerable breakdown of the connective tissue structure and muscle fibers (21–23). Similarly, during the process of carcass fabrication, mechanical processes such as blade tenderization or brine injection introduces bacteria into the deep tissues of the steaks (15, 18, 20, 30), as previous studies have demonstrated, which has the potential to become a considerable risk if the meat is undercooked (31, 35), as seen in the outbreaks previously mentioned. Even though the pathogen implicated on these unfortunate events was *E. coli* O157:H7, *Salmonella* must also be considered and addressed in this type of products, due to the number of infections and deaths that it causes every year.

Previous studies have characterized levels of contamination that can be internalized into the internal muscle of meats with either tenderization (beef) (15, 26, 35) or moisture-enhancement process (pork) (4). Additional studies have analyzed samples that have been tenderized at the processing plant (21) or at the retail level (19, 20), but they have focused mainly on total aerobic counts, coliform counts, and other native meat microflora. As reported by Johnston et al., as far back as 1978 (27) injected meat products have been implicated with outbreaks of salmonellosis. Outbreaks in the last decade have been linked epidemiologically to mechanically tenderized meat contaminated with *E. coli* O157:H7 (12, 29, 40). Clearly, translocation of *Salmonella, E. coli* O157:H7, and other pathogens from contaminated surfaces into the internal muscle of beef or pork products occurs as demonstrated in this and others studies (15, 30, 35), and lead to potentially similar outbreaks and recalls, as they “...are often perceived as whole muscle cuts and are, therefore, prepared to customer specifications...” (37).

In this study, we characterized the effects of three different sprays (LAB, ASC, and LA) as interventions to be

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**TABLE 4. Effects of different interventions applied after an aging period of 14 or 21 days in beef strip loins inoculated with *Salmonella Typhimurium DT 104* and subjected to brine enhancement**

<table>
<thead>
<tr>
<th>Subsection within the meat</th>
<th>14 days of aging</th>
<th>21 days of aging</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL</td>
<td>Lab</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>Top</td>
<td>4.96 (0.2690)</td>
<td>5.03 (0.2690)</td>
</tr>
<tr>
<td>B</td>
<td>4.52 (0.2690)</td>
<td>4.72 (0.2690)</td>
</tr>
<tr>
<td>A</td>
<td>4.89 (0.2690)</td>
<td>4.58 (0.2690)</td>
</tr>
<tr>
<td>CFU per gram</td>
<td>± standard errors of the mean.</td>
<td>± standard errors of the mean.</td>
</tr>
<tr>
<td>Top</td>
<td>1.00 (0.8614)</td>
<td>1.00 (0.8614)</td>
</tr>
<tr>
<td>B</td>
<td>1.84 (0.8274)</td>
<td>1.83 (0.8274)</td>
</tr>
<tr>
<td>A</td>
<td>1.89 (0.8274)</td>
<td>1.89 (0.8274)</td>
</tr>
<tr>
<td>Data within days of aging</td>
<td>a Means with different letters within a row for each aging period differ significantly (P &lt; 0.05).</td>
<td>b Data within days of aging are separate and distinct studies (not the same steer).</td>
</tr>
</tbody>
</table>

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**Notes:**
- CFU: Colony-forming units.
- CTRL: sterile distilled water; LAB, 3% lactic acid; ASC, 1,000 to 1,200 ppm of acidified sodium chlorite; LA, 3% lactic acid. Values are expressed in least-square means log transformation.
- b Additional studies have demonstrated, which has the potential to become a considerable risk if the meat is undercooked (31, 35), as seen in the outbreaks previously mentioned. Even though the pathogen implicated on these unfortunate events was *E. coli* O157:H7, *Salmonella* must also be considered and addressed in this type of products, due to the number of infections and deaths that it causes every year.
- Previous studies have characterized levels of contamination that can be internalized into the internal muscle of meats with either tenderization (beef) (15, 26, 35) or moisture-enhancement process (pork) (4). Additional studies have analyzed samples that have been tenderized at the processing plant (21) or at the retail level (19, 20), but they have focused mainly on total aerobic counts, coliform counts, and other native meat microflora. As reported by Johnston et al., as far back as 1978 (27) injected meat products have been implicated with outbreaks of salmonellosis. Outbreaks in the last decade have been linked epidemiologically to mechanically tenderized meat contaminated with *E. coli* O157:H7 (12, 29, 40). Clearly, translocation of *Salmonella, E. coli* O157:H7, and other pathogens from contaminated surfaces into the internal muscle of beef or pork products occurs as demonstrated in this and others studies (15, 30, 35), and lead to potentially similar outbreaks and recalls, as they “...are often perceived as whole muscle cuts and are, therefore, prepared to customer specifications...” (37).
used to reduce the translocation of *E. coli* O157:H7 and *Salmonella* that occur from inoculated strip loins surfaces into the interior of the muscle after processing with either mechanical tenderization or moisture enhancement. In a previous study (15), we demonstrated that application of these interventions significantly reduced translocation of pathogens due to the mechanical process when applied in a packer setting (i.e., sprayed with the interventions on day 0, or before the subprimals are fabricated into steaks). However, recent outbreaks involving contaminated nonintact steaks are forcing the system to perhaps add more controls to prevent these types of situations. Although not required by current U.S. regulations, application of interventions at the purveyor level could serve as a potential control point to reduce microbial hazards of meat cuts intended for nonintact beef products. Purveyors rely on the controls that packers have in place to control these types of hazards, and usually no additional steps are taken to address this issue other than keeping their products under refrigerated conditions. The outbreaks have prompted consumer groups to demand the FSIS to start a regulatory process in order to implement labeling for mechanically tenderized, nonintact beef products (6), and application of interventions at this point in the food chain could increase the safety of the final product.

The treatment of inoculated subprimals with LAB, both on days 14 and 21, significantly reduced the surface levels (~0.7 and ~1.3 log CFU/g, respectively) of *E. coli* O157:H7 in those pieces subjected to MT and EN (Figs. 2 and 4). LAB was also effective reducing *Salmonella* from the surface of EN pieces (Fig. 5, day 14) while higher reductions were observed with the use of lactic acid on the MT samples (Fig. 3).

Transfer of *E. coli* O157:H7 and *Salmonella* from the surface into the internal muscles occurred at levels of ca. 2.0 to 4.0 log after the use of the mechanical processes. For both mechanical processes, the samples treated only with water (CTRL) consistently presented higher microbial counts in the internal surfaces than those samples treated with the interventions did. Subsections A and B of MT samples inoculated with *E. coli* O157:H7 presented significant reductions after treatment with LAB, ASC, and LA on day 14 (Table 1). On day 21, the highest reductions were observed with the application of LA in these subsections, although the other interventions also presented significant reductions (Table 1). For MT samples inoculated with *Salmonella*, the internal counts of those samples treated with the interventions were also lower than those samples treated with the CTRL. On day 14, LAB, ASC, and LA were effective reducing *Salmonella* counts on subsection A, but only significant reductions were observed on subsection B on those samples treated with LAB (Table 2). On day 21, the highest reductions were observed with the application of LA. Samples subjected to brine injection were also consistently lower when treated with the interventions. On day 14, LAB and LA were the most effective in reducing *E. coli* O157:H7 in subsections A and B, respectively (Table 3); however, ASC was the most effective in these subsections when applied on day 21 (Table 3). When the sprays were applied on day 14 and 21 before EN, internal *Salmonella* counts were reduced significantly from the internal subsections with the use of all the interventions (Table 4).

Results from this study indicate that the effectiveness of the interventions varied with different degrees of efficacy depending on the process (MT or EN), the day in which the intervention is applied, or the sampling location within the piece. In general, *Salmonella* and *E. coli* O157:H7–inoculated samples subjected to mechanical tenderization and treated with LAB and LA presented the lowest internal pathogen counts (subsection B) when compared with the CTRL. Similarly, those pieces subjected to brine enhancement presented the lowest transfer (subsection B) when treated with ASC and LAB (in the case of *E. coli* O157:H7) and LA and ASC (in the case of *Salmonella*). In this experiment, a low-level inoculation study (approximately 2 log CFU/g) was also conducted to mimic what would actually be encountered in the industry, and treated similarly as was the data presented here (data not shown). On some occasions, the internal subsections (A and B) were positive for the pathogens, but at the same time, the top, containing the original inoculated surface, was not (data not shown). It was expected that at low inoculation levels, if the pathogen was not detected on the top because of the interventions, then it would be absent from the internal tissues; however, this was not always the case. The spray system might not cover all the meat’s surface leaving areas untreated and where the microorganisms could survive. Still, one needs to be cautious about negative results for the presence of the pathogens in the internal tissues of the meat, as a lack of detection does not necessarily mean that the pathogens were not transferred into the internal parts of the subprimal, as only small samples per subsection were analyzed and not the entire piece. Because of these results, any additional interventions that can be applied by purveyors will increase the safety of their products. Additionally, establishments that provide cooked, mechanically tenderized–injected beef might need to consider if a warning label or an increase in the minimum cooking temperature should be required, as they are not distinguishable from other intact beef products.

The effects of LAB, ASC, and LA on the sensory characteristics and quality attributes of steaks subjected to brine enhancement or mechanical tenderization have been obtained in further studies at Texas Tech University (13).

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