Effects of Plant-Derived Extracts, Other Antimicrobials, and Their Combinations against *Escherichia coli* O157:H7 in Beef Systems

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ABSTRACT

The antimicrobial effects of thyme oil (TO), grapefruit seed extract (GSE), and basil essential oil, alone or in combination with cetylpyridinium chloride (CPC), sodium diacetate, or lactic acid, were evaluated against *Escherichia coli* O157:H7 in a moisture-enhanced beef model system. The model system was composed of a nonsterile beef homogenate to which NaCl (0.5%) and sodium tripolyphosphate (0.25%) were added, together with the tested antimicrobial ingredients. Beef homogenate treatments were inoculated (ca. 3 log CFU/ml) with rifampin-resistant *E. coli* O157:H7 (eight-strain mixture) and incubated at 15°C (48 h). The most effective individual treatments were TO (0.25 or 0.5%) and GSE (0.5 or 1.0%), which immediately reduced (P < 0.05) pathogen levels by ≥3.4 log CFU/ml. Additionally, CPC (0.04%) reduced initial *E. coli* O157:H7 counts by 2.7 log CFU/ml. Most combinations of the tested plant-derived extracts with CPC (0.02 or 0.04%) and sodium diacetate (0.25%) had an additive effect with respect to antibacterial activity. In a second study, antimicrobial interventions were evaluated for their efficacy in reducing surface contamination of *E. coli* O157:H7 on beef cuts and to determine the effect of these surface treatments on subsequent internalization of the pathogen during blade tenderization. Beef cuts (10 by 8 by 3.5 cm) were inoculated (ca. 4 log CFU/ml) with rifampin-resistant *E. coli* O157:H7 strain mixture and were then spray treated (20 lb/in², 10 s) with water, GSE (5 and 10%), lactic acid (5%), or CPC (5%). Untreated (control) and spray-treated surfaces were then subjected to double-pass blade tenderization. Surface contamination (4.4 log CFU/g) of *E. coli* O157:H7 was reduced (P < 0.05) to 3.4 (5% CPC) to 4.1 (water or 5% GSE) log CFU/g following spray treatment. The highest and lowest transfer rates of pathogen cells from the surface to deeper tissues of blade-tenderized sections were obtained in the untreated control and CPC-treated samples, respectively.

Nonintact beef products are considered high risk for pathogens such as *Escherichia coli* O157:H7 because of the possibility of transfer of cells from the meat surface to the deep muscle tissues through injection of moisture enhancement solutions, mechanical tenderization, marination, or restructuring (8, 9, 27). Recently, there have been *E. coli* O157:H7 outbreaks associated with consumption of nonintact beef (16, 32). The meat industry is, therefore, in need of new and/or better antimicrobial treatments for pathogen control in nonintact beef products.

Essential oils are known to inhibit growth of spoilage microorganisms and have antimicrobial properties against pathogenic bacteria (26). Extracts derived from herbs, such as thyme and basil, are known to have antimicrobial activity (26), whereas grapefruit seed extract (GSE) has been reported to have antibacterial and antiviral properties (15). However, practical use of plant extracts in meat products might be restricted due to their impact on sensory qualities. In general, when an essential oil is added to foods, a higher concentration than that used in vitro is required to achieve an antibacterial effect (3, 14). However, use of essential oils in combination with other antimicrobial compounds may allow for antimicrobial effects to be achieved at lower concentrations of the essential oils, thus minimizing adverse effects to the organoleptic acceptability of treated meat products (22). For example, combinations of lactic acid (LA) or sodium diacetate (SD) with essential oils may exhibit synergistic antimicrobial effects, because lower pH conditions may make essential oils more hydrophobic, which can increase their penetration into cell membranes and improve their binding on lipoproteins (10).

Inclusion of antimicrobials in brining solutions used for moisture enhancement of beef products or application of antimicrobial interventions to the surface of beef subprimal cuts prior to mechanical tenderization may potentially reduce the risk of transfer of pathogen cells into the interior of muscle tissues. Thus, a study was performed to evaluate the antimicrobial effects of plant-derived compounds (i.e., thyme oil [TO], GSE, and basil essential oil [BO]), alone or in combination with cetylpyridinium chloride (CPC), SD, or LA, against *E. coli* O157:H7 in a nonsterile beef homogenate containing salt and phosphate (i.e., a model system simulating moisture-enhanced beef products). Use of...
E. coli

This model system allowed for screening of a larger number of antimicrobial treatments than would have been possible if actual meat cuts had been subjected to moisture enhancement. In a second study, individual treatments of GSA, LA, and CPC were evaluated for their efficacy in reducing surface contamination of E. coli O157:H7 on beef cuts, and the effect of these surface treatments on subsequent internalization of the pathogen during double-pass blade tenderization was determined.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. Eight rifampin-resistant strains of E. coli O157:H7, including American Type Culture Collection strains (ATCC; Rockville, MD) ATCC 43888 (human isolate), ATCC 43895 (raw hamburger meat isolate implicated in a hemorrhagic colitis outbreak), ATCC 43895/ISEHGGP (21), and five strains isolated from cattle feces (C1-057, C1-072, C1-109, C1-154, and C1-158) (25), were separately cultured and subcultured at 35°C for 24 h in 10 ml of tryptic soy broth (Difco, BD, Sparks, MD) plus rifampin (100 μg/ml; Sigma-Aldrich, St. Louis, MO). The strains were subsequently combined, centrifuged at 4,629 × g for 15 min at 4°C (Eppendorf model 5810 R, Brinkmann Instruments Inc., Westbury, NY), and washed with phosphate-buffered saline (PBS; pH 7.4, 0.2 g/liter KH₂PO₄, 1.5 g/liter Na₂HPO₄, 7H₂O, 8.0 g/liter NaCl, and 0.2 g/liter KCl). Then, cell pellets were resuspended and diluted to 5 or 7 log CFU/ml in PBS.

Preparation and inoculation of model system simulating moisture-enhanced beef products. The model system was composed of a nonsterile beef homogenate to which two commonly used ingredients of meat enhancement solutions, namely, NaCl and sodium tripolyphosphate (STP), were added. As previously indicated, the decision to use the model system was based on the large number of treatments that were selected for evaluation. Furthermore, using a nonsterile beef homogenate, with its associated natural meat microflora, was considered a more appropriate substrate than using a sterile broth culture system, which is frequently used for screening studies similar to the one reported here.

Beef knuckles (95% lean) were used for preparation of the moisture-enhanced beef product model system. The beef knuckles were obtained from a local beef slaughter facility (northern Colorado) and were stored vacuum-packaged at −23°C until needed. The meat was thawed at 4°C for 24 h before use. After removing excess fat, the beef knuckles were cut into smaller pieces to facilitate blending. Beef knuckle portions (100 g) were blended (Waring Commercial Laboratory Blender 7012G, Waring Commercial, Torrington, CT) for 90 s with 200 ml of sterile distilled water, and the resulting homogenate was filtered through cheesecloth. Filter-sterilized solutions of NaCl and STP (kindly provided by BK Giulini Corp., Simi Valley, CA) were added to the beef homogenate to achieve 0.5 and 0.25% final concentrations, respectively. After thorough mixing, 10-ml aliquots of the beef homogenate plus NaCl plus STP mixture were aseptically dispensed into sterile test tubes, and the tested antimicrobials were added. A total of 44 antimicrobial treatments (Figs. 1 through 6), including a control (no antimicrobial) and individual and combination treatments of TO (Now Foods, Bloomingdale, IL), GSE (NutriBiotic, Lakeport, CA), BO (WFMED, Burke, VA), CPC (Safe Foods Corporation, North Little Rock, AR), SD (Niacet Corporation, Niagara Falls, NY), and LA (PURAC America Inc., Lincolnshire, IL), were tested for their effects against E. coli O157:H7. Selection of CPC, SD, and LA was based on previous work conducted in our laboratory (1, 25). Note that CPC is approved for use only on poultry carcasses and parts in the United States (33); however, based on previous reports (5, 17, 23, 24, 25, 29) of its antimicrobial activity against foodborne pathogens, including E. coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes, on beef products it was decided to include this antimicrobial in the present study.

All beef homogenate samples were inoculated with 0.1 ml of the prepared 5 log CFU/ml E. coli O157:H7 inoculum mixture to achieve a target inoculation level of approximately 3 log CFU/ml.

FIGURE 1. Mean (log CFU/ml, n = 4) rifampin-resistant E. coli O157:H7 and aerobic plate counts in a beef homogenate containing 0.5% NaCl, 0.25% sodium tripolyphosphate, and different concentrations of thyme oil (TO). The beef homogenate treatments were inoculated with rifampin-resistant E. coli O157:H7 (eight-strain mixture; approximately 3 log CFU/ml) and incubated at 15°C for 48 h. Detection limit: 0.0 log CFU/ml.

FIGURE 2. Mean (log CFU/ml, n = 4) rifampin-resistant E. coli O157:H7 and aerobic plate counts in a beef homogenate containing 0.5% NaCl, 0.25% sodium tripolyphosphate, and different concentrations of grapefruit seed extract (GSE). The beef homogenate treatments were inoculated with rifampin-resistant E. coli O157:H7 (eight-strain mixture; approximately 3 log CFU/ml) and incubated at 15°C for 48 h. Detection limit: 0.0 log CFU/ml.
All samples were incubated at 15°C for 48 h. Note that under normal circumstances moisture-enhanced beef products would not be stored at 15°C; however, for purposes of this study, an incubation temperature at which growth of *E. coli* O157:H7 would be expected was chosen.

Microbiological and pH analyses of beef homogenate samples. Immediately after inoculation (0 h) and after 48 h of incubation at 15°C, beef homogenate samples were diluted in 0.1% buffered peptone water (Difco) and appropriate dilutions were surface plated on tryptic soy agar (TSA; Difco) for enumeration of aerobic bacterial populations and on TSA supplemented with rifampin (100 μg/ml; TSA rif) for enumeration of inoculated *E. coli* O157:H7. Plates were incubated at 35°C for 48 h. The detection limit of the analysis was 1 CFU/ml (0.0 log CFU/ml). After microbial analysis of 0-h samples, pH measurements of the beef homogenates were taken with a digital pH meter fitted with a glass electrode (Denver Instruments, Arvada, CO).

Inoculation, surface treatment, and blade tenderization of beef. For this study, beef eye of round samples obtained from a commercial beef processing facility were cut into portions (10 by 8 by 3.5 cm), vacuum-packaged, and stored at 22°C. Prior to each experiment, frozen round pieces were thawed at 4°C for 18 to 24 h. The beef portions were placed on sterile trays and were inoculated on one of the two flat surfaces with 0.1 ml of the prepared 7 log CFU/ml *E. coli* O157:H7 inoculum mixture. The target pathogen inoculation level was approximately 4 log CFU/g. The inoculum was spread over the meat surface with a sterile glass spreader. Following a 30-min cell attachment period at 4°C, inoculated samples were either left untreated (control) or were placed on a sterile wire mesh surface, with the inoculated surface facing up. Next, samples were sprayed with sterile distilled water, 5% GSE, 10% GSE, 5% CPC, or 5% LA. The spray treatments were applied with a handheld sprayer (RL FLO-Master, Root-Lowell Manufacturing, Lowell, MI) for 10 s at an approximate pressure of 20 lb/in² and from a distance of approximately 15 cm so as to obtain complete coverage of the meat surface with little run-off. After spraying, meat samples were removed from the wire.
bacterial populations were converted to log CFU per milliliter (beef homogenate study) or log CFU per gram (blade tenderization study) before statistical analysis. The data were analyzed (analysis of variance test using the JMP SAS program [version 7.0.2, SAS Institute, Cary, NC]) to determine whether there were significant differences (P < 0.05) between E. coli O157:H7 and aerobic bacterial counts in response to the tested antimicrobial treatments evaluated in the moisture-enhanced beef product model system and whether there were significant differences (P < 0.05) in recovered populations (E. coli O157:H7 and aerobic microflora) from sections A, B, and C of blade-tenderized beef cuts that received a surface antimicrobial spray treatment prior to tenderization.

RESULTS AND DISCUSSION

Antimicrobial effects of various ingredients in a moisture-enhanced beef product model system. Initial (0 h) rifampin-resistant E. coli O157:H7 counts of the inoculated control (i.e., beef homogenate plus NaCl plus STP) were 3.3 ± 0.1 log CFU/ml, and after 48 h at 15°C, pathogen levels were 5.0 ± 0.6 log CFU/ml (Figs. 1 through 6). Corresponding aerobic bacterial populations increased from an initial level of 4.1 ± 0.6 to 8.0 ± 0.4 log CFU/ml following the incubation period. Uninoculated beef homogenate underwent microbial analysis in a preliminary study and was found not to contain (1 CFU/ml [0.0 log CFU/ml] detection limit) any naturally present rifampin-resistant populations on TSA + rif (data not shown).

Beef homogenate samples containing TO at 0.25 and 0.5% showed immediate (0 h) and sustained (up to 48 h at 15°C) bactericidal effects (P < 0.05) against E. coli O157:H7, and counts were below the detection limit (<0.0 log CFU/ml) in all samples (Fig. 1). At the lower concentrations of 0.01, 0.05, and 0.1% TO, initial E. coli O157:H7 counts of 3.2, 3.3, and 3.0 log CFU/ml increased to 5.5, 4.3, and 3.6 log CFU/ml, respectively, in stored samples. Overall, compared with the control, growth of aerobic bacterial populations was only inhibited (P < 0.05) in samples containing 0.25 and 0.5% TO (Fig. 1). In general, essential oils composed of carvacrol and/or its isomer, thymol, have been reported to have strong antimicrobial properties (3, 14, 20). Such components damage the outer membrane of gram-negative bacteria and increase the permeability of the cytoplasmic membrane, leading to ATP leakage or dissipation of the proton motive force (7, 14, 31).

GSE at =0.25% did not (P ≥ 0.05) exhibit any antimicrobial activity against E. coli O157:H7 or the aerobic bacterial flora; whereas, at the higher tested concentrations of 0.5 and 1.0%, pathogen counts were undetectable (<0.0 log CFU/ml) in samples analyzed at 0 h as well as after 48 h (Fig. 2). Initial reductions and growth inhibition of aerobic bacterial populations were also observed in samples containing 0.5 and 1.0% GSE. GSE has been reported to possess antibacterial, antiviral, antifungal, and antiparasitic properties, and its activity is attributed to its polyphenolic compounds (15). Additionally, GSE is known to prevent activation of bacterial enzymes and to weaken the bacterial cell wall and cell membranes (6).
No immediate bactericidal effects against *E. coli* O157:H7 were obtained by any of the tested concentrations of BO; however, at 0.1 and 0.25%, this essential oil effectively (*P* < 0.05) inhibited pathogen growth at 15°C (Fig. 3). Furthermore, at the higher concentration of 0.5%, pathogen levels during incubation were reduced from 3.2 (0 h) to 1.8 (48 h) log CFU/ml. Essential oils extracted from *Ocimum* species have been reported to have high antimicrobial activity against gram-negative bacteria (30). Compared with the antimicrobial activities of the individual TO and GSE treatments tested in the current study, BO had slightly lower antibacterial effects against the pathogen, possibly due to the absence of phenols (11) and the low solubility of BO. The chemical components of plant-derived extracts are known to be dependent on a number of factors, including plant cultivar, climate and geographical origin of the plant, harvesting season, and extraction method (3, 30). Thus, comparison of our results for TO, GSE, and BO with those of previously published data is difficult.

Initial *E. coli* O157:H7 and aerobic plate counts of the 0.02% CPC treatment, alone or in combination with all the tested concentrations of TO, GSE, and BO, were not different (*P* ≥ 0.05) from the corresponding initial counts of the control (Fig. 4). Pathogen growth was, however, inhibited at 15°C in all the combination treatments, and counts were 1.2 (0.02% CPC plus 0.25% GSE) to 2.6 (0.02% CPC plus 0.1% TO) log CFU/ml lower (*P* < 0.05) than pathogen levels of the control treatment after 48 h. CPC at 0.04%, alone and in combination with BO (0.25 and 0.5%), reduced (*P* < 0.05) initial pathogen counts by 2.7 to 3.2 log CFU/ml, whereas all remaining tested combination treatments reduced (*P* < 0.05) initial *E. coli* O157:H7 counts to below the detection limit (Fig. 4). Furthermore, undetectable levels of the pathogen were obtained in all the 0.04% CPC–containing samples incubated for 48 h. Immediate bactericidal effects and growth inhibition of aerobic bacterial populations were also noted in all the tested 0.04% CPC–containing treatments. In a previous study conducted in our laboratory (1), addition of 5.5% CPC to a brine solution, composed of 5.5% NaCl plus 2.75% STP, immediately reduced *E. coli* O157:H7 levels from 3.7 to <1.3 log CFU/ml. CPC is a quaternary ammonium compound, and its antimicrobial activity is attributed mainly to electrostatic interactions. Positively charged cetylpyridinium ions favor the negative charges of bacterial cell wall constituents, which subsequently allow CPC to leak cellular components and damage bacterial membranes (5).

The combination treatments composed of SD (0.25%) and TO (0.25 and 0.5%) or GSE (0.5 and 1.0%) immediately reduced (*P* < 0.05) initial *E. coli* O157:H7 counts to below the detection limit, and the pathogen was not detected in these treatments after 48 h (Fig. 5). Overall, the individual SD treatment and remaining SD combination treatments did not have initial bactericidal effects against the pathogen; however, pathogen growth was inhibited in all these treatments. More specifically, pathogen counts of incubated samples were 1.7 (SD) to 3.3 (SD plus 0.5% BO) log CFU/ml lower than those of the NaCl plus STP control, SD on its own and all its combinations with TO, GSE, and BO also effectively (*P* < 0.05) suppressed growth of the aerobic bacterial flora as compared with the control (Fig. 5). SD is a generally recognized as safe substance used in foods for pH control, flavoring, and antimicrobial properties (2).

In general, the individual and combination treatments of LA (0.3%) evaluated did not (*P* ≥ 0.05) affect the initial *E. coli* O157:H7 and aerobic plate counts (Fig. 6). Inhibition of growth compared with the control was, however, noted for all the LA-containing treatments. Pathogen counts in samples incubated at 15°C were 1.8 (LA) to 2.8 (LA plus 0.1% TO) log CFU/ml lower (*P* < 0.05) than those of the control treatment. Unlike SD, 0.3% LA did not have an additive antimicrobial effect when combined with the plant extracts. LA is one of the most widely utilized antimicrobials for decontamination of beef in the United States and is also used for improving food quality (13, 25).

The pH of the control (beef homogenate plus NaCl plus STP) samples at 0 h was 5.96 pH units, and similar pH values (pH 5.76 to 6.05) were obtained for TO (0.01 to 0.1%), GSE (0.01 to 0.25%), and all 0.02% CPC–containing treatments (data not shown). Addition of ≥0.25% TO, ≥0.5% GSE, or 0.5% BO to the beef homogenate mixture increased the pH to 6.35 to 6.44. Similarly, pH values ranged from 6.35 to 6.44 in all treatments containing 0.04% CPC. LA-containing treatments, on the other hand, had much lower pH values (pH 4.49 to 4.55) than the control, and the pH range of the SD-containing samples was 5.25 (SD plus 0.1% BO) to 5.79 (SD plus 0.5% BO).

Overall, this study showed that individual treatments of TO (0.25 and 0.5%) and GSE (0.5 and 1.0%) effectively reduced initial inoculated levels of *E. coli* O157:H7 by ≥3.4 log CFU/ml in the model system simulating moisture-enhanced beef. Moreover, most combinations of the tested plant-derived extracts with CPC and SD had an additive effect with respect to antibacterial activity.

**Effect of antimicrobial spray treatments on surface contamination levels and subsequent cell internalization in blade-tenderized beef.** Only individual antimicrobial treatments were evaluated in this study. The concentrations of the tested antimicrobials were 5 and 10% GSE, 5% CPC, and 5% LA. Preliminary studies indicated that higher antimicrobial concentrations than those tested in the beef homogenate study were needed for spray treatment of inoculated beef pieces (data not shown). In general, cells attached to the surface of meat are more resistant to antimicrobials than those in suspension because they may be protected by muscle tissue (29). Moreover, interactions between plant extract constituents and food matrix components, such as protein and fat, have been reported to result in lower potency of essential oils on food products than in vitro (10, 14). Although strong bactericidal activity against *E. coli* O157:H7 was obtained with TO in the beef homogenate study (Fig. 1), this essential oil was not selected for surface treatment of beef portions because of the strong odor that was noted in samples containing the effective concentrations of TO (0.25 and 0.5%). The odor would have been even more undesirable at the higher concentrations needed to
elicit antimicrobial effects on the beef tissue surface, and for this reason, TO was excluded from this particular study.

The recovered level of *E. coli* O157:H7 in the topmost 0.2 cm (section A) of inoculated untreated (control) beef samples after blade tenderization was 4.4 log CFU/g and was assumed to be the same as the initial inoculated population (Table 1). Pathogen counts recovered from the corresponding section of beef portions that were spray treated with water, 5% GSE, 10% GSE, LA, or CPC were 0.3, 0.3, 0.5, 0.6, and 1.0 log CFU/g lower (*P* < 0.05), respectively, than that of the untreated control. When comparing pathogen cell recoveries of the antimicrobial-treated samples with those of water-treated samples, however, significantly (*P* < 0.05) lower counts were only obtained for the CPC treatment. CPC has not been approved at this time for decontamination of beef (33); however, a number of studies (5, 17, 24, 25, 29) have reported on its efficacy against *E. coli* O157:H7 on beef products. Spray treatment of inoculated lean and adipose beef carcase tissue with 1% CPC resulted in *E. coli* O157:H7 reductions of ≥6.4 and 4.9 log CFU/cm², respectively (5). In another study, application of 0.5% CPC during spray chilling (30-min intervals, for 10 h) of beef carcase tissue effectively reduced *E. coli* O157:H7 levels by more than 5 log CFU/cm² (29).

In meat sections B and C, analyzed to determine the extent of internalization of surface contamination during blade tenderization, recovered levels of *E. coli* O157:H7 in the untreated control samples were 3.3 and 2.8 log CFU/g, respectively (Table 1). Pathogen levels recovered from sections B and C of all spray-treated samples, except those treated with CPC, were similar (*P* ≥ 0.05) to those of the untreated control. Pathogen counts of CPC-treated samples were 0.5 (section B) and 0.6 (section C) log CFU/g lower (*P* < 0.05) than those of corresponding sections of the control. Therefore, of all the antimicrobial spray treatments evaluated, CPC was the most effective in reducing *E. coli* O157:H7 on the beef tissue surface, and compared with the untreated control, it was the only treatment that had significantly (*P* < 0.05) lower pathogen cell recovery from the deeper tissues of the beef cuts. GSE, which was found to be very effective at much lower concentrations (0.5 and 1.0%) in the moisture-enhanced beef model system, was ineffective (*P* ≥ 0.05) against *E. coli* O157:H7, compared with the water treatment, when applied to the surface of the pieces of eye of round. Under the conditions of this study, 5% LA was also ineffective (*P* ≥ 0.05) compared with the water treatment.

Irrespective of surface antimicrobial treatment, transfer rates of *E. coli* O157:H7 were higher in section B samples than in section C samples (Table 1). Untreated control samples had the highest transfer rates of the pathogen into sections B and C (30.2 and 13.6%, respectively), and CPC-treated samples had the lowest (7.8 and 2.2%, respectively). Pathogen cell transfer rates for the remaining treatments ranged from 11.8% (10% GSE) to 19.4% (5% GSE) in section B samples and from 3.6% (10% GSE) to 6.7% (water) in section C samples. Note that transfer rates were highly variable in some instances, as indicated by the large standard deviations shown in Table 1. However, despite the

**TABLE 1. Recovered levels and translocation of rifampin-resistant *E. coli* O157:H7 and aerobic bacterial flora in three excised sections obtained from beef eye of round portions subjected to different surface spray treatments, followed by double-pass blade tenderization**

<table>
<thead>
<tr>
<th>Bacterial population</th>
<th>Section</th>
<th>Recovered</th>
<th>% Transfer</th>
<th>% Log CFU/g</th>
<th>% Log CFU/g</th>
<th>%Log CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>A</td>
<td>4.1 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td>3.4 ± 0.2A</td>
<td>3.4 ± 0.3A</td>
<td>3.4 ± 0.3A</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3.1 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>2.8 ± 0.3A</td>
<td>2.8 ± 0.3A</td>
<td>2.8 ± 0.3A</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.1 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td>3.4 ± 0.2A</td>
<td>3.4 ± 0.3A</td>
<td>3.4 ± 0.3A</td>
</tr>
<tr>
<td>Aerobic bacterial flora</td>
<td>A</td>
<td>3.3 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
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<tr>
<td></td>
<td>B</td>
<td>4.1 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td>3.4 ± 0.3A</td>
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*Log CFU/g values, within each column, followed by different letters are significantly different (*P* < 0.05).
large standard deviations, transfer rates for all samples that received a spray treatment were lower than those of the untreated control. Overall, similar trends with respect to recovered populations and transfer rates were obtained for the aerobic bacterial flora (Table 1).

Deep tissue microbial contamination as a result of mechanical blade tenderization has been reported by a number of investigators (4, 8, 9, 12, 18, 19, 28). In agreement with our findings, Luchansky et al. (18, 19) reported that pathogen cell transfer rates were highest in the topmost 1 cm of beef subprimal tissues subjected to blade tenderization. The degree of translocation of microorganisms into deep tissues may vary depending on the type of tenderizing equipment and the level of surface contamination (8), as well as on the number of blades, blade tenderization passes, and thickness of the beef cut. In our study, beef portions were only 3.5 cm thick and they were subjected to double-pass tenderization; this possibly explains the high pathogen transfer rates observed.

In summary, under the conditions of the described studies, immediate E. coli O157:H7 reductions of \( \geq 3.4 \) log CFU/ml were obtained by the addition of TO (0.25 and 0.5%) or GSE (0.5 and 1.0%) to a moisture-enhanced beef model system. Additionally, 0.04% CPC reduced inoculated pathogen levels by 2.7 log CFU/ml. When TO, GSE, or BO were combined with CPC (0.02 or 0.04%) or SD (0.25%), additive antibacterial effects were obtained in the beef homogenate. Beef surface spray treatments composed of 10% GSE, 5% LA, or 5% CPC lowered \( (P < 0.05) \) surface contamination of inoculated (4.4 log CFU/g) E. coli O157:H7 by 0.5, 0.6, and 1.0 log CFU/g, respectively. Furthermore, transfer rates of pathogen cells, from the surface to deeper tissues of blade-tenderized samples, were lower in samples that received a surface spray treatment than in those that did not. Further studies are needed to determine factors that contribute to the internalization and survival of E. coli O157:H7 in deep tissues of nonintact beef products.

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