*Journal of Food Protection, Vol. 72, No. 1, 2009, Pages 151–156*

## Research Note

# Effectiveness of 1,3-Dibromo-5,5 Dimethylhydantoin on Reduction of *Escherichia coli* O157:H7– and *Salmonella*-Inoculated Fresh Meat†

**NORASAK KALCHAYANAND,* TERRANCE M. ARTHUR, JOSEPH M. BOSILEVAC, DAYNA M. BRICHTA-HARHAY, MICHAEL N. GUERINI, STEVEN D. SHACKELFORD, TOMMY L. WHEELER, AND MOHAMMAD KOOHMARAIE‡**

*U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA*

MS 08-257: Received 4 June 2008/Accepted 26 August 2008

## ABSTRACT

1,3-Dibromo-5,5-dimethylhydantoin (DBDMH; 25°C) and hot water (85°C) spray treatments were evaluated for efficacy in decontamination of pathogenic bacteria attached to beef carcass surfaces represented by cutaneous trunci (CT) muscle sections and beef hearts. Treatments were evaluated using two different systems, a commercial carcass wash cabinet and a model carcass washer. The effects were measured immediately after treatment and again after 48 h of storage at 4°C. Sections of CT and beef hearts were inoculated with bovine fecal solution containing approximately 6 log CFU/cm$^2$ of *Escherichia coli* O157:H7 and *Salmonella.* After DBDMH or hot water spray treatments, bacterial populations were enumerated immediately and after storage for 48 h at 4°C. DBDMH treatments reduced aerobic plate counts, *Enterobacteriaceae, E. coli* O157:H7, and *Salmonella* by the same or slightly lower amounts relative to hot water treatment. DBDMH reduced aerobic plate counts and *Enterobacteriaceae* by 2.8 to 3.6 log CFU/cm$^2$, *E. coli* O157:H7 by 1.6 to 2.1 log CFU/cm$^2$, and *Salmonella* by 0.7 to 2.3 log CFU/cm$^2$ on CT sections and beef hearts. Hot water treatment reduced aerobic plate counts and *Enterobacteriaceae* by 3.0 to 4.1 log CFU/cm$^2$, *E. coli* O157:H7 by 1.8 to 2.3 log CFU/cm$^2$, and *Salmonella* by 2.5 to 2.8 log CFU/cm$^2$. After 48 h of storage, the reductions of organisms by DBDMH and hot water treatments were not different. This study demonstrated that DBDMH spray washing could be effective as an antimicrobial intervention for beef carcasses and variety meats.

Antimicrobial interventions are a crucial step for food industries to ensure that their products are safe before reaching consumers. Hot water treatment has been found to be effective against pathogens as well as spoilage bacteria *(2, 7, 8, 9, 11, 16, 21),* whereas the use of chlorinated water has shown little or no effect *(6, 12, 24).* The disadvantage of hot water treatment is the high-water-volume use and high cost of maintaining such a high temperature. 1,3-Dibromo-5,5-dimethylhydantoin (DBDMH; $C_5H_6Br_2N_2O_2$), or Bromitize, and other organohalamine derivatives have been widely used as disinfectants for water treatment and for treating industrial or commercial water-cooling systems *(20, 22, 23).* These halogenated hydantoin derivatives have shown considerable efficacy against several species of microorganisms *(13, 15, 26).* In aqueous solution, DBDMH hydrolyzes to hypobromous acid, an active antimicrobial agent, and dimethylhydantoin *(22).* In the poultry industry, DBDMH at a level of 100 ppm has been approved for use as an antimicrobial in chiller water during processing *(25).* Although the use of DBDMH to decontaminate beef carcasses or variety meats has been recently approved, the use of bromine compounds for decontamination of red meat is very limited. The objective of this study was to evaluate the efficacy of DBDMH as an antimicrobial intervention in reducing aerobic bacteria, *Enterobacteriaceae, Escherichia coli* O157:H7, and *Salmonella* on inoculated fresh meat surfaces.

## MATERIALS AND METHODS

**Bacterial cultures.** *E. coli* O157:H7 ATCC 43888 and ATCC 43895 were obtained from the American Type Culture Collection (Manassas, VA). *E. coli* O157:H7 FSIS EL50179, *E. coli* O157:H7 CO50, *E. coli* O157:H7 SSNE1040, *Salmonella* Newport 13324 POH2, *Salmonella* Newport 644AB2, *Salmonella* Typhimurium 11241 PRB1, and *Salmonella* Typhimurium 14218 PRH2 were obtained from the U.S. Meat Animal Research Center (USMARC; Clay Center, NE) culture collection. All strains were maintained in 25% glycerol at −70°C and were propagated individually in tryptic soy broth without dextrose, supplemented with 0.6% yeast extract (TSBY; Difco, Becton Dickinson, Sparks, MD) at 37°C for 18 h.

**Feces screening and preparation of fecal solution.** Fresh bovine fecal samples (10 g) were obtained from cattle at the USMARC feedlot and then screened for the prevalence of *E. coli*

* Author for correspondence. Tel: 402-762-4224; Fax: 402-762-4149; E-mail: norasak.kalchayanand@ars.usda.gov.

† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

‡ Present address: IEH Laboratories & Consulting Group, 15300 Bothell Way N.E., Lake Forest Park, WA 98155, USA.

O157:H7 and *Salmonella* by methods described previously *(1, 14).* Only fecal samples that did not have both pathogens were combined and frozen at −20°C. The fecal solutions used for inoculation were prepared by thawing the screened feces in a refrigerator overnight. Two grams of feces was placed in a filtered stomacher bag (Whirl-Pak, Nasco, Ft. Atkinson, WI), mixed with 98 ml of phosphate buffered tryptic soy broth (Difco, Becton Dickinson), and hand massaged thoroughly.

**Preparation of inoculums.** All bacterial strains were grown in TSBY for 18 h at 37°C. For experiments utilizing the carcass wash cabinet, strain 43888 was used as it does not produce Shiga toxins *(5),* and the wash cabinet did not have containment facilities to prevent environmental release of pathogens. This *E. coli* culture was diluted 10-fold with fecal solution to achieve a final concentration of approximately $5 \times 10^8$ CFU/ml. For the experiments conducted in the model carcass washer installed in a biological containment hood, pathogenic bacteria were used. A four-strain mixture of pathogenic *E. coli* O157:H7 and a four-strain mixture of *Salmonella* were prepared separately by combining 1 ml of each strain into two 10-ml conical tubes. A cocktail mixture containing a combination of four strains of *E. coli* O157:H7 and four strains of *Salmonella* was then prepared by combining the *E. coli* O157:H7 and *Salmonella* mixtures in a 2:1 ratio. This ratio was empirically predetermined to ensure that nearly equal amounts of both pathogens were in the mixture. The cocktail was then diluted 10-fold with fecal solution to the concentration of approximately $5 \times 10^8$ CFU/ml. All inoculums were maintained on ice until used for inoculation onto fresh meats.

**Fresh meat tissue preparation and inoculation.** Fresh cutaneous trunci (CT) muscle and beef hearts from pre-rigor carcasses were obtained during online processing from a commercial processing plant, packed in insulated containers, and transported to the USMARC laboratory. Approximately 30 to 120 min elapsed between collection and use in the laboratory. Two replications were conducted, and each replication was composed of 24 CT sections and 48 beef hearts. For each CT section, 4 squares of 100 $cm^2$ were marked with edible ink and a template (10 by 10 cm). Each 100-$cm^2$ square was then divided into 4 25-$cm^2$ squares for a total of 16 25-$cm^2$ areas for each treatment. The hearts were cut lengthwise in half, and 2 100-$cm^2$ outlines on the outside surface of each half were marked and divided into 4 25-$cm^2$ areas for each heart. Therefore, two hearts were used to provide 16 25-$cm^2$ areas for each intervention treatment. A set of 12 CT sections and 24 beef hearts was inoculated with *E. coli* O157:H7 43888, and another set was inoculated with the pathogenic cocktail mixture. Two milliliters of each inoculum was applied over the marked areas (4 100-$cm^2$ areas) with either a disposable cell spreader or the back of a sterile plastic spoon. The inoculated CT sections and beef hearts were allowed to stand at room temperature for 15 min to allow bacterial attachment before applying any treatment.

**Spray washing cabinets and washing procedures.** Two spray wash cabinets were used to apply hot water or DBDMH in this study. A carcass wash cabinet was used for the *E. coli* O157:H7 43888 inoculation. The cabinet was a top half of a standard commercial beef carcass wash cabinet (Chad Co., Olathe, KS). Four nozzles (SS2510, Spraying Systems Co., Wheaton, IL) were positioned to spray from a 30° angle onto the tissue sample and were oscillated at 80 cycles per min, giving an up-and-down sweeping motion of the flat spray over the tissue sample. For the pathogenic-bacteria inoculation, a model carcass washer installed in a biological containment hood was used to apply treatments. Three spray nozzles (SS2510; Spraying Systems) were oscillated at 60 cycles per minute. For both cabinets, all of the CT sections were spray washed either with hot water (85°C at nozzles) at 20 lb/$in^2$, with a flow rate of 6.8 liters (1.8 gal) per min or DBDMH (25 ± 2°C) for 12 s at 35 lb/$in^2$, while all of the beef hearts were spray washed for 28 s. The 12- and 28-s exposure times were based on carcass chain speed (300 head per hour) and the variety meat chain speed of a typical processing plant, respectively. The spraying pressure was based on the pressure for hot water and organic acid currently used in beef cattle processing plants. The pressure for hot water was 20 lb/$in^2$ in order to maintain the water temperature at 85°C. A higher pressure causes smaller droplets, which exchange temperature rapidly with the environment. The concentration of DBDMH (75, 175, or 270 ppm; Solution BioSciences, Inc., Chatham, NJ) was determined with a bromine pocket colorimeter II test kit (Hach, Loveland, CO). With the carcass wash cabinet, each DBDMH concentration was applied through a vertical spray line via two fixed nozzles (SS9530, Spraying Systems) that delivered 9.8 liters (2.6 gal) per min at 35 lb/$in^2$. With the model carcass washer, beef hearts and CT sections inoculated with a cocktail mixture of pathogens were sprayed with DBDMH at 35 lb/$in^2$, with a flow rate of 7.6 liters (2.0 gal) per min. The pH values for DBDMH were 6.84, 6.76, and 6.71 for 75, 175, and 270 ppm, respectively. For all treatments, the excess liquid was allowed to drip off for 30 s before sampling for microbiological analyses.

**Microbiological and statistical analyses.** Samples from both tissues were collected before (controls) and after applying treatments. To avoid sampling bias, the four 25-$cm^2$ sections in each inoculation area were rotated clockwise during each collection. Before treatment, one of the four 25-$cm^2$ sections was aseptically excised and placed into a sterile filtered Whirl-Pak bag. After treatments, two additional 25-$cm^2$ sections were excised. One section was used for microbiological analyses on the same day. The other section was stored at 4°C for 48 h to determine the residual effect of the treatments. The time between spray treatment and microbiological analyses was approximately 15 min. A 50-ml aliquot of Dey-Engley neutralizing broth (Difco, Becton Dickinson) supplemented with 0.3% Soytone (Difco, Becton Dickinson) and 0.25% sodium chloride (Sigma, St. Louis, MO) was aseptically added into each tissue sample bag. Each bag was homogenized (540 rpm) for 1 min with a stomacher (InterScience, Markham, Ontario, Canada). An aliquot of 0.1 ml of each homogenate from the control bags was used to determine aerobic plate counts (APC) and *Enterobacteriaceae* counts (EBC) by using a Bactometer (bioMérieux, Hazelwood, MO), as described previously *(3).* Each homogenate from treated tissue samples was 10-fold serially diluted and appropriate dilutions were plated on Petrifilm Aerobic and *Enterobacteriaceae* Count Plates (3M Health Care, St. Paul, MN). The plates were incubated as recommended by the manufacture and counted with a Petrifilm plate reader (3M Health Care). The homogenates from both control and treated tissue samples also were enumerated on selective media for *E. coli* O157:H7 and *Salmonella,* as previously described *(3)* by using a spiral plater (Spiral Biotech, Inc., Norwood, MA) with the limit of detection 60 CFU/$cm^2$. The bags from treated tissue samples were enriched by incubating at 25°C for 2 h, 42°C for 6 h, and stored at 4°C overnight in order to test for the recovery of viable but injured microorganisms. Tissue samples that showed no count on selective media were subjected to immunomagnetic separation. One milliliter from the enrichments was subjected to immunomagnetic separation for *E. coli* O157:H7 and *Salmonella* as described previously *(1, 14).* Three presumptive colonies of *E.*

TABLE 1. *Effectiveness of DBDMH on APC, EBC, and* E. coli *O157:H7 43888 compared with hot water treatment of inoculated beef tissues using a commercial carcass wash cabinet*

| Tissue | Treatment[a] | Bacterial population, log CFU/cm$^2$ (log reduction/cm$^2$) | | |
|---|---|---|---|---|
| | | APC | EBC | *E. coli* O157:H7 |
| Cutaneous trunci[b] | Control | 8.6 A | 8.6 A | 6.1 A |
| | Hot water | 5.0 B (3.5 D)[c] | 4.7 B (3.9 D) | 4.3 B (1.8 D) |
| | Control | 8.8 A | 8.2 A | 6.2 A |
| | DBDMH[d], 75 ppm | 5.7 B (3.0 E) | 5.5 B (2.8 E) | 4.5 B (1.6 D) |
| | Control | 8.7 A | 8.2 A | 6.3 A |
| | DBDMH, 175 ppm | 5.8 B (2.9 E) | 5.5 B (2.8 E) | 4.7 B (1.6 D) |
| | Control | 9.0 A | 8.6 A | 6.4 A |
| | DBDMH, 270 ppm | 5.7 B (3.3 DE) | 5.5 B (3.1 E) | 4.6 B (1.8 D) |
| Heart | Control | 8.0 G | 7.7 G | 5.8 G |
| | Hot water | 3.9 H (4.1 X) | 3.6 H (4.1 X) | 3.6 H (2.2 X) |
| | Control | 8.0 G | 7.6 G | 6.0 G |
| | DBDMH, 75 ppm | 4.5 H (3.5 Y) | 4.3 H (3.3 Y) | 4.2 H (1.7 Y) |
| | Control | 8.0 G | 7.4 G | 5.8 G |
| | DBDMH, 175 ppm | 4.5 H (3.6 XY) | 4.2 H (3.2 Y) | 4.2 H (1.7 Y) |
| | Control | 7.9 G | 7.6 G | 6.1 G |
| | DBDMH, 270 ppm | 4.3 H (3.6 XY) | 4.2 H (3.4 Y) | 4.1 H (2.1 X) |

[a] Hot water (85°C) was sprayed at 20 lb/in$^2$ for 12 s for CT sections and 28 s for beef heart. All DBDMH treatments (25 ± 2°C) were sprayed at 35 lb/in$^2$ for 12 s for CT sections and 28 s for beef hearts.

[b] Representing carcass surface tissue.

[c] A and B, and G and H (log CFU per square centimeter) denote means in the same column within the treatment group (control versus treatment), and tissue types bearing the common letter do not differ significantly at $P \leq 0.05$. D and E, and X and Y (log reduction per square centimeter) denote means in the same column within the column, and tissue types across treatments bearing the common letter do not differ significantly at $P \leq 0.05$.

[d] DBDMH, 1,3-dibromo-5,5-dimethylhydantoin.

*coli* O157:H7 and *Salmonella* were confirmed by PCR *(10, 17).* Colony counts were transformed to values expressed in log CFU per centimeter squared from two experimental replications.

One-way statistical analysis of variance was performed using the General Linear Model procedure of SAS (SAS Institute, Inc., Cary, NC). Least-squares means were calculated, and pairwise comparisons of means were determined using Tukey-Kramer test method, with the probability level at $P \leq 0.05$.

## RESULTS AND DISCUSSION

**Spray treatments with a carcass wash cabinet.** In this study, DBDMH was compared with hot water for its efficacy in reduction of inoculated CT sections and beef hearts. To demonstrate the effectiveness of these treatments on the target bacteria, high levels of organisms were inoculated on both tissues. The efficacy of hot water and DBDMH on APC, EBC, and *E. coli* O157:H7 43888 counts is presented in Table 1. Both hot water and DBDMH reduced ($P < 0.05$) all target bacteria on inoculated surfaces of CT sections and beef hearts. Hot water reduced APC and EBC on the CT sections by 3.5 and 3.9 log CFU/cm$^2$, respectively. Hot water treatment produced a 1.8-log reduction of *E. coli* O157:H7 43888–inoculated CT sections. Bosilevac et al. reported that hot water caused 2.7-log reductions of both APC and EBC *(2).* Hot water treatment also caused approximately 2.0-log reductions on *E. coli* O157:H7–inoculated bovine heads *(11).* Treatment with DBDMH at 270 ppm resulted in the same APC reduction as did treatment with hot water. Slightly lower ($P < 0.05$) reductions in APC were obtained with 75 and 175 ppm DBDMH than with 270 ppm DBDMH. All levels of DBDMH had slightly lower ($P < 0.05$) reductions in EBC compared with hot water. DBDMH treatments on CT produced significant reductions in *E. coli* O157:H7 43888, which ranged between a 1.6- and a 1.8-log reduction compared with controls, which did not differ ($P > 0.05$) from reductions due to hot water treatment.

Increasing the concentration of DBDMH from 75 to 270 ppm did not increase the inactivation effect on APC, EBC, or *E. coli* O157:H7 ATCC 43888. This may be due to organic materials that could react with hypobromous acid. Hypobromous acid is a strong oxidant and is highly reactive with heme groups, amines, and amino acids *(4).* The nitrogen–bromine bond is relatively unstable *(27)* and can react further with other biological molecules *(4).* The pH values of DBDMH solution at 75, 175, and 270 ppm were 6.84, 6.76 and 6.71, respectively. The pH values of the solution dropped slightly as the concentration of DBDMH increased. The hydrolysis of DBDMH to hypobromous acid in water was critically dependent on its pH value *(22).* Because hypobromous acid is a very weak acid, the hydrolysis of DBDMH results in the slightly decreased pH *(22).* This suggests that a similar amount of hypobromous acid probably was generated, even though a higher concentration of DBDMH was used.

Hot water significantly reduced APC and EBC on beef hearts by 4.1 log CFU/cm$^2$, and reduced *E. coli* O157:H7 43888 by 2.2 log CFU/cm$^2$ compared with controls (Table 1). DBDMH caused reductions on hearts ranging between

TABLE 2. *Effectiveness of DBDMH on APC, EBC,* E. coli *O157:H7, and* Salmonella *compared with hot water treatment of inoculated beef tissues, using a model carcass washer*

| Tissue | Treatment[a] | Bacterial population, log CFU/cm$^2$ (log reduction/cm$^2$) | | | |
|---|---|---|---|---|---|
| | | APC | EBC | *E. coli* O157 | *Salmonella* |
| Cutaneous trunci[b] | Control | 8.4 A | 7.6 A | 6.3 A | 6.6 A |
| | Hot water | 5.4 B (3.0 D)[c] | 4.3 B (3.3 D) | 3.9 B (2.3 D) | 4.1 B (2.5 D) |
| | Control | 8.5 A | 7.7 A | 6.3 A | 6.6 A |
| | DBDMH,[d] 75 ppm | 6.3 B (2.2 E) | 5.9 B (1.8 E) | 5.2 B (1.1 E) | 5.9 B (0.7 E) |
| | Control | 8.4 A | 7.5 A | 6.2 A | 6.8 A |
| | DBDMH, 175 ppm | 6.2 B (2.2 E) | 5.8 B (1.8 E) | 5.0 B (1.2 E) | 5.7 B (1.1 EF) |
| | Control | 8.5 A | 7.8 A | 6.3 A | 6.8 A |
| | DBDMH, 270 ppm | 6.0 B (2.5 E) | 5.7 B (2.1 E) | 4.8 B (1.5 F) | 5.5 B (1.3 F) |
| Heart | Control | 7.9 G | 6.7 G | 5.6 G | 5.8 G |
| | Hot water | 4.1 H (3.8 X) | 3.3 H (3.4 X) | 3.2 H (2.4 X) | 3.0 H (2.8 X) |
| | Control | 7.8 G | 6.6 G | 5.6 G | 5.8 G |
| | DBDMH, 75 ppm | 4.6 H (3.2 Y) | 4.2 H (2.4 Z) | 3.9 H (1.7 Y) | 3.8 H (2.0 Z) |
| | Control | 7.7 G | 6.7 G | 5.6 G | 5.8 G |
| | DBDMH, 175 ppm | 4.5 H (3.2 Y) | 4.1 H (2.6 YZ) | 3.8 H (1.8 Y) | 3.6 H (2.2 YZ) |
| | Control | 7.7 G | 6.8 G | 5.6 G | 5.9 G |
| | DBDMH, 270 ppm | 4.6 H (3.0 Y) | 4.1 H (2.7 Y) | 3.7 H (1.9 Y) | 3.6 H (2.3 Y) |

[a] Hot water (85°C) was sprayed at 20 lb/in$^2$ for 12 s for CT sections and 28 s for beef heart. All DBDMH treatments (25 ± 2°C) were sprayed at 35 lb/in$^2$ for 12 s for CT sections and 28 s for beef hearts.

[b] Representing carcass surface tissue.

[c] A and B, and G and H (log CFU per square centimeter) denote means in the same column within the treatment group (control versus treatment), and tissue types bearing the common letter do not differ significantly at $P \leq 0.05$. D, E, and F and X, Y, and Z (log reduction per square centimeter) denote means in the same column within the column, and tissue types across treatments bearing the common letter do not differ significantly at $P \leq 0.05$.

[d] DBDMH, 1,3-dibromo-5,5-dimethylhydantoin.

3.2 and 3.6 log cycles for APC and EBC, respectively, and reductions between 1.7 and 2.1 log cycles for *E. coli* O157:H7 43888. The larger reductions for APC, EBC, and *E. coli* O157:H7 43888 by DBDMH on beef hearts can be attributed to a longer exposure time than was used for CT (28 versus 12 s). DBDMH at 175 and 270 ppm provided a similar ($P > 0.05$) reduction in APC on hearts as did hot water. Hot water treatment reduced EBC on hearts more ($P < 0.05$) than did any level of DBDMH. Hot water and 270 ppm DBDMH reduced *E. coli* O157:H7 43888 on hearts more than did 75 and 175 ppm DBDMH. The slight advantage of using hot water is probably due to killing bacteria by denaturing of enzymes and damaging functional and structural components *(18).* Organic and other acids need to enter the bacterial cells through the membrane before killing bacterial cells *(19).*

**Spray treatments with a model carcass washer.** In this study, a cocktail of pathogenic bacteria, *E. coli* O157:H7 and *Salmonella,* was inoculated onto surfaces of CT sections and beef hearts. Spray treatments with hot water or DBDMH significantly reduced all target organisms on inoculated CT sections and beef hearts compared with untreated controls (Table 2). Hot water treatment of CT sections reduced APC, EBC, *E. coli* O157:H7, and *Salmonella* by 3.0, 3.3, 2.3, and 2.5 log, respectively. Log reductions on CT sections treated with DBDMH ranged from 1.8 to 2.5 for APC and EBC, and from 0.7 to 1.5 for *Salmonella* and *E. coli* O157:H7, respectively. Compared with hot water treatment, DBDMH treatments, regardless of level, resulted in smaller ($P < 0.05$) reductions in all target organisms on surfaces of CT sections. Concentration of DBDMH did not alter ($P > 0.05$) its effectiveness against APC or EBC on CT. However, treatment with 270 ppm DBDMH reduced *E. coli* O157:H7 and *Salmonella* on CT more than did treatment with 75 ppm.

For beef hearts, APC, EBC, *E. coli* O157:H7, and *Salmonella* treated with hot water or DBDMH were significantly lower than untreated controls were (Table 2). Hot water treatment resulted in the reduction of all target organisms slightly more ($P < 0.05$) than did any level of DBDMH. Level of DBDMH did not affect ($P > 0.05$) the reduction of APC or *E. coli* O157:H7 on hearts. However, 270 ppm DBDMH reduced EBC and *Salmonella* on hearts more than did 75 ppm DBDMH. In both studies, hot water reduced more target bacteria than DBDMH did.

**Effect of treatments after storage at 4°C.** The third 25-cm$^2$ section of each inoculation was held at 4°C for 48 h to examine the residual activity of the treatments (Table 3). The differences in populations of APC, EBC, and *E. coli* O157:H7 43888 inoculations after DBDMH treatments of CT sections and beef hearts, which was followed by 48 h storage at 4°C, showed no further reduction ($P > 0.05$) compared with hot water treatment. For the CT sections inoculated with a mixture of pathogenic bacteria, DBDMH treatments caused a slightly greater decrease ($P < 0.05$) in APC (all levels) and *Salmonella* (175 ppm) after storage for 48 h at 4°C than did hot water treatment. For hearts inoculated with pathogenic bacteria, DBDMH treatment

TABLE 3. *Reduction of microbiological populations on inoculated beef tissue storage for 48 h at 4°C*[a]

| Tissue | Treatment[c] | Difference in population (log CFU/cm$^2$)[b] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Nonpathogenic inoculation | | | Pathogenic inoculation | | |
| | | APC | EBC | *E. coli* O157:H7 43888 | APC | EBC | *E. coli* O157:H7 | *Salmonella* |
| Cutaneous tunci[d] | Hot water | 0.14 ± 0.76 A[e] | 0.11 ± 0.75 A | 0.03 ± 0.75 A | −0.57 ± 0.72 A | 0.22 ± 0.61 A | 0.16 ± 0.71 A | 0.18 ± 0.69 A |
| | DBDMH,[f] 75 ppm | 0.15 ± 0.44 A | 0.20 ± 0.46 A | 0.04 ± 0.54 A | 0.18 ± 0.31 B | 0.32 ± 0.51 A | 0.24 ± 0.49 A | 0.43 ± 0.41 AB |
| | DBDMH, 175 ppm | 0.18 ± 0.45 A | 0.27 ± 0.54 A | 0.10 ± 0.45 A | 0.24 ± 0.38 B | 0.28 ± 0.55 A | 0.23 ± 0.47 A | 0.53 ± 0.46 B |
| | DBDMH, 270 ppm | 0.19 ± 0.58 A | 0.19 ± 0.69 A | −0.03 ± 0.44 A | 0.25 ± 0.44 B | 0.31 ± 0.63 A | 0.03 ± 0.68 A | 0.47 ± 0.49 AB |
| Heart | Hot water | 0.07 ± 1.24 A | 0.21 ± 1.04 A | 0.24 ± 0.78 A | −0.17 ± 0.52 A | 0.39 ± 0.83 A | −0.29 ± 0.65 A | −0.09 ± 0.69 A |
| | DBDMH, 75 ppm | 0.42 ± 1.19 A | 0.35 ± 0.98 A | 0.37 ± 0.76 A | 0.52 ± 0.65 B | 0.72 ± 0.64 A | 0.63 ± 0.58 B | 0.56 ± 0.72 B |
| | DBDMH, 175 ppm | 0.40 ± 0.83 A | 0.46 ± 0.78 A | 0.54 ± 0.78 A | 0.28 ± 0.48 B | 0.56 ± 0.53 A | 0.58 ± 0.70 B | 0.49 ± 0.62 AB |
| | DBDMH, 270 ppm | −0.06 ± 0.69 A | 0.38 ± 0.72 A | 0.28 ± 0.72 A | 0.44 ± 0.32 B | 0.57 ± 0.62 A | 0.46 ± 0.66 B | 0.29 ± 0.62 AB |

[a] Greater values indicate larger reductions in bacterial populations.
[b] The difference in population was calculated by subtracting the viable population after 48 h storage from 0 h.
[c] Hot water (85°C) was sprayed at 20 lb/in$^2$ for 12 s for CT sections and 28 s for beef heart. All DBDMH treatments (25 ± 2°C) were sprayed at 35 lb/in$^2$ for 12 s for CT sections and 28 s for beef hearts.
[d] Representing carcass surface tissue.
[e] A and B denote means in the same column within the treatment group, and tissue types bearing the common letter do not differ significantly at $P \leq 0.05$.
[f] DBDMH, 1,3-dibromo-5,5-dimethylhydantoin.

caused a slightly greater decrease ($P < 0.05$) in APC and *E. coli* O157:H7 (all levels) and *Salmonella* (75 ppm only) after 48 h storage at 4°C than did hot water. This is probably due to injured bacterial cells that could not be enumerated on selective media. Hypobromous acid has been shown to react with double bonds of unsaturated fatty acids of bacterial membranes, forming bromohydrins, which contribute to disruption of cell membranes *(4).* Thirty-six percent of hot water–treated and 43% of DBDMH-treated samples could not be enumerated for the pathogens (data not shown). These samples were enriched, immunomagnetic separated, and confirmed by PCR. Both pathogens were recovered from all of these samples. This indicated that hot water and DBDMH treatments reduced the number of uninjured or viable cells to a level below the limit of detection of our enumeration assay.

Although hot water treatment has been found to be effective for carcass decontamination, the disadvantage of this treatment is the high-water-volume use and high cost of maintaining such a high temperature. DBDMH requires no additional energy resources for treatment, and wastewater from the wash cabinet can be recirculated. This study demonstrated that spray treatments with the bromine compound DBDMH at 25°C could improve the microbiological safety of beef carcasses and variety meats by killing and inflicting injury to pathogenic bacteria.

## ACKNOWLEDGMENTS

The authors thank the representatives from Solution BioSciences, Inc., for providing the necessary equipment and Bromitize for use in this study. The authors thank Drs. Ray A. Field and Xiangwu Nou for their comments and suggestions of this manuscript. The authors also thank Bruce Jasch, Frank Reno, Greg Smith, Julie Dyer, Bradley Dalrymple, Dallas Duncan, Emily Griess, and Sarah Schumacher for their technical assistance, and Debbie Kummer for secretarial assistance.

## REFERENCES

1. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli,* including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
2. Bosilevac, J. M., X. Nou, G. A. Barkocy-Gallagher, T. M. Arthur, and M. Koohmaraie. 2006. Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and *Enterobacteriaceae* and reduce the prevalence of *Escherichia coli* O157:H7 on preevisceration beef carcasses. *J. Food Prot.* 69:1808–1813.
3. Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2007. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and faecal samples using direct plating methods. *J. Appl. Microbiol.* 103:1657–1668.
4. Carr, A. C., J. M. Jeroen, J. J. van den Berg, and C. C. Winterbourn. 1998. Differential reactivities of hypochlorous and hypobromous acids with purified *Escherichia coli* phospholipids: formation of haloamines and halohydrins. *Biochim. Biophys. Acta* 1392:254–264.
5. Centers for Disease Control and Prevention. 1994. *E. coli* O157:H7: procedure for isolation and identification from stool specimens. Available at: http://aepo-xdv-www.epo.cdc.gov/wonder/PrevGuid/p0000445/P0000445.asp. Accessed 28 July 2008.
6. Cutter, C. N., and G. R. Siragusa. 1995. Application of chlorine to reduce populations *Escherichia coli* on beef. *J. Food Saf.* 15:67–75.
7. Delmore, R. J., J. N. Sofos, G. R. Schmidt, K. E. Belk, W. R. Lloyd,

and G. C. Smith. 2000. Interventions to reduce microbiological contamination of beef variety meats. *J. Food Prot.* 63:44–50.
8. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1997. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua,* and *Clostridium sporogenes. J. Food Prot.* 60:619–624.
9. Gill, C. O., J. Bryant, and D. Bedard. 1999. The effects of hot water pasteurizing treatments on the appearances and microbiological conditions of beef carcasses sides. *Food Microbiol.* 16:281–289.
10. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. *J. Appl. Microbiol.* 87:867–876.
11. Kalchayanand, N., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, T. L. Wheeler, and M. Koohmaraie. 2007. Evaluation of various antimicrobial interventions for the reduction of *Escherichia coli* O157:H7 on bovine heads during processing. *J. Food Prot.* 71:621–624.
12. Kelly, C. A., J. F. Dempster, and A. J. McLoughlin. 1981. The effect of temperature, pressure, and chlorine concentration of spray washing water on numbers of bacteria on lamb carcasses. *J. Appl. Bacteriol.* 51:415–424.
13. Kim, B. R., J. E. Anderson, S. A. Mueller, W. A. Gaines, and A. M. Kendall. Literature review—efficacy of various disinfectants against *Legionella* in water systems. *Water Res.* 36:4433–4444.
14. Nou, X., T. M. Arthur, J. M. Bosilevac, D. M. Brichta, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2006. Improvement of immunomagnetic separation for *Escherichia coli* O157:H7 detection by PickPen magnetic particle separation device. *J. Food Prot.* 69: 2870–2874.
15. Panangala, V. S., L. Liu, G. Sun, S. D. Worley, and A. Mitra. 1997. Inactivation of rotavirus by new polymeric water disinfectants. *J. Virol. Methods* 66:263–268.
16. Phebus, R. K., A. L. Nutsch, D. E. Schafer, R. C. Wilson, M. J. Riemann, J. D. Leising, C. L. Kastner, J. R. Wolf, and R. K. Prasai. 1977. Comparison of steam pasteurization and other methods for reduction of beef carcasses at various locations in processing. *J. Food Prot.* 60:476–484.
17. Rahn, K., S. A. DeGrandis, R. C. Clarke, S. A. McEwen, J. E. Galan, C. Ginocchio, R. Curtiss, and C. L. Gyles. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella. Mol. Cell. Probes* 6:271–279.
18. Ray, B. 2001. Control of microorganisms in food: control by heat. p. 435–445. *In* B. Ray (ed.), Fundamental food microbiology. CRC Press, Boca Raton, FL.
19. Ray, B. 2001. Control of microorganisms in food: control by low pH and organic acids, p. 463–470. *In* B. Ray (ed.), Fundamental food microbiology. CRC Press, Boca Raton, FL.
20. Reed, R. A. 1960. A useful brominating agent, 1,3-dibromo-5,5-dimethylhydantoin. *Chem. Prod.* 23:299.
21. Sofos, J. N., and G. C. Smith 1998. Nonacid meat decontamination technologies: model studies and commercial applications. *Int. J. Food Microbiol.* 44:171–188.
22. Song, S., P. Lui, and Q. J. Song. 2007. Quantification of dibromodimethylhydantoin disinfectants in water by chemiluminescent method. *Anal. Sci.* 23:327–330.
23. Sun, G., L. C. Allen, E. P. Luckie, W. B. Wheatley, and S. D. Worley. 1995. Disinfection of water by *N*-halamine biocidal polymers. *Ind. Eng. Chem. Res.* 34:4106–4109.
24. Titus, T. C., J. C. Acton, L. McCaskill, and M. G. Johnson. 1978. Microbial persistence on inoculated beef plates sprayed with hypochlorite solution. *J. Food Prot.* 41:606–612.
25. U.S. Food and Drug Administration. 2003. Inventory of effective food contact substance (FCS) notifications. FCN no. 334. Available at: http//www.cfsan.fda.gov/~dms/opa-fcn.html. Accessed 12 August 2008.
26. Williams, D. E., S. D. Worley, S. B. Barnela, and L. J. Swango. 1987. Bactericidal activities of selected organic *N*-halamines. *Appl. Environ. Microbiol.* 53:2082–2089.
27. Worley, S. D., D. E. Williams, and S. B. Barnela. 1987. The stabilities of new *N*-halamine water disinfectants. *Wat. Res.* 21:983–988.