Journal of Food Protection, Vol. 68, No. 7, 2005, Pages 1393–1398

Efficacy of Ozonated and Electrolyzed Oxidative Waters To Decontaminate Hides of Cattle before Slaughter†

JOSEPH M. BOSILEVAC,* STEVEN D. SHACKELFORD, DAYNA M. BRICHTA, 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 04-517: Received 15 November 2004/Accepted 28 February 2005

ABSTRACT

The hides of cattle are the primary source of pathogens such as *Escherichia coli* O157:H7 that contaminate preevisceration carcasses during commercial beef processing. A number of interventions that reduce hide contamination and subsequent carcass contamination are currently being developed. The objective of this study was to determine the efficacy of ozonated and electrolyzed oxidizing (EO) waters to decontaminate beef hides and to compare these treatments with similar washing in water without the active antimicrobial compounds. Cattle hides draped over barrels were used as the model system. Ozonated water (2 ppm) was applied at 4,800 kPa (700 lb in^2) and 15°C for 10 s. Alkaline EO water and acidic EO water were sequentially applied at 60°C for 10 s at 4,800 and 1,700 kPa (250 lb in^2), respectively. Treatment using ozonated water reduced hide aerobic plate counts by 2.1 log CFU/100 cm^2 and reduced *Enterobacteriaceae* counts by 3.4 log CFU/100 cm^2. EO water treatment reduced aerobic plate counts by 3.5 log CFU/100 cm^2 and reduced *Enterobacteriaceae* counts by 4.3 log CFU/100 cm^2. Water controls that matched the wash conditions of the ozonated and EO treatments reduced aerobic plate counts by only 0.5 and 1.0 log CFU/100 cm^2, respectively, and each reduced *Enterobacteriaceae* counts by 0.9 log CFU/100 cm^2. The prevalence of *E. coli* O157 on hides was reduced from 89 to 31% following treatment with ozonated water and from 82 to 35% following EO water treatment. Control wash treatments had no significant effect on the prevalence of *E. coli* O157:H7. These results demonstrate that ozonated and EO waters can be used to decontaminate hides during processing and may be viable treatments for significantly reducing pathogen loads on beef hides, thereby reducing pathogens on beef carcasses.

The pathogen *Escherichia coli* O157:H7 has been a concern to the meat processing industry for the last 20 years. In the early 1980s, cases of hemorrhagic colitis caused by *E. coli* O157:H7 were associated with consumption of undercooked ground beef *(25),* and a ground beef-related *E. coli* O157:H7 outbreak caused hundreds of illnesses and four deaths during 1992 and 1993 *(28).* In response to these events, the U.S. Department of Agriculture Food Safety and Inspection Service declared *E. coli* O157:H7 to be an adulterant in ground beef and required meat processors to establish hazard analysis and critical control point plans *(14).* Since then, several interventions that focus on preventing carcass contamination and on decontaminating carcasses have been designed, tested, and put into use. These antimicrobial interventions, combined with strict hygiene practices, have significantly improved microbial quality of beef carcasses and reduced the incidence of *E. coli* O157:H7 in processing plants *(2–4, 12).* However, cattle occasionally present for slaughter with a higher level of contamination than can be removed effectively with the current interventions.

The vast majority of *E. coli* O157:H7 that contaminates beef carcasses during processing originates on the hides of cattle *(4, 6, 21).* During the hide removal process, *E. coli* O157:H7 and other pathogens such as *Salmonella* are transferred from the hide, where they are highly prevalent, to the carcass *(2, 4, 6, 21).* Processes that effectively clean the hides before hide removal have been effective in lowering carcass microbial concentrations *(6, 7, 21).* Chemical dehairing of hide-on carcasses was the first intervention to indicate that the prevalence of *E. coli* O157:H7 on the preevisceration carcass was almost eliminated when bacterial contamination of the hide was greatly reduced before hide removal *(21).* Subsequent studies of an on-line hide washing cabinet revealed that hide washes prior to slaughter were highly effective in reducing carcass contamination during hide removal *(7).* Chemicals and antimicrobial compounds that have been evaluated for use in hide interventions include cetylpyridinium chloride, NaOH, trisodium phosphate, acidified chlorine, and phosphoric acid *(6, 7).* The data reported by Bosilevac et al. *(7)* formed the basis for effective hide-washing systems now installed in all Cargill Meat Solutions beef processing plants *(1).* Because hide interventions may be the most effective means to reduce pathogens on beef, we are attempting to provide as many viable alternatives as possible to increase the rate and ease of implementation of these interventions by all processors.

Ozonated water and electrolyzed oxidizing (EO) water are generally recognized as safe (GRAS) and can be used inside a processing plant. Ozone has been used since the 1940s to disinfect drinking water at many municipal water

* Author for correspondence. Tel: 402-762-4225; Fax: 402-762-4149; E-mail: bosilevac@email.marc.usda.gov.

† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

treatment plants, and most bottled water has been treated with ozone since the 1980s. In 1997, ozone was granted GRAS status, and its use has been investigated in the processing of fresh produce and red meat. EO water is produced by passing a current of electricity through a dilute saltwater solution. One product of the reaction is NaOH and the other is hypochlorous acid, which has a low pH, contains active chlorine, and has a strong oxidation-reduction potential similar to that of ozone. The efficacy of both ozonated water and EO water has been demonstrated against *E. coli* O157:H7 and *Salmonella* Typhimurium *(13, 24)* and other food-related pathogens such as *Listeria monocytogenes (15, 24)*, the same organisms that are of concern to beef processors. No information exists describing the potential utility of ozonated or EO waters as hide-washing compounds. The objective of these studies was to evaluate the use of ozonated or EO waters in wash steps to reduce hide contamination in experiments using a model hide-washing system.

MATERIALS AND METHODS

Experimental protocol. At a beef processing plant, hides were selected randomly from the processing line. The selected hides represented a variety of fed cattle breeds (some with long hair and some with short hair). The hides were collected immediately after removal from the carcasses and before any hide processing steps (e.g., trimming, defleshing, or water immersion) occurred. As a matter of standard operating procedure at the participating processing plant, all animals in holding pens and alley ways had been sprayed using low-pressure tap water to remove as much visible contamination as possible before the animals entered the plant. No hide-directed interventions were used during processing.

To evaluate hide decontamination treatments, whole pulled hides were draped over barrels to simulate hide-on carcasses. One side of each pulled hide was used to collect two sets of data. Control samples were obtained before treatment from the anterior and posterior of each hide (Fig. 1). Then, wash treatments were applied to the anterior end of the hide and a sample was collected. The treatment was then applied to the posterior end of the hide and a sample was collected. Ninety-four hides (46 for EO water treatment and 48 for ozonated water treatment) were sampled during two separate sample collection trips. An additional 72 hides were sampled at a later time to determine the potential effects of plain water washing at similar temperatures, durations, and pressures. Thirty-six hides were used for evaluation of EO water treatments and for ozonated water treatments.

EO water application conditions. EO water was generated with a P-5000 EO water generator (Electric Aquagenics Unlimited, Inc., Lindon, Utah). Both the alkaline and acidic forms of EO water were used. First, alkaline EO water (NaOH) was applied for 10 s at 4,800 kPa (700 lb in^2) with a gasoline-powered pressure sprayer (Briggs & Stratton, Milwaukee, Wis.) that was fitted with a rotating nozzle and an adjustable pressure valve. The alkaline EO water was pH 11.2 and 52°C when applied. A 10-s pause occurred before the 10-s application of the acidic EO water (hypochlorous acid) using a second power sprayer (Electric Aquagenics) at 1,700 kPa (250 lb in^2). Acidic EO water was pH 2.4, 70 ppm chlorine, and 60°C. Before each use, the free chlorine content of the acidic EO water was confirmed by titration. Both the alkaline and acidic EO waters were applied with 10 to 15 side-to-side passes of the pressure sprayer. The sprayer nozzle was maintained at a distance of 65 cm from the hide surface during spraying. A 30-s dwell time for antimicrobial activity was allowed after the final spray before samples were collected for microbial analysis.

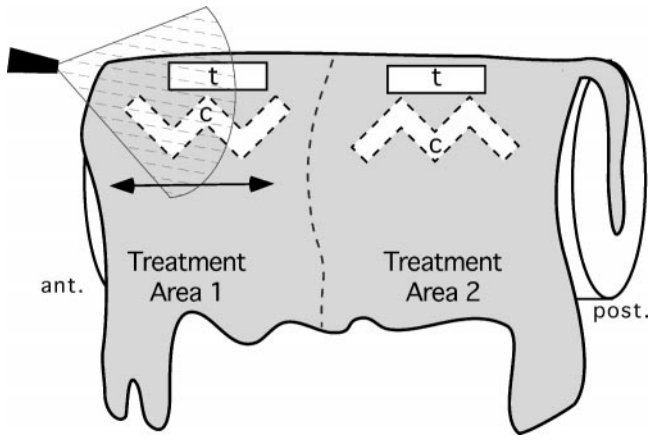

FIGURE 1. *Model hide washing system used to evaluate ozonated and EO water washes. Whole pulled hides were draped over barrels to simulate hide-on carcasses. The hides were taken directly from the processing line immediately after having been pulled from the carcasses and before any hide processing steps had occurred. The anterior (ant.) and posterior (post.) of one side of each hide were used to collect data (treatment area 1 and treatment area 2). Control samples were obtained from each treatment area before spraying. Controls (c) were taken from 500-cm^2 areas of alternating angles across the hide. After control samples were obtained, the wash treatments were applied to the hides, first to one end of the hide and then to the other end, with pauses to allow sample collection. Treated samples (t) were collected from 200-cm^2 areas adjacent to the control areas. All samples were collected using Speci-Sponge Whirl-Pak bags containing 20 ml of Difco neutralization buffer.*

Ozonated water application. Ozonated water was applied using a Mobile Whitewater System (Ozone International, Bainbridge Island, Wash.). This machine generates ozonated water of 2 ± 0.2 ppm and dispenses it at 240 kPa (35 lb in^2) in a stream parallel to a high-pressure water stream. Ozone concentration was monitored periodically during use by visual comparison to optical standards. Atmospheric ozone was also monitored throughout applications to ensure that concentrations did not exceed Occupational Safety & Health Administration standards. Ozonated water was applied to the hide at 4,800 kPa and 15°C with 10 to 15 side-to-side passes for 10 s, then the high-pressure stream was turned off and ozonated water alone was applied at the dispensing pressure (240 kPa) for an additional 5 s. Samples were collected 30 s after the final low-pressure ozonated water spray.

Application of control water washes. To determine the effects of pressure and temperature of the wash procedures, control washes were performed that mimicked the ozonated and EO water wash conditions in pressure, temperature, and duration. A pressure sprayer (model P14030, Precision Industries, Germantown, Wis.) was used for the control washes. Ozonated water controls were applied for 10 s at 15°C and 4,800 kPa with end-to-end passes of the sprayer. A control for the low-pressure 5-s ozonated water treatment was not included because low-pressure washes such as this have had no effect on hide microbial status *(6, 22)*. Samples were collected after a 30-s dwell time. Control EO water washes

consisted of two sequential 10-s washes at 4,800 kPa and 60°C. Samples were collected after a 30-s dwell time.

Sampling. All hide samples were collected using Speci-Sponge Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) containing 20 ml of neutralization buffer (Difco, Becton Dickinson, Sparks, Md.). Controls (nontreated samples) were taken from 500-cm^2 areas of alternated angles across hide (in an ''M'' or ''W'' pattern). Treated samples were collected from 200-cm^2 areas adjacent to the control areas. Samples were collected using 10 bidirectional strokes of the sponge, which was turned over halfway through the process. All samples were thoroughly massaged by hand before being placed on ice and transported to the laboratory to be processed within 6 h.

Bacterial counts. Aerobic plate counts (APC) and *Enterobacteriaceae* counts (EBC) were determined using a Bactometer (bioMérieux, Hazelwood, Mo.) and Petrifilm count plates (3M Microbiology, St. Paul, Minn.). The APC and EBC of hide samples were performed by impedance measurements of 1-ml samples in the Bactometer. Before use, APC samples were diluted 1,000-fold in General Purpose Medium Plus (bioMérieux) supplemented with 18 g/liter dextrose (for a final concentration of 2% dextrose), and EBC samples were diluted 100-fold in Entero Medium (bioMérieux). The Bactometer incubated samples for 16 h at 37°C while measuring the initial detection time for each sample, which was converted to log CFU per milliliter using standard curves derived for each test. The standard curves were determined by performing quadratic regression analysis of initial detection times and log CFU per milliliter, which had been determined using Petrifilm aerobic count plates for APC or Petrifilm *Enterobacteriaceae* count plates for EBC as the standards. The Bactometer in our experiments was therefore calibrated to Petrifilm. The reliable lower limit of detection using the Bactometer is 100 CFU/ml; therefore, all samples from treated hides were also directly plated to Petrifilm to determine APC and EBC. A 1-ml aliquot from each treated sample was serially diluted to 10^{-1} for EBC and 10^{-2} for APC in buffered peptone water. One milliliter of the appropriate dilution was plated to Petrifilm aerobic count plates or Petrifilm *Enterobacteriaceae* count plates. Petrifilm plates were incubated for 16 h at 37°C and colonies were counted manually.

***E. coli* O157 detection.** The procedure for detection of *E. coli* O157 consisted of enrichment, immunomagnetic separation, and plating as described previously *(5, 7)* with minor modifications for plating as follows. Bacterial cells bound to the immunomagnetic separation beads were plated on sorbitol MacConkey agar (Difco, Becton Dickinson) plates supplemented with 0.05 mg/liter cefixime and 2.5 mg/liter potassium tellurite (Dynal, Lake Success, N.Y.) and on *E. coli* O157 chromogenic agar plates. Chromogenic media were either CHROMagar O157 agar (CHROMagar, Paris, France) supplemented with 5 mg/liter novobiocin (Sigma, St. Louis, Mo.) and 1 mg/liter potassium tellurite (Sigma) or Rainbow Agar (Biolog, Hayward, Calif.) supplemented with 10 mg/liter novobiocin and 8 mg/liter potassium tellurite. The use of different chromogenic media was based solely on availability from the manufacturers. All plates were incubated at 37°C for 16 h, and suspect colonies (i.e., sorbitol negative on supplemented sorbitol MacConkey agar, characteristic magenta on supplemented CHROMagar, or characteristic blue on supplemented Rainbow agar) were confirmed as *E. coli* O157 using DrySpot O157 latex agglutination tests (Oxoid, Ogdensburg, N.Y.).

Statistical analyses. APC and EBC of hides were evaluated by analysis of variance using the general linear model procedures of SAS (SAS Institute, Inc., Cary, N.C.). The model included the main effect of treatment. For significant main effects ($P < 0.05$), least squares mean separation was accomplished by the probability of difference (PDIFF) option (a pairwise *t* test). Data for APC and EBC were log transformed before analysis of variance. Pairwise comparisons of frequencies of *E. coli* O157 detection were made using PROC FREQ and Mantel-Haenszel chi-square analysis (SAS).

TABLE 1. *Effects of ozonated water treatment on the microbial status of hides*[a]

	APC (log CFU/100 cm^2)[b]	EBC (log CFU/100 cm^2)[b]	O157 (%)[c]
Control	8.4	6.6	89 (85/96)
Treated[d]	6.3	3.2	31 (30/96)
Reduction	2.1	3.4	65
P[e]	0.0001	0.0001	0.001

[a] Values for APC and EBC are means, $n = 96$. SEM was 0.06 for APC and 0.08 for EBC. Values for *E. coli* O157 prevalence are percentage of positive samples (fraction of positive samples).

[b] All samples were analyzed directly with a Bactometer, and treated samples were also analyzed using Petrifilm.

[c] Prevalence of *E. coli* O157 was determined by selective enrichment and immunomagnetic separation isolation followed by plating on sorbitol MacConkey agar with cefixime and potassium tellurite on O157 chromogenic media. Positive colonies were confirmed by latex agglutination.

[d] Treatment was 10 to 15 end-to-end passes (10 s each) of high-pressure (4,800 kPa or 700 lb in^2) 15°C ozonated water, followed by 5-s application of low-pressure (240 kPa or 35 lb in^2) ozonated water. Samples were collected after a 30-s dwell time.

[e] Values were determined using general linear model procedures of SAS for APC and EBC. The model included the main effect of treatment, and the least squares means separation was accomplished by the probability of difference (PDIFF) option (a pairwise *t* test). Pairwise comparisons of frequencies of *E. coli* O157 detection were made using PROC FREQ and Mantel-Haenszel chi-square analysis.

RESULTS AND DISCUSSION

Recent studies have confirmed that the hide is one of the most significant sites for pathogen intervention in slaughter cattle *(6, 7, 21)*. Not all processors can implement systems such as chemical dehairing *(21)* or on-line hide wash cabinets *(7)*. We continue to investigate viable alternatives that can be applied in an economical fashion. The latest of these are ozonated water and EO water. Both were evaluated using a hide-washing model system (Fig. 1) to determine their efficacy.

Ozonated water washes of hides resulted in significant reductions ($P < 0.05$) of APC and EBC (Table 1). Before treatment, hide APC were 8.4 log CFU/100 cm^2 and hide EBC were 6.6 log CFU/100 cm^2. The ozone wash reduced APC by 2.1 log CFU/100 cm^2 and reduced EBC by 3.4 log CFU/100 cm^2. The prevalence of *E. coli* O157 was also reduced ($P < 0.05$) following ozonated water washing. Before treatment, *E. coli* O157 prevalence was 89%, and after treatment the prevalence was 31%, a reduction of 65%.

Cold (15°C) water applied at 4,800 kPa for 10 s, in the

TABLE 2. *Effects of control water treatments on the microbial status of hides*[a]

	APC (log CFU/100 cm^2)[b]	EBC (log CFU/100 cm^2)[b]	O157 (%)[c]
Ozone water control ($n = 72$)[d]			
Before	7.8	6.8	94 (68/72)
After	7.3	5.9	99 (71/72)
Reduction	0.5	0.9	−5
P[e]	0.001	0.001	0.200
EO water control ($n = 72$)[f]			
Before	7.8	6.2	100 (72/72)
After	6.8	5.3	92 (66/72)
Reduction	1.0	0.9	8
P[e]	0.001	0.001	0.025

[a] Values for APC and EBC are means, SEM ranged from 0.04 to 0.05 for APC and EBC. Values for *E. coli* O157 prevalence are percentage of positive samples (fraction of positive samples).
[b] All samples were analyzed directly with a Bactometer.
[c] Prevalence of *E. coli* O157 was determined by selective enrichment and immunomagnetic separation isolation followed by plating on sorbitol MacConkey agar with cefixime and potassium tellurite and on O157 chromogenic media. Positive colonies were confirmed by latex agglutination.
[d] Treatment was 10 to 15 end-to-end passes (10 s each) of water at 4,800 kPa (700 lb in^2) and 15°C. Samples were collected after a 30-s dwell time.
[e] Values were determined using general linear model procedures of SAS for APC and EBC. The model included the main effect of treatment, and the least squares means separation was accomplished by the probability of difference (PDIFF) option (a pairwise *t* test). Pairwise comparisons of frequencies of *E. coli* O157 detection were made using PROC FREQ and Mantel-Haenszel chi-square analysis.
[f] Treatment was 10 to 15 end-to-end passes (repeated twice, 10 s each) of water at 4,800 kPa (700 lb in^2) and 60°C. Samples were collected after a 30-s dwell time.

TABLE 3. *Effects of electrolyzed water treatment on the microbial status of hides*[a]

	APC (log CFU/100 cm^2)[b]	EBC (log CFU/100 cm^2)[b]	O157 (%)[c]
Control	8.5	6.6	82 (75/92)
Treated[d]	5.0	2.3	35 (32/92)
Reduction	3.5	4.3	57
P[e]	0.0001	0.0001	0.001

[a] Values for APC and EBC are means, $n = 92$. SEM was 0.07 for APC and EBC. Values for *E. coli* O157 prevalence are percentage of positive samples (fraction of positive samples).
[b] All samples were analyzed directly with a Bactometer, and treated samples were also analyzed using Petrifilm.
[c] Prevalence of *E. coli* O157 was determined by selective enrichment and immunomagnetic separation isolation followed by plating on sorbitol MacConkey agar with cefixime and potassium tellurite and on O157 chromogenic media. Positive colonies were confirmed by latex agglutination.
[d] Treatment was two 10-s applications of EO water: alkaline EO water applied at 52°C and 4,800 kPa (700 lb in^2) and acidic EO water applied at 1,700 kPa (250 lb in^2) and 60°C. Samples were collected after a 30-s dwell time.
[e] Values were determined using general linear model procedures of SAS for APC and EBC. The model included the main effect of treatment, and the least squares means separation was accomplished by the probability of difference (PDIFF) option (a pairwise *t* test). Pairwise comparisons of frequencies of *E. coli* O157 detection were made using PROC FREQ and Mantel-Haenszel chi-square analysis.

same fashion as the ozone treatment, reduced APC by 0.5 log CFU/100 cm^2 and reduced EBC by 0.9 log CFU/100 cm^2 (Table 2). We concluded that the effect of ozonated water was therefore at least a reduction of 1.5 log CFU/100 cm^2 for APC and 2.5 log CFU/100 cm^2 for EBC. This water wash control had no effect on the prevalence of *E. coli* O157, so the 65% reduction of *E. coli* O157 following ozonated water washing can be directly attributed to the antimicrobial activity of the ozone.

The results of EO water washing also demonstrated significant effects ($P < 0.05$) on APC, EBC, and the prevalence of *E. coli* O157 (Table 3). The hides used for the EO washing experiments were not different ($P > 0.05$) from those washed with ozonated water. Reductions of APC and EBC were 3.5 and 4.3 log CFU/100 cm^2, respectively. The reduction of *E. coli* O157 was also significant and similar to that observed with ozonated water; 82% of the hide samples contained detectable *E. coli* O157 before EO water treatment, but only 35% of the hides were positive after treatment, a reduction of 57%.

Hot (60°C) water applied for a total of 20 s in two 10-s steps (in the same manner as the EO water treatment) reduced APC by 1.0 log CFU/100 cm^2 and reduced EBC by 0.9 log CFU/100 cm^2 (Table 2). The conditions of the EO water washes were demonstrably more effective than those of the ozonated water washes, possibly because of the length of time and/or the application pressure. The liquid pressure at which a hide is washed affects the efficacy of the washing treatment *(8)*. The difference of 0.5 log CFU/100 cm^2 in APC between wash conditions was significant ($P < 0.05$), but there was no difference in the EBC or the prevalence of *E. coli* O157. In a previous study in which water was applied to hides at 8,300 kPa (1,200 lb in^2) for two 20-s periods, APC were reduced by 0.4 log CFU/100 cm^2 and EBC were reduced by 0.6 log CFU/100 cm^2 *(8)*. Neither of these reductions was significantly different ($P > 0.05$) from those for the controls. In another study, a similar hide-washing treatment using water significantly reduced EBC by 1.6 log CFU/100 cm^2 *(7)*. The findings of the current study and those of the previous studies suggest that water washes have variable effects on the microbial status of hides, possibly because of differences in the hides used in each study.

The observed differences between the EO and ozonated water washes, however, were not solely due to the differences in wash conditions, because when the effect of the hot water washes was taken into account, treatments with EO water still had a greater effect on hide cleanliness than did treatments with ozonated water. Adjusting results

of treatments using EO water to take into account the hot water effects resulted in APC reductions of 2.5 log CFU/100 cm^2 and EBC reductions of 3.4 log CFU/100 cm^2. The hot water wash also had a small (8%) but significant ($P < 0.05$) effect on the prevalence of *E. coli* O157. These findings suggest that the temperature and/or duration of the EO water treatments also contributed to some extent to the antimicrobial effect.

We previously examined other hide decontamination methods using the same model system described here *(7).* Using a similar two-stage application treatment, we evaluated combinations of phosphoric acid, trisodium phosphate, and NaOH as wash and water or acidified chlorine as rinse to reduce hide EBC. The greatest reduction in EBC was observed with the combination of phosphoric acid and acidified chlorine *(7),* and that reduction was similar to that obtained in the present study for EBC following ozonated and EO water washes.

Ozonated water can instantly reduce populations of *E. coli, Salmonella* Typhimurium, and other food-related pathogens by 5 to 6 log CFU/ml *(24).* However, direct applications of ozonated water to various food products have not resulted in such dramatic reductions. When alfalfa seeds were treated with 4 ppm ozone for 2 min to inactivate *E. coli* O157:H7 (inoculated at 10^5 CFU/g), no reduction in *E. coli* O157:H7 numbers was observed *(27).* Further experiments revealed that reductions of 0.51 to 1.75 log CFU/g required 4 to 21 ppm ozone and exposure times of up to 64 min *(27).* Treatment of beef carcass surfaces with ozone and water to reduce populations of inoculated *E. coli* O157:H7 and *Salmonella* Typhimurium resulted in no significant differences between the two treatments *(10).* In our system, ozonated water was much more effective than water controls, and concentrations and exposure times were much lower. The efficacy of ozone treatment is increased when competing organic particles are removed and when a mechanical means is used to dislodge bacteria *(16).* We hypothesize that our results are due in part to the high-pressure application, which satisfies both of these requirements.

In previous studies, acidic EO water reduced *E. coli* O157 and *L. monocytogenes* by 8 log CFU/ml when exposed for 30 and 60 s, respectively *(15). Salmonella* Typhimurium was reduced by 7 log CFU/ml when treated in a similar manner *(13).* In studies similar to those with ozonated water, direct application to food did not result in reductions of such magnitude. EO water applied to tomatoes reduced populations of *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium by 4 log CFU/cm^2 each after exposures of 30 and 60 s *(11).* Our EO water wash lasted a total of 20 s (10 s for each phase) and was as effective as treatments used on produce products, but not nearly as effective as treatments of pathogens suspended in EO water.

The efficacies of ozonated water, EO water, and other solutions to disinfect lettuce, cucumbers, and strawberries have been compared *(17, 18).* In each case, electrolyzed water outperformed ozonated water, but not always to a significant extent. APC were reduced about 1 log CFU/g more by EO water than by ozone. This finding is similar to the difference we observed between the two treatments on hides, which may be due to the difference in antimicrobial action rather than the differences in application time and temperature. EO water and ozone both have a strong oxidation-reduction potential, but only EO water contains free chlorine, an additional bactericide. EO water treatments expose organisms to two extremes of pH (alkaline at pH 11.2 and acid at pH 2.4) that can stress and damage cells to a greater extent than can ozonated water, which is only slightly acidic (pH 6).

The presence of foodborne pathogens on cattle hides has been examined, and the hide is considered a likely source of cross-contamination of beef during the hide removal process because the brisket area of the hide is frequently contaminated with pathogens *(23).* Improved hygienic practices during removal of hides from dirty animals resulted in significant reductions of bacteria on the carcass *(19),* and a direct relationship between the prevalence of *E. coli* O157 on hides and the prevalence of this pathogen on preevisceration carcasses has been established *(1).* Several hide interventions have been used with various degrees of success. Washing the hides of cattle with water did not result in significant reductions in hide or carcass contamination *(9, 20).* The inclusion of an antimicrobial (cetylpyridinium chloride) or a decontamination step (chemical dehairing) was required to obtain improvements in cleanliness of hides and subsequent preevisceration carcasses, but use of antimicrobials *(6, 20)* or chemical dehairing *(26)* did not have a significant effect. Later use of chemical dehairing on a large sample in an industry setting did clearly demonstrate the efficacy of hide decontamination and improved carcass cleanliness *(21).* Other successful hide interventions that followed chemical dehairing included treatment with cetylpyridinium chloride *(6)* and treatment with NaOH and water in an on-line hide wash cabinet *(7).* These treatments have resulted in effective reduction of hide contamination, and the hide intervention resulted in less contamination of the corresponding carcasses compared with carcasses that were processed conventionally without hide intervention *(6, 7, 21).* Ozonated and EO waters are not currently validated for use in hide interventions. These experiments were performed in anticipation of potential on-line applications using either a hide wash cabinet or some alternative equipment. The results of the model system reported here suggest that on-line implementation of processes using either ozonated or EO water will be just as successful in controlling pathogens on carcasses as the currently used hide interventions.

ACKNOWLEDGMENTS

We are grateful to the participating processing plant and its personnel for cooperation in these experiments. We thank the representatives from Ozone International and Electric Aquagenics Unlimited, Inc., for providing the necessary equipment for use in these studies. We also thank Dr. Xiangwu Nou and Dr. Terrance Arthur for their scientific assistance; Bruce Jasch, Ann Goding, and Gregory Smith for their technical support; and Carol Grummert for her secretarial skills.

REFERENCES

1. Allen, D. M. 2005. The kill zone: subjecting animals to a hide-on carcass wash has been shown to dramatically decrease carcass contamination. *Meat Market. Technol.* 13:68–69.

2. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:658–665.
3. Bacon, R. T., K. E. Belk, J. N. Sofos, R. P. Clayton, J. O. Reagan, and G. C. Smith. 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *J. Food Prot.* 63:1080–1086.
4. 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.
5. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
6. Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.* 67:646–650.
7. Bosilevac, J. M., X. Nou, M. S. Osborn, D. M. Allen, and M. Koohmaraie. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *J. Food Prot.* 68:265–272.
8. Bosilevac, J. M., T. L. Wheeler, M. Rivera-Betancourt, X. Nou, T. M. Arthur, S. D. Shackelford, M. P. Kent, D. Jaroni, M. Osborn, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Protocol for evaluating the efficacy of cetylpyridinium chloride as a beef hide intervention. *J. Food Prot.* 67:303–309.
9. Byrne, C. M., D. J. Bolton, J. J. Sheridan, D. A. McDowell, and I. S. Blair. 2000. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. *Lett. Appl. Microbiol.* 30:142–145.
10. Castillo, A., K. S. McKenzie, L. M. Lucia, and G. R. Acuff. 2003. Ozone treatment for reduction of *Escherichia coli* O157:H7 and *Salmonella* serotype Typhimurium on beef carcass surfaces. *J. Food Prot.* 66:775–779.
11. Deza, M. A., M. Araujo, and M. J. Garrido. 2003. Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. *Lett. Appl. Microbiol.* 37:482–487.
12. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
13. Fabrizio, K. A., and C. N. Cutter. 2003. Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella* Typhimurium and *Listeria monocytogenes*. *J. Food Prot.* 66:1379–1384.
14. Federal Register. 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems, final rule. *Fed. Regist.* 61:38805–38989.
15. Kim, C., Y.-C. Hung, and R. E. Brackett. 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of food-borne pathogens. *Int. J. Food Microbiol.* 61:199–207.
16. Kim, J. G., A. E. Yousef, and M. A. Khadre. 2003. Ozone and its current and future application in the food industry. *Adv. Food Nutr. Res.* 45:167–218.
17. Koseki, S., K. Yoshida, S. Isobe, and K. Itoh. 2001. Decontamination of lettuce using acidic electrolyzed water. *J. Food Prot.* 64:652–658.
18. Koseki, S., K. Yoshida, S. Isobe, and K. Itoh. 2004. Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries. *J. Food Prot.* 67:1247–1251.
19. McEvoy, J. M., A. M. Doherty, M. Finnerty, J. J. Sheridan, L. McGuire, I. S. Blair, D. A. McDowell, and D. Harrington. 2000. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Lett. Appl. Microbiol.* 30:390–395.
20. Mies, P. D., B. R. Covington, K. B. Harris, L. M. Jucia, G. R. Acuff, and J. W. Savell. 2004. Decontamination of cattle hides prior to slaughter using washes with and without antimicrobial agents. *J. Food Prot.* 67:579–582.
21. Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
22. Reid, C.-A., S. M. Avery, M. L. Hutchison, and S. Buncic. 2002. Evaluation of sampling methods to assess the microbiological status of cattle hides. *Food Control* 13:405–410.
23. Reid, C.-A., A. Small, S. M. Avery, and S. Buncic. 2002. Presence of food-borne pathogens on cattle hides. *Food Control* 13:411–415.
24. Restaino, L., E. W. Frampton, J. B. Hemphill, and P. Palnikar. 1995. Efficacy of ozonated water against various food-related microorganisms. *Appl. Environ. Microbiol.* 61:3471–3475.
25. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681–685.
26. Schnell, T. D., J. N. Sofos, V. G. Littlefield, J. B. Morgan, B. M. Gorman, R. P. Clayton, and G. C. Smith. 1995. Effects of postexsanguination dehairing on the microbial load and visual cleanliness of beef carcasses. *J. Food Prot.* 58:1297–1302.
27. Sharma, R. R., A. Demirici, L. R. Beuchat, and W. F. Fett. 2002. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. *J. Food Prot.* 65:447–451.
28. Tuttle, J., T. Gomez, M. P. Doyle, J. G. Wells, T. Zhao, R. V. Tauxe, and P. M. Griffin. 1999. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. *Epidemiol. Infect.* 122:185–192.