FOOD QUALITY and CULTURE
Food Quality Culture 2(1): 55~60 (2008)

Evaluating Commercial Spray Applications of Lactic Acid, Hot Water, and Acidified Sodium Chlorite for the Reduction of *Escherichia coli* on Beef Carcasses

Dong-Hyun Kang[1] and Sun-Young Lee[2]*
[1]*Department of Food Science and Human Nutrition, Washington State University, Pullman WA 99164-6376, USA*
[2]*Department of Food and Nutrition, Chung-Ang University, Nae-ri, Daedeok-myeon, Anseong-si, Gyeonggi-do, Korea*

ABSTRACT This study examined the effects of lactic acid spray, hot water spray, or their combined treatment, as well as the effects of acidified sodium chlorite (ASC), for the decontamination of *Escherichia coli* on beef carcass surfaces using a commercial intervention system. With this system, the effects of 2 or 4% lactic acid (v/v), hot water (89±1°C), or their combined treatment, were examined in terms of reducing inoculated *E. coli*. ASC (266 ppm), which was adjusted to pH 2.5 using acetic acid or citric acid, was applied using a hand-held spray system. When the beef carcasses were treated with 2 or 4% lactic acid for 10.4 s, less than 1 log reductions of inoculated *E. coli* were observed. A hot water spray treatment for 9.8 s resulted in a 2.1 log reduction of inoculated *E. coli*. However, when the hot water was followed with either 2 or 4% lactic acid, no difference in *E. coli* reduction was found between the hot water alone or the combined treatment with lactic acid. When ASC was adjusted to pH 2.5 with acetic acid and citric acid, 3.8 and 4.1 log reductions of *E. coli* were observed, respectively. Overall, the lactic acid spray treatment was least effective, and the ASC treatment was most effective, for the *E. coli* decontamination of beef carcasses. Therefore, these data suggest that ASC would be a more effective intervention against *E. coli* than most of the methods currently being used. However, more research is required to evaluate the effects of ASC on other organisms, as well as to identify application methods that will not affect meat quality.

KEYWORDS: beef carcass, *E. coli*, commercial intervention, hot water, acid, chemical

INTRODUCTION

Escherichia coli O157:H7 is a member of the enterohemorrhagic group of pathogenic *E. coli,* which has emerged as a foodborne and waterborne pathogen of major public health concern. This group of organisms causes a spectrum of illnesses that increase in severity, ranging from mild diarrhea to hemorrhagic colitis, hemolytic uremic syndrome, and, in some cases, death (Doyle MP 1991). In the United States (U.S.), an estimated 10,000 to 20,000 cases of *E. coli* infection occur each year (Mead PS et al 1999). A wide variety of foods have been implicated as vehicles of *E. coli* O157:H7 infection, including meat, milk, fruit juices, and vegetables (Buchanan RL and Doyle MP 1997).

*Corresponding author
Tel: 82-31-670-4587
Fax: 82-31-676-8741
E-mail: nina6026@cau.ac.kr

Received August 27, 2007; Accepted May 10, 2008

Beef is an important food source. For example, the average U.S. beef consumption per capita was 67.7 kg in the year 2000 (Minihan D et al 2003). However, beef is also recognized as an important cause of foodborne illness, including *E. coli* O157 infection. Beef carcasses become contaminated from several sources during slaughter operations, such as from hide or hair, and more rarely, from internal organs ruptured during evisceration (Smith CA 1997). One of most feared properties of *E. coli* O157:H7 is its ability to grow in the bovine digestive tract. For this reason, an important focus of pathogen dissemination is *E. coli* O157:H7 contamination of beef carcasses either directly or indirectly from feces. Both the ease and firmness with which bacteria adhere to a carcass surface during slaughter play an important role in the sanitary quality of the resulting meat; attachment is the first step in contamination and may lead to subsequent surface colonization if conditions allow (Delazari I et al 1998).

In 2002, the United States Food Safety and Inspection Service (FSIS) required all raw beef processors to reassess their HACCP plans to ensure that their critical control points

were adequately addressing *E. coli* O157:H7 contamination (Federal Register 2002). Many commercial beef processing plants presently employ several combined interventions, which may include combinations of trimming, steam vacuuming, steam pasteurization, water washes, and organic acids, to achieve large reductions in carcass contamination in accordance with their individual HACCP plans (Bacon RT et al 2000). Recent studies have demonstrated that combined antimicrobial interventions are more effective at reducing surface contamination on beef tissue than individual interventions alone (Phebus RK et al 1997, Castillo A et al 1999, Kang DH et al 2001).

Acidified sodium chlorite (ASC) is an antimicrobial compound that is used as a broad-spectrum disinfectant. It is approved as a direct food additive by the U.S. Department of Agriculture and U.S. Food and Drug Administration for utilization in poultry and red-meat carcass decontamination (Code of Federal Regulations 1998), where it is used at levels sufficient to achieve a pH of 2.3 to 3.2 on red meat, poultry, seafood, and fruits and vegetables (Hwang CA and Beuchat LR 1995). ASC has also been proven to be an effective microbial control agent in meat products as well as a surface disinfectant and sanitizer, resulting from a combination of antimicrobial effects that it offers due to acid content and the antimicrobial properties of chlorine (Lim K and Mustapha A 2004). ASC's antimicrobial mechanism comes from the conversion of chlorite ions to chlorine dioxide that inhibits protein synthesis in bacterial cells (Thiessen GP et al 1984, Villarreal ME et al 1990, Lim K and Mustapha A 2004).

The effects of a variety of antimicrobial solutions have been tested using warm carcasses or pieces of meat inoculated with bacterial cultures or fecal preparations, and most reports show reduced bacterial numbers under such experimental conditions. However, of the antimicrobials that have been considered, only a few are currently used in commercial practice to treat dressed beef carcass sides. Thus, current information does not allow for the confident selection of an antimicrobial compound that would be both effective and commercially acceptable for treating beef carcasses under disparate conditions (Gill CO and Badoni M 2004). Specific interventions during beef slaughter are designed to reduce bacterial contamination on carcasses and usually involve the application of heat, organic acids, or both (Bolton DJ et al 2001). Therefore, this study was undertaken to determine the effectiveness of hot water, lactic acid, or their combination, as antimicrobial interventions for reducing *E. coli* as a surrogate to *E. coli* O157:H7 on the slaughter floor. Additionally, ASC was investigated for its ability to reduce levels of *E. coli* on beef carcasses, as a possible supplemental or replacement treatment.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation

Three surrogate species, *E. coli* ATCC 25922, *E. coli* K-12 2B, and *E. coli* B E4a, were obtained from the Food Science and Human Nutrition Culture Collection at Washington State University (Pullman, WA, USA). Each *E. coli* strain was grown in 50 mL of Tryptic soy broth (TSB; Difco laboratories, Detroit, MI, USA) for 18 hr at 37°C. The cells were harvested by centrifugation at 4,000×*g* for 20 min at 4°C, and washed twice with buffered peptone water. The final pellet was resuspended in buffered peptone water to a concentration calculated to yield $10^{9\text{-}10}$ CFU mL^{-1} and mixed to construct the culture cocktail. This mixed, concentrated culture suspension was stored at 4°C and used as an inoculum within 24 hr.

Preparation of beef samples and inoculation

All experiments were conducted at a commercial slaughter plant (Toppenish, WA, USA). Dressed beef carcasses (1 hr postmortem), which had received a combination treatment of ambient temperature tap water (22±2°C) and 2%(v/v) lactic acid (pretreatment wash) prior to the antimicrobial interventions, were inoculated with *E. coli*. Two randomly selected portions (1×10 cm) within the chuck area of the dressed beef carcass sides (Fig. 1) were marked and inoculated with the prepared inoculum using a sterile cotton swab. Following inoculation, the carcasses were immediately subjected to further treatments.

Spray applications

Hot (89±2°C) water spray, lactic acid [2 or 4%(v/v)] spray, and a combination of both sprays were applied inside spray cabinets (CHAD, Lenexa, KA, USA) used in the slaughter plant that included an organic acid sanitizing spray assembly (SSA-2000) and a hot water pasteurization system (HWPS-1000) designed to spray lactic acid and hot water subsequently (Fig. 1). For the combination treatment, hot water was sprayed followed by lactic acid. Table 1 describes the treatments in detail. Two hundred sixty-six ppm of ASC (working strength Sanova, pH 2.5; Alcide, Redmond, WA, USA) was prepared using two different types of acid: acetic acid and citric acid. Following preparation, the ASC was sprayed onto inoculated portions of the carcasses for 15 sec at ambient temperature using a hand-held spray gun (Flojet pump, Model # D3631V5011A; Flojet Corporation, Foothill Ranch, CA, USA).

Bacterial enumeration

Before or after treatment, the previously marked portions (10×10 cm) on the carcasses were excised by hand using a

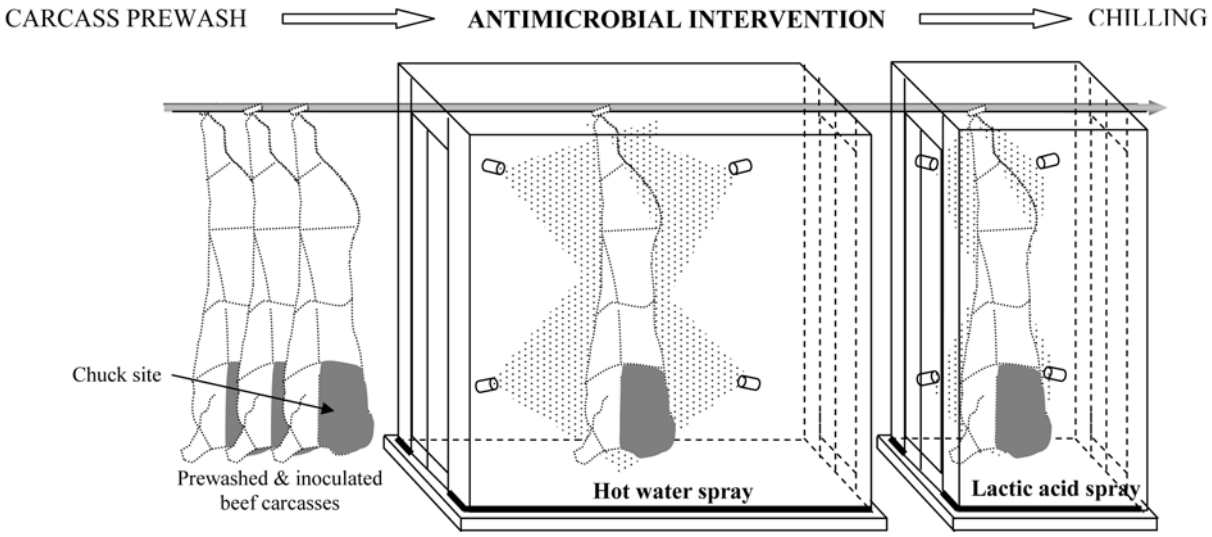

Fig. 1. Diagram of the commercial intervention system (spray cabinets) used in this study. *E. coli* was inoculated onto two different sites in the chuck area of prewashed beef carcasses (see details in the Materials and Methods). One inoculated site was used as a control (non-treated) and the other was used for bacterial enumeration after antimicrobial treatment.

knife sanitized in hot (82.2°C) water. Samples were taken within 30 min following the treatments. Each sample was placed in a stomacher bag containing 100 mL of buffered peptone water (Difco) and was homogenized for 2 min using a stomacher (400 Circulator, Seward, London, UK). Aliquots (1 mL) of the samples were ten-fold serially diluted in 9 mL of sterile buffered peptone water. Finally, one milliliter of sample or diluent was plated onto *E. coli*/Coliform Petrifilm plates (3M Co., Seoul, Korea) and incubated at 37°C for 24 hr, followed by colony enumeration.

Statistical analysis

For each treatment, 10 carcass sides and 2 portions each (10×10 cm) per carcass were tested (n=10). One of the two inoculated portions on a carcass was used as a control and the other was used for the enumeration of surviving *E. coli* following treatment. The average of duplicate plate counts from ten replications was converted to units of $\log_{10}$ CFU cm^{-2}. The data were analyzed by analysis of variance using the GLM procedure of SAS (Version 8.1, SAS Institute Inc., Cary, NC, USA) for a completely randomized block design. The effect of a treatment was tested with block×treatment as an error term. When the main effect was significant (p< 0.05), the means were separated by Duncan's multiple-range test.

Table 1. Summary of intervention treatments used on *E. coli*-inoculated beef carcasses

Treatment	Concentration	Treatment temperature	Treatment pressure	Treatment time	Description
Hot water	-	89±1°C	15 psi	9.8 s	Intervention system (cabinet) One spray bar on each side of the cabinet with 5-6 nozzles
Lactic acid	2 or 4%	RT*	>18 psi	10.4 s	Intervention system (cabinet) Two bars on each side of cabinet with 16 nozzles
Combination of hot water and lactic acid	-	-	-	9.8 s (hot water) and 10.4 s (lactic acid)	Intervention system (cabinet) Hot water treatment followed by lactic acid spray
ASC-citric acid†	266 ppm (pH 2.5)	RT	40 psi	15 s	Hand-held spray gun with single nozzle
ASC-acetic acid†	266 ppm (pH 2.5)	RT	40 psi	15 s	Hand-held spray gun with single nozzle

*Room temperature (22±2°C).
†Acidified sodium chlorite activated by either citric acid or acetic acid.

RESULTS AND DISCUSSION

Specific interventions during beef slaughter are designed to reduce the bacterial contamination of carcasses, and usually involve the application of heat, organic acids, or both (Bolton DJ et al 2001). This study evaluated the effects of commercial hot water and lactic acid spray interventions on beef carcasses prior to chilling. Here, *E. coli* was inoculated onto the beef carcasses at an approximate initial level of 10^{6-7} CFU cm^{-2}. The hot water spray, 2 or 4% lactic acid spray, and a combination of both sprays were tested using commercial spray cabinets. Both the 2 and 4% lactic acid sprays reduced the level of inoculated *E. coli* by less than 1 log (a 0.4 log reduction with 2% lactic acid and a 0.6 log reduction with 4% lactic acid), and there was no significant difference between the treatments ($p>0.05$) (Fig. 2). The hot water spray resulted in a 2.1 log reduction of inoculated *E. coli*. However, when the hot water spray was followed by a lactic acid treatment, there was no significant difference in reduction as compared to the treatment with hot water alone ($p>0.05$) (Fig. 2). Therefore, the hot water spray was more effective at reducing *E. coli* levels on beef carcass surfaces than the lactic acid spray.

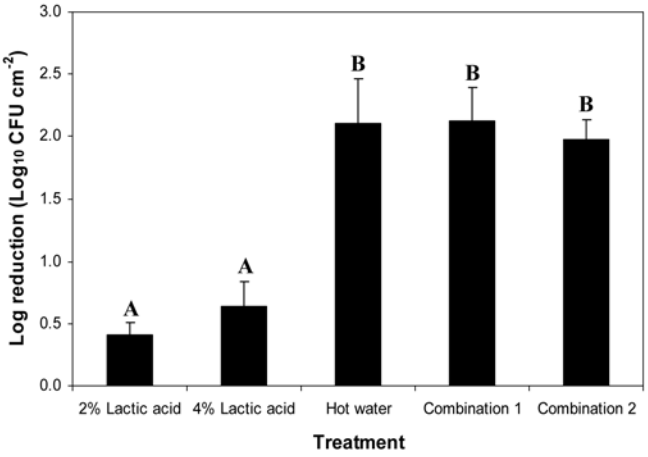

Fig. 2. Reduction of *E. coli* on inoculated beef carcasses following intervention treatments. The combination treatments consisted of hot water followed by treatment with 2% or 4% lactic acid for Combination 1 or Combination 2, respectively. Error bars represent standard deviations of replicates within treatments. Bars with different letters are significantly different ($p < 0.05$).

Organic acids are widely used in the United States for beef carcass decontamination (Smulders FJM and Greer GG 1998). However, varied effectiveness by organic acids against specific target bacterial populations has been reported (Anderson ME et al 1979, Snijders JMA et al 1985, Prasai PK et al 1991, Brackett RE et al 1994, Cutter CN and Siragusa GR 1994, Fratamico PM et al 1996). There is no clear evidence that organic acids have a significant lethal effect on their own, and their effectiveness depends on concentration, temperature, exposure time, mode of application, the type of meat tissue evaluated, and the sensitivity of specific bacterial populations (Anderson ME and Marshall RT 1990). In addition to the antimicrobial effectiveness of organic acids, other considerations affecting their commercial applicability include equipment requirements, employee safety, waste disposal, corrosiveness, and cost (Phebus RK et al 1997). For instance, one study reported that 2% lactic acid was relatively ineffective when it was applied to cooled carcasses; however, substantial reductions in numbers were obtained with a 4% solution (Castillo A et al 2001). Also, Gill CO and Badoni M (2004) reported that the 4% lactic acid treatment of meat resulted in substantial reductions in numbers of aerobes and *E. coli*, in which aerobes were reduced by 1.5 to 2 log units. However, in our study, there was no significant difference between the 2% and 4% lactic acid treatments for *E. coli* reduction. These differences in data may be due to the locations of the microbial inoculations and treatments, since the antimicrobial activity of the treatments could vary depending on the surface characteristics where the microorganisms were attached.

The spraying of beef carcasses with cold or warm water is a commonly practiced intervention method in beef slaughter plants. However, the effects of cold or warm water carcass sprays on bacterial numbers are not clear. Some studies have shown no significant reductions occurring (Sheridan JJ and Sherington J 1984, Gill CO et al 1996, Bell RG 1997), while in other studies, significant reductions were recorded, but only at specific carcass sites (Prasai RK et al 1991, Jericho KWF et al 1995). Therefore, washing with cold or warm water is not considered to be a decontamination step during slaughter as their effects are related solely to improving carcass appearance and not food safety (Bolton DJ et al 2001). In hot water treatments, water of 75-85°C is usually applied to carcasses under pressure as a spray, or by using a deluge system (Bacon RT et al 2000, Gill CO et al 1999). Previous studies have shown that levels of mesophilic flora and *E. coli* were reduced by approximatley 2 log when water was treated at >80°C for 10 sec (Smith MG and Graham A 1978, Davey KR and Smith MG 1989, Gill CO et al 1999). Furthermore, reductions of coliforms and *E. coli* on beef sides using hot water treatments were found to be similar to those reported for commercial treatments using steam (Gill CO and Bryant J 1997a and b, Nutsch AL et al 1997 and 1998). In our study, a slightly higher temperature (89°C) hot water spray produced similar results (2.1 log reduction). However, different results were reported by Barkate ML et al (1993) who used hot water (95°C reduced to 82°C at the carcass surface) to treat freshly slaughtered carcasses either before or after a final carcass wash, demonstrating reductions of 1.3 and 0.8 log CFU cm^{-2}, respectively, when initial populations were 2.4 log CFU cm^{-2}. This difference in data may be due to different initial microbial levels. The main bactericidal effect of hot water is

Table 2. Populations (Log_{10}CFU cm^{-2}) of *E. coli* on beef carcasses at 4ºC following chemical treatments

	Activation with	*E. coli* (Log_{10} CFU cm^{-2})†		
		Initial	After treatment	Reduction
ASC*	Acetic acid	7.37±0.08	3.57±0.30	3.80
	Citric acid	7.31±0.10	3.22±0.26	4.09

*Acidified sodium chlorite (266 ppm, pH 2.5).
†Values are means±standard deviations (n=10).

its thermal effect, although there may also be a physical effect involving the removal of some bacteria as a result of washing (Bolton DJ et al 2001). Hot water is easier and more economical to generate than steam; however, the heat may discolor the carcass surface. Gill CO et al (1999) reported that the treatment of beef carcass sides with 85ºC water for 10 sec substantially reduced (approximate 2 log reduction) natural *E. coli* and coliforms without producing unacceptable damage in product appearance.

Recent studies have demonstrated that combinations of antimicrobial interventions are more effective at reducing surface contamination on beef than individual interventions alone (Phebus RK et al 1997, Kang DH et al 2001, Castillo A et al 1999). And a commercial system of hot water pasteurization followed by organic acid pasteurization has been developed for use in beef slaughter at pre-evisceration and/or prior to chilling (Chad, Olathe, KS; Bolton DJ et al 2001). An earlier study indicated that organic acids, in combination with other treatments such as heating or chilling, may have a beneficial effect (Dickson JS 1992). In the present study, an intervention system designed by CHAD Corporation was used to evaluate the combination treatment of organic acid and hot water. Our results show there was no synergistic effect by combining hot water with either 2 or 4% lactic acid for the reduction of *E. coli* levels on beef carcasses (Fig. 2).

ASC supplemented with acetic acid or citric acid was applied to the beef carcasses using a hand-held spray gun to investigate its effect on *E. coli* decontamination. The average reductions for 10 samples were 3.8 and 4.1 log when ASC was supplemented with acetic acid and citric acid, respectively (Table 2). Both of these ASC formulations significantly reduced *E. coli* on the beef carcasses when compared to the hot water and lactic acid (2 and 4%) sprays, or their combined treatment (p<0.05). Finally, the ASC supplemented with citric acid was more effective at reducing *E. coli* than the ASC supplemented with acetic acid (Table 2).

Recently, several researchers have examined ASC interventions. For example, ASC (0.12%; activated by citric acid) reduced numbers of *E. coli* O157:H7 by 2.5 log CFU/cm^2, and during storage at 4ºC, the level of reduction increased for up to 14 days (Lim K and Mustapha A 2004). Gill CO and Badoni M (2004) investigated the effects of 0·16% ASC (activated by citric acid) and 2 or 4% lactic acid on microflora in chilled beef carcasses and found that ASC was less effective than the 2 or 4% lactic acid (less than 1.5 log reduction). However, in our study, there was less than a 1 log reduction of *E. coli* when 2 or 4% lactic acid were applied and an approximate 4 log reduction when ASC was employed. These conflicting results may be due to different spraying methods, sampling methods, or treatment temperatures.

Our finding that ASC was very effective at reducing *E. coli* on beef carcass surfaces and 2 or 4% lactic acid spray treatments were not, suggests that ASC could replace lactic acid spray in commercial settings. However, treating with high concentrations of ASC can change meat quality, causing discoloration similar to lactic acid sprays due to a low pH. Several studies have reported loss of redness and light color on beef surfaces after ASC treatment (Lim K and Mustapha A 2004, Xiong H et al 1998). Also, the efficacy of antimicrobial sprays on beef surfaces could differ depending on the types of microorganisms present and the treatment conditions such as spraying system, spraying pressure, and treatment time and temperature. Lim K and Mustapha A(2004) investigated the effects of cetylpyridinium chloride (CPC), ASC supplemented with citric acid, and potassium sorbate (PS), on pathogens occurring on fresh beef. They found that ASC was the most effective agent for reducing *E. coli* O157:H7, but CPC was the most effective treatment for reducing *Listeria monocytogenes* and *Staphylococcus aureus*. Therefore, further research is required before ASC can be applied commercially.

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