APPLICATION OF CHLORINE TO REDUCE POPULATIONS OF *ESCHERICHIA COLI* ON BEEF

CATHERINE NETTLES CUTTER[1] and GREGORY R. SIRAGUSA

United States Department of Agriculture, Agricultural Research Service[2]
Roman L. Hruska U.S. Meat Animal Research Center
P.O. Box 166, Clay Center, Nebraska 68933

Received for Publication June 10, 1994
Accepted for Publication November 14, 1994

ABSTRACT

The effects of chlorine against 2 strains of E. coli *attached to the surface of beef carcass tissue (BCT) were examined using a model carcass washer. Lean and adipose BCT with approximately 5 $\log_{10}$ CFU/cm² E. coli bacteria were spray-treated with water and sodium hypochlorite (NaOCl) to give chlorine concentrations of 50, 100, 250, 500, or 800 ppm, incubated for 24 h, 4C, and* E. coli *populations enumerated. Spray treatments with water did significantly ($P < 0.05$) reduce the bacterial populations of either organism attached to lean or adipose BCT, as compared to populations of controls; however, reductions were less than 0.60 $\log_{10}$ CFU/cm². Treatments with 500 and 800 ppm chlorine against* E. coli *ATCC 25922 attached to BCT resulted in the greatest reductions of 1.22 and 1.28 $\log_{10}$ CFU/cm², respectively. At 800 ppm chlorine,* E. coli *O157:H7 ATCC 43895 attached to BCT was reduced by 1.04 $\log_{10}$ CFU/cm², whereas spray treatments with 50, 100, 250, and 500 ppm chlorine resulted in reductions of < 1 $\log_{10}$ CFU/cm². Spray treatments with chlorine from sodium hypochlorite solutions reduced populations of* E. coli, *however, these reductions were not sufficient to completely inactivate the bacteria attached to red meat.*

[1]Author's phone number: 402-762-4386.

[2]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Journal of Food Safety **15** (1995) 67–75. *All Rights Reserved.*

INTRODUCTION

Several studies have examined the use of chlorine compounds, alone or in combination with other methods, to limit the growth of natural microflora on the surface of carcasses (Kotula *et al.* 1974; Kotula 1975; Emswiler *et al.* 1976; Anderson *et al.* 1977a, 1977b; Childers *et al.* 1977; Marshall *et al.* 1977; Stevenson *et al.* 1978; Titus *et al.* 1978; Johnson *et al.* 1979; Kelly *et al.* 1981, 1982; Rose and Kotula 1984; Kuttinarayanan and Soman 1985; Carpenter *et al.* 1986), in ground meat products (Stevenson *et al.* 1978; Johnson *et al.* 1979; Skelley *et al.* 1985) or primal cuts (Stevenson *et al.* 1978; Cacciarelli *et al.* 1983; Fandino *et al.* 1989) produced from spray-treated carcasses. During the 1970's, a chlorine chilling process was implemented in several meat processing facilities to reduce shrinkage and spoilage bacteria on red meat carcasses while maintaining a commercially acceptable red meat product (Hanson *et al.* 1973; Johnson *et al.* 1979). By repeatedly spraying chlorine (50–200 ppm) at predetermined intervals during the chill cycle, aerobic plate counts associated with the carcass surface were reduced 94.5–99.96% (Kelly et al. 1982).

Given that chlorine compounds are used as sanitizers in the food industry for utensils and surfaces as well as for the treatment of public water supplies, and the recent outbreaks of *E. coli* O157:H7 associated with meat products (Anon. 1993), this study was carried out to determine the efficacy of chlorine from sodium hypochlorite solutions against enteropathogenic and nonpathogenic strains of *E. coli* attached to red meat.

MATERIALS AND METHODS

Bacterial Cultures

Escherichia coli ATCC 25922 and *Escherichia coli* O157:H7 ATCC 43895 were obtained from American Type Culture Collection (ATCC; Rockville, MD). Both the nonpathogenic and enteropathogenic strain were maintained in 75% glycerol at −20C. Strains were propagated in tryptic soy broth (TSB; Troy Biologicals, Troy, MI) at 37C for 18 h.

Inoculation of Beef Carcass Tissue

Surface lean and adipose beef carcass tissues (BCT) from the outer surfaces of postrigor beef carcasses were obtained from the Roman L. Hruska U. S. Meat Animal Research Center (RLHUSMARC) abattoir, trimmed to 7.5 cm × 7.5 cm, surface sterilized by ultraviolet light, and stored at −20C, as described previously (Cutter and Siragusa 1994, 1995). Early stationary phase cultures were

diluted 1:100 in sterile physiological saline (pH 7.0) to obtain a viable cell density of approximately 9 $\log_{10}$ CFU/ml. After thawing to 25C, the fascia of individual pieces of lean and adipose BCT were placed in a sterile weigh boat (14 cm × 14 cm) containing 10 ml of the bacterial suspension, incubated for 15 min, 25C, allowed to drip, and subjected to spray treatments with water or hypochlorite solutions. Populations of approximately 5 $\log_{10}$ CFU/cm^2 were obtained using this methodology.

Sodium Hypochlorite Preparation

Sodium hypochlorite (NaOCl; 5% minimum available chlorine; Sigma Chemical Co., St. Louis, MO) was diluted in sterile distilled water to give final concentrations of chlorine of 50, 100, 250, 500, and 800 ppm. Solutions were adjusted to pH 6.5 with hydrochloric acid (Fisher Scientific, St. Louis, MO) and used immediately after preparation. Sterile distilled water for spraying (pH 6.5) was stored at 28C until needed.

Spray Treatments and Experimental Design

The solutions of NaOCl and water were tested against two organisms attached to lean or adipose BCT. A model carcass washer (MCW) located at RLHUSMARC was used to apply water or NaOCl solutions as described previously (Cutter and Siragusa 1994). Operation parameters for the MCW were as follows: spray nozzle oscillation speed, 80 cycles/min; chain speed, 14 m/min; nozzle pressure, 60 psi; flow rate, 4.2 L/min; nozzle distance from sample, 17.8 cm; temperature of solutions, 28C. Following spray treatments with either water or NaOCl solutions, BCT was trimmed to 25 cm^2, and placed into a stomacher bag (Spiral BioTech, Bethesda, MD), to prevent contamination and dehydration of the tissue. To mimic commercial conditions following spray washing, BCT was stored at 4C for 24 h, at which time samples were prepared for bacterial enumeration. BCT inoculated with bacteria but not subjected to spray treatments (untreated samples), were handled similarly. After excising the 25 cm^2 section, remaining pieces of control and treated BCT were used to assess surface pH values (flat electrode, Corning Instruments, Corning, NY) and again after storage at 4C, 24 h.

Bacterial Enumeration

Each 25 cm^2 piece of untreated or spray treated BCT was homogenized for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH) in 50 ml of a neutralizing buffer (Difco, Detroit, MI) consisting of sodium thiosulfate with 0.1% Tween 20 (Fisher Scientific). Homogenates were diluted in 2% buffered peptone water

(Difco) and plated on trypticase soy agar (TSA, Troy Biologicals, Troy, MI) using a Model D Spiral Plater (Spiral Biotech, Bethesda, MD). Plates were enumerated after incubation for 48 h at 37C.

Calculations and Statistical Analyses

Following enumeration, bacterial populations were converted to $\log_{10}$ CFU/cm^2 values. The least squared means (LSM) of bacterial populations were calculated from three experimental replications. Statistical data analysis (ANOVA) was performed using the GLM Procedure of SAS 1989. Inoculum counts were used as a covariant to normalize data from treatment replications. A log reduction factor (LRF) was calculated as the difference between populations of cells attached to meat and cells attached to meat treated with water or NaOCl solutions (LRF = log CFU/cm^2 untreated − log CFU/cm^2 treated). The probability level was $P < 0.05$, unless otherwise noted.

RESULTS

The efficacy of five concentrations of chlorine from sodium hypochlorite solutions against two strains of *E. coli* attached to lean and adipose BCT was ascertained in this study. Figure 1a demonstrates the significant effect ($P < 0.0001$) of treatment against *E. coli* ATCC 25922. When compared to untreated cells, treatments with water, 50, 100, and 250 ppm chlorine did significantly alter populations of *E. coli* ATCC 25922 attached to lean or adipose BCT, however not greater than 0.80 $\log_{10}$ CFU/cm^2. At 500 and 800 ppm chlorine, the organism was maximally reduced by 1.22 and 1.28 $\log_{10}$ CFU/cm^2, respectively. Additional analyses indicated that tissue type did not impact upon the results. No 2-way interaction of treatment × tissue was observed in this experiment.

At 800 ppm chlorine, *E. coli* O157:H7 ATCC 43895 attached to BCT was significantly reduced by 1.04 $\log_{10}$ CFU/cm^2 (Fig. 1b). While spray treatments with water or NaOCl at concentrations of 50 and 250 ppm chlorine did significantly reduce the pathogen, reductions were not greater than 0.80 $\log_{10}$ CFU/cm^2. As demonstrated previously, tissue type did not affect the results (data not shown) of spray washing with these solutions against *E. coli* O157:H7 ATCC 43895. Additionally, no 2-way interaction of treatment × tissue was observed for treatments against the pathogen.

ANOVA of surface pH data did not demonstrate a significant 3-way interaction of treatment × tissue × day; however, individual factors (treatment, tissue, day) were significant ($P < 0.001$). Treatments ($P < 0.0012$) with chlorine solutions of 50, 100, 250, 500, and 800 ppm resulted in surface pH values of 6.38, 6.45, 6.25, 6.23, 6.06, respectively. With regard to tissue type ($P < 0.001$),

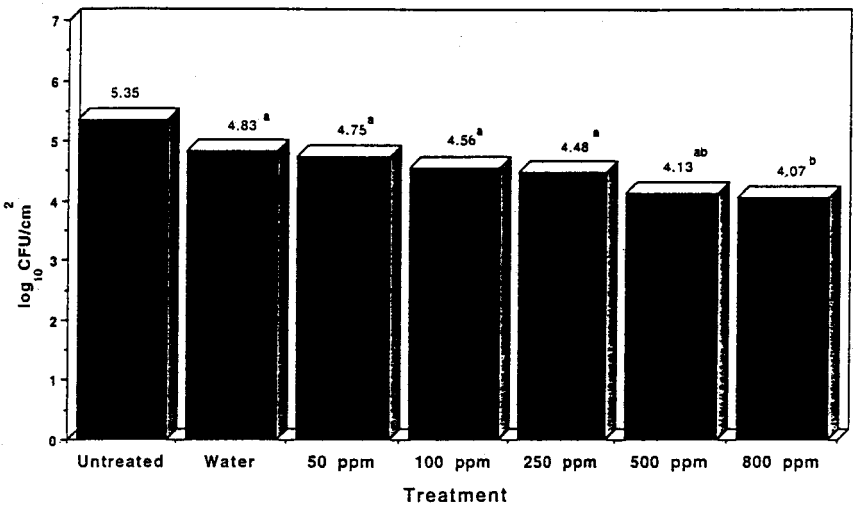

FIG. 1a. THE SIGNIFICANT EFFECT ($P < 0.0001$) OF TREATMENT AGAINST *E. COLI* ATCC 25922 ATTACHED TO BCT FOLLOWING TREATMENTS WITH WATER AND CHLORINE FROM SODIUM HYPOCHLORITE SOLUTIONS AND INCUBATION AT 4C, 24 H

[a,ab,b]Means with the same letter(s) were not statistically different at $P < 0.027$ when compared to control tissues.

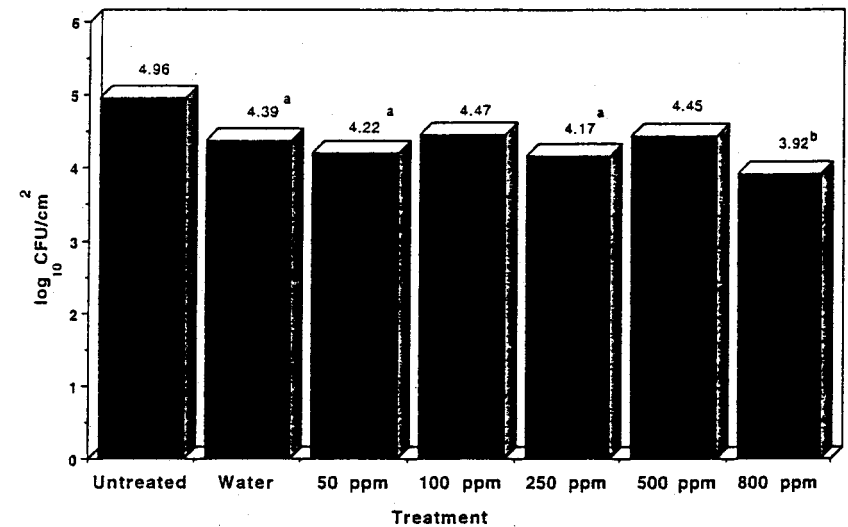

FIG. 1b. THE SIGNIFICANT EFFECT ($P < 0.027$) OF TREATMENT OF *E. COLI* O157:H7 ATCC 43895 ATTACHED TO BCT FOLLOWING TREATMENTS WITH WATER AND CHLORINE FROM SODIUM HYPOCHLORITE SOLUTIONS AND INCUBATION AT 4C, 24H

[a,b]Means with the same letter were not statistically different at $P < 0.05$ when compared to control tissues.

surface pH values of adipose BCT (6.96) were higher than values for lean BCT (5.67), while surface pH values of tissues increased from 6.19 on day 0 to 6.44 on day 1 ($P < 0.001$).

DISCUSSION

For the sanitation of food processing equipment and utensils, the highest acceptable level of chlorine is 200 ppm (FDA 1993). For research purposes, NaOCl solutions with chlorine levels of 50, 100, 250, 500, and 800 ppm chosen in this study represent a range lower and higher than the acceptable limit of 200 ppm.

The effectiveness of chlorine from NaOCl solutions was examined against *E. coli* strains attached to red meat. Spray treatments with increasing concentrations of chlorine solutions resulted in concurrent reductions in bacterial populations on either lean or adipose tissue. The greatest reductions (1.28 $\log_{10}$ CFU/cm^2) in this study were observed when *E. coli* strains were treated with 500 or 800 ppm of chlorine solutions. Spray treatments with water resulted in reductions < 0.60 $\log_{10}$ CFU/cm^2 on either BCT for either organism. As applied in this study, spray treatments with water, as well as lower concentrations of chlorine from NaOCl solutions, may reduce bacterial populations by physically removing bacterial cells from the surface of BCT.

In the present study, data from both tissues indicate that application of NaOCl solutions, possibly due to the presence of hypochlorous acid, initially alters the surface pH values. After 24 h, the lowered surface pH due to acid was diminished on both tissues since pH values returned to pretreatment values.

When NaOCl is mixed in water, three available forms of chlorine may be found: elemental chlorine (Cl_2), hypochlorous acid (HOCl), or hypochlorite ion (OCl^-; Marriott 1985). It is believed that hypochlorous acid is responsible for the lethal reactions associated with the bacterial cell membrane, DNA denaturation, or disruption of protein synthesis (Marriott 1985). Other reports have demonstrated that treatments with sodium or calcium hypochlorite against *E. coli* in aqueous solutions, resulted in reductions of 90% or greater (Gelinas and Goulet 1983). However, in the presence of nitrogenous organic material (i.e., dried beef blood, whole milk, or fish meal), the available chlorine can react to form chloramines, thereby reducing the antimicrobial effectiveness of the chlorine (Gelinas and Goulet 1983). The abundance of organic material associated with red meat may be responsible for the ineffectiveness of chlorine as an antimicrobial agent against *E. coli* strains in the present study. Given the association of chloramine formation with health problems (Dunnick and Melnick 1993), it is unlikely that spray treatments of carcasses with chlorine at four times the legal limit will meet with regulatory approval.

Even at levels near 200 ppm, reductions due to chlorine were not different from reductions from spraying with water. While sodium hypochlorite solutions are ineffective against the growth of *E. coli* on beef, continued investigation of other decontamination methods for reducing the incidence of *E. coli* on carcasses is warranted.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Tsai, Provost, and Kastner for their critical review of this manuscript and to thank Jane Long, Carole Smith, and Kevin Tennill for their technical support of this project.

REFERENCES

ANDERSON, M.E., MARSHALL, R.T., STRINGER, W.C. and NAUMANN, H.D. 1977a. Efficacies of three sanitizers under six conditions of application to surfaces of beef. J. Food Sci. *42*, 326–329.

ANDERSON, M.E., MARSHALL, R.T., STRINGER, W.C. and NAUMANN, H.D. 1977b. Combined and individual effects of washing and sanitizing on bacterial counts of a meat-model system. J. Food Prot. *40*, 668–670.

Anon. 1993. Preliminary report: Foodborne outbreak of *Escherichia coli* O157:H7 infections from hamburgers-western United States. Morbid. Mortal. Week. Rept. *42*, 85–86.

CACCIARELLI, M.A., STRINGER, W.C., ANDERSON, M.E. and NAUMANN, H.D. 1983. Effects of washing and sanitizing on the bacterial flora of vacuum-packaged pork loins. J. Food Prot. *46*, 231–234.

CARPENTER, J.A., REAGAN, J.O., BAUER, F.T. and HARRISON, M.A. 1986. The effects of carcass decontamination on retail case life of pork chops. J. Food Quality *9*, 311–317.

CHILDERS, A.B., KEAHEY, E.E. and KOTULA, A.W. 1977. Reduction of *Salmonella* and fecal contamination of pork during swine slaughter. J. Am. Vet. Med. Assoc. *171*, 1161–1164.

CUTTER, C.N. and SIRAGUSA, G.R. 1994. Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. J. Food Prot. *57*, 97–103.

CUTTER, C.N. and SIRAGUSA, G.R. 1995. Decontamintation of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiol. *11*, 481–489.

DUNNICK, J.K. and MELNICK, R.L. 1993. Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. J. Nat. Cancer Instit. *85*, 817–822.

EMSWILER, B.S., KOTULA, A.W. and ROUGH, D.K. 1976. Bactericidal effectiveness of three chlorine sources used in beef carcass washing. J. Anim. Sci. *42*, 1445–1450.

FANDINO, G., SKELLEY, G.C. and HANDLIN, D.L. 1989. Acceptability, shrinkage, and microbial growth of vacuum packaged pork comparing intact packages, leaking packages, and roasts sprayed with sodium hypochlorite. J. Food. Prot. *52*, 35–40.

FDA. 1993. Substances utilized to control the growth of microorganisms. Food and Drug Admin., Code of federal regulations. April 1, *21*, 316–325.

GELINAS, P. and GOULET, J. 1983. Neutralization of the activity of eight disinfectants by organic matter. J. Appl. Bacteriol. *54*, 243–247.

HANSON, R.R., BARRINGTON, T.A., RAY, L. and REIMER, G.M. 1973. Carcass chilling process. U.S. Pat. 3,745,026. Swift & Company, Chicago, ILL.

JOHNSON, M.G., TITUS, T.C., McCASKILL, L.H. and ACTON, J.C. 1979. Bacterial counts on surfaces of carcasses and in ground beef from carcasses sprayed or not sprayed with hypochlorous acid. J. Food Sci. *44*, 169–173.

KELLY, C.A., DEMPSTER, J.F. and McLOUGHLIN, A.J. 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.

KELLY, C.A., LYNCH, B. and McLOUGHLIN, A.J. 1982. The effect of spray washing on the development of bacterial numbers and storage life of lamb carcasses. J. Appl. Bacteriol. *53*, 335–341.

KOTULA, A.W. 1975. Washing carcasses with chlorinated water. In *Proceedings of the Meat Industry Conferences*, pp.. 274–287. AMI Foundation, Arlington, VA.

KOTULA, A.W., LUSBY, W.R., CROUSE, J.D. and de VRIES, B. 1974. Beef carcass washing to reduce bacterial contamination. J. Anim. Sci. *39*, 674–679.

KUTTINARAYANAN, P. and SOMAN, M. 1985. Effect of chlorine water spraying for reducing bacterial load on carcase. Kerala J. Vet. Sci. *16*, 126–129.

MARRIOTT, N.G. 1985. In *Principles of Food Sanitation*. Van Nostrand Reinhold/AVI, New York.

MARSHALL, R.T., ANDERSON, M.E., NAUMANN, H.D. and STRINGER, W.C. 1977. Experiments in sanitizing beef with sodium hypochlorite. J. Food Prot. *40*, 246–249.

ROSE, B.E. and KOTULA, A.W. 1984. Inhibition of bacterial growth by two chlorine sources in a model system. J. Food Sci. *49*, 931–933.

SAS. 1989. SAS User's Guide: Statistics, ver. 6.06.01, SAS Institute, Cary, NC.

SKELLEY, G.C., FANDINO, G.E., HAIGLER, J.H. and SHERARD, R.C. 1985. Bacteriology and weight loss of pork carcasses treated with a sodium hypochlorite solution. J. Food Prot. *48*, 578–581.

STEVENSON, K.E., MERKEL, R.A. and LEE, L.C. 1978. Effects of chilling rate, carcass fatness and chlorine spray on microbiological quality and case-life of beef. J. Food Sci. *43*, 849–852.

TITUS, T.C., ACTON, J.C., McCASKILL, L. and JOHNSON, M.G. 1978. Microbial persistence on inoculated beef plates sprayed with hypochlorite solutions. J. Food Prot. *41*, 606–612.