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Chlorine Dioxide Spray Washes for Reducing Fecal Contamination on Beef†

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ABSTRACT

The ability of chlorine dioxide (ClO_2) to reduce bacterial populations (i.e., aerobic plate count, APC) on fecally contaminated beef carcass tissue (BCT) was examined in two separate experiments. In the first study, individual pieces of BCT were inoculated with fresh bovine feces to obtain approximately 6.60 log APC/cm^2 and spray treated (10 s; 520 kPa; 16°C) with ClO_2 at tank concentrations ranging from 0 to 20 ppm. Bacterial populations were reduced by no more than 0.93 log CFU/cm^2, regardless of ClO_2 concentration, and were not statistically different ($P \geq 0.05$) from water-treated BCT. In the second study, tap water (16°C) and ClO_2 at a tank concentration of 20 ppm (16°C) were sprayed (690 kPa) for 15, 30, and 60 s onto BCT inoculated with fresh bovine feces to obtain approximately 5.80 log APC/cm^2 and the remaining bacterial populations compared. While spray treatments with ClO_2 or water reduced APC by 1.53 to 2.07 log CFU/cm^2, spray treatments with either water or ClO_2 at 15, 30 or 60 s were not statistically different ($P \geq 0.05$). Similar reductions (1.61 log CFU/cm^2) were observed when BCT was spray treated for 60 s with tap water followed by a 60 s spray wash with ClO_2. These results demonstrate that spray treatments with ClO_2 are no more effective than water for reducing fecal contamination on beef.

Key words: Chlorine dioxide, beef, spray washing, fecal contamination

Chlorine is presently used as a sanitizer in the food industry for utensils and food-contact surfaces as well as for the treatment of public water supplies. It has been reported that chlorine spray washes at high concentrations (800 ppm) did not reduce bacterial populations of *Escherichia coli* O157:H7 on beef carcass tissue by more than 1.3 log CFU/cm^2 (*4*). Greater reductions did not occur, presumably because of the abundance of organic material and nitrogenous compounds associated with red meat and subsequent formation of inactive forms of chlorine (*4*). Another chlorinated compound used to disinfect public water supplies that is finding application in the food industry is chlorine dioxide (ClO_2). ClO_2 is soluble in water, does not react with ammonia or nitrogenous compounds like chlorine, has a greater oxidizing capacity than chlorine, and its lethality towards bacteria is not affected by high pH (*2*). The mechanism of action of the compound against bacteria involves the loss of permeability control with nonspecific oxidative damage to the outer membrane and subsequent destruction of the transmembrane ionic gradient (*3*). Several reports have addressed the use of ClO_2 as a bactericide to reduce bacterial populations both in poultry chiller water (PCW) and on poultry carcasses. Results from these studies demonstrated that ClO_2 at <20 ppm was as effective as higher concentrations (>20 ppm) of chlorine (*5, 6*); ClO_2 treatments extended the shelf life of broilers (*8*); and ClO_2 treatments reduced the incidence of *Salmonella* spp. on poultry carcasses (*9*). Pork carcasses have also been subjected to spray treatments with ClO_2 (*7*); however, there are no reports in which ClO_2 has been examined as a decontaminant of beef. This study describes the application of ClO_2 in sprays to reduce fecal contamination on prerigor beef.

MATERIALS AND METHODS

Generation and analytical determination of chlorine dioxide

Chlorine dioxide (ClO_2) gas was generated in the laboratory using the manufacturer's (Rio Linda, Sacramento, CA) instructions as follows. Concentrated hydrochloric acid (Fisher Scientific Co., St. Louis, MO) was added slowly to 200 ml of 25% sodium chlorite (Rio Linda) that was constantly purged with nitrogen (approximately 50 kPa; 99.9% pure). During generation, the gas was directed to a diffuser through a series of traps (Figure 1). The gas was absorbed into 2 l of deionized water and stored for up to one week in a dark bottle (Nalge Co., Rochester, NY) at 4°C. The concentration and purity of ClO_2 was determined using established titration procedures (*1*). Using the HACH Free Chlorine Kit (Loveland, CO), any available free chlorine was measured in tap water, tank solutions, or sprays and recorded (Table 1). A general-purpose electrode (Corning Instruments, Corning, NY) was used to obtain pH values for all solutions used in the experiments (Table 1).

† 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.

Preparation of tissue and inoculation of feces

Lean beef carcass tissue (BCT) was obtained from the *cutaneous trunci* of prerigor beef carcasses within 20 min of slaughter, trimmed to 10 cm by 10 cm by 0.5 cm pieces, and surface sterilized by using ultraviolet light, [(*4*) 60 Watt germicidal bulbs, at a 51-cm distance from the tissue, 20 min each side]. Bovine feces were obtained from three different heifers fed a corn-silage ration, mixed

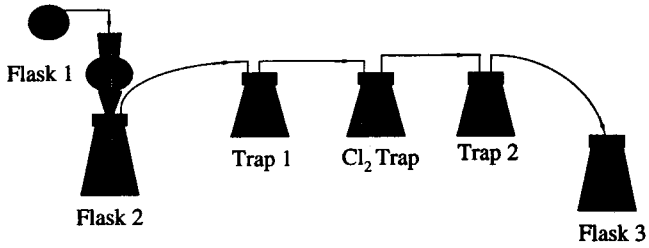

Figure 1. *Schematic diagram of chlorine dioxide generation in the laboratory. N_2 was continually purged into flask 2 at approximately 50 kPa. Flask 1 contained 250 ml of concentrated HCl. Flask 2 contained 200 ml of 25% sodium chlorite. Trap 1 was an empty flask used to prevent acid carryover into the Cl_2 trap, which contained 200 ml of 10% sodium chloride with 2% NaOH. Trap 2 was another empty flask to prevent carryover of chlorite into flask 3. Flask 3, a dark plastic 2-l bottle, contained deionized water, 4°C, in which chlorine dioxide was absorbed.*

together, diluted 1:2 in sterile distilled water, serially diluted, and used within 1 h of defecation. The diluted feces were plated, and bacterial flora were enumerated as described below. Individual pieces of lean BCT were inoculated by applying a layer of the diluted feces (25°C) onto the fascia side of the tissue with a sterile paintbrush and incubating for 15 min at 25°C. Bacterial counts of approximately 6 log CFU/cm^2 were obtained using this methodology.

Experimental design

A pilot-scale model carcass washer (MCW) (*4*) was used to apply ClO_2 in two separate experiments. In the first experiment (A), a stock solution of 1,460 ppm ClO_2 was diluted in 40 l of tap water to obtain ClO_2 concentrations of 0, 2, 5, 8, 10, 12, 15, and 20 ppm. Individual pieces of feces-inoculated lean BCT were subjected to spray treatments with ClO_2 solutions using the following parameters: line pressure, 520 kPa; flow rate, 3.6 l/min; 10 s; temperature of solution in tank, 16°C; spray head oscillation, 60/min; nozzle distance from sample, 17.8 cm. In the second experiment (B), a stock concentration of 2,650 ppm ClO_2 was diluted in tap water to obtain ClO_2 concentrations of 11.5 (chlorine dioxide; cd) and 19 ppm (chlorine dioxide-high; cdh). Individual pieces of feces-inoculated lean BCT were subjected to spray treatments with tap water and ClO_2 solutions as follows: line pressure, 690 kPa; flow rate, 4 l/min; BCT exposed for washes for 0, 15, 30, and 60 s; temperature of solutions in tank, 16°C; spray head oscillation, 60/min. A 60-s spray wash with tap water followed by a 60 s spray wash with 19 ppm ClO_2 (w60-cdh60) as well as a 60 s spray wash with 19 ppm ClO_2 alone (cdh60) were also analyzed.

Bacterial enumeration and surface pH determination

Immediately after spray treatments for either experiment, a 5 by 5 by 0.5 cm (25 cm^2 total surface area) piece was aseptically excised from the treated BCT. BCT inoculated with feces but not subjected to spray treatments (untreated samples, 0 s) was handled similarly. Each 25-cm^2 piece of BCT was stomached for 2 min in 50 ml of 2% buffered peptone water (BPW)(Difco Laboratories, Detroit, MI) with 0.1% Tween 20. Serial dilutions were made in 2% BPW and samples were plated on trypticase soy agar (BBL)(Cockeysville, MD) using a Model D Spiral Plater (Spiral Biotech, Bethesda, MD). Plates were enumerated with the CASBA lll Image Colony Counter (Spiral Biotech, Bethesda MD) after incubation for 36 h at 35°C. After excising the 25-cm^2 section, remaining pieces of untreated and treated BCT were used to assess surface pH values with a flat electrode (Corning Instruments, Corning, NY). The surface pH values (average of three replications) of BCT remained at or near neutrality, regardless of treatment; pH of untreated BCT ranged from 7.04 to 7.37, water-spray-treated BCT pH ranged from 6.91 to 7.32, and ClO_2 spray-treated BCT pH ranged from 7.05 to 7.52.

Data analyses

Following enumeration, bacterial populations of APC (CFU/ml) were converted to log CFU/cm^2 values. The least squared means (LSM) of bacterial populations were calculated from three experimental replications. Data were evaluated by analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS, ver. 6.06.01, 1989 (SAS Institute, Inc., Cary, NC). Statistical significance was defined as $P \leq 0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

In experiment A, spray washes with various ClO_2 concentrations were examined for reducing bacterial populations associated with fecal contamination on beef. During spray washing, the concentration of free chlorine was greatly diminished, compared to tank concentrations (Table 1). Spray treatments with water or ClO_2 significantly reduced the APC (0.93 log CFU/cm^2) from the surface of BCT, as compared to

TABLE 1. *Concentration and pH of ClO_2 in the tank and during spray washes*

Calculated ClO_2 (ppm)	pH of ClO_2 solns in tap water	free chlorine in tank (ppm)	free chlorine during spray (ppm)
0[a]	7.00	0	0
2[a]	6.61	1.8	0.2
5[a]	6.83	5.8	0.9
8[a]	6.93	8.0	0.4
10[a]	7.05	10	5.2
12[a]	7.08	11.8	6.8
15[a]	7.12	14.4	7.4
20[a]	7.12	20	11.4
15[b]	7.43	11.5	6.7
20[b]	7.46	19	9.6

[a] Denotes concentration used in experiment A.
[b] Denotes concentration used in experiment B.

untreated BCT (Figure 2). However, data analyses demonstrated that at tank concentrations up to 20 ppm, spray washing with ClO_2 was no more effective than spray washing with water for reducing bacterial counts. The lack of reduction difference between the APC of water-treated and ClO_2-spray-treated BCT may be due to the dissipation or atomization of ClO_2 from the sprays, which thereby prevented prolonged contact with the BCT.

In view of the short contact time (10 s) and low pressure (520 kPa) in the first experiment, a second experiment (B) was devised to examine whether an extended contact time (15 to 60 s) and increased pressure (690 kPa) would increase the effectiveness of ClO_2 against APC of fecally contaminated BCT. The results from this experiment (Figure 3) demonstrated that spray washing with either water or ClO_2 for up to 60 s significantly reduced ($P \leq 0.05$) the APC by >1.53 log CFU/cm^2, compared to untreated BCT. However, a 60-s water wash followed by a 60-s spray wash with ClO_2 (w60-cdh60) did not significantly reduce ($P \leq 0.05$) the APC associated with fecal contamination any more than 15-s spray washes with water or ClO_2 alone. The observed reductions in APC associated with fecal contamination can be attributed to the physical removal of bacteria that occurs during spray washing rather than any bactericidal action of ClO_2.

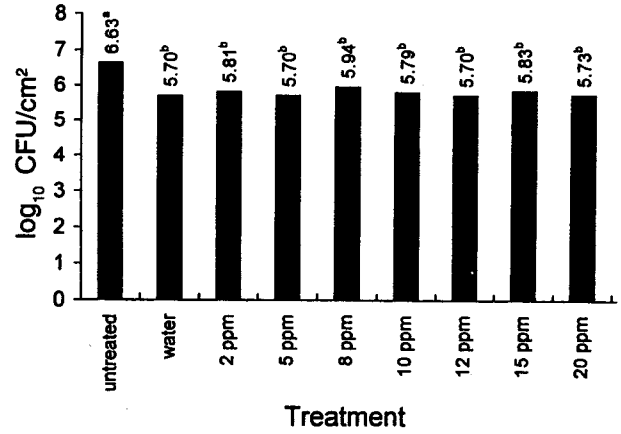

Figure 2. *Bacterial populations (log CFU/cm^2) following spray treatments with water and chlorine dioxide (520 kPa; 10 s; 16°C).* [a,b] *Denote statistical difference between populations* ($P \leq 0.05$).

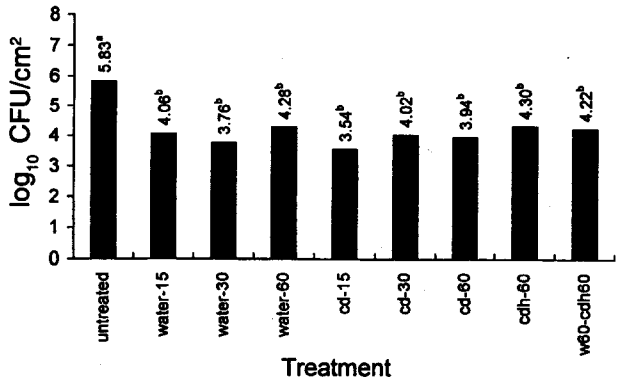

Figure 3. *Bacterial populations (log CFU/cm^2) following spray treatments with water and chlorine dioxide (690 kPa; 15, 30, 60 s; 16°C). cd, ClO_2 at 11.5 ppm in tank/6.65 ppm in spray. cdh, ClO_2 at 19 ppm in tank/9.6 ppm in spray. w60-cdh60, 60 s water followed by 60 s ClO_2 at 19 ppm in tank/9.6 ppm in spray.* [a,b] *Denote statistical difference between populations* ($P \leq 0.05$).

Treatments with ClO_2 have reduced APC approximately 1 log CFU/ml in PCW, >1.21 CFU/g on poultry carcasses (*5*, *6*), and up to 2 log CFU/cm^2 on pork carcasses (*7*). In these cases, the animal carcasses were subjected to prolonged exposure to the compound either by an extended submersion of 40 min in chiller tanks (*5*, *6*) or during intermittent sprays over a 24-h chilling period (*7*). Since water controls were not implemented in the patent claim for pork, bacterial reductions associated with ClO_2 spray treatments may be due, in part, to the physical effects of washing and/or dripping. The results from the present study indicate that spray washes with ClO_2 of up to 1 min were no more effective than water as a decontaminant of beef. Because of the cost of lost product associated with trimming red meat carcasses and a continued interest in reducing fecal contamination, researchers and industry are continuing to investigate the use of antimicrobial compounds such as ClO_2 or other methods, for decontaminating carcasses. Despite the ineffectiveness of ClO_2 against bacteria associated with fecal contamination, other decontamination methods for carcasses are warranted.

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