Comparison of Electrolyzed Oxidizing Water with Various Antimicrobial Interventions to Reduce *Salmonella* Species on Poultry

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ABSTRACT Foodborne pathogens in cell suspensions or attached to surfaces can be reduced by electrolyzed oxidizing (EO) water; however, the use of EO water against pathogens associated with poultry has not been explored. In this study, acidic EO water [EO-A; pH 2.6, chlorine (CL) 20 to 50 ppm, and oxidation-reduction potential (ORP) of 1,150 mV], basic EO water (EO-B; pH 11.6, ORP of -795 mV), CL, ozonated water (OZ), acetic acid (AA), or trisodium phosphate (TSP) was applied to broiler carcasses inoculated with *Salmonella* Typhimurium (ST) and submerged (4 C, 45 min), spray-washed (85 psi, 25 C, 15 s), or subjected to multiple interventions (EO-B spray, immersed in EO-A; AA or TSP spray, immersed in CL). Remaining bacterial populations were determined and compared at Day 0 and 7 of aerobic, refrigerated storage. At Day 0, submersion in TSP and AA reduced ST 1.41 $\log_{10}$, whereas EO-A water reduced ST approximately 0.86 $\log_{10}$. After 7 d of storage, EO-A water, OZ, TSP, and AA reduced ST, with detection only after selective enrichment. Spray-washing treatments with any of the compounds did not reduce ST at Day 0. After 7 d of storage, TSP, AA, and EO-A water reduced ST 2.17, 2.31, and 1.06 $\log_{10}$, respectively. ST was reduced 2.11 $\log_{10}$ immediately following the multiple interventions, 3.81 $\log_{10}$ after 7 d of storage. Although effective against ST, TSP and AA are costly and adversely affect the environment. This study demonstrates that EO water can reduce ST on poultry surfaces following extended refrigerated storage.

(*Key words*: electrolyzed oxidizing water, *Salmonella*, poultry, chlorine)

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INTRODUCTION

Organic acids, chlorinated compounds [chlorine (CL), chlorine dioxide], trisodium phosphate (TSP), heat, steam, or hot water are generally recognized as safe (GRAS) interventions and are used extensively by the meat and poultry industries to reduce bacterial contamination on carcass surfaces. These interventions have been found to be effective for immediately reducing foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium (ST), and *Listeria monocytogenes* associated with meat or poultry surfaces (Lillard, 1980; Thiessen et al., 1984; Sorrells et al., 1989; Quintavalla and Campanini, 1991; Cygnarowicz et al., 1994; Lillard, 1994). Other novel compounds that have been or are currently being investigated to reduce microbes on meat and poultry under commercial conditions include hydrogen peroxide and acidified sodium chlorite, also known as Alcide (Mulder et al., 1987; Villarreal et al., 1990; Russell, 1998). Despite the availability of these compounds and potential effectiveness of these interventions, researchers continually investigate the use of other novel, antimicrobial agents or preservatives to reduce or inhibit pathogenic or spoilage organisms associated with fresh meat and further processed meats and are doing it more effectively and economically.

Chlorine is the most commonly used antimicrobial agent in food processing due its availability, relative low cost, and efficacy (Tsai et al., 1992). CL is known to be most active in its hypochlorous acid form where it will penetrate the bacterial cell wall (Lillard, 1980) and react with key enzymes to prevent normal respiration (Banwart, 1989b). One major disadvantage of CL is its ability to bind to organic materials, rendering it ineffective in a relatively short period of time, which requires its constant replenishment (Lillard, 1980; Tsai et al., 1992). Additionally, CL is known to produce chloramines, which may

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Abbreviation Key: GRAS = general recognized as safe; EO = electrolyzed oxidizing; ORP = oxidation reduction potential; AA = acetic acid; OZ = ozonated water; TSP = trisodium phosphate; ST = *Salmonella* Typhimurium; UV = ultraviolet; APC = aerobic plate counts; XLD = xylose lysine deoxycholate.

interfere with the chlorinated compound's activity against bacterial populations (Gelinas and Goulet, 1983). Despite these limitations, CL is a compound that is better suited to prevent cross-contamination between poultry carcasses or as a disinfectant for food- and nonfood contact surfaces, rather than exclusively reducing pathogens during prolonged exposure, as is seen with immersion chilling (Thomas et al., 1979; James et al., 1992; Tsai et al., 1992).

In 1984, the USDA approved the use of ozone in recycling water used in poultry chill tanks (Kim et al., 1999). A U.S. expert committee deemed ozone GRAS and encouraged its use over a broader spectrum of foods in 1997 (Kim et al., 1998). More recently the Food and Drug Administration (FDA) approved the use of ozone as an antimicrobial for use on meat and poultry in aqueous or gaseous phases (Federal Register, 2001). The production of ozone usually involves the radiation of oxygen in the air by a 185-nm wavelength emitted by a high-transmission ultraviolet lamp (Kim et al., 1999). Other methods of production include corona discharge, chemical, thermal, chemonuclear, and electrolytic methods. Ozone acts as a powerful oxidizing agent that is 1.5 times stronger than CL (Xu, 1999) and is more effective against a variety of microorganisms, including vegetative and spore-forming Gram-negative and Gram-positive bacteria, fungi, viruses, and protozoa (Kim et al., 1999). Some limitations include its human health hazards, instability and rapid degradation, which requires generation at the point and time of use (Kim et al., 1999). The instability of ozone is also considered an advantage as it decomposes rapidly to the form of oxygen without leaving any residual ozone. As a result, ozone is considered a process rather than a chemical additive (Pryor and Rice, 1999).

Organic acids, such as lactic, acetic and citric acids, have been used to reduce the pH of food systems and to control the growth of microorganisms. At low pH levels, the membrane of the microorganism is saturated with hydrogen ions, which influences cell permeability, ultimately affecting its ability to reproduce (Banwart, 1989a). There are additional concerns by the food industry in using organic acids, primarily due to the emergence of acid-resistant pathogens as well as the disposal and environmental effects of wastewater. Another disadvantage to the use of acids is the adverse effects on organoleptic properties, such as appearance and texture of poultry carcasses (Dickens and Whittemore, 1994; Dickens et al., 1994).

TSP has been used in the United States since 1992 when it was granted GRAS status by the FDA (Coppen et al., 1998). Whether bacteria are subjected to low or high pH, the mechanisms required for cell growth or survival require a neutral pH (Jay, 2000). The extreme alkaline pH (10 to 13) of TSP is detrimental to microorganisms since they are not able to adjust and carry out cellular functions. In much the same way that organic acids can be hazardous to the environment, TSP also poses a similar problem with its high pH and phosphate removal, making disposal difficult and expensive.

Electrolyzed oxidizing (EO) water is a relatively new antimicrobial agent that has been shown to be effective against pathogens attached to cutting boards (Venkitanarayanan et al., 1999a), in cell suspensions (Venkitanarayanan et al., 1999b), and against spoilage organisms associated with vegetables (Izumi, 1999). The only chemical used in its production is sodium chloride. Therefore, adverse effects on the environment may be reduced (Kim et al., 2000). When a current is passed across the electrodes, two types of water are generated: the cathode produces an electrolyzed basic aqueous solution [pH 11.6 and oxidation-reduction potential (ORP) of –795 mV] that contains dilute sodium hydroxide, while the anode produces an electrolyzed acidic solution composed of dilute hypochlorous acid (Kim et al., 2000). It is the acidic solution that has gained the attention of the food industry with its pH range of 2.4 to 2.7, 1,150 mV of ORP, and approximately 50 ppm (±10 ppm) free CL. Recently, Kim et al. (2000) demonstrated that the effectiveness of EO water may be due to the ORP, rather than pH or CL content. The ORP is defined as the ability to gain or lose electrons (Jay, 2000). Positive ORP value indicates electron acceptance, while negative values designate electron donation. The acidic EO water (1,150 mV) sequesters electrons from the cellular membrane rendering it unstable. This phenomenon allows the antimicrobial to move into the cell, affect other metabolic compounds, and ultimately inactivate the cell (Jay, 2000).

During poultry processing, spray washing with antimicrobial compounds may be carried out immediately after evisceration to reduce the microbial load and to prevent cross-contamination prior to immersion chilling in cold (4 C), chlorinated (20 ppm) water (Conner et al., 2001). Immersion chilling is used to reduce carcass temperature to 4 C within 4 h following exsanguination (Sams, 2001). Multi-stage immersion chillers may be equipped with paddles or augers, which are used to slowly move the carcasses through the process. Carcasses may spend between 45 and 60 min in the immersion chillers, which is considered an ideal time for application of antimicrobials to reduce microbial contaminants.

The objective of this study is to compare the effectiveness of EO water with chlorine, aqueous ozone, acetic acid, and TSP in immersion chilling and spray washing experiments against populations of *E. coli* biotype 1, coliforms, and ST on fecally contaminated poultry surfaces. The effectiveness of basic EO water spray followed by immersion in acidic EO water will also be compared against spray treatments of either acetic acid or TSP followed by immersion in chlorinated water against populations of *E. coli* biotype 1, coliforms, and ST. The information from this study may provide poultry processors with an additional antimicrobial intervention to reduce undesirable microbial contaminants on broiler carcasses.

MATERIALS AND METHODS

Bacterial Cultures

ST ATCC 13311[2] was obtained from the Penn State Food Microbiology culture collection and stored at –70

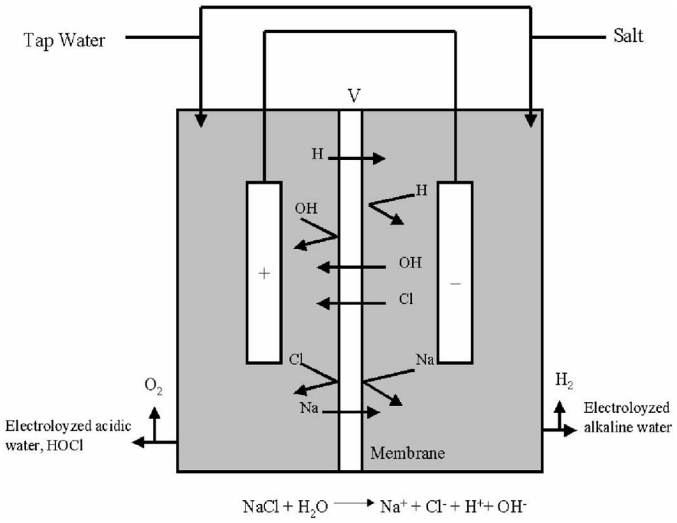

FIGURE 1. A schematic representation of the electroloyzed oxidizing reaction. (Adapted from Kim et al., 2000).

C in trypticase soy broth[3] containing 10% glycerol. Prior to experiments, ST was propagated in trypticase soy broth at 37 C for 18 h. Prior to inoculation in poultry feces (see below), overnight cultures were serially diluted in buffered peptone water[3] to obtain a viable cell population of approximately 7 $\log_{10}$ cfu/mL.

Electrolyzed Oxidizing Water Preparation

The generation of EO water involved electrolysis of NaCl in a cell containing inert positively charged and negatively charged electrodes separated by a bipolar membrane. A salt solution (12% NaCl) and deionized water were pumped into the EO water generator[4] (Figure 1). According to the manufacturer, by subjecting the platinum electrodes to direct current voltage [14 Amp, 10 V], two types of water possessing different characteristics were generated: an electrolyzed basic aqueous solution (pH 11.6) and ORP of –795 mV containing dilute NaOH is produced by the cathode, and an electrolyzed acidic solution containing dilute hypochlorous acid (HOCl) was produced from the anode side with an ORP of 1,150 mV, 2.6 pH, and approximately 50 ppm free CL. Small amounts of oxygen and hydrogen gas were also produced during this reaction. HOCl was also produced during electrolysis, with the amount of HOCl increasing in response to the amount amperage applied.

Ozonated Water Preparation

Ozone gas was generated by a laboratory-scale ozone generator[5]. Gaseous ozone was purged through distilled water at 4 C with a 10 μm pore size stainless steel sparger. Excess ozone was collected and passed through 1% potassium iodide solution to prevent ozone from being released into the environment. During ozonation, the water was maintained at approximately 4 C by surrounding the container with ice. The purging through 20 L of deionized water was done at a flow rate of 12 standard cubic feet per hour for 2 h to obtain an aqueous ozone concentration of 10 ppm.

Concentration of ozone in the water was measured by spectrometric analysis, which involves direct measurement of its ultraviolet (UV) absorption at 258 nm (Badger and Hoigne, 1981; Grunwell et al., 1983; Gordon et al., 1988). The absorption of ozonated water samples was measured in 1-cm cuvets. The formula used for calculating ozone concentration in the water (ppm or mg/L) is given below:

$$c = A \times b/\varepsilon \quad [1]$$

where, c = concentration of ozone in water (ppm or mg/L), A = absorbance value at 258 nm UV, b = length of path of light = width of quartz cuvet (cm), and ε = molar absorptivity = 2,900 M^{-1} cm^{-1}.

Broiler Carcasses and Fecal Samples

Freshly eviscerated broiler carcasses, approximately 6 to 7 wk of age, were obtained from the processing facilities of the Poultry Science Department at Pennsylvania State University (University Park, PA). Whole (Experiments 1 and 3) and halved (Experiment 2) carcasses were placed into plastic bags and transported to the laboratory and stored in portable insulated coolers to maintain body temperature until inoculated and chilled or sprayed within 30 min.

Poultry fecal samples were obtained from pathogen-free layers (Experiments 1 and 3) or broilers (Experiment 2) housed in the facilities of the Poultry Science Department at Pennsylvania State University. Feces were collected from freshly cleaned pans and transported to the laboratory in Whirl-Pak bags[6]. Feces from three birds were mixed manually and serially diluted in BPW. Diluted ST inoculum was added to fecal slurries to obtain approximately 5 $\log_{10}$ cfu/mL and transferred to sterile spray bottles for inoculation of carcasses.

Immersion Chilling Experiment (Experiment 1)

The surfaces of the carcasses were UV light-treated in a biological safety hood to reduce the amount of background microflora. Surfaces were evenly exposed to UV light by turning carcasses every 10 min for a total time of no more than 30 min (Cutter and Siragusa, 1994). UV-treated carcasses were spray-inoculated with approximately 30 mL of a poultry fecal suspension containing 5 $\log_{10}$ cfu/mL ST using a sterile spray bottle. Inoculated carcasses were allowed to attach for 15 min at room tem-

[2]American Type Culture Collection, Manassas, VA.
[3]Difco, Detroit, MI.
[4]ROX 20TA EOWater Generator, Hoshizaki Electric Co. Ltd., Sakae, Toyoake, Aichi, Japan.
[5]Model No. H-50, Hess Machines International, Ephrata, PA.
[6]Nasco, Fort Atkinson, WI.

perature under a biological safety hood, to obtain approximately 3 $\log_{10}$ cfu of pathogen/mL of rinsate.

Following inoculation and attachment, carcasses were submerged in 5-gallon buckets containing the antimicrobial of interest [distilled water, EO water (pH 2.4 to 2.7, 1,150 mV ORP, 50 ppm free CL), 2% acetic acid,[7] 10% TSP,[8] 20 ppm sodium hypochlorite,[7] or 10 mg/L ozonated water (OZ)] and chilled to 4 C by surrounding the bucket with ice. Antimicrobials were circulated continuously around carcasses using a Barnant Series 10 Mixer[9] with 4-blade (Model 700-5400) propeller.

Treated and untreated carcasses were evaluated for remaining bacterial populations immediately after immersion chilling (Day 0) or following aerobic storage for 7 d at 4 C. All carcasses were loosely wrapped in individual polypropylene bags[10] to prevent cross-contamination and stored at 4 C until sampled. For enumeration, individual carcasses were subjected to whole-carcass rinses (Lillard, 1988; Cason et al., 1999) with 400 mL of BPW. Rinsates were transferred to sterile tubes and serially diluted in BPW. For enumeration of mesophilic aerobic plate counts (APC), diluted rinsates were spiral-plated in duplicate on trypticase soy agar[3] (TSA) using the Autoplate 4000[11] and incubated at 35 C for 36 h. For enumeration of *E. coli* biotype 1 and coliforms, rinsates were plated onto 3*M E. coli*/coliform Petrifilm[12] according to the manufacturer's instructions and incubated for 48 h at 35 C. Enumeration of *Salmonella* spp. was performed by spiral-plating rinsates onto xylose lysine deoxycholate agar[3] (XLD) and incubating for 24 to 48 h at 35 C. Typical colony morphology was identified on XLD and verified serologically using the Oxoid *Salmonella* latex test[13]. All plates were enumerated manually or with the Q-count image analyzer.[11] The lowest level of detection of organisms was 1.30 $\log_{10}$ cfu/mL of rinsate using spiral-plating procedures.

To ensure detection of low levels of *Salmonella* on the carcasses following immersion chilling, 5 mL of undiluted rinsate was pre-enriched in 20 mL of lactose broth[3] and incubated for 24 h at 35 C. Additional samples were taken from the immersion chill water to test for the presence/absence of the pathogen by adding 5 mL rinse water to 20 mL lactose broth and incubating for 24 h at 35 C. After 24 h of pre-enrichment, 1 mL lactose broth was transferred to 9 mL selenite cystine broth3 (SC), another 1 mL was transferred to 9 mL tetrathionate broth[2] and all tubes incubated for 24 h at 35 C. After incubation, samples were taken from selenite cystine broth and tetrathionate broth, streaked for isolation onto XLD agar, and incubated for 48 h at 35 C. Typical colony morphology was identified on XLD and verified serologically using the Oxoid *Salmonella* latex test[13].

Spray-Washing Experiment (Experiment 2)

Due to size constraints in the spray washer, eviscerated broiler carcasses were split in half. Carcasses were then inoculated with 10 mL poultry fecal suspension containing 5 $\log_{10}$ cfu/mL of ST using a sterile spray bottle and allowed to attach for 15 min at 25 C as described previously. Carcasses were sprayed with the antimicrobial of interest [distilled water, EO water (pH 2.4 to 2.7, 1,150 mV ORP, 50 ppm free CL), 2% AA, 10% TSP, 20 ppm sodium hypochlorite, or 10 mg/L OZ] for 15 s at 85 psi (100 oscillations per min) using a carcass washer[14]. Untreated and treated carcasses were transported to the laboratory for enumeration in portable, insulated coolers within 15 min of treatment.

Carcass rinse procedures and microbiological analyses for enumeration and detection of bacterial populations were carried out as described previously. Samples were processed immediately following treatment (Day 0) and again after 7 d of aerobic storage at 4 C.

Multiple Intervention Experiment (Experiment 3)

Surfaces of whole broiler carcasses were UV-treated to reduce the amount of microflora present and inoculated as described for Experiment 1. After bacteria were allowed to attach for 15 min, the carcasses were sprayed with approximately 25 mL of either basic EO water (pH 11.6, –795 mV ORP), 2% AA, or 10% TSP with sterile, hand-held spray bottles. The carcasses were allowed to remain undisturbed for 20 min before they were submerged in 5-gallon buckets containing the antimicrobial of interest. Carcasses sprayed with basic EO water were submerged in acidic EO water (pH 2.4 to 2.7, 1,150 mV ORP, 50 ppm free CL), and carcasses sprayed with either AA or TSP were submerged in 50 ppm sodium hypochlorite and chilled to 4 C by surrounding the bucket with ice. Antimicrobials were circulated continuously around carcasses using a Barnant Series 10 Mixer (Model 700-5400) with 4-blade propeller[9].

Free CL Determination

Free CL content of the CL and EO water treatments was measured using Hach DPD = N,N-diethyl-p-phenylenediamine-ferrous ethylene diammonium sulfate method[15] as described by the manufacturer. Briefly, 25-mL sample, diluted 10-fold with sterile distilled water, was transferred into an Erlenmeyer flask. A DPD-free CL powder pillow was added to the sample and swirled to mix. The sample was titrated using 0.00564 *N* ferrous ethylenediammonium sulfate to a colorless endpoint. Free CL was calculated from the number obtained following titration, inclusive of the dilution factor (1:10).

[7]Sigma, St. Louis, MO.
[8]Rhone-Poulenc, Cranbury, NJ.
[9]Barnant, Barrington, IL.
[10]Seward, London, UK.
[11]Advanced Instruments, Norwood, MA.
[12]3M Microbiology Inc., Minneapolis, MN.
[13]Oxoid, Inc., Ogdensburg, NY.
[14]CHAD, Inc., Lenexa, KS.
[15]Hach Company, Loveland, CO.

TABLE 1. Effect of immersion chilling in distilled water, electrolyzed oxidizing water, chlorine (20 ppm), ozone (10 ppm), 2% acetic acid, or 10% trisodium phosphate on populations (log_{10} cfu/mL of rinsate) of aerobic, mesophilic plate counts (APC), *Salmonella* Typhimurium (ST), *Escherichia coli* (EC), and total coliforms (TC) on broiler carcasses immediately following treatments and after 7 d of aerobic storage at 4 C

Treatment	Organism	Day 0	Day 7
Untreated	APC	5.49 ± 0.64^{A}	7.08 ± 1.94^{A}
Distilled water	APC	4.78 ± 0.39^{AB}	6.56 ± 0.58^{A}
EO[1] water	APC	4.15 ± 0.56^{BC}	5.06 ± 0.99^{AB}
Chlorine	APC	4.32 ± 0.26^{BC}	5.98 ± 1.43^{A}
Ozone	APC	4.55 ± 0.43^{B}	6.43 ± 1.67^{A}
Acetic acid	APC	3.53 ± 0.17^{C}	3.30 ± 0.00^{B}
Trisodium phosphate	APC	3.33 ± 0.29^{C}	2.93 ± 0.79^{B}
Untreated	ST	2.71 ± 0.52^{A}	2.28 ± 0.96^{A}
Distilled water	ST	3.21 ± 0.90^{AB}	1.93 ± 0.81^{A}
EO water	ST	1.88 ± 0.36^{AC}	1.30 ± 0.00^{A}
Chlorine	ST	2.76 ± 0.31^{ABC}	2.00 ± 0.94^{A}
Ozone	ST	1.97 ± 0.55^{AC}	1.30 ± 0.00^{A}
Acetic acid	ST	1.30 ± 0.01^{C}	1.30 ± 0.00^{A}
Trisodium phosphate	ST	1.30 ± 0.00^{C}	1.30 ± 0.00^{A}
Untreated	EC	4.04 ± 0.97^{A}	4.91 ± 1.21^{A}
Distilled water	EC	3.33 ± 0.27^{A}	2.89 ± 0.59^{AB}
EO water	EC	2.93 ± 0.55^{A}	1.35 ± 1.56^{BC}
Chlorine	EC	3.42 ± 0.80^{A}	2.29 ± 1.80^{B}
Ozone	EC	3.26 ± 0.68^{A}	2.62 ± 0.51^{B}
Acetic acid	EC	1.20 ± 1.39^{B}	0.00 ± 0.00^{C}
Trisodium phosphate	EC	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}
Untreated	TC	4.13 ± 0.99^{A}	5.16 ± 1.05^{A}
Distilled water	TC	3.70 ± 0.47^{A}	3.56 ± 0.61^{B}
EO water	TC	3.00 ± 0.52^{A}	2.90 ± 0.52^{B}
Chlorine	TC	3.54 ± 0.72^{A}	3.49 ± 0.63^{B}
Ozone	TC	3.32 ± 0.70^{A}	2.95 ± 0.57^{B}
Acetic acid	TC	1.13 ± 1.39^{B}	0.00 ± 0.00^{C}
Trisodium phosphate	TC	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}

$^{A–C}$Means within a column for a given organism on a given day sharing the same letter are not significantly different ($P > 0.05$).

[1]Electrolyzed oxidizing.

Determination of pH and ORP

Measurements of pH were taken from the antimicrobial solutions using a pH meter.[16] ORP measurements of antimicrobial solutions were obtaining with a pH meter with an ion electrode.[17]

Statistical Analysis

Means of bacterial populations (log_{10} cfu/mL per carcass rinsate) from each treatment were calculated from four replications for each experiment. Data were analyzed using Minitab 13 statistical package.[18] One-way ANOVA was performed to note differences ($P < 0.05$) among treatments. Comparisons of means were performed using Tukey's HSD multiple comparison test.

RESULTS

Of the treatments evaluated during immersion chilling experiments, TSP and AA were the most effective treatments against ST with a 1.41 log_{10} reduction (Table 1). Acidic electrolyzed oxidizing (EO-A) water was as effective as ozone (OZ), while distilled water and CL were ineffective at reducing ST during immersion chilling. After 7 d of aerobic storage at 4 C, all the treatments were able to reduce ST, although the reductions were not statistically different. OZ, EO-A, TSP, and AA reduced the pathogen to nearly undetectable levels by Day 7, since the organism was only found after selective enrichment. APC of treated carcasses taken immediately after antimicrobial treatments were also significantly reduced by AA and TSP treatments while, EO-A, AA, and TSP significantly reduced APC after 7 d of refrigerated aerobic storage. TSP and AA effectively reduced populations of *E. coli* biotype 1 and coliforms on Days and 7 approximately 4 log_{10} cfu/mL rinsate. AA effectively reduced approximately 3 log_{10} cfu/mL *E. coli* biotype 1 and coliforms in the rinsate on Day 0 and virtually 5 log_{10} on Day 7, while EO-A, CL, and OZ treatments did not.

Average free CL levels of the chill water for CL and EO-A treatments were measured before treatment and found to be 30.9 and 39.6 ppm, respectively. Immediately following treatments of carcasses, free CL was found to be 0.90 and 0.25 ppm for CL and EO-A, respectively. Average pH readings of the solutions from the immersion chilling experiment are presented in Table 2.

Spray washing of broiler carcasses with each of the compounds afforded a slight and immediate reduction in APC and ST; however, *E. coli* biotype 1 and total coliforms were not significantly reduced (Table 3). By Day 7, there appeared to be some residual antimicrobial activity of spray washes with AA and TSP on broiler carcasses since ST was reduced significantly.

All multiple intervention treatments showed a significant reduction of ST immediately following the treatment (Table 4). EO-A and basic electrolyzed oxidizing (EO-B) water used in concert demonstrated a 2.11 log_{10} reduction of the pathogen. Following 7 d of aerobic storage at 4 C, EO-B water was statistically different from all other treatments with a 3.81 log reduction against ST. The results for EO water, AA, and TSP against APC were 2.36, 1.40, and 1.20 logs reduction, respectively. All three treatments were statistically different from untreated after refrigerated storage. On Day 0 and 7, all treatments were statistically different from untreated with regard to popu-

TABLE 2. pH measurements of treatment solutions taken before and after immersion treatment of poultry carcasses[1]

Treatment	Before	After
Distilled water	5.42[2]	6.96
EO water	2.36	2.45
Chlorine	8.97	6.71
Acetic acid	2.28	2.41
TSP	12.2	12.61
Ozone	3.48	4.05

[1]Treatment solutions: distilled water, electrolyzed oxidizing water (EO water), chlorinated water (20 ppm), acetic acid (2%), 10% trisodium phosphate (TSP), 10 mg/L ozonated water.

[2]Values represent the average of four replications.

[16]Corning Inc., Corning, NY.
[17]Orion Research Inc., Beverly, MA.
[18]Minitab, State College, PA.

TABLE 3. Effect of spray-washing distilled water, electrolyzed oxidizing water, chlorine (20 ppm), ozone (10 ppm), 2% acetic acid, or 10% trisodium phosphate on populations (log_{10} cfu/mL of rinsate) of aerobic, mesophilic plate counts (APC), *Salmonella* Typhimurium (ST), *Escherichia coli* (EC), and total coliforms (TC) on broiler carcasses immediately following treatments and after 7 d of aerobic storage at 4 C

Treatment	Organism	Day 0	Day 7
Untreated	APC	6.56 ± 0.13^{A}	8.54 ± 0.30^{A}
Distilled water	APC	6.09 ± 0.28^{AB}	8.25 ± 0.28^{A}
EO water[1]	APC	6.37 ± 0.21^{AB}	8.44 ± 0.26^{A}
Chlorine	APC	6.26 ± 0.26^{AB}	8.50 ± 0.12^{A}
Ozone	APC	6.01 ± 0.43^{B}	8.50 ± 0.16^{A}
Acetic acid	APC	6.19 ± 0.14^{AB}	6.52 ± 0.28^{B}
Trisodium phosphate	APC	6.11 ± 0.19^{AB}	8.39 ± 0.39^{A}
Untreated	ST	5.84 ± 0.21^{A}	6.92 ± 0.30^{A}
Distilled water	ST	4.97 ± 0.17^{B}	5.27 ± 0.28^{B}
EO water	ST	5.25 ± 0.28^{B}	5.86 ± 0.26^{C}
Chlorine	ST	5.01 ± 0.37^{B}	5.56 ± 0.17^{BC}
Ozone	ST	5.25 ± 0.29^{B}	5.32 ± 0.23^{BC}
Acetic acid	ST	5.01 ± 0.12^{B}	4.61 ± 0.06^{D}
Trisodium phosphate	ST	4.94 ± 0.18^{B}	4.75 ± 0.28^{D}
Untreated	EC	5.63 ± 0.23^{A}	6.15 ± 0.40^{A}
Distilled water	EC	5.41 ± 0.23^{A}	5.39 ± 0.48^{A}
EO water	EC	5.92 ± 0.19^{A}	6.40 ± 0.06^{A}
Chlorine	EC	5.79 ± 0.07^{A}	6.18 ± 0.61^{A}
Ozone	EC	5.27 ± 0.49^{A}	5.60 ± 0.31^{A}
Acetic acid	EC	5.62 ± 0.26^{A}	5.50 ± 0.58^{A}
Trisodium phosphate	EC	5.12 ± 0.11^{A}	6.32 ± 0.34^{A}
Untreated	TC	5.85 ± 0.13^{A}	6.30 ± 0.38^{A}
Distilled water	TC	5.56 ± 0.24^{A}	5.51 ± 0.44^{B}
EO water	TC	6.09 ± 0.13^{A}	6.54 ± 0.05^{A}
Chlorine	TC	5.93 ± 0.11^{A}	6.30 ± 0.56^{A}
Ozone	TC	5.48 ± 0.49^{A}	6.10 ± 0.20^{AB}
Acetic acid	TC	5.68 ± 0.26^{A}	5.60 ± 0.48^{AB}
Trisodium phosphate	TC	5.68 ± 0.13^{A}	6.39 ± 0.34^{A}

$^{A–C}$Means within a column for a given organism on a given day sharing the same letter are not significantly different ($P > 0.05$).

[1]Electrolyzed oxidizing.

TABLE 4. Multiple intervention experiment: Effect of basic electrolyzed oxidizing (EO) water spray treatment followed by immersion in acidic EO water, 2% acetic acid spray treatment followed by immersion in chlorinated water (50 ppm), or 10% trisodium phosphate spray treatment followed by immersion in chlorinated water (50 ppm) on populations (log_{10} cfu/mL of rinsate) of aerobic, mesophilic plate counts (APC), *Salmonella* Typhimurium (ST), *Escherichia coli* biotype 1 (EC), and total coliforms (TC) on broiler carcasses immediately following treatments and after 7 d of aerobic storage at 4 C

Treatment	Organism	Day 0	Day 7
Untreated	APC	6.65 ± 0.41^{A}	9.03 ± 0.05^{A}
EO water	APC	4.29 ± 0.26^{B}	6.25 ± 0.58^{B}
Acetic acid	APC	5.25 ± 0.15^{AB}	6.73 ± 0.81^{B}
Trisodium phosphate	APC	5.27 ± 0.19^{AB}	7.37 ± 0.58^{B}
Untreated	ST	4.55 ± 0.58^{A}	5.88 ± 0.71^{A}
EO water	ST	2.44 ± 0.51^{B}	2.07 ± 0.46^{C}
Acetic acid	ST	2.55 ± 0.91^{B}	2.75 ± 0.56^{BC}
Trisodium phosphate	ST	2.60 ± 0.67^{B}	3.21 ± 0.32^{B}
Untreated	EC	5.52 ± 0.35^{A}	5.91 ± 0.48^{A}
EO water	EC	4.00 ± 0.75^{B}	2.89 ± 0.71^{B}
Acetic acid	EC	3.95 ± 0.11^{B}	3.27 ± 0.66^{B}
Trisodium phosphate	EC	3.76 ± 0.26^{B}	2.62 ± 0.33^{B}
Untreated	TC	5.61 ± 0.27^{A}	6.08 ± 0.47^{A}
EO water	TC	4.06 ± 0.77^{B}	3.00 ± 0.67^{B}
Acetic acid	TC	4.07 ± 0.21^{B}	3.48 ± 0.46^{B}
Trisodium phosphate	TC	3.88 ± 0.23^{B}	2.83 ± 0.38^{B}

$^{A–C}$Means within a column for a given organism on a given day sharing the same letter are not significantly different ($P > 0.05$).

lations of *E. coli* biotype 1 and coliforms; however, they were not statistically significant from each other. In all cases, the greatest reduction was seen with TSP, which demonstrated a 2 log_{10} reduction on Day 0 and greater than 3 log_{10} reduction after 7 d.

Average free CL levels were measured before submersion of the carcasses and found to be 64.1 ppm for EO-A and 25.1 ppm for CL. Immediately following treatment, the average free CL levels fell to 31 ppm for EO-A and 0 ppm for CL. Average pH values from the multiple intervention experiment are presented in Table 5.

DISCUSSION

Various antimicrobial treatments are currently being used during poultry processing to reduce pathogens and to improve the quality of broiler carcasses. In this study, the effectiveness of OZ, AA, CL, and TSP was compared with the newly recognized antimicrobial agent, EO water. During immersion chilling, EO-A was more effective than CL immediately after treatment and again after 7 d of refrigeration storage against all bacterial populations evaluated (Table 1).

It has been documented that chlorine is more suitable for preventing cross-contamination of pathogens in solution, rather than reducing ST on meat and poultry carcasses (Thomas et al., 1979; Tsai et al., 1992; James et al., 1992). Theories suggest that the free CL becomes bound to the high organic load associated with immersion chilling of broiler carcasses, resulting in formation of chloramines. In this study, the free CL available following the immersion experiment for both EO-A and CL was less than 1 ppm. Even after selective enrichment of rinse water from both treatments, no ST was detected.

The immersion chilling experiment demonstrates the prolonged effectiveness of EO-A, as well as TSP, OZ and AA, for reducing microbial populations associated with broiler carcasses. We hypothesize that the effectiveness was due to the effect of refrigeration temperatures on potentially injured cells and effectiveness of residual antimicrobials. Other researchers have demonstrated the effectiveness over time of OZ (Yang and Chen, 1979), AA (Dickens and Whittemore, 1995; Tamblyn and Conner, 1997), and TSP (Bender, 1992; Lillard, 1994; Coppen et

TABLE 5. pH measurements of treatment solutions taken before and after multiple intervention treatment of poultry carcasses[1,2]

Treatment	Before	After
Acetic acid	2.74	*[3]
Trisodium phosphate	12.73	*
Basic EO water	11.61	*
Acidic EO water	2.73	3.15
Chlorine	9.08	7.27

[1]Treatment solutions: acetic acid (2%), 10% trisodium phosphate, acidic electrolyzed oxidizing water (EO water) sprays followed by basic electrolyzed oxidizing water (EO water), chlorinated water (50 ppm) immersion.

[2]Values represent the average of four replications.

[3]No measurements were taken after treatment.

al., 1998; Kim and Marshall, 1999) against pathogens associated with poultry surfaces. The current study is the first to report the immediate and long-term effectiveness of EO water against ST on poultry carcasses.

Of all the treatments applied via spray washing, there were no statistically significant differences observed immediately following application (Table 3). This observation is not surprising since the contact time for each treatment was only 15 s; whereas immersion chilled carcasses were subjected to treatments for 45 min. Increased contact time might improve the effectiveness of EO-A as a spray wash antimicrobial.

The use of multiple interventions to combat foodborne pathogens is not a new concept. L. Leistner coined the phrase "hurdle technology," which involves exploiting the intrinsic and extrinsic parameters required for bacterial growth (Jay, 2000). As previously described, two types of water are generated during the production of EO water:acidic and basic solutions. Many studies have described the use of both pH extremes being used to inhibit or destroy foodborne pathogens (Bender, 1992; Dickens and Whittemore, 1994; Tamblyn and Conner, 1997). In the poultry industry, carcasses are sprayed with either an organic acid (AA) or an alkaline compound (TSP) followed by immersion in chlorinated chill water. In this study, the solutions produced during the production of EO water were used in concert and found to reduce ST to the same degree or better than the interventions currently used by industry (Table 4). After 7 d of refrigerated storage, the EO water solutions performed better than AA and TSP sprays followed by immersion in CL with nearly a 4 $\log_{10}$ reduction of ST. EO water was also found to be more effective than AA and TSP for reducing populations of APC immediately following treatment and after 7 d of refrigerated storage; however, the differences were not statistically significant. In this experiment, free CL was detectable after immersion chilling of the carcasses with EO water (31 ppm). The effectiveness of the EO water treatment could be attributed to the injury sustained to the cells as well as the residual free CL still present on the carcass surface.

EO water is simple to derive, reasonably priced, and is less harmful to the environment than some other antimicrobial compounds (Kim et al., 2000). While previously published studies clearly illustrate that EO water is an effective antimicrobial for reducing foodborne pathogens in water and on cutting boards, no studies have been conducted to determine whether EO water is more or less effective than other antimicrobial compounds currently used by the poultry industry to reduce or to inhibit pathogens associated with broiler carcasses. Our findings suggest that EO water may provide poultry establishments with an inexpensive and easy alternative to CL treatments during processing to control growth of pathogenic bacteria.

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