Efficacy of Electrolyzed Water in the Prevention and Removal of Fecal Material Attachment and Its Microbicidal Effectiveness During Simulated Industrial Poultry Processing

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ABSTRACT This study was undertaken to investigate the efficacy of alkaline and acidic electrolyzed (EO) water in preventing and removing fecal contaminants and killing *Campylobacter jejuni* on poultry carcasses under simulated industrial processing conditions. New York dressed and defeathered chicken carcasses spot-inoculated with cecal material or *C. jejuni* were subjected to spraying treatment with alkaline EO or 10% trisodium phosphate (TSP) water or combinations of spraying and immersion treatments with acidic EO and chlorinated water, respectively. Prespraying chicken carcasses with alkaline EO water significantly lowered cecal material attachment scores (3.77) than tap water (4.07) and 10% TSP (4.08) upon treatment of the dorsal area. Combinations of pre- and postspraying were significantly more effective than postspraying only, especially when using alkaline EO water in removing fecal materials on the surface of chicken carcasses. Although treatment by immersion only in EO and chlorinated water significantly reduced the initial population (4.92 $\log_{10}$ cfu/g) of *C. jejuni* by 2.33 and 2.05 $\log_{10}$ cfu/g, respectively, combinations of spraying and immersion treatment did not improve the bactericidal effect of sanitizers. The results indicated that alkaline EO water might provide an alternative to TSP in preventing attachment and removal of feces on the surface of chicken carcasses. The results also suggested that chicken carcasses containing pathogenic microorganisms may contribute to the cross-contamination of whole batches of chickens during processing in the chiller tank and afterward.

(*Key words*: electrolyzed water, chicken, cecal material, *Campylobacter jejuni*)

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INTRODUCTION

Contamination of poultry with foodborne pathogens can potentially occur as a result of exposure of carcasses to feces and ingesta during and after slaughter (Windham et al., 2001). The Food Safety Inspection Services of the US Department of Agriculture has established a zero tolerance policy that does not allow fecal contaminants on the surface of poultry carcasses (USDA, 1994). Increased consumption of poultry products has been accompanied by an increase in foodborne illnesses associated with these foods (Alterkruse et al., 1999). Because of the relatively high frequency of contamination of poultry with *Campylobacter jejuni*, raw poultry products have been perceived to be responsible for a significant amount of human illness (White et al., 1997).

Although numerous decontaminant processes including cetylpyridinium chloride (Xiong et al., 1998), acidified sodium chlorite (Kemp et al., 2000), ozone (Kim et al., 1999), chlorine dioxide and peroxyacetic acid (Morris, 1999), hydrogen peroxide (Lillard and Thomson, 1983), γ-irradiation (Katta et al., 1991), microwaves (Göksoy et al., 2000), and chilling (Vivien et al., 2000) have been applied to reduce carcass contamination, most of these processes have not been completely acceptable due to induction of product quality deterioration, chemical residues, discoloration of chicken carcasses, high cost, or limited effectiveness (Farkas, 1998; Smulders and Greer, 1998; Sofos and Smith, 1998; Capita et al., 1999a,b). One of the current methods, trisodium phosphate (TSP) wash, has been reported to assist in prevention of foodborne illnesses by removing a thin layer of lipids during defeathering (Vareltzis et al., 1997; Capita et al., 1999b). In other words, TSP may prevent lipid smearing, which protects bacteria lodged in open feather follicles and subsequently contributes to eliminate bacteria that have not yet firmly adhered to the surface of the chicken skin. Sampathkumar et al. (2003) also demonstrated that TSP treatment permeabilizes and disrupts the cytoplasmic and outer membranes of bacterial cells because of the alkaline pH, which in turn leads to a release of intracellu-

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lar contents and eventual cell death. However, use of TSP is undesirable in that it produces a significant amount of phosphate released in the waste stream making disposal difficult and expensive (Fabrizio et al., 2002) and enhances water retention in the carcasses.

Therefore, the development of a reliable alternative to current methods is crucial in preventing the attachment of fecal contaminants to carcasses and in removing the contaminants from the surface of poultry and hence to reduce or eliminate *C. jejuni* from poultry products.

Generated and controlled on the site of production, electrolyzed (EO) water is formed by electrolyzing a dilute salt (NaCl) solution that is subsequently separated into a basic fraction and an acidic fraction (Hoshizaki Electric Co., 1997). Basic EO water obtained from the cathode has a pH of approximately 11 and an oxidation-reduction potential (ORP) of approximately −80 mV, indicating it could have a similar effect as TSP in removing lipid smearing and killing bacterial cells on the surface of chicken skin (Capita et al., 2002). In the meantime, acidic EO water obtained from the anode has a pH of approximately 2.6, an ORP of approximately 1,100 mV, and a residual chlorine concentration in the range 10 to 100 mg/L (Hoshizaki Electric Co., 1997).

Although numerous studies (Hayashibara et al., 1994; Izumi, 1999; Venkitanarayanan et al., 1999; Kim et al., 2000a,b; Len et al., 2000; Park et al., 2001, 2002; Fabrizio et al., 2002; Bari et al., 2003; Koseki et al., 2003; Yang et al., 2003; Hsu et al., 2004) on the application of EO water have reported that acidic EO water is a bactericidal agent and has potential as a method for disinfection, the possible usage of alkaline EO water instead of disposing of it has not been reported. In addition, the application of EO water appears to be an attractive nonthermal process as it is very effective, easy to operate, relatively inexpensive, and environmentally friendly due to the production of the disinfectant using pure water with no added chemicals except NaCl; thus there is no need for handling potentially dangerous concentrated chemicals such as chlorine (Hsu et al., 2004).

Hence, the study reported herein was undertaken to investigate the efficacy of alkaline and acidic EO water in preventing and removing fecal contaminants and reducing populations of *C. jejuni* on poultry carcasses under simulated industrial processing conditions.

MATERIALS AND METHODS

Warm body New York dressed and defeathered chicken carcasses (broilers, 1.8 to 2.0 kg/carcass) at approximately 5 to 6 wk of age were obtained from a commercial processing plant. Carcasses were transported to the Poultry Research Center at the University of Georgia (Athens, GA).

Treatment Water

Alkaline EO and a 10% trisodium phosphate (TSP, Fisher Scientific Co., Fair Lawn, NJ) solution were used for the evaluation of their efficacy in preventing and removing fecal contaminants on poultry carcasses, whereas acidic EO and chlorinated water were used for the investigation in reducing populations of *C. jejuni*. Alkaline and acidic EO water were produced using an EO water generator (ROX 20 TA, Hoshizaki Electric Co., Ltd., Toyoake, Aichi, Japan) at a setting of 14 A. Trisodium phosphate was diluted with tap water to 10% and used to compare its effectiveness in preventing and removing the attachment of fecal contaminants on chicken carcass with alkaline EO water. In the meantime, chlorinated water was prepared to match the residual chlorine (about 40 mg/L) of the acidic EO water by diluting to a level of 5 to 6% NaOCl (Fisher Scientific Co.) using tap water. The final concentration of chlorine in the chlorinated water was, however, approximately 70 mg/L due to the difficulties of precisely adjusting the ratio of large volumes (80 L) of tap water and NaOCl concentrate in addition to the influence of byproducts in tap water, which was used as a diluent. Acidic EO and chlorinated water was then stored in a cold room at 3°C, because they were used for the simulation of industrial immersion processing in a chiller tank. However, alkaline EO and TSP water were maintained at room temperature (24 ± 2°C) until they were used.

The properties (pH, ORP, and residual chlorine) of the solutions used for treatment were determined immediately before and after treatment using a dual-scale pH meter (Accumet model 15, Fisher Scientific Co.) with pH and ORP electrodes. The residual chlorine was determined with an iodometric method using a chlorine test kit (Hach Co., Ames, IA). Tap water was used as a control for both studies on cecal material removal and bacterial elimination.

Prevention and Removal of Fecal Contaminants

Fecal Sampling. To obtain cecal material for the study, chicken carcasses were hand-eviscerated, and material from the cecum portion of the viscera was collected in a sterile stomacher bag. To homogenize, the cecal material was pummeled for 60 s at a medium speed using a stomacher (model LabBlender 400, Dynatech Laboratories Inc., Alexandria, VA), collected in a syringe (Becton Dickinson & Co. Franklin Lakes, NJ) and stored at 4°C until used in 2 d.

Removal of Fecal Material. After 4 h of holding at room temperature (24 ± 2°C) to allow the cecal material to warm up to room temperature, 0.1 g of cecal material was smeared on the skin surface (2 by 4 cm on lower dorsal and breast areas of each chicken) of warm chicken carcasses while the carcass was inverted and hung vertically on the shackle of a conveyor. Chicken carcasses were then held for 1 min at 24 ± 2°C to allow for attachment of fecal contaminants before being subjected to the spray treatment at 40 psi for 1.5 s with 6 L of tap, alkaline EO, or 10% TSP water using a commercial-style spray cabinet with 5 nozzles at about 18 to 22 bird/min on processing

Figure 1. A 5-point hedonic scale used for visual evaluation of cecal material remaining on the surfaces of chicken carcasses.

shackle line. To evaluate the effectiveness of treatment water for preventing and removing fecal contaminants on the surface of chicken carcasses, alkaline EO, 10% TSP, and tap water were sprayed before (prespraying) and after (postspraying) the application of cecal material. Five trained panelists visually evaluated the removal of feces from the surface of 15 chicken carcasses after each spraying treatment by using a 5-point hedonic scale (Figure 1, where 1 = none, no feces; 2 = slight, smear tint only; 3 = moderate, more than a smear tint up to 50% wash off; 4 = severe, less than 50% wash off; and 5 = extreme, no removal).

Inactivation of *Campylobacter jejuni*

Inoculum. Five strains of wild type *C. jejuni* (obtained from the Poultry Microbiology Safety Research Unit, USDA, Athens, GA) were used in this study. Strains were isolates from outbreak linked to poultry product. Each strain was grown on *Campylobacter* selective agar (Blaser-Wang Agar, Difco Laboratory, Detroit, MI.) that contained 5% horse blood and *Campylobacter* selective supplement (SR98, Oxoid Ltd., Basingstoke, Hampshire, UK) at 42°C for 48 h under a microaerobic condition (5% O_2, 10% CO_2, and 85% N_2). Equal volumes of 5 cultures were mixed and then diluted in sterile 0.1% peptone water to give an inoculum (10^8 cfu/mL) containing approximately equal numbers of cells of each strain of *C. jejuni*.

Inoculation and Treatments. A total of 260 chicken carcasses with 2 independent replicate trials were used in this study. Individual chicken carcasses were spot-inoculated on the dorsal areas with 0.1 mL of the mixture of *C. jejuni* (approximately 8 $\log_{10}$ cfu/mL), smeared using a bent glass rod, and then held for 30 min at 24 ± 2°C to allow for microorganism attachment. To evaluate the effectiveness of treatment, each of 5 birds was treated with either immersion only or the combination of spraying and immersion. To simulate industrial washing processes and to investigate any possible cross-contamination from inoculated samples, 15 birds from the same batch with no inoculate were also immersed along with 5 inoculated birds in a chiller tank containing approximately 80 L of treatment water (tap, acidic EO, and chlorinated waters) for 40 min. During the immersion treatment, fresh treatment water was added at a rate of 1 L/min. For the spraying treatment, each inoculated chicken was inverted and hung vertically on the shackle of a conveyor and sprayed with 6 L of water at 40 psi using a commercial style spray cabinet as described previously (Figure 2).

Bacterial Enumeration. After the treatment, each carcass was placed in a sterile stomacher bag, and 1 L of neutralizing buffer (5.2 g/L, neutralizing buffer, Difco, Sparks, MD) containing mixture of 0.0043% monopotassium phosphate, 0.016% sodium thiosulphate, and 0.5% aryl sulfonate complex was added and then rubbed by gloved hand with firm pressure for 1 min. The neutralizing buffer solution was serially diluted in sterile 0.1% peptone water and surface-plated (0.1 mL) in duplicate on Blaser-Wang agar. Presumptive *Campylobacter* colonies were counted after incubating plates under microaerobic conditions at 42°C for 48 h. Moreover, 1 mL of each sample solution after treatment was inoculated into 10 mL of *Brucella* broth (BBL, Becton Dickinson and Co., Cockeysville, MD) for enrichment to detect the presence of the low number of survivors that would not be detected by direct plating. Colonies formed on the media from samples of peptone water, which was from untreated and treated chicken carcass, were randomly picked and subjected to *C. jejuni* latex agglutination test (Dryspot *Campylobacter* test, Oxoid Ltd.) and microscopic observation for confirmation. Under the microscopic observation, colonies with curved spiral form were considered as *C. jejuni*. Samples treated with tap water serving as a control were also placed into 1 L of sterile neutralizing buffer solution and then analyzed for *Campylobacter* as described above. Two independent replicates with 5 birds for each treatment were executed.

Data Analysis

Trials in preventing and removing fecal contaminants on chicken carcass were conducted with each reported value representing a mean of 15 sample values. Results of 5-point hedonic scale data for the prevention and removal of fecal contaminants were analyzed by GLM and the least significance difference test at the confidence level of 95% to determine if significant differences in the prevention and removal of cecal contaminants existed among mean values of treatments (SAS User's Guide, 2001, version 8.2, SAS Institute, Cary, NC.).

Two independent replicate trials composed of total 260 chicken carcasses were used for the microbial inactivation

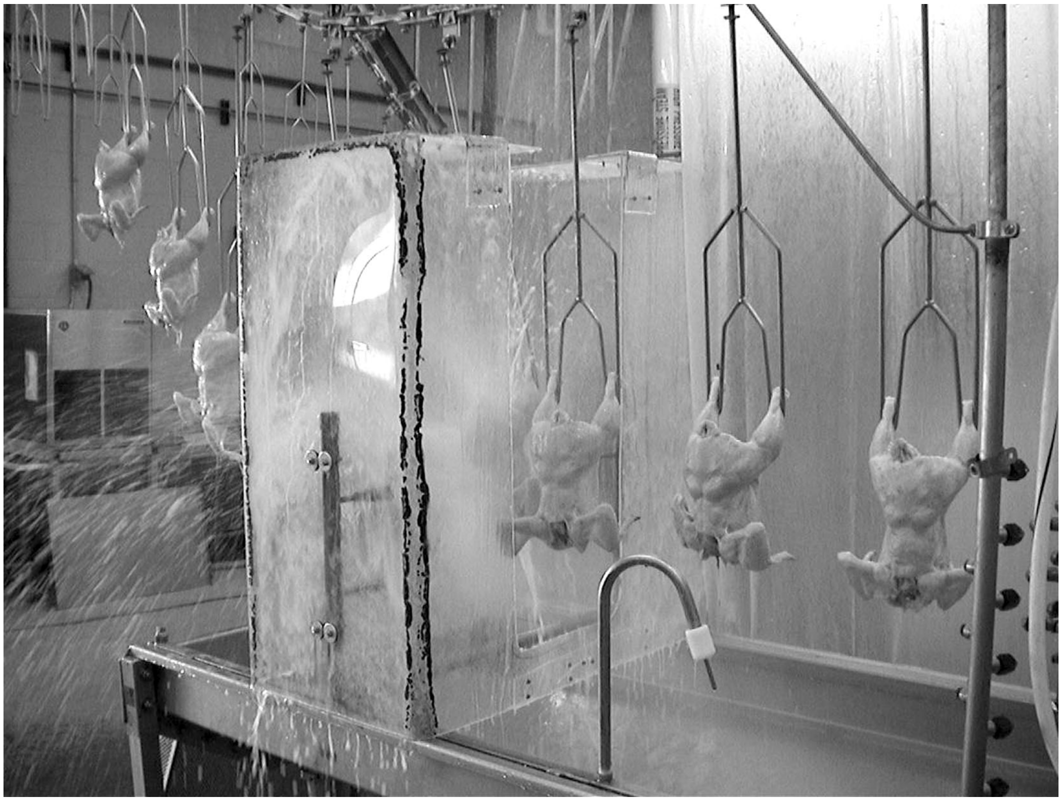

Figure 2. Acidic electrolyzed water spraying treatment used in killing *Campylobacter jejuni* in chicken carcasses during simulated industrial poultry processing.

study. Each replicate involving inoculated and uninoculated 30 and 90 birds consisted of each set of 5 inoculated and 15 uninoculated birds, respectively, were subjected to 6 different treatment (immersion only of tap, chlorinated and acidic EO solution, and immersion in combination with spraying of tap, chlorinate, and acidic EO solution). Each set of 5 birds was also used to determine the background population of *C. jejuni* before the inoculation and the bacterial population survived on birds after inoculation, respectively. All quantitative microbiological data were transformed to $\log_{10}$ colony-forming units per gram of chicken carcass prior to statistical analysis. Ninety-five percent confidence intervals were calculated for count data. Counts were compared by ANOVA using Duncan's multiple range test to determine if significant differences ($P \leq 0.05$) in the populations of microorganisms existed among mean values of treatments (SAS Institute)

RESULTS AND DISCUSSION

The properties of tap water, 10% TSP, and alkaline EO water used for the prevention and removal of fecal materials on the surface of chicken carcasses are presented in Table 1. Both alkaline EO and 10% TSP treatment waters had a relatively high pH and low ORP compared with tap water.

Effects of combinations of pre- and postspraying treatments on the prevention and removal of cecal material on chicken carcasses are presented in Table 2. The values represent the difference in the hedonic scores between cecal material applied and remained on the carcass after treatment. In general, a greater difference represented a more effective treatment in removal of cecal material. Results of prespraying treatment alone in the column of after prespraying and application of cecal material in Table 2 indicated that alkaline EO water was the most effective treatment in the prevention of attachment of fecal materials on the surface of chicken carcasses. In other words, carcasses presprayed with alkaline EO water had a significantly lower score (3.77) of cecal material attachment than tap water (4.07) and 10% TSP (4.08) for the dorsal area; whereas, the difference on the breast area was not significant. Although the mechanism of alkaline EO water is not well known, the presence of NaOH in alkaline EO water may play a role in the prevention of cecal material attachment.

Table 1. Properties of treatment waters used for the prevention of attachment and removal of cecal material on chicken carcass surface[1]

Treatment	pH	ORP (mV)	Chlorine (mg/L)
Tap water	7.4	580	0.7
10% TSP water	12.5	60.3	Not detected
Alkaline EO water	11.3	–84	Not detected

[1]TSP = trisodium phosphate; EO = electrolyzed water; ORP = oxidation-reduction potential.

Table 2. Effect of pre- and postspraying or postspraying only treatment on the removal of cecal material on chicken carcasses

Treatment	Hedonic scale score of visual evaluation: After prespraying and application of cecal material (I)		After postspraying (II)		Reduction of cecal material attachment (I-II)[1]	
	Dorsal	Breast	Dorsal	Breast	Dorsal	Breast
Combinations of pre- and postspraying[2]						
Tap[3] + tap	4.07[a]	4.17[a]	1.53	1.39	2.54[a]	2.78[ab]
Tap + TSP[4]	3.98	4.27	1.43	1.39	2.55[a]	2.88[ab]
TSP + TSP	4.08[a]	4.02[a]	1.48	1.27	2.60[a]	2.75[ab]
Tap + alkaline EO[5]	4.13	4.08	1.60	1.15	2.53[a]	2.93[a]
Alkaline EO + alkaline EO	3.77[b]	4.00[a]	1.35	1.39	2.42[a]	2.61[bc]
Postspraying only						
Tap	4.43	3.80	1.93	1.17	2.50[a]	2.63[abc]
TSP	3.97	3.85	1.70	1.47	2.27[ab]	2.38[cd]
Alkaline EO	4.03	3.88	2.03	1.68	2.00[b]	2.20[d]

[a-d]Values with the same letter in the same column are not significantly different ($P > 0.05$).

[1]Values represent the difference of hedonic scale between cecal material remaining on the carcass after treatment and cecal material applied on the carcass before treatment.

[2]Combinations of spraying treatment before and after cecal material application; chemical preceding '+' symbol was for prespraying, cecal material was then applied and chemical after '+' symbol was for postspraying.

[3]Tap water.

[4]Water with 10% trisodium phosphate.

[5]Electrolyzed water.

The 3 test solutions (i.e., tap water, TSP, and alkaline EO solutions) applied to carcasses by a method that combines pre and postspraying (Table 2) did not differ ($P > 0.05$) in their abilities to prevent or remove cecal material from carcasses. When test solutions were applied to carcasses by postspraying alone (Table 2), tap water was the most effective ($P \leq 0.05$) in removing fecal material from carcasses. However, results in Table 2 seem to indicate that application of test solutions (tap water, TSP, and alkaline EO water) by combined pre- and postspraying of test solutions was more effective than postspraying alone. For example, applying alkaline EO by combined pre- and postspraying removed more ($P \leq 0.05$) fecal material from carcasses compared with when alkaline EO was applied by postspraying alone (Table 2). Postspraying alone was not as good as combinations of pre- and postspraying treatment in removing cecal material probably due to the fact that prespraying increased moisture content on the surface of the carcass and acted as a surface barrier to prevent cecal material attachment. Combinations of pre and postspraying treatments were able to remove cecal material on the surface of chicken carcasses from more or less severe (score of 4) to less than smear tint only (score of 2). In the end, in both spraying methods (combined pre- and postspraying of test solutions vs. postspraying alone), alkaline EO was equally effective as 10% TSP in preventing the attachment of feces and in removal of feces from chicken carcass.

A preliminary study conducted following the same procedure as described previously to compare the effectiveness of immersion vs. spray treatments on the reduction of bacterial populations on chicken carcasses revealed that the immersion treatment was more effective than the spraying treatment in reducing bacterial populations on chicken carcasses by 0.67 $\log_{10}$ cfu/g for deionized water and 1.72 $\log_{10}$ cfu/g for EO water, respectively (Table 3), whereas EO water spraying alone did not significantly affect the reduction of bacterial populations on the carcasses compared with deionized water. Hence, spray alone treatment was excluded for the following studies. Fabrizio et al. (2002) also indicated that various treatments (EO, chlorine, ozone, 2% acetic acid, and 10% TSP water) applied via spray washing were not particularly effective due to short contact time (15 s), but longer contact time with immersion (45 min) contributed to the improvement of the effectiveness of treatments on the reduction of microorganisms.

Treatments with 40 min of immersion only and the combination of 1.5 s of spraying and 40 min of immersion were, henceforth, investigated in the current study for reducing bacterial populations on chicken carcasses. The properties of tap, EO, and chlorinated water used for killing *C. jejuni* on the surface of chicken carcasses before and after immersion treatments are presented in Table 4. Table 4 indicated that although there was no significant

Table 3. Comparison of the effectiveness of EO water immersion vs. spray treatments[1,2]

Treatment	pH	ORP (mV)	Chlorine (mg/L)	Mode	Surviving population ($\log_{10}$ cfu/g)
Deionized water	4.7	375	None	Spray	3.73
				Immersion	3.06
EO water	2.5	1140	47.2	Spray	3.61
				Immersion	1.89

[1]The population of *Campylobacter jejuni* on chicken carcasses after inoculation was 4.68 $\log_{10}$ cfu/g.

[2]EO = electrolyzed water; ORP = oxidation-reduction potential.

Table 4. Properties of water used for killing *Campylobacter jejuni* on chicken carcass before and after immersion treatment[1]

	Before treatment			After treatment		
Treatment	pH	ORP (mV)	Chlorine (mg/L)	pH	ORP (mV)	Chlorine (mg/L)
Tap water	6.8	610	1.1	7.4	352	Not detected
Acidic EO	2.8	1165	39.5	2.9	1092	21.2
Chlorinate	9.1	685	73.1	8.6	651	41

[1]EO = electrolyzed water; ORP = oxidation-reduction potential.

difference of ORP values in EO and chlorinated water between before and after treatment, concentrations of total residual chlorine in EO and chlorinated water were reduced by 46.3 and 43.9% due to the immersion treatment, respectively.

The simulated industrial washing process with EO and chlorinated water immersion only treatment significantly reduced the initial population (4.92 $\log_{10}$ cfu/g) of *C. jejuni*, which was spot-inoculated on the lower dorsal area of chicken carcasses, by 2.33 and 2.05 $\log_{10}$ cfu/g, respectively (Table 5). In contrast, Fabrizio et al. (2002) reported that although multiple intervention treatments with acidic (50 mg/L of chlorine) and basic EO water, chlorine (50 mg/L of chlorine), 2% acetic acid, and 10% TSP water were effective in killing *Escherichia coli*, *Salmonella*, and total coliforms on broiler carcasses, treatment with acidic EO water alone was not able to significantly reduce bacterial populations evaluated. On the other hand, Park et al. (2002) reported findings that are similar to the results of this study. They reported that acidic EO water was very effective, not only in reducing the population of *C. jejuni* on chicken but also for prevention of cross-contamination of processing environments, demonstrating that acidic EO water reduced populations of *C. jejuni* by about 3 $\log_{10}$ cfu/g on chicken samples. Results from Park et al. (2002) also demonstrated that no viable cells of *C. jejuni* were recovered in acidic EO water after treatment.

Combinations of spraying and immersion treatment did not significantly improve bactericidal effect of sanitizers compared with the immersion only treatment. However, EO water was as effective as chlorinated water in reducing microorganisms. Furthermore, results in Table 5 suggest that chicken carcasses contaminated with pathogenic microorganisms may also contribute to the cross-contamination of whole batches of chickens during processing in the chiller tank and afterwards.

In summary, this study suggests that alkaline EO water may provide an alternative to TSP in preventing and removing attachment of feces on the surface of chicken carcasses. Information collected from this study suggests that prespraying washing with alkaline EO water may be carried out before defeathering and evisceration to reduce and prevent the load of cross-contaminants on poultry. Although reductions of numbers of microorganisms on chicken carcasses were significant, none of the treatments used for this study can completely eliminate pathogenic bacteria on chicken carcasses. Results from this study may, however, provide guidelines for the development of effective application of alkaline and acidic EO water in preventing and removing attachment of fecal contaminants and thus, reducing foodborne pathogens associated with chicken carcasses during an industrial washing process.

Table 5. Populations of *Campylobacter jejuni* survived on chicken surfaces after immersion only and the combinations of spraying and immersion treatment[1]

Treatment	Surviving population ($\log_{10}$ cfu/g)
Immersion only	
Tap water	3.66[a]
Chlorinated water	2.87[bc]
Acidic EO water	2.59[c]
Cross contamination on carcasses after immersion[2]	
Tap water	3.50[a]
Chlorinated water	2.94[bc]
Acidic EO water	2.58[c]
Combinations of spraying and immersion	
Tap water	3.59[a]
Chlorinated water	2.63[c]
Acidic EO water	2.88[bc]
Cross contamination on carcasses after combinations of spraying and immersion[2]	
Tap water	3.56[a]
Chlorinated water	2.64[c]
Acidic EO water	3.15[ab]

[a-c]Values with the same letter in the same column are not significantly different ($P > 0.05$).

[1]Populations of *C. jejuni* on chicken carcasses before and after inoculation were 2.25 (control) and 4.92 $\log_{10}$ cfu/g.

[2]Chicken carcass samples immersed in the treatment water along with inoculated carcasses to investigate any possible cross-contamination from inoculated samples.

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