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Evaluation of Ozonated Water as a Potential Intervention to Reduce *Salmonella* and Indicator Organisms on Raw Chicken Wing Sections

ABSTRACT

The objective of this study was to evaluate the effectiveness of ozonated water to reduce *Salmonella* and aerobic plate counts (APCs) on raw chicken wing sections. Samples were inoculated with a cocktail of five poultry-borne *Salmonella* strains (mean 6.3 ± 0.3 $\log_{10}$ CFU/ml rinsate). The chicken wing sections (51.3 ± 12.4 g) were treated with ozonated water at three concentrations (2.5, 5.0, and 10.0 ppm) and three exposure times (15, 30, and 45 s) by either immersion or direct application (directly from a hose). Tap water was used as control, and the experiments were performed in triplicate with three wings per treatment. *Salmonella* reductions with ozonated water ranged from 0.3 ± 0.1 to 0.7 ± 0.1 $\log_{10}$ CFU/ml rinsate. APC reductions with ozonated water ranged from 0.3 ± 0.1 to 1.8 ± 0.7 $\log_{10}$ CFU/ml rinsate. Reductions were numerically higher, although not significantly, with ozonated water than with tap water. Ozone concentration had a significant effect (P = 0.003) on *Salmonella* reductions in immersion treatment. Although *Salmonella* reductions on chicken wing sections were considered marginal, future work should focus on evaluating longer exposure times and ozone concentrations on the microbiological quality of raw chicken parts, possibly as part of a multihurdle antimicrobial approach.

INTRODUCTION

Chicken meat is the most consumed animal protein in the United States at 97.6 lb (44.3 kg) per capita in 2020, ahead of beef (58.4 lb [26.5 kg]) and pork (52.0 lb [23.6 kg]) *(22)*. More pounds of chicken are marketed as parts (40%) or processed products (49%) than as whole carcasses (11%) *(21)*. According to U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS), *Salmonella* prevalence in chicken parts (legs, breasts, and wings) is often higher than in chicken carcasses *(28)*. Risk model evaluations have shown that no single antimicrobial intervention can reduce *Salmonella* to levels that achieve the current U.S. performance standards *(14)*. Therefore, the industry relies on multiple interventions applied sequentially in prechill tanks, chill tanks, and inside-out washers, among other steps *(14)*. In the

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last 10 years, postchill tanks, located after the primary chiller, have become a popular step for the application of antimicrobial interventions. These tanks resemble a traditional chiller but with lower volume ranges (400–600 gal [1.5–2.3 kl], compared with 20,000–50,000 gal [75.7–189.3 kl]), resulting in shorter product residence times *(20)*. Typical residence times in chill tanks range from 1.5 to 2 h, whereas contact time in postchill tanks can be less than 30 s *(8)*. Therefore, higher concentrations of antimicrobials can be used in postchill tanks with fewer safety or quality concerns *(8)*. In addition, postchill tanks have lower levels of organic matter buildup, which may increase the efficacy of antimicrobials *(8)*. Currently approved antimicrobials for postchill tanks, including ozone-based applications, are listed in USDA-FSIS Directive 7120.1 *(29)*.

Gaseous and aqueous ozone treatments have been proposed as effective antimicrobials with fewer quality and environmental concerns than other chemicals *(3)*. Ozone is highly reactive and has strong oxidizing power *(3)*, thus disrupting sulfhydryl groups, polysaccharides, unsaturated fatty acids, and nucleotides in bacterial cell membranes, cell envelopes, cytoplasm, and spore coats, as well as in virus capsids *(3, 17)*. In addition, ozone readily decomposes into hydroxyl, hydroperoxyl, and superoxide radicals, which eventually turn into oxygen, thus minimizing residues on the surface of the product *(17, 18)*. Current aqueous ozone systems generate ozone gas from ambient air and inject the ozone into water streams *(10)*. Because of its reactivity and rapid decomposition, ozone cannot be accumulated, so it is generated continuously as needed, which means that there are no chemical storage concerns *(3)*.

The use of ozone and ozonated water for food safety applications in the food industry has already been reviewed *(3, 5, 17, 23, 24)*. Factors that influence application efficacy include treatment factors, such as physical state, exposure time, and ozone concentration; bacterial factors, such as cell wall composition and physiological state; and matrix or environmental factors, such as temperature, pH, and presence of ozone-consuming compounds *(3, 17, 23)*. For chicken parts specifically, fat content, presence of skin or bones, ozone dose (combination of exposure time and ozone concentration), and bacterial attachment appear to be important *(5)*. However, few studies have focused on ozonated water as an antimicrobial intervention for poultry parts or compared the effect of each of these factors on the efficacy of ozonated water *(15, 19, 30)*. This is an important knowledge gap that affects the implementation of ozone-based treatments in the poultry industry. Therefore, the objective of this study was to determine the efficacy of ozonated water to decontaminate *Salmonella* and background microbiota on raw chicken wing sections under simulated product and process conditions.

MATERIALS AND METHODS

Sample preparation

Fresh raw chicken wing sections in the form of party-mix trays, containing both drums or drumettes and flats or wingettes, were purchased at a local retailer in Lincoln, Nebraska. A single commercial brand was consistently procured for all experiments. Preliminary studies (data not shown) confirmed aerobic plate count (APC) < 100 CFU/ml rinsate and nondetectable *Salmonella* in fresh chicken wings. Samples were maintained at 4°C in the laboratory and used within 72 h of the sell-by date to ensure complete product thawing before artificial inoculation with *Salmonella*, as well as sufficient and consistent growth of indigenous microflora to estimate APC populations. Three chicken wing sections from each replicate were reserved to enumerate initial APC values.

Inoculum preparation and sample inoculation

Five *Salmonella* serovars isolated from chicken products were provided by the University of Nebraska–Lincoln Food Processing Center's Food Microbiology Service Laboratory. *Salmonella* serovars Braenderup ($n = 1$), Enteritidis ($n = 2$), Hadar ($n = 1$), and Typhimurium ($n = 1$) were stored in tryptic soy broth (TSB; Remel, Lenexa, KS) with 20% glycerol at −80°C. For the experiment, isolates were removed from frozen storage, streaked onto individual plates of xylose lysine deoxycholate agar (XLD; BD, Franklin Lakes, NJ), and incubated at 37°C for 18–24 h. One isolated colony of each strain was inoculated into 10 ml of TSB and incubated at 37°C for 18–24 h. For each culture, 1 ml of suspension was then transferred to a bottle of 200 ml of TSB. The five bottles were incubated at 37°C for 18–24 h. After incubation, the five cultures were combined in a sterile stainless steel container (4.5 qt [4.25 liters]; Syscoware, Houston, TX) to yield 1 liter of working inoculation cocktail *(25)*. Chicken wing sections were immersed in the bacterial suspension for 30 s and then placed on stainless steel racks in a biosafety cabinet for 20 min to allow for bacterial attachment. Afterward, the wings were stored at 4°C for 18–24 h before the experiments were conducted. Three chicken wing sections from each replicate were reserved to enumerate the initial concentration of *Salmonella*.

Ozonated water treatment of chicken wing sections

Ozonated water was generated using a prototype TetraClean aqueous ozone system (TetraClean, Omaha, NE). The system generates ozone from oxygen gas in the air. The ozone gas is then bubbled into tap water (ca. 20°C). The ozonated water is recirculated and infused with additional ozone gas to increase the gas levels until the desired concentration is achieved. The nominal ozone concentration range of the equipment is 0.0–20.0 ppm; however, the ozone concentration in water is most stable in the 0.0- to 10.0-ppm range. Ozonated water flow

rate was set to 3 liters/min per equipment design and delivered from a hose with a 3/8-in. (9.525 mm) diameter. Three concentrations of ozone were used: 2.5, 5.0, and 10 ppm. The ozone levels were monitored and displayed by a Q46H-64 Dissolved Ozone Monitor (Analytical Technologies Industries, Collegeville, PA), which was integrated into the aqueous ozone system. In addition, ozone concentration was corroborated with a CHEMets ozone measuring kit (K-7404; CHEMetrics, Midlands, VA). Two treatment configurations were tested. For the first set of experiments, or immersion treatment, ozonated water was used to fill a covered stainless steel container (4.5 qt [4.25 liters]; Syscoware, Houston, TX). Chicken wing sections were immersed in the ozonated water for 15, 30, or 45 s. These times were chosen based on industry input to simulate realistic product postchill interventions. The container was kept covered to decrease the loss of ozone into the atmosphere, and the flow of ozonated water was kept constant to avoid depleting the ozone inside the container. Three wing sections (51.3 ± 12.4 g each) were individually treated for each of the time and ozone concentration combinations. The used ozonated water was discarded and the container was refilled with fresh ozonated water for each wing section. The experiment was repeated using tap water as a control.

For the second set of experiments, or direct application treatment, chicken wing sections were held with sterile tongs under the opening of a hose with the ozonated water for 15, 30, or 45 s and turned halfway through the treatment time so that both sides were exposed to the ozone treatment. The wing sections were placed 1 cm below the hose opening. The flow of ozonated water was kept at 3 liters/min. The direct application treatment was repeated with tap water as a control, using the same flow rate and a hose of the same diameter (9.525 mm). Three wing sections (51.3 ± 12.4 g each) were individually treated for each of the times and ozone concentration combinations described earlier. After treatment, wing sections were placed individually in sterile Whirl-Pak bags for microbial analysis.

Microbiological analysis

Buffered peptone water (BPW, 100 ml; Sigma-Aldrich, St. Louis, MO) was added to each bag containing one chicken wing section, and the samples were manually massaged for 30 s to detach bacteria, as previously described *(18, 25)*. Appropriate serial decimal dilutions were prepared in 0.1% BPW. For samples artificially inoculated with *Salmonella*, dilutions were plated onto XLD agar and then incubated at 37°C for 18–24 h before counting colonies. For noninoculated samples, dilutions were spread onto Petrifilm APC plates (3M, Saint Paul, MN) and incubated at 35°C for 48 h. The experiments were repeated in triplicate, and results were recorded as $\log_{10}$ CFU/ml rinsate to reflect changes in surface contamination because of the treatments.

Statistical analysis

Each microbiological response (*Salmonella* or APC) and treatment combination application (immersion or direct application) was analyzed separately. Data were analyzed as a randomized complete trial with two factors (exposure time and ozone concentration) at three levels each for a total of nine treatments. Mean $\log_{10}$ CFU/ml rinsate was compared through double-factor analysis of variance on Excel ($P < 0.05$; Microsoft, Redmond, WA), and means were separated using the Tukey post hoc method. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

The initial mean *Salmonella* counts were 6.3 ± 0.3 $\log_{10}$ CFU/ml rinsate, whereas the average starting concentration of indigenous microflora (APC values) in noninoculated samples was 4.3 ± 0.7 $\log_{10}$ CFU/ml rinsate. Chicken wings inoculated with *Salmonella* were stored at 4°C for 18–24 h to simulate the worst-case scenario for microbial contamination, where the bacteria have attached to the matrix and adapted to the cold temperatures.

Salmonella counts for chicken wings treated with ozonated and tap water are presented in *Table 1*. When treating chicken wing sections with tap water, counts ranged from 6.0 ± 0.1 to 6.2 ± 0.1 $\log_{10}$ CFU/ml rinsate, which corresponds to reductions of 0.1 ± 0.1 to 0.3 ± 0.1 $\log_{10}$ CFU/ml rinsate. From initial counts of 6.3 ± 0.3 $\log1_0$ CFU/ml rinsate, ozonated water treatment achieved reductions that ranged from 0.3 ± 0.2 to 0.6 ± 0.1 $\log_{10}$ CFU/ml rinsate. There was a significant effect of concentration ($P = 0.003$) but no significant effect of time of application ($P = 0.635$) on the observed reductions. The reductions with higher ozone concentrations (5 and 10 ppm) were significantly greater than those with tap water and the lower (2.5 ppm) ozone treatment. However, the differences between reductions were less than 0.5 $\log_{10}$ CFU/ml rinsate, and reductions in all treatments were lower than 1.0 $\log_{10}$ CFU/ml rinsate. Reductions lower than 1 log may not be considered of practical application for industry *(2)*. *Salmonella* reductions of 0.74 $\log_{10}$ CFU/ml rinsate were observed in carcasses treated with 10-ppm ozonated water for 45 min *(11)*. Megahed et al. *(19)* found *Salmonella* reductions of 1.2 $\log_{10}$ CFU/cm^2 on chicken drumsticks soaked in 8-ppm ozonated water for 4 min. Agirdemir et al. *(1)* immersed chicken carcasses inoculated with *Salmonella* in 1.5-ppm ozonated water for 5, 10, and 15 min and achieved reductions of 1.21, 1.43, and 1.13 $\log_{10}$ CFU/ml rinsate, respectively. Based on our results and other findings, it seems that longer ozonated water treatments are required to observe practical

TABLE 1. ***Salmonella*** **counts (log_{10} CFU/ml rinsate ± standard error of the mean) on chicken wing sections after treatment by immersion in ozonated water**			
	Application time (s)		
Ozone concentration (ppm)	15	30	45
0	$6.2 \pm 0.1^{a,x}$	$6.2 \pm 0.1^{a,x}$	$6.0 \pm 0.1^{b,x}$
2.5	$6.2 \pm 0.1^{a,x}$	$6.0 \pm 0.0^{b,y}$	$6.0 \pm 0.0^{b,x}$
5	$5.8 \pm 0.1^{a,y}$	$5.9 \pm 0.0^{a,y}$	$5.9 \pm 0.0^{a,x}$
10	$5.9 \pm 0.1^{a,y}$	$5.9 \pm 0.0^{a,y}$	$5.9 \pm 0.0^{a,x}$

^{a,b}Means with different superscripts in the same row were significantly different ($P < 0.05$).

^{x,y}Means with different superscripts in the same column were significantly different ($P < 0.05$).

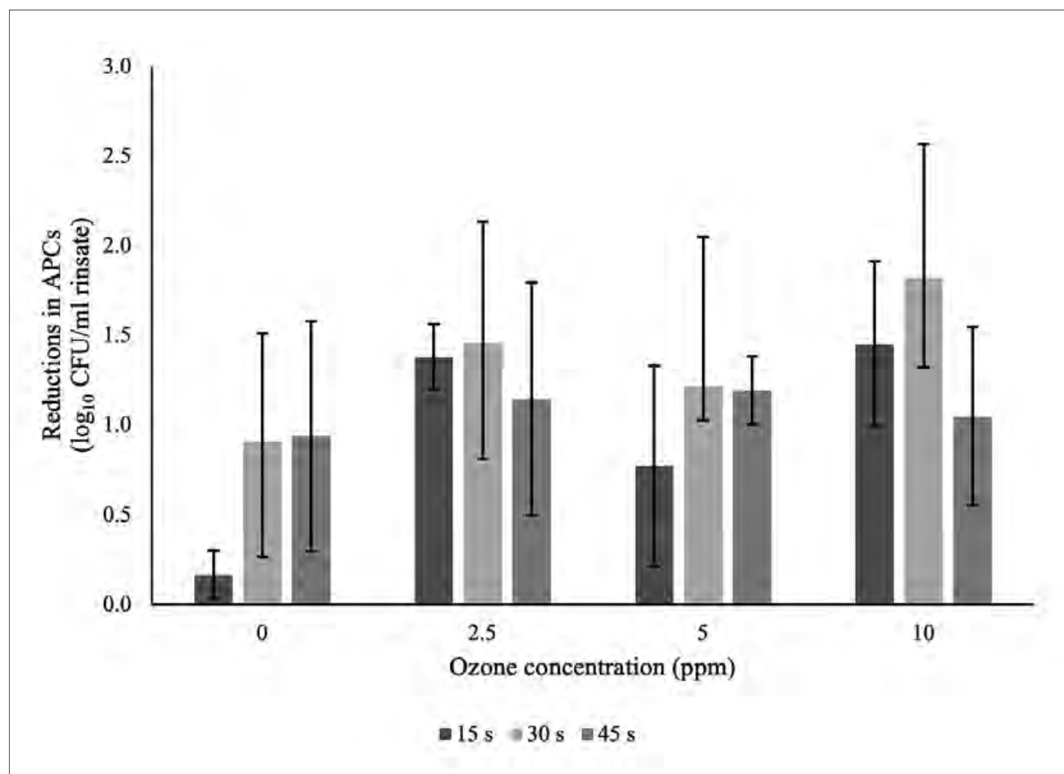

Figure 1. Reductions in APCs on chicken wing sections after treatment by immersion in ozonated water. Error bars represent standard error of the mean.

reductions in *Salmonella* counts, because this would achieve a higher ozone dose. However, this may not be feasible for poultry processors, because product residence times in tanks is typically 30–90 s.

For APC, reductions with tap water (0 ppm of ozone) ranged from 0.2 ± 0.1 to 0.9 ± 0.6 log_{10} CFU/ml rinsate across all control application times *(Fig. 1)*. Reductions with ozonated water ranged from 0.8 ± 0.6 to 1.8 ± 0.7 log_{10} CFU/ml rinsate for APC across all concentrations and application times. Ozonated water treatments achieved higher numerical reductions than tap water treatments; however, the difference was not statistically significant ($P = 0.584$). The APC reductions observed with ozonated water are similar to those previously reported for skin-on chicken products (0.2–1.06 log_{10} CFU/ml rinsate) and bone-in chicken products (0.2–2.1

TABLE 2. *Salmonella* counts ($\log_{10}$ CFU/ml rinsate ± standard error of the mean) on chicken wing sections after direct application of ozonated water

	Application time (s)a,b		
Ozone concentration (ppm)	15	30	45
0	5.9 ± 0.2[a,x]	5.7 ± 0.[a,x]	5.8 ± 0.1[a,x]
2.5	6.0 ± 0.1[a,x]	5.9 ± 0.1[a,x]	5.8 ± 0.1[a,x]
5	5.9 ± 0.0[a,x]	5.8 ± 0.1[a,x]	5.7 ± 0.1[a,x]
10	5.8 ± 0.1[a,s]	5.7 ± 0.1[a,x]	5.6 ± 0.1[a,y]

[a]Means with different superscripts (a, b) in the same row were significantly different ($P < 0.05$).

[b]Means with different superscripts (x, y) in the same column were significantly different ($P < 0.05$).

$\log_{10}$ CFU/g) *(6, 11, 12, 13, 15, 26, 30)*. Fabrizio et al. *(11)* found low reductions in APC (0.8 $\log_{10}$ CFU/ml rinsate) when immersing chicken carcasses in 10-ppm ozonated water for 45 min. In the present study, there was no effect of exposure time ($P = 0.709$) or ozone concentration ($P = 0.584$) on the observed reductions. Other studies have reported an effect of ozone concentration on microbial counts. For example, Gertzou et al. *(12)* reported that treating chicken legs with 10 ppm of gaseous ozone for 1 h significantly extended the shelf life, compared with 2 or 5 ppm of gaseous ozone. Cantalejo et al. *(6)* reported a similar observation when comparing the effect of 0.4, 0.6, and 0.72 ppm of gaseous ozone on aerobic counts of skinless chicken breasts. However, the treatment times for these interventions were longer (1 h and 30 min, respectively) than the ones used in the current study and applied gaseous ozone instead of ozonated water, which may account for the difference.

The second part of the study focused on direct application of ozonated water to the chicken wing sections. This was carried out to avoid the dissipation of ozone gas into the environment or the effects of accumulated organic matter in the immersion container. The initial mean *Salmonella* counts were 6.3 ± 0.1 $\log_{10}$ CFU/ml rinsate, whereas the average starting concentration of indigenous microflora (APC values) in noninoculated samples was 4.7 ± 0.8 $\log_{10}$ CFU/ml rinsate. For the direct application study, *Salmonella* counts with tap water ranged from 5.7 ± 0.1 to 5.9 ± 0.2 $\log_{10}$ CFU/ml rinsate, which corresponds to reductions from 0.4 ± 0.1 to 0.6 ± 0.0 $\log_{10}$ CFU/ml rinsate *(Table 2)*. Reductions of *Salmonella* with ozonated water ranged from 0.3 ± 0.1 to 0.7 ± 0.1 $\log_{10}$ CFU/ml rinsate across application time and ozone concentration. However, there were no significant differences in reductions between treatments. There was no effect of exposure time ($P = 0.145$) or ozone concentration ($P = 0.381$) on the observed reductions, although reductions seemed to increase with longer exposure time and higher ozone concentrations. Dittoe et al. *(10)* inoculated chicken carcasses with *Salmonella* and applied an ozonated water spray at 10 ppm for 20 s, achieving a reduction of 0.1 $\log_{10}$ CFU/ml rinsate, which is lower than the results in this study. However, the researchers applied only 500 ml of ozonated water spray, whereas this study used higher volumes of ozonated water (750–2,250 ml). However, Fabrizio et al. *(11)* observed *Salmonella* reductions of 0.59 $\log_{10}$ CFU/ml rinsate in half-carcasses treated with 10-ppm ozonated water for 15 s at 81 psi (558,475 Pa) using a carcass washer, which was not significantly different from reductions obtained from spraying tap water (0.87 $\log_{10}$ CFU/ml rinsate) or other common antimicrobials.

For APC, reductions with tap water ranged from 0.2 ± 0.1 to 0.7 ± 0.2 $\log_{10}$ CFU/ml rinsate across all application times *(Fig. 2)*. Reductions in APC with ozonated water ranged from 0.3 ± 0.1 to 0.9 ± 0.2 $\log_{10}$ CFU/ml rinsate across all time points. However, the reductions achieved with each treatment were not significantly different from each other. There was no effect of exposure time ($P = 0.918$) or ozone concentration ($P = 0.944$) on the estimated reductions. Fabrizio et al. *(11)* used a carcass washer to spray half-carcasses with ozonated water (10 ppm, 15 s, and 81 psi [558,475 Pa]) and observed significant APC reductions ($P < 0.05$) of 0.55 $\log_{10}$ CFU/ml rinsate. Spray-washing with ozone was the only intervention that significantly reduced APC on half-carcasses compared with electrolyzed oxidizing water, chlorine, acetic acid, and trisodium phosphate. In line with the present study, there was no significant difference between a water treatment (reduction of 0.47 $\log_{10}$ CFU/ml rinsate) and the ozone treatment. Yoder et al. *(31)* also compared an ozonated water spray (3 ppm and 30 s) with a tap water spray to treat beef plates and did not find a significant difference between the reductions obtained with ozone (0.23 log CFU/cm^2) and those obtained with water (0.44 log CFU/cm^2). In these cases, it seems

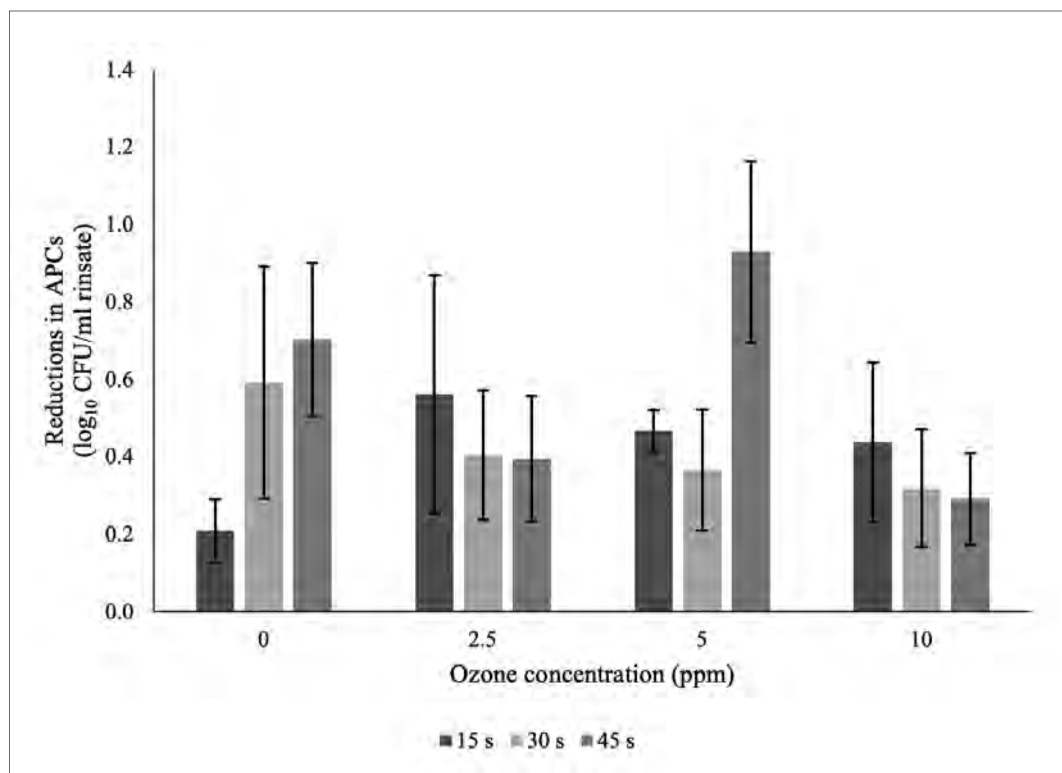

Figure 2. Reductions in APCs on chicken wing sections after direct application of ozonated water. Error bars represent standard error of the mean.

that the antimicrobial activity of ozone contributes less to microbial reductions than the physical removal of bacteria because of spraying *(4)*. However, spraying ozone has shown promise in other applications with longer exposure times and/or with a controlled ambient temperature *(16)*.

The efficacy of antimicrobial interventions depends on ensuring full coverage of the product for the full exposure time, which can be more challenging for spray interventions than for immersion treatments *(4)*. This could explain the lower reductions in APC and *Salmonella* achieved by direct application in this study compared with immersion application. However, satisfactory coverage for spray applications can be achieved with multiple spray nozzles or sequential spray interventions *(9, 19)*. This could close the gap in reductions between the two types of application. Ozone does not share the occupational, environmental, and product quality concerns of other antimicrobials, such as the production of unwanted chlorine derivatives, changes in color and appearance of poultry because of organic acids, wastewater effects of peroxyacetic acid, and amount of water spent in rinsing carcasses after cetylpyridinium chloride application *(7, 25, 27, 29)*, so its potential practical applications should continue to be explored.

CONCLUSIONS

The reductions in *Salmonella* and APC populations attributed to the ozonated water immersion treatment were only marginal and not significantly different from the water control, suggesting that higher ozone doses, concentrations, and exposure times are needed to observe a consistent practical improvement in the microbiological quality of raw chicken wing sections and potentially other chicken parts. Additional studies with ozonated water may focus on unconventional raw chicken products, such as hearts and livers, for which processing operating speeds may not be as great of a concern as for chicken parts. As ozone-based applications continue to improve, studies should focus on simulating larger industry processing volumes to determine scalability to real-life scenarios, as well as use in tandem with other antimicrobial interventions as part of a multihurdle approach.

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