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# Stability of Electrolyzed Oxidizing Water and Its Efficacy against Cell Suspensions of *Salmonella* Typhimurium and *Listeria monocytogenes*

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## ABSTRACT

Electrolyzed oxidizing (EO) water has proved to be effective against foodborne pathogens attached to cutting boards and poultry surfaces and against spoilage organisms on vegetables; however, its levels of effectiveness against *Listeria monocytogenes* and *Salmonella* Typhimurium in cell suspensions have not been compared with those of other treatments. In this study, the oxidation reduction potentials (ORPs), chlorine concentrations, and pHs of acidic and basic EO water were monitored for 3 days at 4 and 25°C after generation. There were no differences between the pHs or ORPs of acidic and basic EO waters stored at 4 or 25°C. However, the free chlorine concentration in acidic EO water stored at 4°C increased after 24 h. In contrast, the free chlorine concentration in acidic EO water stored at 25°C decreased after one day. Cell suspensions of *Salmonella* Typhimurium and *L. monocytogenes* were treated with distilled water, chlorinated water (20 ppm), acidified chlorinated water (20 ppm, 4.5 pH), acidic EO water (EOA), basic EO water (EOB), or acidic EO water that was "aged" at 4°C for 24 h (AEOA) for up to 15 min at either 4 or 25°C. The largest reductions observed were those following treatments carried out at 25°C. EOA and AEOA treatments at both temperatures significantly reduced *Salmonella* Typhimurium populations by >8 $\log_{10}$ CFU/ml. EOA and AEOA treatments effectively reduced *L. monocytogenes* populations by >8 $\log_{10}$ CFU/ml at 25°C. These results demonstrate the stability of EO water under different conditions and that EO water effectively reduced *Salmonella* Typhimurium and *L. monocytogenes* populations in cell suspensions.

Agents currently used to reduce *Salmonella* spp. or *Listeria monocytogenes* in food systems include chlorinated compounds, organic acids, trisodium phosphate, steam, and heat. Despite the availability and effectiveness of these agents, researchers are continually investigating other compounds with which to reduce these and other pathogens more effectively and economically.

Traditionally, chlorine has been the antimicrobial agent of choice because of its availability, efficacy, and relatively low cost. Chlorine is most active in its hypochlorous acid form, which predominates when the pH of a solution is lowered to 4.5 *(4).* However, adding chlorine directly to water raises the pH to ca. 9, so that hypochlorous acid makes up only 4% of the solution. To compensate for this pH fluctuation, many processors have acidified chlorinated water with organic acids to make it more effective, thus increasing costs.

Lactic, acetic, and citric acids are organic acids currently used in the food industry for their antimicrobial activity. Organic acids effectively reduce the pH of the surrounding environment, making it difficult for the bacteria to equilibrate, ultimately causing cellular death. More specifically, the membrane of the cell becomes saturated with hydrogen ions at the low pH. This phenomenon affects the permeability of the cell, leading to inactivation. However, when organic acids are used at the concentrations that are most effective against pathogens, adverse effects on organoleptic properties have been observed for both poultry *(5)* and beef trimmings *(6).*

In a number of studies, trisodium phosphate (TSP) has been shown to be effective against populations of *Salmonella* spp. associated with poultry. Although the mode of action for this effect is not completely understood, it has been speculated that at raised pH levels, the microorganism is not able to maintain homeostasis and carry out cellular functions owing to disruption of the cellular membrane *(12).* Despite the promising nature of TSP as a potential carcass decontaminant, it is expensive to purchase, and disposal issues associated with its extremely high pH and phosphate content make it cost-prohibitive.

Electrolyzed oxidizing (EO) water is a novel system that was developed in Japan. Several studies have shown that EO water is capable of reducing pathogens and/or spoilage organisms attached to cutting boards *(14),* poultry carcasses *(7, 13),* and vegetables *(8).* To produce EO water, a salt solution is passed across a charged bipolar membrane, resulting in two solutions: an acidic solution containing hypochlorous acid (pH 2.6) and having an oxidation reduction potential (ORP) of 1,150 mV and a free chlorine concentration of ca. 50 ppm, and a basic solution containing sodium hydroxide (pH 11.6) and having an ORP of −795 mV. Although the acidic EO water (EOA) has gained the attention of the food industry, basic EO water (EOB) also has antimicrobial attributes that warrant exploration.

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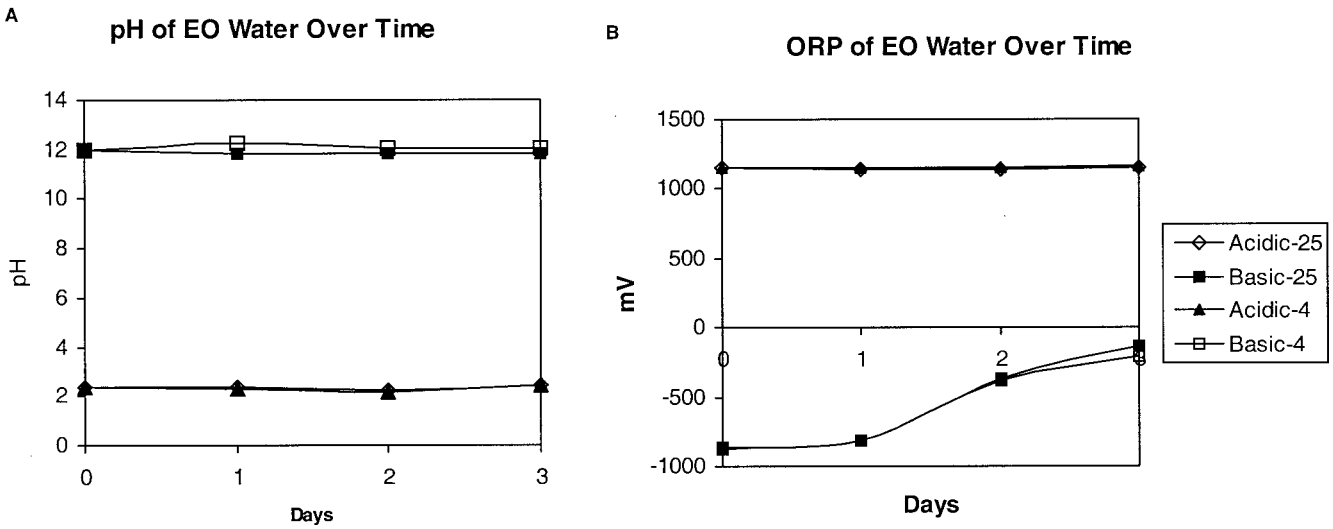


FIGURE 1. *pH (A) and ORP (B) stability of EOA and EOB over 3 days of storage at 4 and 25°C.*

It has been speculated that the effectiveness of EO water is due primarily to its ORP *(10).* Jay *(9)* defines ORP as the ability to gain or lose electrons. Bacteria require certain ORP ranges for growth. Anaerobic bacteria grow in a range of −200 to −400 mV, while aerobic bacteria grow in a range of 200 to 800 mV. When bacteria are subjected to extreme ORPs, either the membrane is saturated with ions (basic solution) or ions are sequestered (acidic solution). For either high or low ORPs, the cellular membrane is rendered unstable, thereby allowing antimicrobial agents to disrupt metabolic processes, ultimately inactivating the cell *(9).*

The present study was designed to determine the stability of EO water over time at different temperatures and to compare its levels of effectiveness against populations of *Salmonella* Typhimurium and *L. monocytogenes* in cell suspensions with those of other commercially available compounds.

## MATERIALS AND METHODS

**Bacterial cultures and media.** *Salmonella* Typhimurium ATCC 13311 and *L. monocytogenes* Scott A were obtained from the culture collection at the Pennsylvania State University and stored at −70°C in Trypticase soy broth (TSB; Difco Laboratories, Sparks, Md.) containing 10% glycerol. Prior to the experiments, *Salmonella* Typhimurium and *L. monocytogenes* were propagated separately in 25 ml of TSB at 37°C for 18 h. Following incubation, 10 ml of each culture was centrifuged (4,000 × *g* for 20 min), washed, and resuspended in 10 ml of buffered peptone water (Difco). Bacterial populations were determined by spread plating serial dilutions on tryptic soy agar (TSA; Difco). Plates were incubated at 35°C for 24 to 48 h. An overnight culture of each pathogen was determined to contain ca. $10^9$ CFU/ml.

**EO water preparation.** The generation of EO water involved the electrolysis of sodium chloride (NaCl) in a cell containing inert positively charged and negatively charged platinum electrodes separated by a bipolar membrane. The membrane was



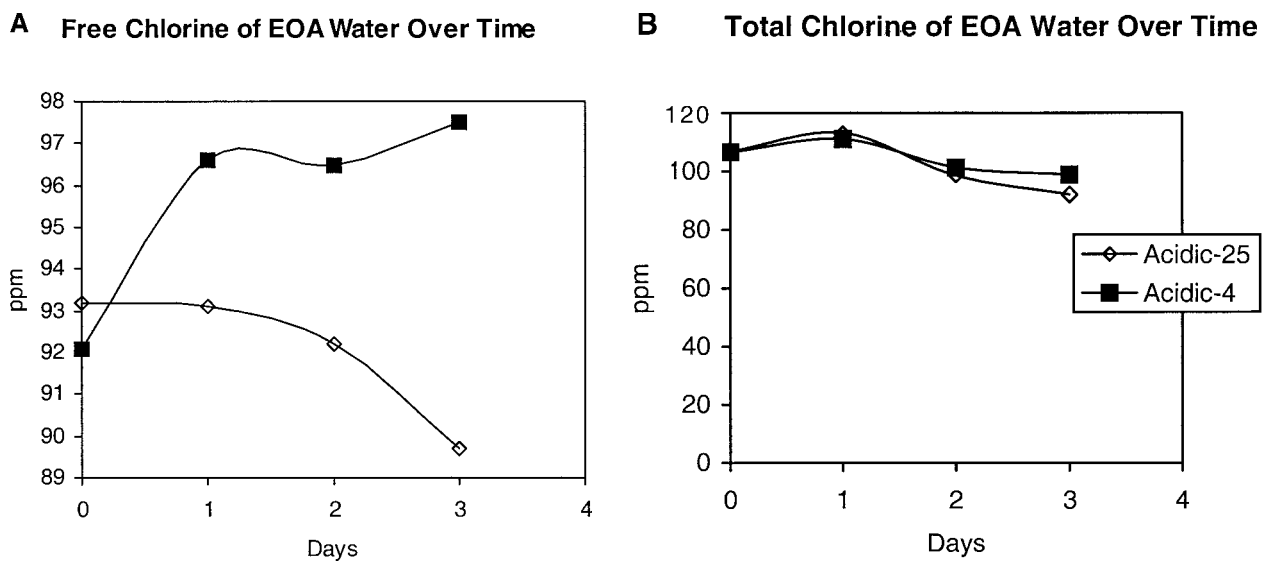


FIGURE 2. *Free (A) and total (B) chlorine levels EOA over 3 days of storage at 4 and 25°C.*

composed of vinylidine polyfluoride. A salt solution (12% NaCl) and deionized water were pumped into the EO water generator (ROX Water Electrolyzer, Hoshizaki America, Inc., Peachtree City, Ga.). According to the manufacturer's directions, by subjecting the electrodes to direct-current voltage of 19 A, two types of waters possessing different characteristics were generated: an electrolyzed basic aqueous solution (pH 11.6) containing dilute sodium hydroxide (NaOH) and having an ORP of −795 mV was produced by the cathode, and an electrolyzed acidic solution (pH 2.6) containing dilute hypochlorous acid (HOCl) and ca. 50 ppm of free chlorine and having an ORP of 1,150 mV was produced by the anode. Small amounts of oxygen and hydrogen gas were also produced during this reaction.

**Stability of EO water.** EOA and EOB were generated at 19 A. Portions (500 ml each) were produced and stored in sterile Pyrex bottles (no. 1395) at either 4 or 25°C. Measurements of free and total chlorine concentrations, pH, and ORP were taken on days 0, 1, 2, and 3. Initial experiments were conducted to determine the combination of storage time and storage temperature that was most detrimental to bacterial populations, and this combination was used in subsequent cell suspension experiments.

**Inoculation and treatments.** For each pathogen, 1 ml of an overnight culture was added to 9 ml of test solution (distilled water [DI; control], EOA, EOB, EOA "aged" for 24 h at 4°C [AEOA; 100 ppm of free chlorine, pH 2.2, 1,170-mV ORP], chlorinated water [CL; 30 ppm of free chlorine, pH 9], acidified chlorinated water [CLA; 30 ppm of free chlorine, pH 4.5]). Chlorinated water was acidified with 0.1 N hydrochloric acid (HCl; Sigma Chemical Co., St. Louis, Mo.). Each treatment was carried out at 25 and at 4°C. Samples were taken for each treatment after 0, 5, 10, and 15 min of exposure, and samples were serially diluted in buffered peptone water. Dilutions were spread plated on TSA in duplicate and incubated at 35°C for 24 to 48 h.

To ensure the detection of low levels of *Salmonella* Typhimurium following treatment, 1-ml samples were also preenriched in 9 ml of lactose broth (Difco) and incubated for 24 h at 35°C. After 24 h of preenrichment, 1 ml of lactose broth was transferred to 9 ml of selenite cystine broth (Difco), another 1 ml was transferred to 9 ml of tetrathionate broth (Difco), and all tubes were incubated for 24 h at 35°C. After incubation, samples were taken from selenite cystine broth and tetrathionate broth, streaked onto xylose lysine decarboxylase (Difco) agar plates for isolation, and incubated for 48 h at 35°C. Typical colony morphology was identified on xylose lysine decarboxylase agar (black colonies) and verified serologically with the Oxoid *Salmonella* latex test (Oxoid, Inc., Ogdensburg, N.Y.). To ensure the detection of low levels of *L. monocytogenes* following treatment, 1-ml samples were enriched in 9 ml of Fraser broth (Difco) for 24 h at 35°C. Following incubation, samples were streaked onto Oxford agar plates for isolation and incubated at 35°C for 48 h. Typical *L. monocytogenes* morphological characteristics (black colonies on Oxford agar) were verified serologically with the Visual Immunoassay for *Listeria* (TECRA Diagnostics, Roseville, Australia).

**Free chlorine determination.** The free chlorine contents of the CL and EO water treatments were measured with the use of the Hach DPD-FEAS digital titrator method (Hach Co., Loveland, Colo.) as described by the manufacturer. Briefly, a 25-ml sample, diluted 10-fold with sterile distilled water, was transferred into an Erlenmeyer flask. A DPD Free Chlorine Powder Pillow was added to the sample and swirled to mix. The sample was titrated with 0.00564 N ferrous ethylenediammonium sulfate (FEAS) to a colorless end point. The free chlorine concentration was calculated from the number obtained following titration, inclusive of the dilution factor (1:10).

**Total chlorine determination.** Total chlorine measurements were taken for the CL and EO water treatments with the use of the Hach DPD-FEAS digital titrator method according to the manufacturer's directions. Briefly, a 10-ml sample was transferred into a 250-ml Erlenmeyer flask. One Dissolved Oxygen 3 Powder Pillow and one Potassium Iodide Powder Pillow were added and swirled to mix. One dropper (1 ml) of starch indicator was added and swirled to mix, producing a dark blue color. The solution was titrated with 0.113 N sodium thiosulfate to a colorless end point. Total chlorine content (ppm) was calculated on the basis of the number obtained following titration and the digit multiplier supplied by Hach.

**Determination of pH and ORP.** The pHs of the antimicrobial solutions were measured with a Corning pH meter (Corning Inc., Corning, N.Y.). The ORPs of the antimicrobial solutions were measured with the use of a Corning pH meter with an Orion ion electrode (Orion Research Inc., Beverly, Mass.).

**Statistical analysis.** Means of bacterial populations from each treatment were calculated from values obtained in three replications for each experiment. Data were analyzed with the SPSS statistical package (SPSS Inc., Chicago, Ill.). The general linear model with repeated measures (with time being held constant) was used to determine differences ($P < 0.05$) between means for treatments. Means were compared with Tukey's honestly significantly different multiple-comparison test.



TABLE 1. *Treatment of* Salmonella *Typhimurium cell suspensions at 25°C for 0, 5, 10, and 15 min with various antimicrobial compounds*

| Treatment[a] | *Salmonella* Typhimurium count (CFU/ml) after treatment time[b] | | | |
|---|---|---|---|---|
| | 0 min | 5 min | 10 min | 15 min |
| DI | 8.32 ± 0.2 A | 8.35 ± 0.3 A | 8.40 ± 0.1 A | 8.34 ± 0.1 A |
| CL | 8.31 ± 0.1 A | 8.33 ± 0.0 A | 8.32 ± 0.2 A | 8.34 ± 0.1 A |
| CLA | 8.35 ± 0.1 A | 8.40 ± 0.1 A | 8.36 ± 0.1 A | 8.34 ± 0.1 A |
| EOB | 8.27 ± 0.1 A | 8.03 ± 0.5 A | 7.61 ± 0.7 A | 7.59 ± 0.5 A |
| EOA | 5.11 ± 1.6 B | 3.46 ± 1.4 B | 0.0 ± 0.0 B | 0.0 ± 0.0 B |
| AEOA | 4.54 ± 1.0 B | 0.75 ± 1.3 B | 0.82 ± 1.4 B | 0.63 ± 1.1 B |

[a] DI, distilled water; CL, 30 ppm of free chlorinated water; CLA, 30 ppm of free acidified chlorinated water; EOB, basic electrolyzed oxidizing water; EOA, acidic electrolyzed oxidizing water; AEOA, "aged" acidic EO water. Mean square error = 0.37.

[b] Means with the same letter in the same column are not significantly different ($P > 0.05$).

TABLE 2. *Treatment of* Salmonella *Typhimurium cell suspensions at 4°C for 0, 5, 10, and 15 min with various antimicrobial compounds*

| Treatment[a] | *Salmonella* Typhimurium count (CFU/ml) after treatment time[b] | | | |
|---|---|---|---|---|
| | 0 min | 5 min | 10 min | 15 min |
| DI | 8.36 ± 0.1 A | 8.47 ± 0.1 A | 8.26 ± 0.1 A | 8.39 ± 0.0 A |
| CL | 8.29 ± 0.1 A | 8.16 ± 0.1 A | 8.23 ± 0.1 A | 8.36 ± 0.1 A |
| CLA | 8.30 ± 0.1 A | 8.32 ± 0.1 A | 8.23 ± 0.2 A | 8.23 ± 0.2 A |
| EOB | 8.13 ± 0.2 A | 7.98 ± 0.3 A | 7.97 ± 0.4 A | 7.81 ± 0.4 A |
| EOA | 5.20 ± 1.0 B | 5.13 ± 1.2 B | 3.37 ± 0.7 B | 3.32 ± 0.5 B |
| AEOA | 3.35 ± 0.5 C | 2.11 ± 1.8 C | 0.47 ± 0.8 C | 0.39 ± 0.7 C |

[a] DI, distilled water; CL, 30 ppm of free chlorinated water; CLA, 30 ppm of free acidified chlorinated water; EOB, basic electrolyzed oxidizing water; EOA, acidic electrolyzed oxidizing water; AEOA, "aged" acidic EO water. Mean square error = 0.25.
[b] Means with the same letter in the same column are not significantly different ($P > 0.05$).

## RESULTS

When both EOA and EOB characteristics were monitored over time, it was determined that EO water stored at 4°C was more stable than that stored at 25°C (Fig. 1). The pHs of both EOA and EOB remained fairly consistent over the 3 days of storage (Fig. 1A). The ORP began to increase for EOB after day 0, while the ORP of EOA remained relatively unchanged (Fig. 1B). Free chlorine levels for EOA stored at 25°C were consistent until day 3, when levels decreased (Fig. 2A). The total chlorine concentration for EOA was initially high at 106.9 ppm, increased to 113.2 ppm after 24 h of storage, and consistently decreased thereafter (Fig. 2B). EOA stored at 4°C demonstrated an increase in free chlorine over the 3 days of storage; the total chlorine concentration for EOA stored at 4°C increased after 24 h of storage. The ORP and the free and total chlorine concentrations for EOA stored at 4°C for 24 h were consistently higher than those for EOA stored at 25°C.

Cell suspensions of *Salmonella* Typhimurium were treated at 25 and 4°C for 0, 5, 10, and 15 min with CL, CLA, EOB, EOA, and AEOA. At 25°C, EOA and AEOA significantly reduced bacterial populations for all times examined (Table 1). Immediately following the addition of the test solutions, EOA and AEOA afforded *Salmonella* Typhimurium reductions of 3.21 and 3.78 $\log_{10}$ CFU/ml, respectively. After 5 min, AEOA treatment afforded the largest reduction (7.6 $\log_{10}$ CFU/ml). Cells treated with EOA were rapidly inactivated after 10 min of treatment. DI, CL, CLA, and EOB treatments did not significantly reduce bacterium levels for any of the treatment times.

At 4°C, *Salmonella* Typhimurium populations remaining after the EOA and AEOA treatments were significantly different from those remaining after the control treatments (Table 2). AEOA was most effective in reducing populations of *Salmonella* Typhimurium. EOA effectively reduced >5 $\log_{10}$ CFU of the pathogen per ml within 15 min of treatment. As demonstrated previously, DI, CL, CLA, and EOB were not effective in reducing *Salmonella* Typhimurium in cell suspensions.

Populations of *L. monocytogenes* remaining after treatments with DI, CL, CLA, and EOB for 0, 5, 10, and 15 min were not statistically different from each other at 25 or 4°C (Tables 3 and 4). Of the treatments carried out at 25°C, AEOA was the most effective at time 0 (Table 3). After 5 min of treatment, EOA significantly reduced levels of the pathogen by >6 $\log_{10}$ CFU/ml. The decline in the levels of viable cells continued after treatments with EOA such that after 10 min, no cells were detected following enrichments. The decline in viable *L. monocytogenes* cells following initial treatment with AEOA represented the most dramatic reduction.

When treatments were carried out at 4°C, the most significant reductions (>5 $\log_{10}$ CFU/ml) were those obtained with AEOA, regardless of time (Table 4). EOA also reduced levels of *L. monocytogenes* by >4 $\log_{10}$ CFU/ml up to 15 min posttreatment, and the reductions obtained with



TABLE 3. *Treatment of* Listeria monocytogenes *cell suspensions at 25°C for 0, 5, 10, and 15 min with various antimicrobial compounds*

| Treatment[a] | *L. monocytogenes* count (CFU/ml) after treatment time[b] | | | |
|---|---|---|---|---|
| | 0 min | 5 min | 10 min | 15 min |
| DI | 8.75 ± 0.1 A | 8.71 ± 0.1 A | 8.82 ± 0.1 A | 8.76 ± 0.1 A |
| CL | 8.69 ± 0.0 A | 8.45 ± 0.2 A | 8.37 ± 0.3 A | 8.17 ± 0.5 A |
| CLA | 8.44 ± 0.6 A | 8.51 ± 0.2 A | 8.51 ± 0.1 A | 8.29 ± 0.3 A |
| EOB | 8.72 ± 0.2 A | 8.71 ± 0.1 A | 8.75 ± 0.1 A | 8.69 ± 0.0 A |
| EOA | 5.10 ± 1.4 B | 2.66 ± 1.1 B | 0.0 ± 0.0 B | 0.0 ± 0.0 B |
| AEOA | 2.03 ± 3.5 B | 1.86 ± 3.2 B | 1.24 ± 2.1 B | 0.59 ± 1.0 B |

[a] DI, distilled water; CL, 30 ppm of free chlorinated water; CLA, 30 ppm of free acidified chlorinated water; EOB, basic electrolyzed oxidizing water; EOA, acidic electrolyzed oxidizing water; AEOA, "aged" acidic EO water. Mean square error = 1.11.
[b] Means with the same letter in the same column are not significantly different ($P > 0.05$).

TABLE 4. *Treatment of* Listeria monocytogenes *cell suspensions at 4°C for 0, 5, 10, and 15 min with various antimicrobial compounds*

| Treatment[a] | *L. monocytogenes* count (CFU/ml) after treatment time[b] | | | |
|---|---|---|---|---|
| | 0 min | 5 min | 10 min | 15 min |
| DI | 8.73 ± 0.1 A | 8.73 ± 0.1 A | 8.71 ± 0.1 A | 8.74 ± 0.1 A |
| CL | 8.75 ± 0.0 A | 8.77 ± 0.0 A | 8.48 ± 0.1 A | 8.57 ± 0.2 A |
| CLA | 8.70 ± 0.1 A | 8.49 ± 0.1 A | 8.53 ± 0.2 A | 8.43 ± 0.2 A |
| EOB | 8.70 ± 0.1 A | 8.69 ± 0.1 A | 8.67 ± 0.2 A | 8.77 ± 0.0 A |
| EOA | 5.89 ± 0.4 AB | 5.36 ± 0.8 AB | 5.12 ± 0.8 AB | 4.60 ± 1.1 AB |
| AEOA | 2.11 ± 3.6 B | 1.98 ± 3.4 B | 2.00 ± 3.5 B | 1.98 ± 3.4 B |

[a] DI, distilled water; CL, 30 ppm of free chlorinated water; CLA, 30 ppm of free acidified chlorinated water; EOB, basic electrolyzed oxidizing water; EOA, acidic electrolyzed oxidizing water; AEOA, "aged" acidic EO water. Mean square error = 2.12.
[b] Means with the same letter in the same column are not significantly different ($P > 0.05$).

this agent were statistically different from those obtained with DI, CL, CLA, or EOB.

The characteristics of compounds used in cell suspension experiments are presented in Table 5.

The statistical analysis identified time as a linear response for all treatments at all temperatures, indicating that as time increased, the amount of the pathogen decreased accordingly.

## DISCUSSION

The stability of EO water over time at various temperatures has not previously been explored. It was expected that EO water would be very unstable because of its extreme ORP values; however, that was not the case. The ORPs and pHs of the waters stored at both temperatures remained very consistent, with the exception of the ORP of EOB, which began to increase after day 1. Len et al. *(11)* observed fluctuations in ORP, pH, and chlorine levels following storage of EO water under different light, agitation, and packaging conditions. These authors attributed these fluctuations to the storage conditions. In this study, after the generation of EO water, samples were stored in airtight glass bottles at 4 and 25°C. The most surprising result was that for the free chlorine concentrations in EOA. When EOA was stored at 25°C, the free chlorine concentration began to decrease after 24 h, as did the total chlorine concentration (Fig. 2A and 2B). A decrease in chlorine levels could have been due to evaporative loss (since the bottles were opened daily for measurements) or self-decomposition of the chlorine, as Len et al. *(11)* hypothesized for their closed system. Free chlorine concentrations in EOA stored at 4°C increased from 92 to 97 ppm after 24 h of storage (Fig. 2A). This increase may have continued past the 3 days of storage; however, the total chlorine concentration began to drop after 24 h of storage.

In the present study, the cell suspension treatments were carried out at two temperatures, 4 and 25°C. For *Salmonella* Typhimurium, we observed a larger, more rapid decrease in viable cells when treatments were carried out at 25°C. This observation may be attributed to the makeup of the cell membrane. At 25°C, the membrane of a gram-negative bacterium is more fluid owing to its high phospholipid composition. When the membrane becomes more fluid at increased temperatures, the antimicrobial agent can enter the cell much faster and easier than it can at reduced temperatures, when the membrane is more rigid *(9)*.

Following treatment with EOA at 25°C, *Salmonella* Typhimurium and *L. monocytogenes* populations were reduced to undetectable levels. This observation is consistent with the results of a previously published study in which *Escherichia coli* O157:H7, *L. monocytogenes,* and *Salmonella* Enteritidis were treated with EOA at 4, 23, 35, and 45°C *(15)*.

In the present study, treatment with various compounds at 4°C did not result in the total inactivation of *L. monocytogenes*. This observation may be attributed to the fluidity of the outer surface of the organism at refrigeration temperatures, as well as the composition of the cell membrane. The outer surfaces of gram-positive organisms are made up



TABLE 5. *Characteristics of compounds used against populations of* Salmonella *Typhimurium and* L. monocytogenes *in cell suspension experiments*

| Treatment[a] | pH | ORP (mV) | Free Cl concn (ppm) | Total Cl concn (ppm) |
|---|---|---|---|---|
| EOA | 2.3 ± 0.3 | 1,155.5 ± 15.9 | 83.4 ± 30.4 | 86.3 ± 30.6 |
| AEOA | 2.2 ± 0.4 | 1,167.8 ± 10.1 | 107.4 ± 5.6 | 109.9 ± 13.5 |
| EOB | 11.5 ± 0.4 | −862.8 ± 14.6 | ND[b] | ND |
| CL | 9.3 ± 0.3 | 499.8 ± 36.1 | 26.6 ± 3.2 | 28.8 ± 0.6 |
| CLA | 4.4 ± 0.3 | 889.0 ± 39.6 | 28.6 ± 6.4 | 35.1 ± 19.2 |

[a] EOA, acidic electrolyzed oxidizing water; AEOA, acidic electrolyzed oxidizing water that was "aged" for 24 h at 4°C; EOB, basic electrolyzed oxidizing water; CL, chlorinated water; CLA, acidified chlorinated water.
[b] ND, not determined.

almost entirely of peptidoglycan, which imparts resistance to a number of environmental stresses.

One noteworthy trend in the data presented is the ability of AEOA at 4°C to reduce both pathogens to a greater extent than AEOA at 25°C does. Similarly, EOA generated immediately prior to treatment and used at 25°C reduced pathogen populations to a greater extent than did AEOA used at 25°C. In both cases, the only difference observed between the results for the treatments was with respect to the amount of detectable chlorine.

Many poultry processors acidify chlorinated water to increase its effectiveness against pathogens associated with broiler carcasses. At a decreased pH (4.5), hypochlorous acid, which is the most active form of chlorine, is formed. When sodium hypochlorite is added to water, the resulting pH is ca. 8 to 9 and the concentration of hypochlorous acid is only 4% *(4).* Thus, we expected to see an increase in the effectiveness of acidified chlorinated water compared with that of chlorinated water. However, this trend was not observed; there was no significant difference between the remaining populations of either pathogen when either of these two treatments was applied for up to 15 min.

The results of the present study clearly demonstrate the effectiveness of an acidic EO water treatment against *Salmonella* Typhimurium and *L. monocytogenes* in cell suspensions at different temperatures. These results also demonstrate that AEOA increased the concentration of chlorine and, subsequently, its effectiveness against populations of *Salmonella* Typhimurium and *L. monocytogenes* at reduced temperatures. Additional studies should be conducted to further validate these findings for cells attached to a variety of surfaces, including meat, poultry, and vegetables.

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