



Effects of water hardness and pH on efficacy of chlorine-based sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*

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ABSTRACT

The effects of hardness and pH of water used to prepare electrolyzed oxidizing (EO) water and bleach solutions on the bactericidal activity of sanitizer prepared from the water were examined. EO water and bleach solutions were prepared with hard water of 0, 50, 100, and 200 mg/l as CaCO₃ at pH 5, 6, 7, and 8. Increased water hardness tended to increase free chlorine and oxidation–reduction potential (ORP) and decrease pH of EO water. Chlorine levels also increased with water pH. Water hardness and pH only had minor effect on the pH of bleach solutions. Increasing hardness to 50 mg/l increased antimicrobial effect of EO water against *Escherichia coli* O157:H7, but reduced when water hardness further increased to 100 mg/l or higher. Water pH had no effect on EO water produced against *E. coli* O157:H7. Water hardness had no significant effect on bactericidal activity of EO water against *Listeria monocytogenes* but elevated water pH decreased bactericidal activity of EO water produced against *L. monocytogenes*. Bleach solution prepared using hard water at 200 mg/l or at pH 7 or higher had significant lower efficacy in inactivating *E. coli* O157:H7, but had no effect on the inactivation of *L. monocytogenes*. Results indicate that increasing the hardness or pH of water used to prepare EO water or bleach solutions will decrease the bactericidal activity of sanitizers prepared from the water.

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1. Introduction

Minimally processed fresh produce is typically washed or treated with chlorinated water containing 50–200 ppm active chlorine to kill or reduce microbial contamination. At home or food service operations, washing with chlorine bleach solution is also a convenient and inexpensive practice to kill and eliminate foodborne pathogens attached to the surfaces of produce. Bleach at dilution of one teaspoon to one tablespoon per gallon of water (ca. 65–200 ppm active chlorine) is considered to be safe to use on fresh produce before consumption (Bourquin, 2008; McGlynn, 2010). However, washing with chlorinated water may not be effective in reducing microorganisms on fruits and vegetables (Parish et al., 2003, chap. 5; Wu and Kim, 2007). Likewise, washing steps generally practiced at household or food service kitchens have been shown to be ineffective in removing all pathogenic bacteria from produce (Parish et al., 2003, chap. 5).

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Electrolyzed oxidizing (EO) water produced by electrolysis of a mild salt solution has been demonstrated as a novel sanitizer for inactivation of various foodborne pathogens. Extensive studies on EO water conducted at the University of Georgia have demonstrated the efficacy of EO water to inactivate *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, and *Bacillus cereus* (Kim, Hung, & Bracket, 2000; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999). Several studies have shown that EO water is effective in killing or reducing foodborne pathogens attached to the surface of lettuce (Koseki, Yoshida, Kamitani, & Itoh, 2003; Pangloli and Hung, 2011; Park, Hung, Doyle, Ezeike, & Kim, 2001; Park, Alexander, Taylor, Costa, & Kang, 2008a; Yang, Swem, & Li, 2003), cabbage (Inatsu, Bari, Kawasaki, Ishiki, & Kawamoto, 2005; Lin, Wu, Yeh, & Saalia, 2005), spinach (Park et al., 2008a), leafy green (Stopforth, Mai, Kottapalli, & Samadpour, 2008), broccoli and strawberries (Hung, Tilly, & Kim, 2010), tomatoes (Bari, Sabina, Isobe, Uemura, & Isshiki, 2003; Deza, Araujo, & Garrido, 2003; Park, Alexander, Taylor, Costa, & Kang, 2008b), alfalfa sprouts (Kim, Hung, Bracket, & Lin, 2003), and green onions (Park et al., 2008b). Application of EO water under simulated food service operation conditions had shown to be effective in killing or reducing *E. coli* O157:H7 on various produces and no bacteria survived in the wash solutions after use also help to prevent cross-contamination during

preparation of foods in kitchens (Pangloli, Hung, Beuchat, King, & Zhao, 2009).

Small and relatively low cost EO water generators are now commercially available, marketed as water ionizers or alkalizers under various brand names, and could be adopted for household use. EO water generated from these devices has been claimed to be effective in killing microorganisms. Therefore, application of these novel devices to generate sanitizer for washing produce prior to consumption could be considered as an additional protection to ensure food safety at home or food service operations. However, scientific information on the efficacy of low cost EO water home unit in killing bacterial pathogens is limited. Additionally, the qualities of water (hardness and pH) used to generate such sanitizer may affect EO water properties and therefore affecting its antimicrobial activities.

According to the U.S. Geological Survey (USGS, 2012a), water hardness varies widely across the United States and is classified into different categories: soft water (0–60 mg/l CaCO₃), moderately hard (61–120 mg/l), hard (121–180 mg/l), and very hard (more than 180 mg/l). Soft waters are mostly found in New England, the South Atlantic Gulf, and the Pacific Northwest states and Hawaii. On the other hand, the hardest waters (>1000 mg/l) are located in Texas, New Mexico, Kansas, Arizona, and Southern California. Leenerts (1959) also reported that water hardness in the United States ranged from 21 mg/l in Maine to 403 mg/l in South Dakota. Similarly, the pH values of water in the U. S. vary widely and range from 4.1 to 6.1 (USGS, 2012b). The USEPA recommends a pH between 6.5 and 8.5 for drinking water and a survey found 14% of US domestic wells had a water pH less than 6.5 and 2% higher than 8.5 (DeSimone, Hamilton, & Gilliom, 2009, 48 p.). The pH of water determines the solubility and biological availability of chemicals and heavy metals. Therefore, the pH may affect the properties of EO water and sanitizers it dilutes.

Hard water can cause a variety of problems such as decreased efficiency of laundry detergents and water heaters and decreased life of plumbing and appliances (Wilson, Parrot, & Ross, 2009). According to Leenerts (1959), synthetic detergents prepared from surface-active agents produce more voluminous and stable foam in hard water than in soft water. Hinton and Holser (2009) reported that high concentrations of calcium and magnesium can reduce capability of water in removing bacteria from the skin of broiler chicken.

No report was found on the effects of water hardness and pH on the properties of EO water or other chlorine-based sanitizers and their efficacy in activating foodborne pathogens. Thus, the objective of this study was to evaluate the effects of water hardness and pH on the properties of EO water generated by a home-use device and its efficacy in killing *E. coli* O157:H7 and *L. monocytogenes* compared to bleach solution.

2. Materials and methods

2.1. Source and preparation of inocula

Five stains of *E. coli* O157:H7 and *L. monocytogenes* were used in this study. The *E. coli* O157:H7 strains were CDC-658 (isolated from human feces in a cantaloupe-associated outbreak), E-19 (calf feces isolate), F-4546 (human feces, alfalfa sprout-associated outbreak), H-1730 (human feces, lettuce-associated outbreak), and LJH-557 (apple cider isolate) and the five strains of *L. monocytogenes* were F8027 (serotype 4b, celery isolate), F8255 (1/2b, peach), F8369 (1/2a, corn), H0222 (1/2a, potato), and LCDC 81-861 (4b, raw cabbage). *E. coli* O157:H7 strains were grown individually in tryptic soy broth (TSB) (Bacto/Difco, Becton Dickinson, Sparks, MD) at 37 °C for 24 h and maintained on tryptic soy agar (TSA) (Difco, Becton Dickinson,

Sparks, MD) slants at 4 °C as working cultures. *L. monocytogenes* strains were grown in brain heart infusion (BHI) broth (Bacto/BBL) at 37 °C for 24 h and maintained on brain heart infusion agar (BHIA) (Bacto or BBL) slants at 4 °C. Working cultures were occasionally transferred to new slants (not more than 4 wk) after reculturing in respective broth (TSB or BHI).

Before experiments, the strains of *E. coli* O157:H7 and *L. monocytogenes* were grown in 10 ml of TSB and BHI, respectively, at 37 °C for 24 h, individually transferred into a 15-ml sterile centrifuge tube, and centrifuged at 2000 × g at 22 °C for 15 min (Centra-CL3, International Equipment Co., Needham Heights, MA). The supernatant was discarded and cells were resuspended in 10 ml of 0.1% peptone water (Difco). Equal volumes of each strain were combined to obtain a cocktail inoculum containing approximately 9 log CFU/ml and equal populations of each strain. Populations in the individual cultures and cocktail inoculum were determined by spread plating on TSA or BHIA.

2.2. Preparation of hard water

Total hardness (as mg/l CaCO₃) of water used to generate EO water and prepare bleach solutions consisted of 4 levels: 0 (control), 50, 100, and 200 mg/l and each hardness level consisted of 4 pH levels: 5, 6, 7, and 8. Hard water was prepared on a 4-1 basis by mixing three parts of calcium carbonate (CaCO₃) and one part of magnesium carbonate (MgCO₃) with deionized water (ration 3:1) in 4-1 Nalgene polymethylpentene beakers with stirring bars. The pH of solutions was lowered to about 4–5 with 1 N HCl to help dissolve CaCO₃ and MgCO₃. After all CaCO₃ and MgCO₃ completely dissolved which was indicated by clear solution, the pH of hard water was adjusted to the desirable levels using 0.1 or 1 N NaOH or HCl. Hard water was prepared at least two days in advance of each experiment. The pH of hard water was measured and adjusted if needed to the designated levels before it was used to prepare EO water and bleach solutions.

2.3. Preparation of EO water

EO water was generated by electrolyzing NaCl solution (0.085%) using a home-use generator (model BTM-3000, Bion-Tech Co., Ltd., Seoul, Korea) according to the manufacturer's instructions with some modification. The results of preliminary experiments indicated that 0.085% NaCl solution prepared with deionized water and processed for 20 min produced EO water with about 40 mg/l free chlorine. NaCl solution (0.085%), prepared with hard water at the designated hardness and pH levels, was placed in both anode and cathode chambers of generator (2 l/chamber) and electrolyzed for 20 min. EO water (9.0 or 9.9 ml) was immediately dispensed into screw-cap test tubes for treatment of test pathogens. The pH and ORP of EO water were measured using a dual channel ACCUMET meter (model AR50, Fisher Scientific, Pittsburgh, PA). Free chlorine levels were determined using the DPD-FEAS method (Hach Co., Loveland, CO).

2.4. Preparation of bleach solution

Regular bleach (Everyday Living, Inter-American Products, Cincinnati, OH) containing ca. 6.0% sodium hypochlorite was obtained from a local supermarket. Bleach solution was prepared by diluting 0.60 ml of regular bleach in 499.4 ml of designated hard water to obtain solution of ca. 65 mg/l free chlorine. This free chlorine level was based on the recommendations for washing fresh produce (Bourquin, 2008) and guidelines for the use of chlorine bleach as sanitizer in food processing operations (McGlynn, 2010). Bleach solution (9.0 or 9.9 ml) was dispensed into screw-cap test tubes and

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