Effectiveness of low concentration electrolyzed water to inactivate foodborne pathogens under different environmental conditions

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ABSTRACT

Strong acid electrolyzed water (SAEW) has a very limited application due to its low pH value (<2.7) and corrosive characteristics. Thus, we developed new low concentration electrolyzed water (LcEW). The efficacy of LcEW under various treatment conditions for the inactivation of different foodborne pathogens in pure culture was evaluated and compared with SAEW. The efficiency of LcEW and SAEW for the inactivation of predominant foodborne pathogens (Escherichia coli O157:H7, Listeria monocytogenes, Staphylococcus aureus and Salmonella Typhimurium) with different dipping times (1, 3, 5, 7 and 10 min), pH values (2.5, 4.0, 5.0, 6.0 and 9.0) and temperatures (4, 15, 23, 35 and 50 °C) were determined. Reductions of bacterial populations of 1.7 to 6.6log CFU/mL in various treated conditions in cell suspensions were observed after treatment with LcEW and SAEW, compared to the untreated control. Dip washing (1 min at 35 °C) of lettuce leaves in both electrolyzed water resulted in 2.5 to 4.0log CFU/g compared to the unwashed control. Strong inactivation effects were observed in LcEW, and no significant difference (p>0.05) was observed between LcEW and SAEW. The effective form of chlorine compounds in LcEW was almost exclusively hypochlorous acid (HOCl), which has strong antimicrobial activity and leaves no residuals due to the low concentration of residual chlorine. Thus, LcEW could be widely applied as a new sanitizer in the food industry.

1. Introduction

A gradual increase in the world population and changes in lifestyles has resulted in greater demands for food safety. Listeria monocytogenes, Escherichia coli O157:H7, Staphylococcus aureus and Salmonella Typhimurium are common foodborne pathogens of major public health concern worldwide that can cause illness and death (Mead et al., 1999). A variety of foods, including poultry, eggs, meat, milk, fruits, and vegetables, have been implicated as vehicles of one or more of these pathogens in outbreaks of foodborne illness (Beuchat, 1995; D’Aoust, 1997; Doyle et al., 1997). The Centers for Disease Control and Prevention (CDC) considers E. coli O157:H7 and L. monocytogenes to be of great concern because of the severity and number of illnesses they cause (Wilkinson, 1997).

The Pathogen Reduction Program of the U.S. Department of Agriculture Food Safety and Inspection Service recommends antimicrobial treatments as a method for reducing or inactivating pathogenic bacteria in foods (FSIS, USDA, 1995). Many commercial disinfecting cleaning agents, such as potassium persulfate, isopropanol, hydrogen peroxide, sodium dichloroisocyanurate, ethanol and phenol derivatives (Aarnisalo et al., 2000), quaternary ammonium compounds, and chlorine (Tuncan, 1993) have been shown to be effective against foodborne pathogens in suspension tests. Despite the availability and effectiveness of these agents, researchers are continually investigating other compounds with which to reduce these and other pathogens more effectively, economically and safely.

Strong acid electrolyzed water (SAEW), which is generated by the electrolysis of a dilute salt (NaCl) solution, has been proven to exhibit strong bactericidal activity for the inactivation of many pathogens (Venkitanarayanan et al., 1999; Kim et al., 2000a; Park et al., 2004; Fabricio and Cutter, 2005; Huang et al., 2008; Cao et al., 2009). However, the potential application of SAEW is limited because of its low pH values (≤2.7) and corrosive characteristics. At low pH, dissolved Cl₂ gas can be rapidly lost due to volatilisation, adversely affecting human health and the environment. Moreover, the high acidity of SAEW may cause the corrosion of equipment and consequently limit its practical application (Abadias et al., 2008; Guentzel et al., 2008). Our newly developed low concentration electrolyzed water (LcEW) with a pH value of 6.2–6.5 and a low concentration of free chlorine (2–5 mg/L) is produced by electrolysis of a dilute NaCl solution in a chamber without a membrane. At a pH of 6.0–6.5, the effective form of the chlorine in the LcEW is almost all hypochlorous acid (HOCl), which has strong antimicrobial activity (Yoshifumi, 2003; Cao et al., 2009). Hypochlorous acid is 80 times more effective as a sanitizing agent than an equivalent concentration of the hypochlorite ion (Anonymous, 1997). Hypochlorous acid, the most effective form of chlorine compounds, kills microbial cells by inhibiting glucose oxidation by chlorine-oxidizing sulfhydryl groups.
of certain enzymes important in carbohydrate metabolism (Water Review Technical Briefs, 1997). Thus, the application of widely used SAEW might be replaced by LcEW, which may improve the bactericidal activity while maximizing the use of hypochlorous acid, reducing the corrosion of surfaces, and minimizing human health and safety issues from Cl2 off-gassing (Guentzel et al., 2008).

The objectives of this study were as follows: (1) to evaluate the inactivation effect of LcEW as a new sanitizer against four different foodborne pathogens (L. monocytogenes, E. coli O157:H7, S. aureus, S. Typhimurium); (2) to determine the effect of pH, treatment (dipping) time and temperature on bactericidal activity of LcEW; (3) to find the inactivation effect of LcEW on food (lettuce leaves) and (4) to compare the efficiency of LcEW and SAEW for this inactivation effect.

2. Materials and methods

2.1. Bacterial cultures

Stock cultures of L. monocytogenes ATCC 19115 (L.M), S. Typhimurium ATCC 14028 (S.T), E. coli O157:H7 ATCC 43894 (E.C) and S. aureus ATCC 12598 (S.A) were transferred into tryptic soy broth (TSB) and incubated for 24 h at 35 °C. Following incubation, 10 mL of each culture was sedimented by centrifugation (3000×g for 10 min), washed twice with 0.85% sodium chloride solution and resuspended in 10 mL of the same solution to obtain a final cell concentration of 10^9 CFU/mL. The bacterial population in each culture was confirmed by plating 0.1 mL portions of appropriately diluted culture on tryptic soy agar (TSA) plates (Difco Laboratories, Becton, Dickinson and Company, Sparks, MD 21152, USA) and incubating the plates at 35 °C for 24 h.

2.2. Preparation of electrolyzed water solutions

The low concentration electrolyzed water (LcEW) used in this study had a pH of 6.2, an oxidation reduction potential (ORP) of 500–520 mV and an available chlorine concentration (ACC) of 5 mg/L (≈95% HOCl). It was produced using an EO generator (A2-1000, Korean E&S Fist Inc, Seoul, Seward, London, UK). After homogenization, 1-mL aliquots of the sample were serially diluted in 9 mL of sterile 0.85% sodium chloride solution and 0.1 mL of sample or diluent was spread-plated onto each selective medium. Baird Parker agar (BPA) was used for enumeration of S. aureus, Eosin methylene blue (EMB) agar was employed for enumeration of L. monocytogenes and xylose lysine deoxycholate (XLD) agar was used for enumeration of S. Typhimurium. All plates were incubated at 37 °C for 24 h and microbial count was expressed as log CFU/g. The untreated lettuce sample was used as control.

2.4. Preparation and inoculation of lettuce leaves

RTE iceberg lettuce (Lactuca sativa var. capitata) samples were purchased from a local supermarket in Chuncheon, Korea, and then quickly transported to laboratory and stored at 4 °C. Uneatable, wilted, and damaged portions were trimmed. Lettuce leaves were cut into 3 × 3 cm slices using a sterile knife. Each trimmed leaf was placed on sterile aluminum foil in a biosafety hood. For inoculation, 0.1 mL of each pathogen cocktail (10^6 CFU/mL) was applied to the abaxial-side of each leaf surface by depositing droplets at 20 locations with a micropipettor followed by drying in a laminar flow hood for 1 h at room temperature (23 ± 2 °C) to allow for bacterial attachment to the leaf surfaces. This procedure resulted in initial pathogen inocula levels of approximately 6–7 log CFU/g.

2.5. Sanitizing treatment of lettuce and microbiological analysis

Washing treatments of inoculated lettuce were performed by immersing inoculated shredded lettuce leaves (10 g) in 200 mL of each treatment solution (DW, LcEW and SAEW) in a sterile bag for 1 min at 35 °C. At the end of each treatment, lettuce leaves were drained and washed immediately with 200 mL of sterile neutralizing solution (0.85% NaCl containing 0.5% Na2S2O3) for 1 min to remove residual DW, LcEW, and SAEW. Then all treated samples were transferred into new stomacher bag (Nasco Whirl-Pak, Janesville, WI, USA) containing 90 mL of buffered peptone water (BPW; Difco, Sparks, MD, USA) and homogenized for 2 min with a Seward stomacher (400 Circulator, Seward, London, UK). After homogenization, 1-mL aliquots of the sample were serially diluted in 9 mL of sterile 0.85% sodium chloride solution and 0.1 mL of sample or diluent was spread-plated onto each selective medium. Baird Parker agar (BPA) was used for enumeration of S. aureus, Eosin methylene blue (EMB) agar was employed for L. monocytogenes, and xylose lysine deoxycholate (XLD) agar was used for enumeration of S. Typhimurium. All plates were incubated at 37 °C for 24 h and microbial count was expressed as log CFU/g. The untreated lettuce sample was used as control.

2.6. Statistical analysis

Means of bacterial populations (log CFU/mL and log CFU/g) from each treatment were calculated from three replications for each experiment. Data were analysed using an SPSS statistical package (SPSS Inc., Chicago, IL).

3. Results

3.1. Effect of dipping time on bactericidal efficiency of LcEW and SAEW

The properties (pH, ORP, and available chlorine concentration) of the treatment solutions (distilled water, LcEW and SAEW) used in this study are presented in Table 1. pH, available chlorine concentration (ACC) and oxidation reduction potential (ORP) values for tested solutions (DW, LcEW and SAEW) at various pH (2.5–9.0) and temperatures (4–50 °C) when the pathogens were added have been
The reduction of foodborne pathogens was studied at room temperature (23 ± 2 °C) in 1 min dipping with pH adjusted DW, LcEW and SAEW. After treatment with LcEW, a reduction of L. monocytogenes was recorded to be about 5.40, 5.20, 5.10 and 1.90 log CFU/mL; E. coli O157:H7 were reduced by 5.30, 5.10, 5.0, 4.90 and 2.02 log CFU/mL; and S. aureus were reduced by 6.40, 6.30, 6.20, 6.20 and 1.80 log CFU/mL for pH values of 2.5, 4, 5, 6 and 9, respectively (Fig. 2). More or less similar reduction patterns were found for all foodborne pathogens: populations of S. Typhimurium were reduced by approximately 5.40, 5.20, 5.10 and 1.90 log CFU/mL; E. coli O157:H7 were reduced by 5.30, 5.10, 5.0, 4.90 and 2.02 log CFU/mL; and S. aureus were reduced by 6.40, 6.30, 6.20, 6.20 and 1.80 log CFU/mL for pH values of 2.5, 4, 5, 6 and 9, respectively, compared to the unwashed control. Washing with distilled water (DW) resulted in a reduction of 0.23 to 1.49 log CFU/mL at various pHs (2.5–9.0) for all pathogens.

Table 2 shows the comparative inactivation efficacy of LcEW and SAEW at pH 2.5. The reductions in bacterial count for samples treated with LcEW were about 4.98, 4.80, 4.70 and 4.50 log CFU/mL for L. monocytogenes, S. Typhimurium, E. coli O157:H7 and S. aureus, respectively. On the other hand, bacterial counts for samples treated with SAEW were reduced by approximately 4.90, 4.80, 4.70 and 4.50 log CFU/mL for L. monocytogenes, S. Typhimurium, E. coli O157:H7 and S. aureus, respectively.

### Table 2

Available chlorine concentration (ACC) and oxidation reduction potential (ORP) values for tested solutions (DW, LcEW and SAEW) at different adjusted pH level.

<table>
<thead>
<tr>
<th>pH (±SD)</th>
<th>ACC (mg/L)</th>
<th>ORP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DW</td>
<td>LcEW</td>
</tr>
<tr>
<td>2.5±0.20d</td>
<td>0.9±0.04a</td>
<td>6.8±0.10a</td>
</tr>
<tr>
<td>4.0±0.10c</td>
<td>0.7±0.02ab</td>
<td>6.2±0.30ab</td>
</tr>
<tr>
<td>5.0±0.05bc</td>
<td>0.6±0.03b</td>
<td>5.6±0.20b</td>
</tr>
<tr>
<td>6.0±0.04b</td>
<td>0.5±0.08b</td>
<td>5.0±0.10b</td>
</tr>
<tr>
<td>9.0±0.03a</td>
<td>0.2±0.05c</td>
<td>3.2±0.20c</td>
</tr>
</tbody>
</table>

* Values are the means of three measurements ± standard deviation, values with different letters in the same column differ significantly at p < 0.05.

### Table 3

Oxidation reduction potential (ORP) values for tested solutions (DW, LcEW and SAEW) at different adjusted temperatures.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>pH (±SD)</th>
<th>ACC (mg/L)</th>
<th>ORP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>LcEW</td>
<td>SAEW</td>
<td>DW</td>
</tr>
<tr>
<td>4 cd</td>
<td>6.81±0.25a</td>
<td>6.7±0.07a</td>
<td>2.54±0.13a</td>
</tr>
<tr>
<td>15c</td>
<td>6.72±0.05a</td>
<td>6.5±0.11a</td>
<td>2.44±0.03a</td>
</tr>
<tr>
<td>23bc</td>
<td>6.68±0.09a</td>
<td>6.3±0.20ab</td>
<td>2.43±0.02a</td>
</tr>
<tr>
<td>35b</td>
<td>6.57±0.22a</td>
<td>6.0±0.05b</td>
<td>2.41±0.03a</td>
</tr>
<tr>
<td>50a</td>
<td>6.49±0.18a</td>
<td>5.7±0.12c</td>
<td>2.37±0.05a</td>
</tr>
</tbody>
</table>

* Values are the means of three measurements ± standard deviation, values with different letters in the same column differ significantly at p < 0.05.

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decimal dilution of the samples and 0.1 mL placed on the plates, the limit of detection was 100/mL, i.e. 2 log CFU/mL and in the case of 1.0 mL placed on the plates, the limit of detection was 1 log CFU/mL on direct plate count.

Fig. 3 shows the comparative inactivation efficacy of LcEW and SAEW at 35 °C. The reductions of bacterial count for samples treated with LcEW were about 6.20, 6.30, 6.01 and 6.70 log CFU/mL for *L. monocytogenes*, *S. Typhimurium*, *E. coli* O157:H7 and *S. aureus*, respectively. On the other hand, bacterial counts for samples treated with SAEW were reduced by approximately 6.0, 6.10, 6.0 and 6.60 log CFU/mL for *L. monocytogenes*, *S. Typhimurium*, *E. coli* O157:H7 and *S. aureus*, respectively.

3.4. Inactivation of foodborne pathogens on lettuce leaves using LcEW

Variable results were obtained from the 1 min washing of lettuce leaves at 35 °C using DW, LcEW and SAEW. Dipping inoculated lettuce leaves in DW reduced bacterial counts by 0.67–1.02 log CFU/g for all four organisms. Bacterial counts were reduced by 2.49–3.99 log CFU/g for the LcEW and SAEW treatments, for all organisms tested (Table 4).

4. Discussion

Reductions in bacterial counts ranged from 3.77 to 6.21 log CFU/mL with different dipping times. As dipping time increased, the rate of log reduction significantly decreased (p < 0.05). The ACC reduced with an increase in dipping time (Fig. 4) which could have resulted in lower reductions at increased dipping times. It was found that 1 min dipping time showed a higher log reduction in each bacterium than for 3, 5, 7 or 10 min. Results in this study indicated that LcEW containing 5 ppm of residual chlorine was more effective (p < 0.05) than that of 50 ppm SAEW in reducing populations of bacterial strains, regardless of dipping time (Fig. 1). Among the four pathogens, *S. aureus* showed the highest reduction in bacterial count while *E. coli* O157:H7 showed...
lowest reduction in bacterial count with 1 min dipping. Our results revealed that *E. coli* O157:H7, *L. monocytogenes* and *S. Typhimurium* were comparatively more resistant than *S. aureus* to LeEW and SAEW. In contrast, Kim et al. (2000a) reported that *L. monocytogenes* was slightly more resistant (about 1 log CFU/mL) than *E. coli* O157:H7 to EO water and chlorinated water, probably due to the difference in cell wall structure between Gram-negative and Gram-positive bacteria. At 4 or 23 °C, an exposure time of 5 min with electrolyzed oxidizing (EO) water reduced the populations of *E. coli* O157:H7, *Salmonella enteritidis* and *L. monocytogenes* in the treatment samples by approximately 7 log CFU/mL, with complete inactivation by 10 min of exposure (Venkitanarayanan et al., 1999).

The results obtained in this work showed that the surviving populations of all pathogens increased with increasing pH of the LeEW because ACC and ORP of EW reduced with the increase of pH from the acidic (pH 2.5) to the alkaline (pH 9.0) region. From our results, we also observed that the LeEW with the original pH (6.2–6.5) always gave a higher reduction in bacterial populations than SAEW having original pH (2.5–2.7) in the case of all foodborne pathogens. When the pH was increased to 9.0, inactivation was significantly decreased (p < 0.05) for all organisms. The pH of the solution has important effects on the form of chlorine compounds present (OCl−, Cl2 or HOCl). Chlorine is most active in its hypochlorous acid form, which predominates when the pH of a solution is 5.0–6.5. HOCl dissociates to hypochlorite ions (OCl−) at high pH or chlorine gas (Cl2) at low pH (Fig. 5). Above pH 7.5, very little chlorine exists as the active hypochlorous acid (HOCl), but rather as the inactive hypochlorite ion (ClO−). The pH of the solution should be kept between 6.0 and 7.5 to ensure chlorine activity (Zagory, 2000). Park et al. (2004) demonstrated that the bactericidal activity of EO water increased with decreasing pH for *E. coli* O157:H7 and *L. monocytogenes*, a result similar to that from our work. However, they achieved complete inactivation of both pathogens with >2 mg/L residual chlorine at a pH range between 2.6 and 7.0.

In the present study, it was shown that treatment of *L. monocytogenes*, *S. Typhimurium*, *E. coli* O157:H7 and *S. aureus* at 50 °C for 1 min with LeEW resulted in a complete elimination (reduction of approximately 7.42 to 8.02 log CFU/mL) of these bacteria. As dipping temperature increased, the rate of log reduction significantly increased (p < 0.05). On the other hand, the above-mentioned organisms were more greatly inactivated by LeEW than SAEW at 35 °C. Several studies have been conducted on the efficacy of EO water at different temperatures. Fabrizio and Cutter (2003) showed the efficacy of EO water against *S. Typhimurium* and *L. monocytogenes* at 4 or 25 °C. The highest reductions (≥8 log CFU/mL) were observed with treatments carried out at 25 °C. A mildly heated (50 °C) pre-treatment with alkaline electrolyzed water (AlEW) for 1 min followed by treatment with acidic electrolyzed water (AcEW, 4 °C) resulted in a 2.7 log CFU/g reduction for both pathogens of *E. coli* O157:H7 and *Salmonella* spp. inoculated on lettuce by a dipping procedure (Koselj et al., 2004). Besides, Ding et al. (in press) reported that log reductions of 1.88–2.17 for *L. monocytogenes* were found in lettuce treated with 50 ppm electrolyzed oxidizing water (EOW) for 1 min when the temperature ranged from 15 to 35 °C.

The antimicrobial mechanism of SAEW is not yet fully understood (Suzuki and Watanabe, 2000). SAEW may contain chlorine gas (Cl2), HOCl, and OCl− ions, the collection of which is referred to as the ACC. Some researchers believe that the antimicrobial activity of SAEW is due to the presence of chlorine species, while others believe that the low pH is responsible. A few studies have suggested that this activity is due to its high ORP (Kim et al., 2000b; Liao et al., 2007). The fact remains, however, that SAEW possesses strong bactericidal and virucidal and moderate fungicidal properties (Al-Haq et al., 2005). Acidic electrolyzed water (AEW or AcEW), a popular disinfectant, has been determined to have a strong bactericidal effect on most known pathogenic bacteria (Venkitanarayanan et al. 1999; Kim et al., 2000a, 2000b).

![Fig. 3. Inactivation of different foodborne pathogens treated with LeEW (3 ppm) at different dipping temperatures. Vertical bars represent means of three replications ± SE. Bars labelled with different letters indicate significant difference (p < 0.05). The initial populations of LM, S.T, E.C and S.A used in this study were 7.42, 7.68, 8.02 and 7.74 log CFU/mL, respectively.](image-url)

Table 4

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Unwashed control</th>
<th>Reductions (log10 CFU/g)</th>
<th>DW^b</th>
<th>LeEW^c</th>
<th>SAEW^d</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>6.37 ± 0.21±</td>
<td>1.02 ± 0.02c</td>
<td>3.76 ± 0.09b</td>
<td>3.68 ± 0.23b</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>7.03 ± 0.17a</td>
<td>0.91 ± 0.05c</td>
<td>3.64 ± 0.12b</td>
<td>3.53 ± 0.25b</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>6.79 ± 0.19a</td>
<td>0.67 ± 0.03c</td>
<td>3.40 ± 0.10b</td>
<td>2.50 ± 0.09b</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>7.07 ± 0.13a</td>
<td>0.98 ± 0.07c</td>
<td>3.99 ± 0.19b</td>
<td>3.76 ± 0.12b</td>
<td></td>
</tr>
</tbody>
</table>

^a Log reductions (log10 CFU/g) reported as means of triplicate determinations ± standard deviation. Different letters within the same row differed significantly (p < 0.05).

^b Distilled water.

^c Low concentration electrolyzed water, 5 ppm.

^d Strong electrolyzed water, 50 ppm.
needs low voltage (3 V) and current (1.17 A) for 75 s). Due to its slightly acidic pH value, LcEW can be used as a sanitizer in the food processing industry. Further studies should be elucidated to validate these findings for cells attached to a variety of surfaces, including meat, poultry, fruits and other vegetables as well as the need for further studies with more strains of these pathogens.

Acknowledgements

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Kim, C., Hung, Y.-C., Brackett, R.E., 2000a. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. International Journal of Food Microbiology 61, 199–207

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