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

S.M.E. Rahman, Tian Ding, Deog-Hwan Oh *

Department of Food Science and Biotechnology and Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon, Gangwon 200-701, Republic of Korea

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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 6.6 $\log_{10}$ 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 4.0 $\log_{10}$ 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 persulphate, 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; Fabrizio 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 it corrosive characteristics. At this low pH, dissolved Cl_2 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

* Corresponding author. Tel.: +82 33 250 6457; fax: +82 33 241 0508.
E-mail address: deoghwa@kangwon.ac.kr (D.-H. Oh).

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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 Cl_2 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 by electrolysis of a dilute NaCl solution (0.9%) in a chamber without a membrane using an electrolysis device (model D-7, Dolki Co. Ltd., Wonju, Korea) at a setting of 3 V. For comparison with LcEW, SAEW with a pH of 2.54 and an ORP of 1100–1120 mV was generated using an EO generator (A2-1000, Korean E&S Fist Inc, Seoul, Korea) with a small amount of salt solution (0.1%) and tap water at a setting of 12 A to give a ACC of about 50 mg/L (>95% Cl_2). The pH, ORP and ACC of the treatment solutions (LcEW and SAEW) were measured immediately before treatment with a dual-scale pH meter (Accumet model 15, Fisher Scientific Co., Fair Lawn, NJ) bearing pH and ORP electrodes. The ACC was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corp., Saitama, Japan). The detection range for this measurement was 0–300 mg/L.

2.3. Treatment of pure culture

Volumes of 9 mL of SAEW, LcEW or sterile deionized water (control) were transferred to separate, sterile, screw-capped tubes, and the caps were tightly closed. One millilitre of each bacterial culture (approximately 8.0 $\log_{10}$ CFU/mL) was added to each tube at different treated conditions (five dipping times: 1, 3, 5, 7 and 10 min; five pH levels: 2.5, 4.0, 5.0, 6.0 and 9.0; five temperatures: 4, 15, 23, 35 and 50 °C) and the tubes were mixed immediately. Before adding bacteria pHs were adjusted with 0.1 N HCl and NaOH solutions to make the tested solutions acidic and alkaline, respectively; temperatures were adjusted using incubators and water bath. Following each treatment, 1 mL of each sample was transferred to a tube containing 9 mL of neutralizer (0.85% NaCl containing 0.5% $Na_2S_2O_3$). Serial ten-fold dilutions were performed in 0.85% saline solution and the surviving population of bacteria was determined by plating 0.1 mL of each dilution in duplicate on TSA plates. Colonies of the pathogen were enumerated on TSA plates after incubation at 35 °C for 24 h.

Enrichment was performed to detect the presence of the lower numbers of survivors that would not be detected by direct plating. For enrichment, 1 mL of each sample solution after treatment was transferred to a 150-mL Erlenmeyer flask containing 20 mL of sterile TSB and incubated at 35 °C for 24 h. Following enrichment, the culture was streaked on TSA plates, and the plates were incubated at 35 °C for 24 h before counting. The whole experiment was replicated three times.

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 (10^9 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% $Na_2S_2O_3$) 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 *E. coli*, *Listeria* selective agar 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

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Table 1
Physicochemical properties of tested solutions.

Tested solutions	pH	ORP (mV)	ACC[a] (ppm)
DW[b]	6.63 ± 0.05a	410 ± 12c	0.50 ± 0.08c
SAEW[c]	2.60 ± 0.10b	1100 ± 20a	50 ± 2.2a
LcEW[d]	6.30 ± 0.20a	500 ± 20b	5 ± 0.1b

Values are the means of three measurements ± standard deviation, values with different letters in the same column differ significantly at $p<0.05$.
[a] Available chlorine concentration.
[b] Distilled water.
[c] Strong acid electrolyzed water.
[d] Low concentration electrolyzed water.

reported in Tables 2 and 3.The initial populations of *L. monocytogenes*, *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus* used in this study were approximately 7.42, 7.68, 8.02 and 7.74 log CFU/mL, respectively. The effect of dipping time on the reduction of foodborne pathogens was observed at room temperature (23 ± 2 °C) at the original pH value of the DW, LcEW and SAEW (Fig. 1). After treatment with LcEW, a reduction of *L. monocytogenes* was recorded as about 5.20, 5.18, 4.91, 4.62 and 4.23 log CFU/mL at 1, 3, 5, 7 and 10 min, respectively. More or less similar reduction patterns were found for all foodborne pathogens: populations of *S.* Typhimurium were reduced by approximately 5.15, 5.03, 4.82, 4.55 and 4.18 log CFU/mL; *E. coli* O157:H7 were reduced by 4.90, 4.88, 4.57, 4.34 and 3.89 log CFU/mL; *S. aureus* were reduced by 6.21, 6.15, 5.83, 5.65 and 5.26 log CFU/mL at 1, 3, 5, 7 and 10 min, respectively, compared to the unwashed control. Washing with distilled water (DW) resulted in a reduction of 0.32 to 1.23 log CFU/mL over different dipping time for all pathogens. Significant difference ($p<0.05$) in log reduction was observed in 1 min dipping compared to 10 min dipping for all tested pathogens and *S. aureus* showed highest log reduction ($p<0.05$) compared to other pathogens in 1 min dipping.

Fig. 1 shows the comparative inactivation efficacy of LcEW and SAEW with 1 min dipping. The reduction in bacterial count through treatment with LcEW was about 5.20, 5.15, 4.90 and 6.21 log CFU/mL for *L. monocytogenes*, *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus*, respectively. On the other hand, the bacterial counts for samples treated with SAEW were reduced by approximately 4.92, 4.82, 4.70 and 5.72 log CFU/mL for *L. monocytogenes*, *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus*, respectively.

3.2. Effect of pH on bactericidal efficiency of LcEW and SAEW

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, 5.30, 5.20, 5.20 and 2.23 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, 5.0, 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 1.8 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.

Fig. 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 5.40, 5.40, 5.30 and 6.40 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 5.70 log CFU/mL for *L. monocytogenes*, *S.* Typhimurium, *E. coli* O157:H7 and *S. aureus*, respectively.

3.3. Effect of temperature on bactericidal efficiency of LcEW and SAEW

The reduction of foodborne pathogens was found for 1 min dipping with the original pH value of temperature adjusted DW, LcEW and SAEW. After treatment with LcEW, reductions of *L. monocytogenes* were recorded to be about 4.98, 5.0, 5.20, 6.20 and 7.42 log CFU/mL at 4, 15, 23, 35 and 50 °C, respectively (Fig. 3). More or less similar reduction patterns were found for all foodborne pathogens: populations of *S.* Typhimurium were reduced by approximately 4.91, 4.94, 5.10, 6.30 and 7.68; *E. coli* O157:H7 were reduced by 4.69, 4.73, 4.90, 6.01 and 8.02; and *S. aureus* were reduced by 6.12, 6.16, 6.20, 6.70 and 7.74 log CFU/mL at 4, 15, 23, 35 and 50 °C, respectively, compared to the unwashed control. Washing with distilled water (DW) resulted in a reduction of 0.81 to 1.82 log CFU/mL at various temperatures (4–50 °C) for all pathogens. However, the bacterial counts in all treatment samples decreased to undetectable levels (evidenced by a direct plating procedure and enrichment) at 50 °C. When there was a

Table 2
Available chlorine concentration (ACC) and oxidation reduction potential (ORP) values for tested solutions (DW, LcEW and SAEW) at different adjusted pH level.

pH[a]	ACC[a] (mg/L)			ORP[a] (mV)		
	DW	LcEW	SAEW	DW	LcEW	SAEW
2.5 ± 0.20d	0.9 ± 0.04a	6.8 ± 0.10a	50 ± 2.20a	610 ± 10a	740 ± 20a	1110 ± 20a
4.0 ± 0.10c	0.7 ± 0.02ab	6.2 ± 0.30ab	47 ± 1.10ab	555 ± 15ab	642 ± 14b	1020 ± 18b
5.0 ± 0.05bc	0.6 ± 0.03b	5.6 ± 0.20b	45 ± 1.30b	498 ± 12b	585 ± 12bc	950 ± 13bc
6.0 ± 0.04b	0.5 ± 0.08b	5.0 ± 0.10b	43 ± 1.40b	445 ± 17b	520 ± 20c	890 ± 11c
9.0 ± 0.03a	0.2 ± 0.05c	3.2 ± 0.20c	37 ± 2.10c	315 ± 25c	389 ± 18d	795 ± 15d

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

Temp. (°C)	pH[a]			ACC[a] (mg/L)			ORP[a] (mV)		
	DW	LcEW	SAEW	DW	LcEW	SAEW	DW	LcEW	SAEW
4 cd	6.81 ± 0.25a	6.7 ± 0.07a	2.54 ± 0.13a	0.38 ± 0.04b	4.8 ± 0.09c	48 ± 0.05a	360 ± 15d	500 ± 11c	1130 ± 12b
15c	6.72 ± 0.05a	6.5 ± 0.11a	2.44 ± 0.03a	0.40 ± 0.06b	4.9 ± 0.03c	49 ± 0.10a	375 ± 11 cd	510 ± 8c	1142 ± 9ab
23bc	6.68 ± 0.09a	6.3 ± 0.20ab	2.43 ± 0.02a	0.44 ± 0.08b	5.0 ± 0.01bc	50 ± 2.0a	397 ± 3c	520 ± 20c	1157 ± 6ab
35b	6.57 ± 0.22a	6.0 ± 0.05b	2.41 ± 0.03a	0.66 ± 0.14a	5.2 ± 0.11b	51 ± 0.08a	431 ± 8b	555 ± 17b	1164 ± 2a
50a	6.49 ± 0.18a	5.7 ± 0.12c	2.37 ± 0.05a	0.78 ± 0.11a	5.5 ± 0.05a	53 ± 0.03a	465 ± 5a	610 ± 13a	1170 ± 5a

[a] 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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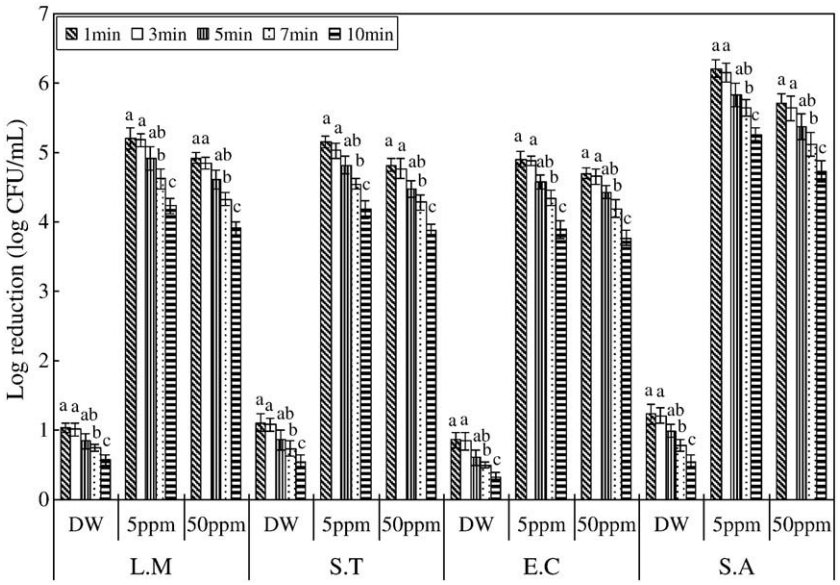

Fig. 1. Inactivation of different foodborne pathogens treated with LcEW (5 ppm) for different dipping times. Vertical bars represent means of three replications ± SE. Bars labelled with different letters indicate significant difference ($p<0.05$). The initial populations of L.M, S.T, E.C and S.A used in this study were 7.42, 7.68, 8.02 and 7.74 log CFU/mL, respectively.

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

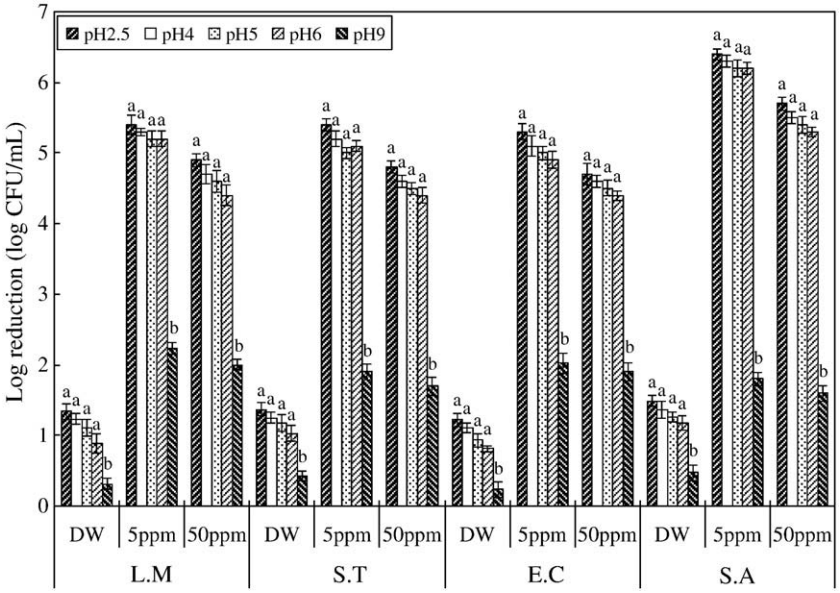

Fig. 2. Inactivation of different foodborne pathogens treated with LcEW (5 ppm) at different pH level. Vertical bars represent means of three replications ± SE. Bars labelled with different letters indicate significant difference ($p<0.05$). The initial populations of L.M, S.T, E.C and S.A used in this study were 7.42, 7.68, 8.02 and 7.74 log CFU/mL, respectively.

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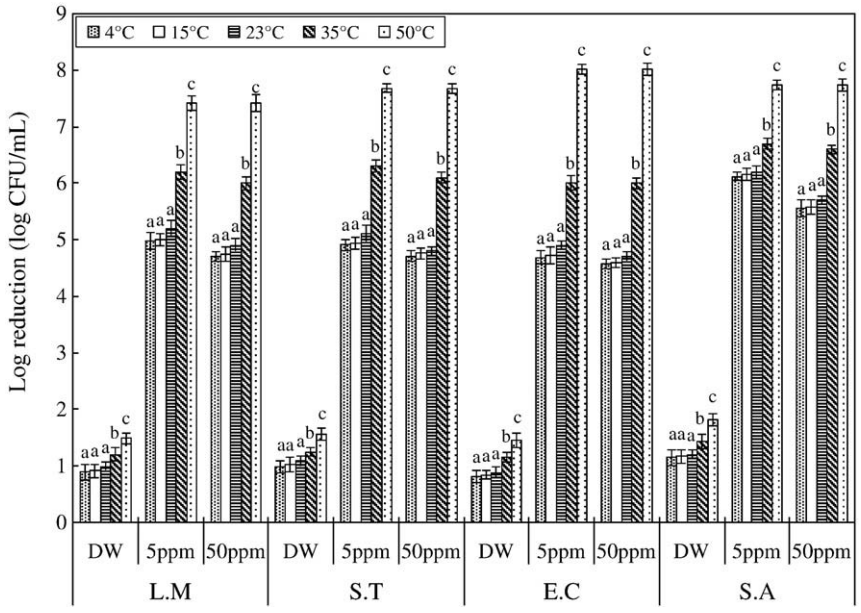

Fig. 3. Inactivation of different foodborne pathogens treated with LcEW (5 ppm) at different dipping temperatures. Vertical bars represent means of three replications ± SE. Bars labelled with different letters indicate significant difference ($p<0.05$). The initial populations of L.M, S.T, E.C and S.A used in this study were 7.42, 7.68, 8.02 and 7.74 log CFU/mL, respectively.

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 LcEW 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 LcEW 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 LcEW 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^-, Cl_2 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 (Cl_2) 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 LcEW 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 LcEW 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 (Koseki 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 (Cl_2), 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,

Table 4
Reductions in the population of inoculated pathogens on lettuce leaves treated with treatment solutions in 1 min dipping at 35 °C.

Pathogens	Unwashed control	Reductions (log_{10} CFU/g)[a]		
		DW[b]	LcEW[c]	SAEW[d]
Listeria monocytogenes	6.97 ± 0.21a	1.02 ± 0.02c	3.76 ± 0.09b	3.68 ± 0.23b
Salmonella Typhimurium	7.03 ± 0.17a	0.91 ± 0.05c	3.64 ± 0.12b	3.53 ± 0.25b
Escherichia coli O157:H7	6.79 ± 0.19a	0.67 ± 0.03c	2.49 ± 0.16b	2.50 ± 0.09b
Staphylococcus aureus	7.07 ± 0.13a	0.98 ± 0.07c	3.99 ± 0.19b	3.76 ± 0.12b

[a] Log reductions (log_{10} 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 acid electrolyzed water, 50 ppm.

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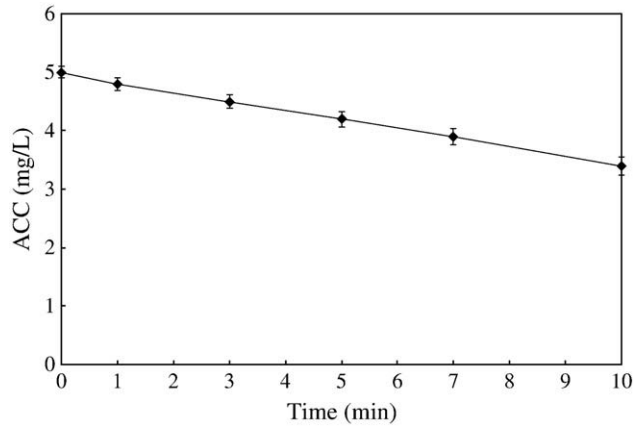

Fig. 4. Changes in available chlorine concentration (mg/L) in low concentration electrolyzed water with time (min).

b; Len et al., 2002; Park et al., 2002a, b, 2004; Fabrizio and Cutter, 2003; Koseki et al., 2003, 2004; Hricova et al., 2008; Cao et al., 2009; Ding et al., 2010). Many studies have been conducted on the bactericidal effect of SAEW, while few reports are available on the use of slightly acidic electrolyzed water (SlAEW) and neutral electrolyzed water (NEW). But no report has been published yet on the use of LcEW. SAEW with low pH (<2.7), high ORP (>1100 mV) and containing free chlorine is produced by the electrolysis of dilute NaCl solution in a cell separated by a membrane and is obtained from the anode side. However, the strong acidity of SAEW causes the corrosion of surfaces and rapid chlorine (Cl_2) loss due to the evaporation of dissolved chlorine gas and ensuing HOCl decomposition, resulting in a reduction in the biocidal effectiveness of the solutions (Guentzel et al., 2008). Under open conditions, the chlorine in SAEW was completely lost after 30 h when agitated and 100 h when not agitated (Len et al., 2002). These disadvantages of SAEW limit its practical application in food industries.

Meanwhile, LcEW with a pH value of 6.2–6.5, also known as nearly neutral EO water, is commonly produced by electrolyzing a dilute salt solution (0.9% NaCl) in a non-membrane electrolytic cell. This electrolytic process converts chloride ions and water molecules into chlorine oxidants (Cl_2, HOCl/ClO^-). At a near-neutral pH (pH 6.2–6.5), the predominant chemical species is the highly biocidal hypochlorous acid species (HOCl, approximately 95%) (Yoshifumi, 2003). This system is more effective and convenient over using other electrolyzed water systems as freshly produced LcEW contain low concentration of chlorine (~5 mg/L) and nearly neutral pH. Also to produce LcEW needs low voltage (3 V) and current (1.15–1.17 A) and minimum electrolysis time (75 s). Due to its slightly acidic pH value, LcEW does not contribute as aggressively as SAEW to corrosion of processing equipment or irritation of hands (Abadias et al., 2008), phytotoxicity in plants or safety issues from Cl_2 off-gassing (Guentzel et al., 2008). Thus, LcEW can be particularly effective for practical applications as a natural sanitizer in the food industry.

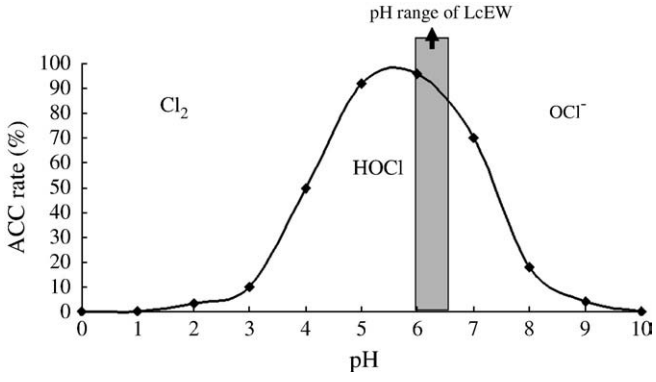

Fig. 5. Changes in available chlorine concentration rate (%) in low concentration electrolyzed water with pH.

This study concluded that LcEW with a near-neutral pH exhibits an equivalent or higher bactericidal activity against foodborne pathogens when compared to SAEW. The advantage of LcEW is numerous: non-corrosive due to near-neutral pH, low current and minimum time required to produce it, it doesn't leave residuals to food due to low content of ACC, comparatively inexpensive, and a less potential health hazard to the worker due to the lack of Cl_2 off-gassing. To produce LcEW, an apparatus is required that utilizes common salt and an electric source. LcEW can be produced at site, as the size of the machine is quite small. Therefore, the widely used EO/SAEW might be replaced by LcEW as an effective and environmentally friendly sanitizer in the food processing industry. Food safety issues have propagated the development of new sanitizers to eliminate pathogenic organisms on foods. This study provides the foundation for further application of LcEW as sanitizing agent in the food 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.

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