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# Bactericidal Activity of Slightly Acidic Electrolyzed Water Produced by Different Methods Analyzed with Ultraviolet Spectrophotometric

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## Abstract

Slightly acidic electrolyzed water (SAEW) as a novel antimicrobial agent is generated by electrolysis of dilute hydrochloric acid (HCl) and/or sodium chloride (NaCl) solution in a cell with or without a separating membrane. The ultraviolet absorption spectra were used to determine the concentration of hypochlorous acid (HClO) and hypochlorite ion ($ClO^-$) in SAEW generated by four different methods and their bactericidal efficiency for inactivation of *Escherichia coli O157:H7* and *Salmonella enteritidis* was evaluated. During the production of equivalent available chlorine in SAEW, more HClO was produced by electrolysis of HCl solution in a non-membrane generator and mixing the acid and alkaline electrolyzed water generated in a generator with membrane, compared with the methods of adding HCl to neutral electrolyzed water (NEW) and electrolyzing the mixture of NaCl and HCl solution in a non-membrane cell. At the 10 mg/L available chlorine concentration, SAEW produced by the methods with more HClO generation had significantly higher ($p<0.05$) bactericidal efficiency for inactivation of both pathogens.

**KEYWORDS:** slightly acidic electrolyzed water, ultraviolet spectra, bactericidal activity, electrolysis methods

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# 1. INTRODUCTION

Food-borne pathogens which can cause human illness and death have continued to be of major public concern in recent years. *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Staphylococcus aureus* are considered to be common of them (Mead et al., 1999). A number of outbreaks of illnesses have been associated with *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Staphylococcus aureus* in fresh produce (Beuchat, 1995).

Acidic electrolyzed water (AEW), also known as electrolyzed oxidizing water, is generated by electrolysis of a dilute sodium chloride (NaCl) solution in a chamber with anode and cathode electrodes separated by a membrane, and obtained from the anode side. AEW has been reported to have strong bactericidal activity against many food-borne pathogens (Venkitanarayanan et al., 1999; Kim et al., 2000; Park et al., 2004; Huang et al., 2008). However, the use of AEW has limited potential for long-term applications because of its strong acidity (pH < 2.7), which causes the corrosion of equipments and chlorine gas ($Cl_2$) off-gassing (Guentzel et al., 2008, Cui et al., 2009).

Slightly acidic electrolyzed water (SAEW), with a pH of 5.0 to 6.5 containing a high concentration of hypochlorous acid (HClO; approximately 95%), could be produced by different methods, such as electrolysis of diluted hydrochloric acid (HCl) and/or NaCl solution in a non-membrane electrolytic cell (Cao et al., 2009; Koide et al., 2009), and redirecting the product formed at the anode into the cathode chamber during electrolysis of NaCl solution in a cell with a separating membrane (Pernezny et al., 2005; Guentzel et al., 2008). SAEW has been considered as an alternative antimicrobial agent in food processing (Abdulsudi et al., 2010; Quan et al., 2010). At the pH of 5.0-6.5, more HClO consists in the SAEW than hypochlorite ion ($ClO^-$), and HClO is the main factor in bactericidal activity of SAEW (Len et al., 2000; Honda, 2003; Cao et al., 2009; Rahman et al., 2010).

Several studies have demonstrated that SAEW has a strong bactericidal activity to inactivate a wide variety of pure foodborne pathogens and their contaminated foods (Cao et al., 2009; Koide et al., 2009; Zhang et al., 2010). A 2-min treatment of SAEW (ACC > 4 mg/L) and a 3-min SAEW (ACC 15 mg/L) treatment resulted in a reduction of 8.2 $\log_{10}$ CFU/mL of *Salmonella enteritidis* and a reduction of 6.5 $\log_{10}$ CFU/g of *Salmonella enteritidis,* respectively (Cao et

al., 2009). SAEW (pH of 6.1 and ACC of 20 mg/L) used with fresh cut cabbage reduced total aerobic bacteria by 1.5 $\log_{10}$ CFU/g and yeasts and molds by 1.3 $\log_{10}$ CFU/g (Koide et al., 2009). Compared to untreated treatment, SAEW treatment significantly ($p$<0.05) reduced the total aerobic mesophilic bacteria from Chinese celery, lettuce and daikon sprouts by 2.7, 2.5 and 2.45 $\log_{10}$ CFU/g, respectively (Abdulsudi et al., 2010). These SAEWs were produced by electrolysis of a mixture of aqueous dilute solution of HCl (2%) and tap water in a SAEW generator without separating membrane. Zhang et al. (2011) demonstrated that SAEW (ACC of 20 and 80 mg/L) treatment on mung bean seeds and sprouts resulted in a reduction of 1.32-1.78 $\log_{10}$ CFU/g and 3.32-4.24 $\log_{10}$ CFU/g for *Escherichia coli* O157:H7, while 1.27-1.76 $\log_{10}$ CFU/g and 3.12-4.19 $\log_{10}$ CFU/g for *Salmonella enteritidis*, respectively. This SAEW was produced by electrolysis of a mixture of NaCl and HCl solution in a non-membrane electrolytic chamber. Based on these studies, SAEW can be generated by different methods. However, limited information is available on the ultraviolet spectrophotometric characteristics and bactericidal efficiency of SAEW generated by different methods.

The objectives of this study were to compare the ultraviolet spectrophotometric characterization of SAEW generated by four different methods at the equivalent pH value and ACC, and to evaluate the efficiency of the SAEW for inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis* at the different ACCs.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of SAEW

SAEW (pH 6.04-6.08, ACC of 32 mg/L) was generated by four different methods: (a) As shown in Fig. 1a, neutral electrolyzed water (NEW) with a pH of 8.02 was prepared by electrolysis of 1 g/L NaCl solution at 18 V for 3 min in a generator (Model CWD-A, Shenyang DongyuXinbor Technology Company Ltd., Shenyang, China) that basically consists of a non-membrane electrolytic chamber with anode and cathode electrodes (dimension of 10 × 10 $cm^2$, distance between the electrodes was 1 cm), and then the pH of NEW was adjusted to 6.08 by adding the diluted HCl (1%) to obtain SAEW1; (b) SAEW2 with a pH of 6.04 was generated by electrolyzing the mixture of 1 g/L NaCl and diluted HCl (1%) solution at 18 V for 3 min in the same generator as above (Fig. 1b); (c) SAEW3 (pH 6.07) was

produced by electrolysis of 9% HCl solution in a SAEW generator (Purester MP-600T, Morinaga Engineering Co., Ltd., Tokyo, Japan) (Fig. 1c); and (d) SAEW4 was prepared by electrolysis of 1 g/L NaCl solution in the generator (model ZSJ-1, Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China) with an electrolysis cell where anode and cathode electrodes (dimension of 15 × 10 $cm^2$, distance between the electrodes was 15 cm) were separated by a membrane (Fig. 1d), mixing the acid electrolyzed water (pH 2.5, ACC 56 mg/L) from the anode side and alkaline electrolyzed water (pH 11.5, ACC of 0 mg/L) from the cathode side to obtain SAEW4 with a pH of 6.05. The mixing ratio was 10:8 (v/v) approximately.

SAEW with an ACC of 10 mg/L were also prepared by the four methods: (a) neutral electrolyzed water (NEW) with a pH of 8.0 was prepared by electrolysis of 1 g/L NaCl solution at 18 V for 1 min in the same generator as above (Fig. 1a), and then the pH of NEW was adjusted to 6.0 by adding the diluted HCl (1%) to obtain SAEW1; (b) SAEW2 with a pH of 6.0 was generated by electrolyzing the mixture of 1 g/L NaCl and HCl (1%) solution at 18 V for 1 min in the same generator as above (Fig. 1b); (c) SAEW3 (pH about 6.0) was produced by electrolysis of 9% HCl solution at a lower electric current in the same SAEW generator as above (Fig. 1c); and (d) SAEW4 was prepared by electrolysis of 1 g/L NaCl solution in the same generator as above (Fig. 1d), mixing the acid electrolyzed water (pH 2.8, ACC 18 mg/L) from the anode side and alkaline electrolyzed water (pH 11.0, ACC of 0 mg/L) from the cathode side to obtain SAEW4 with an approximate pH of 6.0. The mixing ratio was 10:8 (v/v) approximately.

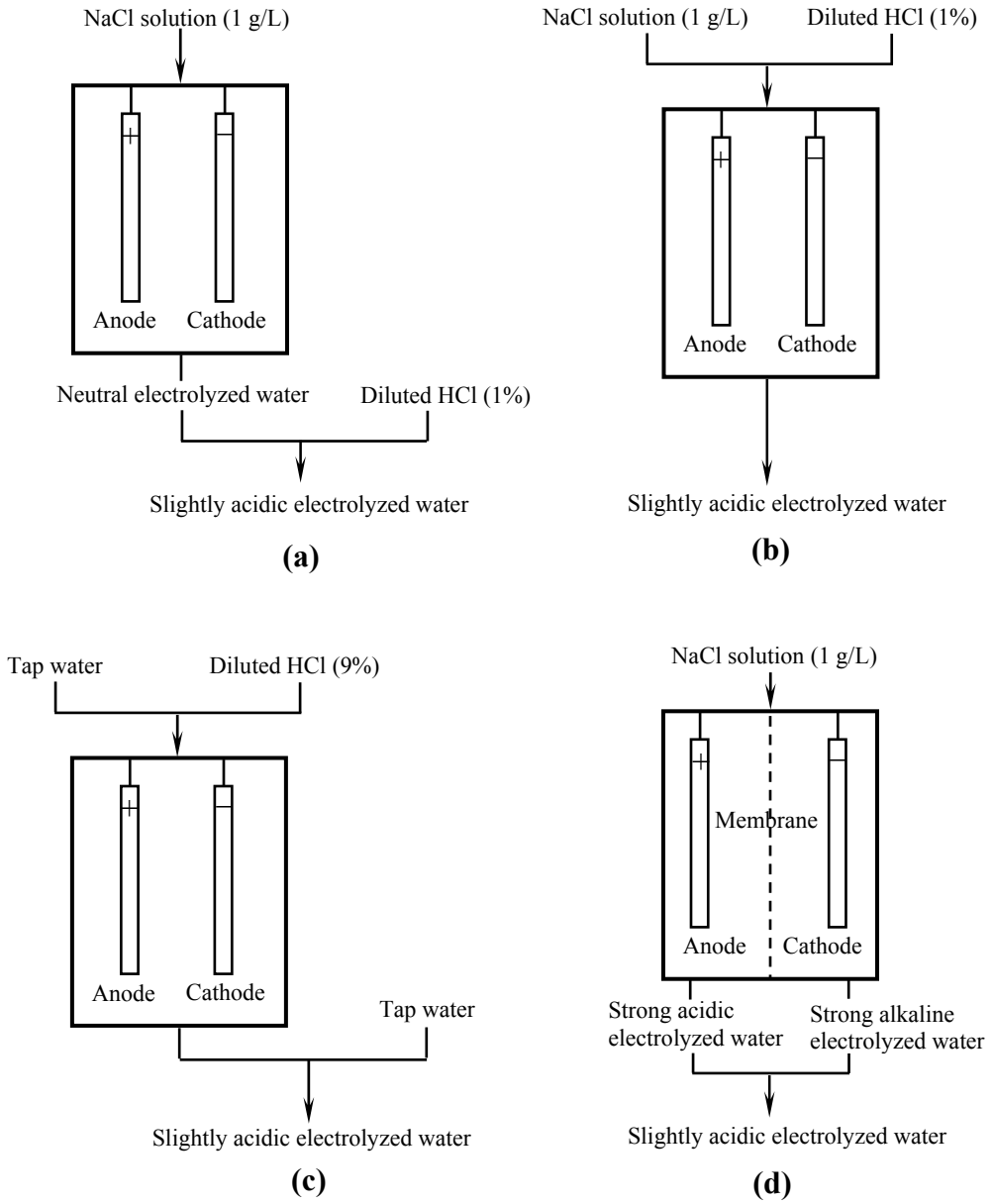


**Fig. 1.** Schematic diagram of slightly acidic electrolyzed water (SAEW) production in four different methods.

## 2.2. Bacterial cultures

Freeze-dried pure cultures of *Escherichia coli* O157:H7 (human feces isolate) and *Salmonella enteritidis* (human gastroenteritis cases isolate) were obtained from

the China Veterinary Culture Collection (CVCC, Beijing, China). Cultures were hydrated according to manufacturer's directions and enriched in sterile tryptic soy broth supplemented with 0.6% (w/v) yeast extract (TSB-YE, CVCC, Beijing, China) for 24 h at 37°C. The viable counts were obtained by plating 0.1 mL tenfold serial dilution of broth cultures onto sterile tryptic soy agar supplemented with 0.6% (w/v) yeast extract (TSA-YE, CVCC, Beijing, China) and incubating the plates at 37°C for 24 h (Cui et al., 2009). The population of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in each culture was approximately 7.0-8.0 $\log_{10}$ CFU/mL.

### 2.3. Analytical measurement of SAEW

The properties of NEW and SAEW were measured immediately before experiments. The pH and oxidation reduction potential (ORP) were measured using a dual scale pH/ORP meter (HM-30 R, DKK-TOA Corporation, Tokyo, Japan) with a pH electrode (GST-5741C) ranged from 0.0 to 14.0 and ORP electrode (PST-5721C) ranged from 0 to 2000 mV. The pH meter was calibrated using commercial standard buffers with pH of 4.01 and 6.86 supplied by the manufacturer. The calibration of the ORP electrode was conducted using the above standard buffers and quinhydrone $C_6H_4(OH)_2$ solution according to manufacturer's instruction. The available chlorine concentration (ACC) in electrolyzed water was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corporation, Saitama, Japan), which was used by Rahman et al. (2010). The detection limit is 0–300 mg/L and the resolution is 1 mg/L.

Ultraviolet spectrophotometric characteristics (200-380 nm) of NEW and SAEW (ACC of 32 mg/L) were tested within 10 minutes after prepared using an ultraviolet-visible spectrophotometer (TU-1810S, Beijing Purkinje Instrument Co. Ltd., China). Ultraviolet spectra were measured at 25 °C in 1 cm quartz cells and deionized water was used as the reference. Absorbance at 234 nm and 292 nm was used to calculate the chlorine concentrations of HClO and $ClO^-$ in mg/L, respectively, according to the following Lambert-Beer's law equation and mass concentration equation (Johnson and Melbourne, 1996; Morris, 1966; Len et al., 2000).

$$A=\varepsilon lc \quad (1)$$

$$\rho_{Cl} = 1000(mg/g)cM_{Cl} \quad (2)$$

Where: $A$ is absorbance at 234 nm or 292 nm, $\varepsilon$ is the molar absorptivity at 234 nm or 292 nm, which is 100 L/mol·cm and 350 L/mol·cm, respectively, $l$ is the length of the light path through the sample in cm, $c$ is the concentration of the compound in solution of mol/L, $\rho_{Cl}$ is the chlorine concentrations in HClO and $ClO^-$ in mg/L, and $M_{Cl}$ is the chlorine molar mass of 35.5 g/mol. All measurements were operated in triplicate.

## 2.4. Evaluation of bactericidal activity of SAEW

Sterile screw-cap tubes containing a volume of 9 mL of SAEW generated by the four different methods and NEW at ACCs of 10 and 32 mg/L were prepared, respectively. Sterile deionized water was used as control. One milliliter of *Escherichia coli* O157:H7 or *Salmonella enteritidis* (approximately 7.0-8.0 $\log_{10}$ CFU/mL) was individually added to the prepared tubes at an ambient temperature of 25 ± 2 $^oC$, mixed and continuously hand-shaken for 20 s. Then 1 mL of each treated sample was immediately transferred to a sterile tube containing 9 mL of neutralizing buffer solution (0.5% sodium thiosulphate + 0.03 M phosphate buffer solution, pH 7.2-7.4). The tubes were shaken by a platform shaker at 150 rpm (MIR-S100, Sanyo Electric Biomedical Co., Ltd., Osaka, Japan). After 5 min of neutralization, the viable count of each pathogen was determined by plating 0.1 mL portions directly or after serially diluted (1:10) in sterile 0.1% peptone water on triplicate TSA-YE (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) plates. Colonies of the inoculated pathogen were enumerated on the plates to determine the bacterial population after incubation at 37$^oC$ for 48 h. An enrichment experiment was further carried out to determine the presence of low numbers of survivals that might not be detected by direct plating. One milliliter of the suspension was transferred to 50 mL of sterile TSB-YE in a 100 mL flask. After incubation at 37$^oC$ for 24 h, the culture solution was streaked on TSA-YE plates, and the plates were incubated at 37$^oC$ for 48 h before counting (Park et al., 2004). Each treatment was replicated in triplicate.

## 2.5. Statistical analysis

For each treatment, mean value and standard deviation of the data from independent replicate measures and trials were determined. Statistical analysis was performed using the SAS8.0 software (SAS Institute Inc. Cary, NC, USA). Tukey's studentized range (honestly significantly different) test was used to determine the significant differences among the means at the 5% probability level.

# 3. RESULTS AND DISCUSSION

## 3.1. Spectroscopic characteristics of SAEW generated by different methods

Ultraviolet absorption spectrograms of the NEW (pH 8.02) and SAEW (pH 6.04-6.08) generated by four different methods at an ACC of 32 mg/L were illustrated in Fig. 2. There were two absorption peaks: one at a wavelength of 234 nm and another one at 292 nm, corresponding to the wavelength maximal previously found for HClO and $ClO^-$, respectively (Morris, 1966; Johnson and Melbourn, 1996; Len et al., 2000). As the data shown in Fig. 2, SAEW3 generated by electrolyzing 9% HCl solution in a non-membrane electrolytic cell and diluted with tap water after electrolysis (Fig. 1c), had the maximum absorbance at 234 nm, followed by SAEW4 produced by electrolyzing NaCl solution in a cell with a membrane (Fig. 1d), and SAEW1 (electrolysis of NaCl solution) and SAEW2 (electrolysis of the mixture of NaCl and HCl solution), but all SAEW samples had similar absorbance at 292 nm. NEW had the minimum absorbance at 234 nm and the maximum absorbance at 292 nm.

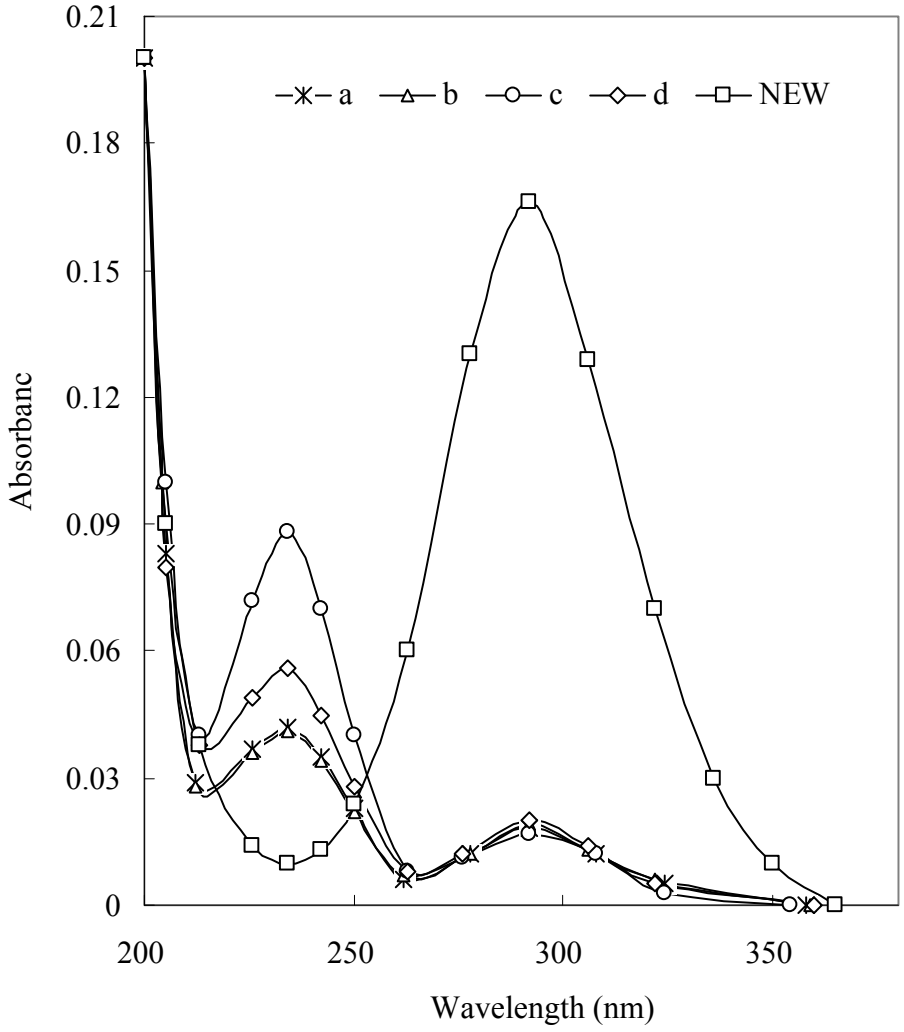


**Fig. 2.** Ultraviolet spectra of neutral electrolyzed water (NEW) and slightly acidic electrolyzed water (SAEW) generated by four different methods. a, SAEW1 produced by adding HCl after electrolysis of NaCl solution in a cell without a membrane; b, SAEW2 produced by electrolysis of the mixture of NaCl and HCl solution; c, SAEW3 produced by electrolyzing HCl solution; d, SAEW4 produced by electrolyzing NaCl solution in a cell with a membrane.

**Table 1** Properties of neutral electrolyzed water (NEW) and slightly acidic electrolyzed water (SAEW) generated by four different methods

| Solutions | ACC (mg/L) | pH | ORP (mV) | HClO concentration ($10^{-4}$ mol/L) | $ClO^-$ concentration ($10^{-4}$ mol/L) | Chlorine concentration (mg/L) | |
|---|---|---|---|---|---|---|---|
| | | | | | | HClO | $ClO^-$ |
| NEW | 32 ± 1 | 8.02 ± 0.08[a] | 815 ± 9[b] | 1.0 ± 0.1[d] | 4.70 ± 0.15[a] | 3.6 ± 0.4[d] | 16.8 ± 0.5[a] |
| SAEW1 | 32 ± 1 | 6.08 ± 0.05[b] | 915 ± 10[a] | 4.1 ± 0.4[c] | 0.51 ± 0.06[b] | 14.6 ± 1.3[c] | 1.8 ± 0.2[b] |
| SAEW2 | 32 ± 2 | 6.04 ± 0.06[b] | 910 ± 12[a] | 4.0 ± 0.3[c] | 0.54 ± 0.05[b] | 14.2 ± 0.9[c] | 1.9 ± 0.2[b] |
| SAEW3 | 32 ± 1 | 6.07 ± 0.04[b] | 907 ± 7[a] | 8.2 ± 0.5[a] | 0.46 ± 0.06[c] | 29.1 ± 1.9[a] | 1.6 ± 0.2[c] |
| SAEW4 | 32 ± 2 | 6.05 ± 0.05[b] | 923 ± 8[a] | 5.4 ± 0.3[b] | 0.57 ± 0.10[b] | 19.2 ± 0.9[b] | 2.0 ± 0.4[b] |

Values reported as the means of triplicate measurements ± standard deviation.

SAEW1 was produced by adding HCl after electrolysis of NaCl solution in a cell without a membrane; SAEW2 was produced by electrolysis of the mixture of NaCl and HCl solution; SAEW3 was produced by electrolyzing HCl solution, and SAEW 4 was produced by electrolyzing NaCl solution in a cell with a membrane.

[a-d] means in the same column followed by different superscripts are significantly different as determined by Tukey's studentized range test ($p<0.05$).

Table 1 shows the pH, ORP, ACC, HClO and $ClO^-$ concentrations of NEW and SAEW generated by four different methods. The pH value of NEW (8.02) was significantly higher than that of SAEW (6.04-6.08) ($p<0.05$), but the ORP of NEW was lower than that of SAEW at an ACC of 32 mg/L. The ORP values of SAEW generated by four different methods had no remarkable differences ($p>0.05$), but it was significantly higher than that of NEW ($p<0.05$). SAEW3 had the highest HClO concentration ($8.2\times10^4$ mol/L) and lowest $ClO^-$ concentration ($0.46\times10^4$ mol/L), followed by SAEW4, SAEW1, SAEW2 and NEW (Table 1). The HClO concentrations in SAEW3 and SAEW4 were significantly higher than that in SAEW1 and SAEW2 ($p<0.05$). It shows that more HClO in SAEW could be generated using method (c) and (d) than method (a) and (b) during the generation of equivalent available chlorine. The properties of SAEW1 and SAEW2 had no remarkable difference ($p<0.05$), which means on difference between adding HCl after electrolysis of NaCl solution and electrolyzing the mixture of NaCl and HCl solution to produce SAEW. The HClO and $ClO^-$ concentrations added up to ACC only in SAEW3. It indicates that the available chlorine be produced by method (c) almost was HClO and the available chlorine compounds such as $Cl_2$ could be produced in the SAEWs generated by

method (a), (b) and (d). Further studies are needed to investigate the available chlorine forms in SAEWs produced by method (a), (b) and (d).

### 3.2. Bactericidal efficiency of SAEW generated by different methods

The bactericidal efficiency of SAEW generated by the 4 different methods and NEW at ACCs of 10 mg/L and 30 mg/L for inactivation of *Escherichiacoli* O157:H7 and *Salmonella enteritidis* is shown in Table 2. The treatments were conducted at 25 ± 2$^{o}$C for 20 s. At an ACC of 10 mg/L, the populations of both pathogens in the treated samples were significantly reduced by all SAEW solutions and NEW compared to the control ($p$<0.05) and the bactericidal activity of SAEW was markedly stronger than that of NEW ($p$<0.05) (Table 2). SAEW3 had the highest bactericidal activity, followed by SAEW4, SAEW1 and SAEW2 A reduction of 6.90 log CFU/mL for *E. coli* O157:H7 and 5.9 log CFU/mL for *S. enteritidis* was resulted by SAEW3 generated by electrolysis of HCl solution in a SAEW generator (Fig. 1c). Treatment of SAEW produced by other three methods reduced the population of *E. coli* O157:H7 by 3.33-3.79 log CFU/mL and 2.63-3.01 log CFU/mL for *S. enteritidis*, respectively. SAEW produced by the methods with more HClO generation had significantly higher ($p$<0.05) bactericidal efficiency for inactivation of both *Escherichia coli* O157:H7 and *Salmonella enteritidis* because of higher HClO concentrations present (Table 1).

**Table 2** Bactericidal efficiency of neutral electrolyzed water (NEW) and slightly acidic electrolyzed water (SAEW) generated by four different methods

| Solutions | Available chlorine concentration of 10 mg/L | | | | Available chlorine concentration of 32 mg/L | | | |
|---|---|---|---|---|---|---|---|---|
| | *Escherichia coli* O157:H7 ($log_{10}$ CFU/mL) | | *Salmonella enteritidis* ($log_{10}$ CFU/mL) | | *Escherichia coli* O157:H7 ($log_{10}$ CFU/mL) | | *Salmonella enteritidis* ($log_{10}$ CFU/mL) | |
| | Initial | Surviving | Initial | Surviving | Initial | Surviving | Initial | Surviving |
| Control | 8.42 ± 0.06 | 8.11 ± 0.18[a] | 7.86 ± 0.16 | 7.63 ± 0.25[a] | 7.38 ± 0.12 | 7.11 ± 0.16[a] | 7.97 ± 0.15 | 7.73 ± 0.11[a] |
| NEW | 8.41 ± 0.13 | 6.71 ± 0.13[b] | 7.86 ± 0.19 | 6.26 ± 0.07[b] | 7.37 ± 0.26 | 2.66 ± 0.07[b] | 7.97 ± 0.28 | 2.54 ± 0.08[b] |
| SAEW1 | 8.42 ± 0.25 | 5.01 ± 0.05[c] | 7.86 ± 0.12 | 5.16 ± 0.20[c] | 7.38 ± 0.19 | ND[A,c] | 7.97 ± 0.19 | ND[c] |
| SAEW2 | 8.42 ± 0.19 | 5.09 ± 0.08[c] | 7.86 ± 0.26 | 5.23 ± 0.13[c] | 7.38 ± 0.20 | ND[c] | 7.97 ± 0.11 | ND[c] |
| SAEW3 | 8.42 ± 0.29 | 1.52 ± 0.17[e] | 7.86 ± 0.35 | 1.96 ± 0.24[e] | 7.38 ± 0.13 | ND[c] | 7.97 ± 0.23 | ND[c] |
| SAEW4 | 8.42 ± 0.17 | 4.63 ± 0.11[d] | 7.86 ± 0.20 | 4.85 ± 0.09[d] | 7.38 ± 0.25 | ND[c] | 7.97 ± 0.20 | ND[c] |

Values reported as the means ± standard deviation with n = 9. The ACC of the control was 0 mg/L.The ambient temperature was 25 ± 2 oC and the treatment time was 20 s.

SAEW1 was produced by adding HCl after electrolysis of NaCl solution in a cell without a membrane; SAEW2 was produced by electrolysis of the mixture of NaCl and HCl solution; SAEW3 was produced by electrolyzing HCl solution, and SAEW 4 was produced by electrolyzing NaCl solution in a cell with a membrane.

[A] Negative by enrichment and no detectable survivors by a direct plating procedure.

[a-e] means in the same column followed by different superscripts are significantly different by Tukey's studentized range test ($p<0.05$).

Treatment of SAEW generated by the four different methods at an ACC of 32 mg/L resulted in a complete inactivation of both pathogens (a reduction of 7.38 log CFU/mL for *E. coli* O157:H7 and 7.97 log CFU/mL for *S. enteritidis*), but NEW with a same ACC reduced the population of *E. coli* O157:H7 by 4.71 log CFU/mL and 5.43 log CFU/mL for *S. enteritidis*. The population of the two pathogens in the control samples was almost no reduction. Results show that $ClO^-$ in NEW has a lower bactericidal activity for both pathogens compared to HClO in SAEW at an equivalent ACC. As shown in Table 1, the concentration of HClO in SAEW was significantly higher than that in NEW, while the concentration of $ClO^-$ in NEW was significantly higher than that in SAEW ($p<0.05$). The bactericidal activity of chlorine-related solutions depends on the amount of HClO present in the solutions (Zagory, 2000). The effective form of chlorine compounds in SAEW at a pH value of 6.0-6.5 is almost the HClO with strong antimicrobial activity (Honda, 2003). HClO is 80 times more effective as a

sanitizer than an equivalent concentration of $ClO^-$ for inactivating *Escherichia coli* at a set contact time (Anonymous, 1997). The available chlorine in SAEW may attribute the most important role in killing bacteria. Therefore, selection of the appropriate electrolyzing methods is important to obtain a maximum bactericidal efficiency of SAEW with a higher HClO concentration.

## 4. CONCLUSIONS

Physicochemical properties and bactericidal activity of SAEW generated by four different methods with different electrolytes and electrolyzing systems (with or without a membrane) were investigated. More HClO was produced by electrolysis of HCl solution in a non-membrane generator and mixing the acid and alkaline electrolyzed water generated in a generator with membrane, compared with the methods of adding HCl to neutral electrolyzed water (NEW) and electrolyzing the mixture of NaCl and HCl solution in a non-membrane cell. There are no difference between the method of adding HCl to neutral electrolyzed water (NEW) and electrolysis the mixture of NaCl and HCl solution in a non-membrane chamber. The primary components responsible in the SAEW and NEW for inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis* were HClO and $ClO^-$, respectively. The bactericidal activity of SAEW was higher than that of NEW at an equivalent ACC. At the ACC of 10 mg/L, SAEW *p*roduced by the methods with more HClO generation had significantly higher ($p<0.05$) bactericidal efficiency for inactivation of *Escherichia coli* O157:H7 and *Salmonella enteritidis*.

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