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Efficiency of slightly acidic electrolyzed water for inactivation of *Salmonella enteritidis* and its contaminated shell eggs

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ABSTRACT

The efficiency of slightly acidic electrolyzed water (SAEW) at different temperatures (4, 20 and 45 °C) for inactivation of *Salmonella enteritidis* and it on the surface of shell eggs was evaluated. The bactericidal activity of SAEW, sodium hypochlorite solution (NaClO) and acidic electrolyzed water (AEW) to inactivate *S. enteritidis* was also compared. SAEW with a pH value of 6.0–6.5 used was generated by the electrolysis of a dilute hydrochloric acid (2.4 mM) in a chamber without a membrane. Although the pH value of SAEW was greatly higher than that of AEW (pH2.6–2.7), SAEW had a comparative powerful bactericidal activity at the same available chlorine concentrations. The efficiency of SAEW for inactivation of pure *S. enteritidis* cultures increased with increasing the available chlorine concentration and treatment time at the three different temperatures. The *S. enteritidis* counts decreased to less than 1.0 $\log_{10}$ CFU/ml at available chlorine of 2 mg/l and 100% inactivation (reduction of 8.2 $\log_{10}$ CFU/ml) was resulted in using SAEW with available chlorine more than 4 mg/l at 4, 20 and 45 °C after 2 min treatment, whereas no reduction was observed in the control samples. Moreover, SAEW was also effective for inactivating the *S. enteritidis* inoculated on the surface of shell eggs. A reduction of 6.5 $\log_{10}$ CFU/g of *S. enteritidis* on shell eggs was achieved by SAEW containing 15 mg/l available chlorine for 3 min, but only a reduction of 0.9–1.2 $\log_{10}$ CFU/g for the control samples. No survival of *S. enteritidis* was recovered in waste wash SAEW after treatment. The findings of this study indicate that SAEW may be a promising disinfectant agent for the shell egg washing processing without environmental pollution.

1. Introduction

Salmonella infection has been recognized as one of the most common bacterial cause of human gastroenteritis worldwide (Baggesen et al., 1997). The risk of a salmonellosis outbreak from consuming contaminated foods, particularly those from animal origin, has continued to be of major public and governmental concern in recent years. More than 75% of the reported salmonellosis cases are caused by contaminated eggs (Bialka et al., 2004). There are approximately 2300 serotypes of *Salmonella* that have been identified, but the serotype *Salmonella enteritidis* has been linked to over 20% of salmonellosis outbreaks (CDC, 2002). *Salmonella enteritidis* is recognized as one of the most frequently isolated serotypes and is closely associated with eggs and egg products (McKellar and Knight, 2000).

In commercial processing, eggs are usually washed in an alkaline detergent and then rinsed with an approved chemical sanitizer to remove dirt and pathogenic microorganisms. Chlorine and chlorine-containing compounds are the most currently used as antimicrobial agents in egg processing due to its availability, relative low cost and efficacy. The strength of the sanitizing should be no less than 50 mg/l nor more than 200 mg/l of available chlorine or its equivalent (USDA, 2001). High levels of chlorine can be detrimental to the quality of the egg (Bialka et al., 2004) and have not been completely acceptable because of the chemical residues, limited effectiveness and adverse environmental impacts. Therefore, developing an effective method to reduce or eliminate *Salmonella enteritidis* on eggs is crucial to the food safety and human health.

Acidic electrolyzed water (AEW), also named electrolyzed oxidizing water, is one of the potential alternatives with environmentally friendly broad spectrum microbial decontamination. AEW is usually generated by electrolysis of a dilute NaCl solution in a chamber with anode and cathode electrodes which separated by a membrane and obtained from the anode side. AEW with lower pH values (<3.0), high oxidation reduction potential ORP (>1000 mV), and containing free chlorine (20–60 mg /l) has been proved to exhibit strong bactericidal activity for inactivating many pathogens including *Escherichia coli* O157:H7 (Venkitanarayanan et al., 1999; Kim et al., 2000; Ozer and Demirci, 2006), *Listeria monocytogenes* (Park et al., 2004; Fabrizio and Cutter, 2005), *Campylobacter jejuni* (Park et al., 2002), *Salmonella enteritidis* (Fabrizio et al., 2002; Park et al., 2005). Several studies have demonstrated that AEW could be used as a disinfectant in food processing (Fabrizio and Cutter, 2004; Kim et al., 2005, Liu and Su, 2006; Huang et al., 2006, 2008). The efficiency of AEW with a pH of 2.1–2.7 and ORP of 1150 mV to decontaminate *S. enteritidis* and *Escherichia coli* on shell eggs has been

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investigated (Russell, 2003; Bialka et al., 2004). Results indicated that AEW was effective in reducing the populations of pathogenic on the surface of shell eggs. However, the potential application of AEW has limited because of its lower pH values (≤2.7). At this low pH, dissolved Cl_2 gas can be rapidly lost due to volatilization, decreasing the bactericidal activity of the solution with time (Len et al., 2000) and adversely affecting human health and environment. Moreover, the high acidity of AEW may cause the corrosion of equipments and consequently limit its practical application.

Slightly acidic electrolyzed water (SAEW) with a pH value of 5.0–6.5 is produced by electrolysis of a dilute hydrochloric acid in a chamber without a membrane. At a pH of 5.0–6.5, the effective form of chlorine compounds in SAEW is almost the hypochlorous acid (HOCl) having strong antimicrobial activity (Yoshifumi, 2003). Hypochlorous acid is 80 times more effective as a sanitizer than an equivalent concentration of the hypochlorite ion ClO^- for inactivating *Escherichia coli* at a set contact time (Anonymous, 1997). The application of SAEW may improve the bactericidal activity with maximizing the use of hypochlorous acid, reduce corrosion of surfaces, and minimize human health and safety issues from Cl_2 off-gassing (Guentzel et al., 2008). Nakayama et al. (2003) showed that the counts of *Bacillus* spores and *Leuconostoc* sp. decreased by about 10^4–10^7 CFU/ml using SAEW containing 15 mg /l of available chlorine at 80 °C for 10 min. However, little information is available on the efficiency of SAEW to inactivate microorganisms on the poultry-related products.

The objectives of the present study were: (1) to evaluate the bactericidal efficiency of slightly acidic electrolyzed water for inactivation of *S. enteritidis* and artificially inoculated shell eggs; (2) to compare the efficiency of SAEW and other disinfectants to inactivate *S. enteritidis*; (3) to determine the effect of available chlorine, treatment time and temperature on bactericidal activity of SAEW.

2. Materials and methods

2.1. Bacterial culture

Freeze-dried pure cultures of *Salmonella enteritidis* (isolated from chicken feces) were obtained from the China Veterinary Culture Collection (CVCC, Beijing, China). Cultures were hydrated according to manufacturer's directions and grown in sterile tryptic soy broth supplemented with 0.6% yeast extract (TSB-YE, CVCC, Beijing, China) at 37 °C for 24 h. 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% yeast extract (TSA-YE, CVCC, Beijing, China) and incubating the plates at 37 °C for 24 h. The population in each culture of *S. enteritidis* was approximately 8.0 $\log_{10}$ CFU/ml.

2.2. Preparation of treatment solutions

Slightly acidic electrolyzed water was generated using a SAEW generator (Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China) basically consisting of an electrolytic cell with anode and cathode electrodes and no separating membrane. SAEW with a pH of 6.12, ORP of 206.1 mV and available chlorine concentration of 15 mg/l used in this study was produced by electrolysis of a dilute hydrochloric acid (about 2.4 mM in tap water) in the SAEW generator at a voltage of 40 V for 10 min. The SAEW generated above was diluted in sterile deionized water to obtain different available chlorine concentrations of 2 to 12 mg/l.

In the meantime, a NaClO solution containing 38 mg/l of available chlorine was diluted in sterile deionized water to obtain 2, 6, 12 and 15 mg/l available chlorine for the experiments. AEW was generated by electrolysis of 0.1% NaCl solution in an experimental AEW generator (model ZSJ-1, Shenyang Dongyu Xinbor Technology Company Ltd., Shenyang, China) with an electrolysis cell where anode and cathode electrodes were separated by a membrane. AEW with a pH of 2.51 and ORP of 1106.6 mV and 15 mg/l of available chlorine was collected from the anode side of the AEW generator at voltage of 20 V for 10 min and then diluted in sterile deionized water to obtain the final different available chlorine concentrations. Moreover, sterile deionized water was used as control. All chemicals used were an analytical grade.

The pH, ORP and available chlorine concentration of treatment solutions were measured immediately before each bactericidal experiment. The pH and ORP values were measured using a dual scale pH/ORP meter (HM-30R, DKK-TOA Corporation, Tokyo, Japan) with a pH electrode (GST-5741C) or an ORP electrode (PST-5721C). The available chlorine was determined by a colorimetric method using a digital chlorine test kit (RC-2Z, Kasahara Chemical Instruments Corp., Saitama, Japan). The detection limit is 0–300 mg/l.

2.3. Treatment of pure culture

To investigate the effect of available chlorine on inactivation of *S. enteritidis*, SAEW with 15 mg/l of available chlorine was diluted in sterile deionized water to obtain different available chlorine concentrations of 2 to 8 mg/l. A volume of 9 ml of SAEW (treatment) and sterile deionized water (control) was separately placed into sterile screw-cap tubes, tightly sealed and then stored at 4 and 20 °C, or in a pre-heated water bath (HHS1-N1, Beijing Changan Scientific Instrument Plant, Beijing, China) at 45 °C. One milliliter of *S. enteritidis* culture (approximately 8.0 $\log_{10}$ CFU/ml) was individual added to the prepared tubes, mixed and kept for 2 min.

The effect of treatment time on bactericidal activity was performed by adding 1 ml of *S. enteritidis* strain (approximately 8.0 $\log_{10}$ CFU/ml) into the sterile screw-cap tubes which containing 9 ml of SAEW with 6 mg/l of available chlorine (treatment) or sterile deionized water (control) kept at 4 and 20 °C, or in a pre-heated water bath at 45 °C for 0, 0.5, 1, 2 and 5 min, respectively.

Following treatment, inactivation experiments were stopped by transferring 1 ml of each treated sample 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) and the tubes was shaken using a platform shaker at 150 rpm (MIR-S100, Sanyo Electric Biomedical Co., Ltd., Osaka, Japan). After 5 min of neutralization, the viable count of *S. enteritidis* in each sample 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 plates. The plates were incubated at 37 °C for 24 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 a 150 ml flask containing 50 ml of sterile TSB-YE, and incubated at 37 °C for 24 h. Following enrichment, the culture solution was streaked on TSA-YE plates, and the plates were incubated at 37 °C for 48 h before counting (Park et al., 2004).

Table 1
Efficiency of sodium hypochlorite solution (NaClO), acidic electrolyzed water and slightly acidic electrolyzed water for inactivation of pure *S. enteritidis* cultures at 20 °C

Treatment	Available chlorine (mg/l)	pH	ORP (mV)	Surviving population ($\log_{10}$ CFU/ml)
Control	0	6.06±0.05[a]	392.5±6.0	8.3±0.2
NaClO solution	2	12.85±0.08	421.2±5.0	<1.0[b]
	6	12.80±0.06	462.8±9.0	ND
Acidic electrolyzed water	2	2.76±0.02	1065.6±3.0	1.2±0.3
	6	2.65±0.03	1096.8±6.0	ND[c]
Slightly acidic electrolyzed water	2	6.53±0.04	238.4±3.0	<1.0
	6	6.41±0.06	265.2±7.0	ND

[a] Values reported as the means of triplicate measurements ± standard deviation.
[b] Positive by enrichment and no detectable survivors by a direct plating procedure.
[c] Negative by enrichment and no detectable survivors by a direct plating procedure.

Table 2
Inactivation of pure *S. enteritidis* cultures by slightly acidic electrolyzed water with different available chlorine and temperatures

Temperature (°C)	Available chlorine (mg/l)	pH	ORP (mV)	Surviving population (log_{10} CFU/ml)
4	0 (Control)	6.05±0.03[a]	398.5±7.0	8.2±0.2
	2	6.55±0.04	236.0±4.0	1.1±0.3
	4	6.52±0.01	252.2±8.0	ND[b]
	6	6.43±0.05	266.6±5.0	ND
	8	6.36±0.02	297.2±9.0	ND
20	0 (Control)	6.02±0.05	395.2±6.0	8.2±0.5
	2	6.53±0.04	238.4±3.0	<1.0[c]
	4	6.51±0.02	251.0±4.0	ND
	6	6.41±0.06	263.4±3.0	ND
	8	6.34±0.05	296.1±5.0	ND
45	0 (Control)	6.07±0.03	396.2±2.0	8.3±0.2
	2	6.52±0.05	236.4±3.0	<1.0
	4	6.50±0.02	256.2±9.0	ND
	6	6.41±0.05	265.9±6.0	ND
	8	6.33±0.07	295.5±4.0	ND

[a] Values reported as the means of triplicate measurements±standard deviation.
[b] Negative by enrichment and no detectable survivors by a direct plating procedure.
[c] Positive by enrichment and no detectable survivors by a direct plating procedure.

2.4. Preparation and inoculation of shell eggs

Shell egg samples were purchased at a local supermarket and stored in a refrigerator at 4 °C for no more than 3 days. The eggs were equilibrated to room temperature before testing, then sequentially washed with tap water and a commercially chlorine-based sanitizer at available chlorine of 20 mg/l (Beijing Kelin Rongan Medical Technology Co. Ltd, Beijing, China) for 1 min, rinsed in sterile deionized water to completely remove the sanitizer, and allowed to dry.

For inoculation, eggs were individually immersed into the inoculum prepared by placing 0.1 ml of approximately 10^8 CFU/ml *S. enteritidis* suspension into 200 ml of sterile 0.1% peptone water for 10 min, and sterilely air-dried under a laminar flow safety hood for 1 h at a room temperature of 20 °C to allow the bacteria attaching (Russell, 2003).

2.5. Treatment and bacteriological analysis of shell eggs

The initial population of *S. enteritidis* on the surface of shell eggs was determined by fully swabbing the surface of an inoculated air-dried egg with a sterile cotton swab moistened with 5 ml of sterile 0.1% peptone water (Deza et al., 2003), then the cotton swab was rinsed in 100 ml of sterile 0.1% peptone water. Appropriate dilutions of this suspension were plated on triplicate TSA-YE plates and incubated at 37 °C for 24 h. Inoculated shell eggs were individually placed in a sterile plastic bag containing 500 ml of SAEW, AEW, NaClO solution at 12 and 15 mg/l available chlorine, or sterile deionized water (control). The bags were shaken vigorously by hands at a room temperature of 20±2 °C, or in a pre-heated water bath at 45±2 °C for 3 min, respectively (± is the standard deviation). After treatment, the egg sample was immediately removed and placed into a sterile plastic bag containing 100 ml of sterile neutralizing buffer solution and shaken vigorously for 1 min. The viable bacterial population in washed treatment solutions and neutralizing buffer solution was serially diluted in sterile 0.1% peptone water and then plating 0.1 ml of each dilution in triplicate on TSA-YE plates. The plates were incubated at 37 °C for 24 h before counting. An enrichment experiment was also conducted to ensure detection of low levels of bacteria following treatments.

The weight of the shell was measured to determine the colony-forming unit of per gram of eggshell+membrane (CFU/g) by the method reported by Bialka et al. (2004). At the end of treatment, the egg sample was cracked and the contents were removed. The shell and membrane were rinsed with deionized water and dried overnight at room temperature and then weighed. Three shell eggs were used for each treatment. All treatments were conducted in triplicates at temperatures of 20±2 and 45±2 °C.

2.6. Statistical analysis

All experiments had three replications for each treatment and measurement. Mean values of bacterial populations, pH, ORP and free chlorine concentration were calculated from the independent triplicate trials. Statistical analysis was performed using the SAS software (SAS Institute Inc. Cary, NC, USA).

3. Results

3.1. Efficiency of SAEW and other treatment solutions for inactivation of *S. enteritidis*

The pH, ORP and available chlorine concentration of NaClO solution, AEW, SAEW and sterile deionized water (control) and its bactericidal efficiency for inactivation of pure *S. enteritidis* cultures are shown in Table 1. The pH (6.41–6.53) of SAEW is near-neutral and its ORP is largely less than other tested solutions. At available chlorine of 2 mg/l, the populations of *S. enteritidis* achieved to less than 1.0 log_{10} CFU/ml (detected by the enrichment) for treated with NaClO solution and SAEW, and reduced by more than 7.0 log_{10} CFU/ml for treated with AEW at 20 °C for 2 min. However, the bacterial counts in all treatment samples decreased to undetectable levels (evidenced by a direct plating procedure and enrichment) at 6 mg/l of available chlorine. The population of *S. enteritidis* in the control samples had no reduction.

3.2. Inactivation of *S. enteritidis* by SAEW with different available chlorine and temperatures

Table 2 shows the pH and ORP of SAEW with different available chlorine concentrations and its bactericidal activity for pure *S. enteritidis* cultures at temperatures of 4, 20 and 45 °C for 2 min. The available chlorine of SAEW containing 15 mg/l was diluted in deionized water to obtain 2, 4, 6, and 8 mg/l for treatment and the control (sterilized deionized water) had no chlorine. The pH (6.33–6.55) of SAEW was a little higher than that of control (pH 6.02–6.07), whereas the population of *S. enteritidis* in the treated samples was greatly reduced at the three different temperatures compared to control samples (Table 2).

As can be seen the data in Table 2, the bactericidal efficiency of SAEW increased with increasing available chlorine at the three different temperatures. The initial population of *S. enteritidis* was approximately 8.2 log_{10} CFU/ml. At an available chlorine concentration of 2 mg/l, SAEW decreased *S. enteritidis* counts by about 7.0 log_{10} CFU/ml at 4 °C and less than 1.0 log_{10} CFU/ml at 20 and 45 °C, respectively. 100% inactivation of *S. enteritidis* (reduction of approximately 8.2 log_{10}

Table 3
Inactivation of pure *S. enteritidis* cultures by slightly acidic electrolyzed water with different treatment time and temperatures

Temperature (°C)		Surviving population (log_{10} CFU/ml)[a]				
		0 min	0.5 min	1 min	2 min	5 min
4	Treated	8.2±0.2	3.6±0.2	1.3±0.3	ND[b]	ND
	Control	8.2±0.2	8.3±0.1	8.3±0.1	8.3±0.2	8.2±0.3
20	Treated	8.0±0.1	2.3±0.5	1.1±0.2	ND	ND
	Control	8.0±0.1	8.2±0.2	8.1±0.1	8.1±0.1	8.1±0.1
45	Treated	8.4±0.2	2.1±0.3	<1.0[c]	ND	ND
	Control	8.4±0.2	8.3±0.1	8.4±0.2	8.4±0.1	8.3±0.2

[a] Surviving population (log_{10} CFU/ml) reported as means of triplicate determinations±standard deviation.
[b] Negative by enrichment and no detectable survivors by a direct plating procedure.
[c] Positive by enrichment and no detectable survivors by a direct plating procedure.

Table 4
Inactivation of *S. enteritidis* on the surface of shell eggs by slightly acidic electrolyzed water and other solutions at 20 and 45 °C

Treatment	Initial population on eggs (log_{10} CFU/g)	Available concentration (mg/l)	Surviving population on eggs (log_{10} CFU/g)		Surviving population in waste wash solutions (log_{10} CFU/ml)	
			20 °C	45 °C	20 °C	45 °C
Control	6.5±0.2[a]	0	5.4±0.5	5.3±0.2	5.6±0.3	5.6±0.2
NaClO solution	6.5±0.2	12	1.2±0.6	1.1±0.3	ND	ND
	6.5±0.2	15	ND[b]	ND	ND	ND
Acidic	6.5±0.3	12	1.6±0.3	1.5±0.2	ND	ND
electrolyzed water	6.5±0.3	15	ND	ND	ND	ND
Slightly acidic	6.5±0.2	12	1.2±0.1	1.1±0.5	ND	ND
electrolyzed water	6.5±0.2	15	ND	ND	ND	ND

[a] Bacterial populations reported as means of triplicate determinations±standard deviation.
[b] Negative by enrichment and no detectable survivors by a direct plating procedure.

CFU/ml) was resulted in using SAEW with available chlorine more than 4 mg/l at the three different temperatures.

3.3. Effect of treatment time on bactericidal efficiency of SAEW

The surviving population of pure *S. enteritidis* cultures treated with SAEW (pH 6.35, ORP 238.2 mV and 6 mg/l of available chlorine) for 0, 0.5, 1, 2 and 5 min, respectively at temperatures of 4, 20 and 45 °C is given in Table 3. At time of 0 min, the bacterial counts in the control and treated samples were 8.0–8.4 log_{10} CFU/ml. Treatment of *S. enteritidis* for 0.5 min resulted in a reduction of 4.6 log_{10} CFU/ml at 4 °C, 5.7 log_{10} CFU/ml at 20 °C, and 6.3 log_{10} CFU/ml at 45 °C, respectively. At 1 min of treatment, the *S. enteritidis* counts in the treated samples were decreased by about 7.0 log_{10} CFU/ml at 4 and 20 °C and reduced to less than 1.0 log_{10} CFU/ml at 45 °C. The bacteria in all treated samples were completely killed (more than 8.0 log_{10} CFU/ml reduction) with treatment time equal to or greater than 2 min at 4, 20 and 45 °C. No reduction in *S. enteritidis* counts was observed in the control samples at different treatment time and temperatures.

3.4. Inactivation of S. enteritidis on the surface of shell eggs by SAEW and other solutions

Table 4 illustrates the survival characteristics of *S. enteritidis* inoculated on the surface of shell eggs, respectively treated by NaClO solution, AEW and SAEW with available chlorine of 12 and 15 mg/l at 20 and 45 °C. The initial population of *S. enteritidis* inoculated on the surface of shell egg samples was approximately 6.5 log_{10} CFU/g. The bacterial colonies on the surface of shell eggs reduced by 5.3–5.4 log_{10} CFU/g for NaClO solution and SAEW, and 4.9–5.0 log_{10} CFU/g for AEW with an available chlorine concentration of 12 mg/l at 20 and 45 °C for 3 min, respectively. A completely inactivation (reduction of 6.5 log_{10} CFU/g) of *S. enteritidis* on the surface of shell egg samples was resulted by treated with NaClO solution, AEW and SAEW at 15 mg/l of available chlorine. However, the population of *S. enteritidis* on the surface of shell eggs was only reduced by 0.9–1.2 log_{10} CFU/g at 20 and 45 °C for the control.

The viable cell of *S. enteritidis* in waste wash solutions are also shown in Table 4. No viable cells was detected in waste wash NaClO solution, AEW and SAEW after treatment, but there were approximately 5.6 log_{10} CFU/ml counts recovered in waste wash water for the control. Results indicated that SAEW was effective not only in decreasing the population of *S. enteritidis* on shell eggs, but also preventing cross-contamination of processing environments due to no survival in the waste wash solutions.

4. Discussion

The results obtained in this work showed that pure *S. enteritidis* cultures were completely inactivated (reduction of 8.2 log_{10} CFU/ml) by SAEW with an available chlorine concentration more than 4 mg/l, pH of 6.3–6.5, ORP of 251.0–297.2 mV at 4, 20 and 45 °C for 2 min. The bactericidal activity of SAEW with a near-neutral pH and low ORP value had similar bactericidal activities with AEW (pH 2.6–2.7, ORP>1000 mV) and sodium hypochlorite solution (pH 12.8, ORP of 460 mV) at a same available chlorine concentrations and contact time. *S. enteritidis* is a common food borne pathogen reported to be present on poultry, meat and vegetables (Russell, 2003; Park et al., 2005). Egg shell can serve as a vehicle for transmission of human *Salmonella* pathogens. Our results demonstrated that 100% inactivation (6.5 log_{10} CFU/g reduction) of *S. enteritidis* on the artificially inoculated shell eggs was achieved by SAEW containing an available chlorine concentration of 15 mg/l for 3 min at 20 and 45 °C. Findings from the current study indicate that SAEW may be used as an alternative disinfectant to reduce or eliminate the population of pathogens on shell eggs.

Several studies have been conducted on the bactericidal effect of AEW, but few reports are available on the use of SAEW, especially in poultry-related products. Venkitanarayanan et al. (1999) observed that pure *E. coli* O157:H7, *S. enteritidis*, and *Listeria monocytogenes* strains treated by AEW (pH 2.5 and 82 mg/l free chlorine) resulted in a complete elimination of these bacteria for 10 min. Bialka et al. (2004) investigated the efficacy of AEW (pH of 2.7, an ORP of 1150 mV and free chlorine level of 70–80 mg/l) for inactivating *S. enteritidis* and *Escherichia coli* K12 on artificially inoculated shell eggs and its effect on the egg quality. Treatment of eggs resulted in a reduction more than 2.1 log_{10} CFU/g for *S. enteritidis* and 2.3 log_{10} CFU/g for *E. coli* K12 on the shell, respectively. AEW did not significantly affect albumen height or eggshell strength; however, there were significant affects on cuticle presence. AEW with low pH (<3.0), high ORP (>1000 mV) and containing free chlorine is produced by electrolysis of dilute NaCl solution in a cell separated by a membrane and obtained from the anode side. But strong acidity of AEW causes corrosion of surfaces, rapidly chlorine (Cl_2) loss due to the evaporation of dissolved chlorine gas and ensuing HOCl decomposition, thus reducing the biocidal effectiveness of the solutions (Guentzel et al., 2008). Under open conditions, the chlorine in AEW was completely lost after 30 h when agitated and 100 h when not agitated (Len et al., 2002). These disadvantages of AEW limit its practical application in food industries.

SAEW with a pH value of 5.0–6.5, also named as near-neutral electrolyzed water (NEW), referred to as mixed oxidants having bactericidal activity, are commonly produced by two different types of the systems. One type electrolyzes diluted hydrochloric acid or NaCl solution in a non-membrane electrolytic cell (Gómez-López et al., 2007; Koide et al., 2009), which is used in this study. This system is more effective, convenient and less expensive than other electrolyzed water systems. Another type electrolyzes dilute NaCl solution in a cell with a separating membrane, and part of the product formed at the anode is then redirected into the cathode chamber during electrolysis (Pernezny et al., 2005; Guentzel et al., 2008). The SAEW or NEW with a near-neutral pH value in which the most effective form of chlorine compounds is almost all hypochlorous acid (HOCl, approximately 95%) which is active bactericidal agent (Yoshifumi, 2003). Due to its neutral pH, SAEW does not contribute as aggressively as AEW to the corrosion of processing

equipment or irritation of hands (Abadias et al., 2008), phytotoxicity in plant and the safety issues from Cl_2 off-gassing (Guentzel et al., 2008). Therefore, SAEW is particularly attractive for practical applications. Horiba et al. (1999) observed that the factor responsible for the bactericidal effect of SAEW is more stable than the corresponding factor in AEW. This may be related to the fact that dissolved chlorine does not decrease as much with time in SAEW as in AEW. Pernezny et al. (2005) reported that mixed-oxidant electrolyzed oxidizing water with a pH of 7.0, ORP of −5.1 mV and available chlorine of 50 mg/l reduced the bacteria on the leaf of vegetables from $\log_9$ to $\log_{10}$ CFU/ml to undetectable levels after 1 min exposure. Gómez-López et al. (2007) observed that the shelf-life of minimally processed cabbage treated with neutral electrolyzed water containing 40 mg/l of free chlorine for 5 min could be extended more than 5 and 3 day s at 4 °C and 7 °C, respectively. Guentzel et al. (2008) treated five pure cultures of pathogenic organism for 10 min using NEW with total residual chlorine concentrations of 20, 50, 100 and 120 mg/l, and reported 100% inactivation (reduction of 6.1–6.7 $\log_{10}$ CFU/ml) of all the pathogens.

In the present study, SAEW with pH values approximately 6.3–6.5, low ORP values of 250–265 mV and containing available chlorine also had very strong bactericidal activity (Tables 1 and 2). The ORP of SAEW was greatly lower than that of other disinfectants such as AEW and NaClO solution at the same available chlorine concentrations, but the bactericidal activity of SAEW was comparative or above (Table 1). A solution with a higher positive ORP indicates a stronger oxidizing ability. It is generally recognized that AEW have an effective bactericidal activity due to its lower pH and higher ORP values and in combination with available chlorine (Venkitanarayanan et al., 1999; Kim et al., 2000; Park et al., 2004). However, with a comparative activity of inactivation for microorganisms, the pH and ORP of SAEW in this study was near 6.5 and less than 300 mV, respectively. The antimicrobial activity of chlorine-related solutions depends on the amount of hypochlorous acid (HOCl) present in the water (Guentzel et al., 2008). The pH of the solution has important effects on the form of chlorine compounds (ClO^-, Cl_2 or HOCl). Above pH 7.5 very little chlorine occurs as active hypochlorous acid (HOCl), but rather as inactive hypochlorite ion (ClO^-). The pH of the solution should be kept between 6.0 and 7.5 to ensure chlorine activity (Zagory, 2000). The available chlorine in SAEW may attribute the most important role in killing bacteria.

There was no viable cell of microorganisms recovered in SAEW, but the bacterial population of about 5.6 $\log_{10}$ CFU/ml was found in the deionized water after washing treatment (Table 4). This is very promising and indicates that SAEW could prevent cross-contamination of egg processing. Similar results for chicken treated with AEW (pH 2.55, ORP 1083 mV, 50 mg/l free chlorine) were reported by Park et al. (2002).

In conclusion, this study demonstrated that SAEW with a near-neutral pH value exhibits an equivalent or higher bactericidal activity for shell eggs compared to AEW and sodium hypochlorite solution (NaClO), and is effective not only in reducing or eliminating *S. enteritidis* on shell eggs, but also could prevent cross-contamination of processing environments due to no viable cells in the SAEW after washing treatment. The advantage of SAEW is non-corrosive, more stable to storage, inexpensive, and a less potential health hazard to the worker due to the lack of Cl_2 off-gassing. SAEW could be used instead of sodium hypochlorite as an effective disinfectant for shell egg washing processing. Further studies are required to determine the characteristics of components in SAEW responsible for the bactericidal activity on microorganisms, and carry out to simulate typical commercial conditions and then lead to the fruitful applications of SAEW in food industries.

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