# The synergistic effects of slightly acidic electrolyzed water and UV-C light on the inactivation of *Salmonella enteritidis* on contaminated eggshells

Sh. Bing, Y. T. Zang,<sup>1</sup> Y. J. Li, and D. Q. Shu

Jiangxi Agricultural University, Jiangxi 330045, China

ABSTRACT Salmonella enteritidis (S. enteritidis) infection has been recognized as one of the most common bacterial causes of human gastroenteritis worldwide and is closely associated with eggs. Slightly acidic electrolyzed water (SAEW) is an emerging environmentally friendly technology for disinfecting eggshell surfaces to remove dirt and pathogenic microorganisms. However, the efficiency of SAEW could be affected by the presence of manure. UV-based advanced oxidation processes have been studied to improve the microorganism's inactivation effect of disinfection. Therefore, in this study, the synergistic bactericidal efficacy of SAEW and UV-C light (ultraviolet lamp,  $\lambda = 254$  nm) for inactivation of S. enteritidis on artificially inoculated eggshells with or without manure was evaluated, and

the bactericidal efficacy of different combination treatments of SAEW and UV-C light was compared. Without manure interference, complete inactivation (reduction of  $6.54 \log_{10} \text{CFU/g}$ ) of *S. enteritidis* on the surface of eggshells was achieved following a 4-min treatment with SAEW+UV at an available chlorine concentration (ACC) of 20 mg/L. In the presence of manure, a 3.02 log reduction was achieved following a 4-min treatment with SAEW+UV at an ACC of 30 mg/L. Simultaneous treatment with SAEW and UV light exhibits higher bactericidal activity for eggshells than other combination process methods with UV and SAEW. The results suggest that the combined treatment of SAEW+UV is a novel method to enhance the microbial safety of eggshells.

Key words: slightly acidic electrolyzed water, shell eggs, UV-C light, Salmonella enteritidis

### INTRODUCTION

Salmonella enteritidis (S. enteritidis) is a serious pathogen of animals and humans causing a variety of infectious diseases (Martelli and Davies, 2012). Most cases of salmonellosis were previously thought to be attributed to consuming contaminated foods originating from animals and particularly poultry products. Contaminated eggs are responsible for more than 75% of the reported salmonellosis cases (Bialka et al., 2004). Eggs are likely to be contaminated with S. enteritidis from the hen's intestinal tract, feces, and the surrounding environment (De Reu et al., 2006, 2008).

Currently, chemical sanitization systems are often used to decontaminate eggshell surfaces prior to packaging. Some of these decontamination procedures include treatments with chlorine, boiling water, or hydrogen peroxide (Favier et al., 2001; Cox et al., 2002). However, none of these chemical solutions are widely accepted due to the chemical residue, limited effectiveness or adverse environmental impacts (Cao et al., 2009). In addition, another aspect is the deterioration of the cuticle when eggshells were intensive washed by some chemhttp://dx.doi.org/10.3382/ps/pez454 tizers, which creates conditions for bacterial

2019 Poultry Science 98:6914-6920

ical sanitizers, which creates conditions for bacterial penetration through the shell. Therefore, developing an effective method to reduce or eliminate *S.enteritidis* on eggs is crucial to the food safety and human health.

Slightly acidic electrolyzed water (SAEW) with a pH value of 5.0 to 6.5, produced by the electrolysis of a dilute hydrochloric acid in a chamber without a membrane, is widely accepted as an environmentally friendly sanitization method. Currently, SAEW is being met with increasing interest in poultry science as an egg surface decontamination method (Cao et al., 2009; Ni et al., 2014). For example, Cao et al. (2009) demonstrated that SAEW could be used as a disinfectant in egg processing. They reported that a reduction of  $6.5 \log_{10} \text{ CFU/g}$  of S. enteritidis on shelled eggs was obtained by SAEW at the available chlorine concentration (ACC) of 15 mg/L following a 3-min treatment. Ni et al. (2014) also asserted that the bactericidal activity of SAEW on shelled eggs toward S. enteritidis was significantly higher than that of chlorine dioxide and NaClO solution at an ACC of 80 or 100 mg/L. Moreover, Zang et al. (2019) reported that relative to acidic electrolyzed water and NaClO solution, the SAEW can reduce corrosion of egg surfaces, and potentially had a less amount of water and CO<sub>2</sub> escaping from eggshell pores. These findings indicate that SAEW is an alternative disinfectant in the control of S. enteritidis

<sup>© 2019</sup> Poultry Science Association Inc.

Received May 16, 2019.

Accepted July 25, 2019.

<sup>&</sup>lt;sup>1</sup>Corresponding author: zangyitian1@126.com

on eggshells. However, it was shown that SAEW efficiency could be affected by the presence of organic matter, and single antimicrobial treatments of SAEW need longer washing and treatment times, and/or a higher ACC in the poultry industry (Hao et al., 2013; Zang et al., 2015; Zang et al., 2017). Therefore, to overcome these drawbacks, combining the effect of two or more decontamination methods with SAEW in lower quantities and lower treatment times could be applied.

UV-C light (ultraviolet lamp,  $\lambda = 254$  nm) has been shown to be effective at reducing various microbial populations on the surface of eggshells (Goerzen and Scott, 1995; Turtoi and Borda, 2014). Kuo et al. (1997) reported that a significant reduction of bacteria on eggshells is obtained with increasing UV exposure time. Chavez et al. (2002) investigated the effects of UV-C light on the total aerobic plate count (**APC**) of eggshells at 7.35 mW/cm<sup>2</sup> for different treatment times. APC was significantly reduced with the exposure of eggshells to UV-C for 30 and 60 seconds compared to the untreated eggs.

UV-based advanced oxidation processes (AOPs), such as  $UV+H_2O_2$ ,  $UV+Cl_2$ , and UV+ozone, have been studied as methods to improve the inactivation of microorganisms (Rodriguez-Romo and Yousef, 2005; Li et al., 2018). Zyara et al. (2016) reported that  $UV+Cl_2$ treatment was more effective at inactivating seventeen different coliphages than chlorine alone. Li et al. (2018) introduced the synergistic inactivation effect of combined UV-LED and chlorine treatment on *Bacillus subtilis* spores. They found that the addition of  $4.0 \,\mathrm{mg/L}$ of free chlorine with UV irradiation at  $125 \,\mathrm{mJ/cm^2}$  resulted in an additional 1.8-log reduction in  $UV_{265}+Cl_2$ and a 1.5-log reduction in  $UV_{280}+Cl_2$ . Rodrigues-Romo and Yousef (2005) found that the use of UV light followed by gaseous ozone treatment produced a strong synergistic antimicrobial action against S. enteritidis on eggshells. Wells et al. (2010) treated eggs with different concentrations of  $H_2O_2$  solution (0.5, 1, 1.5, 2, 2.5, and 3%) with and without UV or with dry UV or wet UV (UV + sterile water) for 2, 4, and 8 min. The maximum reduction of the bacterial count (1.00)to 4.00 log CFU/egg) was obtained for 1.5% H<sub>2</sub>O<sub>2</sub> and UV light treatment for 8 min. Some studies also reported that  $UV+Cl_2$  may disinfect more efficiently than  $UV+H_2O_2$  due to the hydroxyl radical (•OH) production from photolysis of hypochlorous acid/hypochlorite ions (HOCl/OCl-) (Sun et al., 2016). The effective form of chlorine compounds in SAEW is typically HOCl (Huang et al., 2008). Therefore, SAEW+UV treatment may accelerate the formation of •OH and thus has stronger antimicrobial activity than SAEW alone. However, little information is available on the combination treatment efficacy of UV-C light and SAEW on decontaminating eggshell surfaces in the presence of feces.

Therefore, the objectives of this study were (1) to evaluate the synergistic bactericidal efficacy of SAEW and UV-C light for inactivation of *S. enteritidis* on artificially inoculated eggshells with or without manure, and (2) to compare the bactericidal efficacy of different combination treatments of SAEW and UV-C light.

#### MATERIAL AND METHODS

### Preparation of Bacterial Cultures

The strain of S. enteritidis (CVCC 2184) was obtained from the China Veterinary Culture Collection (CVCC, Beijing, China). The bacterium was hydrated according to the manufacturer's instructions and cultured in tryptic soy broth (TSB; CVCC, Beijing, China) at 37°C for 24 h. Following incubation, 10 mL of culture was poured into a sterile centrifuge tube and centrifuged at 4000  $\times$  g and 4°C for 10 min. The supernatant was decanted, and the pellet was resuspended in 10 mL of 0.1% buffered sterile peptone water (**BPW**; Beijing Land Bridge Technology Company Ltd., Beijing, China), washed 3 times and resuspended in 10 mL of the same solution to obtain a final cell concentration of approximately  $8 \log_{10} \text{ CFU/mL}$ . The bacterial population in each culture was confirmed by plating 0.1-mL portions of appropriately diluted culture on tryptic soy agar (**TSA**; Beijing Land Bridge Technology Company Ltd., Beijing, China) plates and then incubating the plates at 37°C for 24 h. The prepared cultures were then used in subsequent experiments.

#### Preparation of Manure Mixtures

To prepare manure for disinfection experiments, a 20% solution of liquid manure was prepared by the addition of 100 g of chicken manure (obtained from poultry with no bedding) to 500 mL of sterile distilled water and then inactivated by autoclaving (YXQ-LS-18SI, Shanghai Boxun Industrial Co., Ltd., Shanghai China). The liquid manure solution was shaken and then mixed with equal portions of the prepared culture mixtures to obtain final populations of contaminated cultures of approximately  $10^8$  CFU/mL and 10% concentration.

#### Preparation of the Treatment Solutions

SAEW was produced using a nonmembrane generator (Ruiande Biosafety Technology Co., Ltd., Beijing, China) to electrolyze a NaCl solution (1 g/L) containing HCl (100  $\mu$ g/L). The SAEW generated was diluted in sterile deionized water to obtain different ACCs (Table 1). The pH, ORP, and ACC of the treatment solutions were measured immediately before each experiment. The pH and ORP values were measured with a dual scale pH/ORP meter (CON60, Trans-Wiggens, Singapore). The ACC was determined by a digital chlorine test system (RC-2Z, Kasahara Chemical Instruments Co., Saitama, Japan). The detection range was 0 to 320 mg/L.

**Table 1.** Physicochemical properties of slightly acidic electrolyzed water (SAEW) solutions.

Solutions	$ACC^{1}$ (mg/L)	pH	$ORP^2 (mV)$
Slightly acidic electrolyzed water	10	$6.53~\pm~0.01$	$645.5 \pm 3.0$
v	20	$6.44 \pm 0.03$	$664.2 \pm 3.0$
	30	$6.36~\pm~0.01$	$689.9~\pm~6.0$

Values are reported as the means of triplicate measurements  $\pm$  standard deviation.

<sup>1</sup>Available chlorine concentration.

<sup>2</sup>Oxidation reduction potential.

## Preparation of Shelled Eggs

Eggs weighing 55 to 60 g were purchased at a local supermarket and stored in a refrigerator at 4°C for no more than 3 d. Eggs were first equilibrated to room temperature before testing and then sequentially washed with tap water and a commercial chlorine-based sanitizer (Beijing Zhenhe Medical Technology Co. Ltd., Beijing, China) at an ACC of 30 mg/L for 1 min, washed with sterile deionized water to completely remove the sanitizer, and then air-dried under a biosafety hood (DH-920, Beijing East Union Hall Instrument Manufacturing Co., Ltd., Beijing, China).

For inoculation, eggs were individually soaked in the inoculum, prepared by placing 0.1 mL of approximately 8  $\log_{10}$  CFU/mL *S. enteritidis* suspension or prepared contaminated culture mixtures into 200 mL of sterile 0.1% peptone water for 10 min, and sterilely air-dried under the biosafety hood for 60 min at a room temperature of 25°C to allow bacterial attachment.

Samples for each treatment were prepared and sampled at least twice.

## Preparation of UV Light

The UV-C treatments were performed in a chamber  $(85 \text{ cm} \times 75 \text{ cm} \times 45 \text{ cm})$  equipped with 2 sets of 2 unfiltered germicidal emitting lamps (253.7 nm, Philips, Co., Netherlands). One set of lamps was placed on the

left and the other one on the right of the radiation cabinet. Each lamp was 40 cm tall. All UV experiments were conducted at a fixed initial UV intensity (10.2  $\pm$ 0.3 W/cm<sup>2</sup>), which was measured by a radiometer (UVX-254, Ultraviolet Products, California, USA). Before each experiment, the UV lamp was turned on for approximately 20 min to achieve stable irradiation intensity. Contaminated eggs, prepared as previously described, were aseptically transferred to the base of sterile glass petri plates and placed on a net positioned midway between the UV-C lamps. To achieve the combined effect, the treatments with SAEW were carried out in the order shown in Tables 2–3.

## **Bacteriological Analysis of Shelled Eggs**

Determination of S. enteritidis on the eggshell surface was carried out by following a previously reported method (Cao et al., 2009). Inoculated shelled eggs were individually placed in a sterile plastic bag containing 600 ml of SAEW at an ACC of 10, 20, and 30 mg/L or sterile deionized water (control). Samples in plastic bags were aseptically transferred to the base of sterile glass petri plates and placed on a net positioned midway between the UV-C lamps for 1, 2, 3, and 4 min. After treatment, the egg sample was placed into a sterile plastic bag, which contained 50 mL of sterile neutralizing buffer solution, and shaken vigorously for 1 min. The viable bacterial population in the washed treatment and neutralizing buffer solutions was serially diluted with sterile 0.1% BPW. A volume of 0.1 mL of each sample was plated in triplicate on TSA plates and Violet Red Bile with Glucose Agar (Qingdao Hope Bio-Technology Co. Ltd., Qingdao, China) and incubated at 37°C for 24 h. The shell was also weighed to determine the colony-forming units per gram of eggshell + membrane (CFU/g) by following a previously reported method (Cao et al., 2009).

Inactivation of *S. enteritidis* on eggshells by different combination treatments of SAEW and UV radiation in the presence of manure test was performed.

**Table 2.** Inactivation of *S. enteritidis* on the surface of eggshells by slightly acidic electrolyzed water (SAEW) and UV radiation.

		Surviving population of S. enteritidis on eggs (log CFU/g)			
Treatment	$ACC^1 (mg/L)$	1 min	2 min	3 min	4 min
Control	0	$5.63 \pm 0.21^{\rm a}$	$5.02 \pm 0.13^{a}$	$4.41 \pm 0.13^{\rm a}$	$3.90 \pm 0.12^{\rm a}$
SAEW	10	$4.50 \pm 0.12^{\rm b}$	$3.39 \pm 0.12^{\rm b}$	$2.17 \pm 0.13^{\rm b}$	$1.12 \pm 0.11^{\rm b}$
	20	$3.95 \pm 0.12^{\rm b}$	$2.53 \pm 0.15^{\rm b}$	$1.06 \pm 0.11^{\rm b}$	ND <sup>2</sup>
	30	$3.06 \pm 0.14^{\rm b}$	$1.59 \pm 0.02^{\rm b}$	ND	ND
UV	0	$4.71 \pm 0.22^{\circ}$	$3.96 \pm 0.15^{\circ}$	$3.09 \pm 0.13^{\circ}$	$2.02 \pm 0.12^{\circ}$
SAEW+ UV	10	$3.71 \pm 0.02^{\rm d}$	$2.79 \pm 0.08^{\rm d}$	$1.51 \pm 0.05^{\rm d}$	$0.47 \pm 0.12^{\rm d}$
	20	$3.16 \pm 0.07^{\rm d}$	$1.15 \pm 0.09^{d}$	$0.16~\pm~0.08^{ m d}$	ND
	30	$2.51 \pm 0.14^{\rm d}$	$0.81~\pm~0.08^{\rm d}$	ND	ND

The data are expressed as the means  $\pm$  standard deviations.

Within the same column of different treatments at the same available concentration, values with different lower-case letters in superscripts (a-d) within a column were significantly different (P < 0.05).

<sup>1</sup>Available chlorine concentration.

 $^2\mathrm{Means}$  not detected.

**Table 3.** Inactivation of *S. enteritidis* on the surface of eggshells by slightly acidic electrolyzed water (SAEW) and UV radiation in the presence of organic matter.

		Surviving population of $S.$ enteritidis on eggs (log CFU/g)			
Treatment	$ACC^1 (mg/L)$	1 min	2 min	3 min	4 min
Control SAEW	0 10 20	$\begin{array}{r} 5.93 \ \pm \ 0.05^{\rm a} \\ 5.80 \ \pm \ 0.11^{\rm b} \\ 5.45 \ \pm \ 0.15^{\rm b} \end{array}$	$\begin{array}{r} 5.82 \ \pm \ 0.03^{\rm a} \\ 5.62 \ \pm \ 0.11^{\rm b} \\ 5.29 \ \pm \ 0.13^{\rm b} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{r} 5.63 \ \pm \ 0.03^{\rm a} \\ 5.42 \ \pm \ 0.08^{\rm b} \\ 4.95 \ \pm \ 0.11^{\rm b} \end{array}$
UV SAEW+ UV	$30 \\ 0 \\ 10 \\ 20 \\ 30$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$5.12 \pm 0.02^{\text{b}}$ $5.82 \pm 0.18^{\text{a}}$ $5.59 \pm 0.12^{\text{c}}$ $5.09 \pm 0.07^{\text{c}}$ $4.59 \pm 0.09^{\text{c}}$	$\begin{array}{rrrr} 4.81 \ \pm \ 0.13^{\rm b} \\ 5.79 \ \pm \ 0.14^{\rm a} \\ 5.31 \ \pm \ 0.15^{\rm c} \\ 4.76 \ \pm \ 0.12^{\rm c} \\ 4.03 \ \pm \ 0.16^{\rm c} \end{array}$	$\begin{array}{r} 4.36 \ \pm \ 0.04^{\rm p} \\ 5.68 \ \pm \ 0.17^{\rm a} \\ 5.07 \ \pm \ 0.14^{\rm c} \\ 4.27 \ \pm \ 0.15^{\rm c} \\ 3.51 \ \pm \ 0.09^{\rm c} \end{array}$

The data are expressed as the means  $\pm$  standard deviations.

Within the same column of different treatments at the same available concentration, values with different lower-case letters in superscripts (a-c) within a column are significantly different (P < 0.05).

<sup>1</sup>Available chlorine concentration.

The test was divided into 4 groups: SAEW-UV (SAEW treatment at ACC of 50 mg/L for 2 min followed by UV treatment for 2 min, UV-SAEW (UV treatment for 2 min followed by SAEW treatment at ACC of 50 mg/L for 2 min), SAEW+UV (SAEW treatment at ACC of 50 mg/L simultaneous with UV treatment for 4 min) and DW (sterilized distilled water treatment for 4 min).

#### Statistical Analysis

All experiments had 3 replications for each treatment and measurement. Mean values of all parameters were calculated from the independent triplicate trials. Statistical analysis was performed using Origin (Version 9.0, OriginLab Cor., Hampton, USA). Differences between variables were assessed by Tukey's test. Results with P < 0.05 were considered statistically significant.

#### **RESULTS AND DISCUSSION**

## Inactivation of S. Enteritidis on Eggshells by Simultaneous Treatment with SAEW at Different Available Chlorine Contents and UV-C Light

Table 2 shows the UV, SAEW, and UV+SAEW with different available concentrations and their bactericidal activity for *S. enteritidis* on eggshells at different times. The initial population of *S. enteritidis* was  $6.54 \pm 0.11 \log_{10} \text{ CFU/g}$ , and the bactericidal efficiency of all solutions increased with increasing available concentrations and time. The populations of *S. enteritidis* were reduced to undetectable levels with SAEW at an ACC of 20 mg/L after 4 min of treatment. Similar results were reported by Cao et al. (2009). They showed complete inactivation (reduction of  $6.5 \log_{10} \text{ CFU/g}$ ) of *S. enteritidis* on the surface of eggshells after treatment with SAEW at 15 mg/L of available chlorine for 3 min. When eggs were treated with UV-C light at a fluence of 10 W/cm<sup>2</sup> for 1, 2, 3, and 4 min, reductions from 1.93 to 4.62 logs were obtained. Several studies also demonstrated that UV-C light is effective at reducing various microbial populations on the surface of eggshells (Chavez et al., 2002). Holck et al. (2017) reported that UV-C light treatments can be used to decontaminate eggshells.

As can be seen from Table 2, UV+SAEW combined treatments showed higher inactivation of *S. enteritidis* compared to SAEW at the same ACC (P < 0.05). This result indicated that the combination of SAEW and UV may be more effective at reducing *S. enteritidis* survival on eggshells than SAEW or UV independently.

## Inactivation of S. Enteritidis on Eggshells by Simultaneous Treatment with SAEW at Different Available Chlorine Contents and UV-C Light in the Presence of Manure

The initial population of S. enteritidis was 6.53  $\pm$  $0.11 \log_{10} \text{ CFU/g}$  in the samples. The antimicrobial effects of SAEW and UV treatment at different conditions against S. enteritidis on eggshells are listed in Table 3. UV treatment achieved only a 0.85 log reduction of the eggs in the presence of manure for the 4-min treatment. This result is mainly caused by the presence of feces on the eggshell surface, which shields cells by preventing the UV light penetration (De Souza and Fernández, 2011). De Souza et al. (2011) found a similar result. They investigated the inactivation of Ascaris *lumbricoides* eggs in soil by UV light and found that UV treatment achieved negligible inactivation of the eggs in soil. Many studies reported that UV light does not penetrate well through organic matter, such as protein and other organic matrices (Guerrero-Beltrn and Barbosa-C, 2004; Gomez-Lopez et al., 2007; Mun et al., 2009). As shown in Table 2 and 3, SAEW treatment exhibited a different antimicrobial effect with or without manure on the eggshell surface. It was speculated that SAEW



Figure 1. Inactivation effect of various decontamination treatments on *S. enteritidis* on eggshell surfaces in the presence of manure. DW, sterilized distilled water; UV-SAEW, ultraviolet lamp treatment (2 min) followed by slightly acidic electrolyzed water (50 mg/L 2 min) treatment; SAEW-UV, slightly acidic electrolyzed water treatment (50 mg/L 2 min) followed by ultraviolet lamp treatment (2 min); SAEW+UV, slightly acidic electrolyzed water treatment (50 mg/L 2 min) followed by ultraviolet lamp treatment (2 min); SAEW+UV, slightly acidic electrolyzed water treatment (50 mg/L) simultaneous with UV treatment (4 min). Values with different lowercase letters (a-d) were significantly different (P < 0.05).

efficiency could be affected by the presence of manure. However, SAEW combined with UV gave a higher S. enteritidis inactivation than SAEW single treatment (P < 0.05). A 3.02 log reduction was obtained after a 4min treatment with SAEW+UV at an ACC of 30 mg/L in the presence of manure. These findings showed that UV+SAEW treatment effectively disinfected S. en*teritidis* on eggshells even under the interference of manure. Some studies have also demonstrated that UV is more efficient when combined with other disinfectants (Ukuku and Geveke, 2010; Wells et al., 2010; Al-Ajeeli et al., 2016). Wells et al. (2010) determined that the combination of  $H_2O_2$  and UV ( $H_2O_2+UV$ ) is more effective at reducing eggshell bacterial counts than  $H_2O_2$  or UV independently. Al-Ajeeli et al. (2016) also reported that eggshell sanitization with the  $H_2O_2+UV$ treatment produced the greatest reduction in eggshellcontaminating aerobic bacteria compared to chlorine. UV-based AOP may be the most likely reason that UV+SAEW treatment was more effective at inactivating S. enteritidis inoculated on the surface of eggshells than SAEW or UV treatment alone.

## Inactivation of S. Enteritidis on Eggshells by Different Combination Treatments in the Presence of Manure

The initial population of S. enteritidis was 6.57  $\pm$  0.13 log<sub>10</sub> CFU/g in the samples. The antimicrobial effects of DW (control), SAEW followed by UV (SAEW-UV), UV followed by SAEW (UV-SAEW), and SAEW simultaneous with UV (SAEW+UV) treatments against *S. enteritidis* on eggshells are shown in Figure 1. The antimicrobial effects of combination treatments were significantly greater than those of the control (P < 0.05). As shown in Figure 1, the

SAEW+UV treatment caused an approximately 4.48 log CFU/g reduction in S. enteritidis, and it is significantly greater than those of the SAEW-UV and UV-SAEW treatments (P < 0.05). As can be seen from Figure 2, this result may be due to the formation of •OH, which was generated from the photooxidation of chlorine by UV irradiation (Cho et al., 2006). Some studies have demonstrated that chlorine photolysis under UV irradiation could vield •OH, and it may directly or indirectly enhance bacterium inactivation (Mamane-Gravetz et al., 2005; Sun et al., 2016; Chuang et al., 2017). Sun et al. (2016) examined UV+peroxydisulfate treatment for water disinfection and found that •OH showed the highest disinfection efficacy. As can be seen from Figure 2, the primary •OH mechanism may disrupt cell integration by oxidizing the membrane and then facilitating diffusion of the disinfectant into the cell to inactivate enzymes and damage intracellular components (Mamane-Gravetz et al., 2005). Therefore, •OH can accelerate HClO and ClO<sup>-</sup> diffusion to the inner membrane, thus enhancing S. enteritidis inactivation by the SAEW.

The efficacy of the SAEW+UV treatment may also be due to the formation of ozone (O<sub>3</sub>), which results from the photolysis of OCl<sup>-</sup> by UV wavelengths. Forsyth et al. (2013) asserted that O<sub>3</sub> would be formed during the solar irradiation of chlorine and would play a significant role in enhancing *Bacillus subtilis* spore inactivation. Many studies have been demonstrated that O<sub>3</sub> exhibits a high antimicrobial efficacy on the surface of eggshells (Khadre et al., 2001; Ragni et al., 2010; Yüceer et al., 2016; Yang et al., 2019).

#### CONCLUSION

In conclusion, without manure interference, reductions from 1.93 to 4.62 logs were obtained when eggs were treated with UV-C light at a fluence of  $10 \text{ W/cm}^2$ for 1, 2, 3, and 4 min. a complete inactivation (reduction of 6.54  $\log_{10}$  CFU/g) of S. enteritidis on the surface of eggshells resulted by treating with SAEW+UV at an ACC of 20 mg/L for 4 min or SAEW single treatment at an ACC of 20 mg/L for 3 min. In the presence of manure, UV or SAEW single treatment exhibited a different antimicrobial effect with or without manure on the eggshell surface. UV or SAEW single treatment achieved only 0.85 or 2.17 log reduction of the eggs in the presence of manure for 4 min. However, 3.02 log reductions were achieved following a 4-min treatment with SAEW+UV at an ACC of 30 mg/L. The results from this study demonstrated the beneficial effects of combined UV and SAEW treatments on the inactivation of S. enteritidis on eggshells compared to SAEW and UV alone with or without manure. Furthermore, simultaneous SAEW and UV treatment exhibits higher bactericidal activity for eggshells than other combination methods with UV and SAEW. Overall, effective S. enteritidis inactivation



**Figure 2.** Model representing the germicidal activity of SAEW+UV. The formed •OH during the UV+SAEW process could damage the membrane of *S. enteritidis* and then accelerate chlorine diffusion to the inner membrane, thus enhancing *S. enteritidis* inactivation. SAEW+UV, slightly acidic electrolyzed water treatment simultaneous with UV treatment.

on eggshells could be provided by combining UV and SAEW.

#### ACKNOWLEDGMENTS

The author gratefully acknowledges the financial support from the National Natural Science Foundation of China (31860665).

### **CONFLICT OF INTEREST STATEMENT**

The authors declare that there are no conflicts of interest.

#### REFERENCES

- Al-Ajeeli, M. N., T. M. Taylor, C. Z. Alvarado, and C. D. Coufal. 2016. Comparison of eggshell surface sanitization technologies and impacts on consumer acceptability. Poult. Sci. 95:1191–1197.
- Bialka, K. L., A. Demirci, S. J. Knabel, P. H. Patterson, and V. M. Puri. 2004. Efficacy of electrolyzed oxidizing water for the microbial safety and quality of eggs. Poult. Sci. 83:2071–2078.
- Cao, W., Z. Zhu, Z. Shi, C. Wang, and B. Li. 2009. Efficiency of slightly acidic electrolyzed water for inactivation of Salmonella enteritidis and its contaminated shell eggs. Inter. J. Food Microbiol. 130:88–93.
- Chavez, C., K. D. Knape, C. D. Coufal, and J. B. Carey. 2002. Reduction of eggshell aerobic plate counts by ultraviolet irradiation. Poult. Sci. 81:1132–1135.
- Cho, M., J. H. Kim, and J. Yoon. 2006. Investigating synergism during sequential inactivation of Bacillus subtilis spores with several disinfectants. Water Res. 40:2911–2920.
- Chuang, Y. H., S. Chen, C. J. Chinn, and W. A. Mitch. 2017. Comparing the UV/monochloramine and UV/free chlorine Advanced Oxidation Processes (AOPs) to the UV/hydrogen peroxide AOP under scenarios relevant to potable reuse. Enviro. Sci. Technol. 51:13859–13868.
- Cox, N. A., M. E. Berrang, J. S. Bailey, and N. J. Stern. 2002. Bactericidal treatment of hatching eggs V: Efficiency of repetitive immersions in hydrogen peroxide or phenol to eliminate Salmonella from hatching eggs. J. Appl. Poult. Res. 11:328–331.
- De Reu, K., K. Grijspeerdt, L. Herman, M. Heyndrickx, M. Uyttendaele, J. Debevere, F. F. Putirulan, and N. M. Bolder. 2006. The

effect of a commercial UV disinfection system on the bacterial load of shell eggs. Lett. Appl. Microbiol. 42:144–148.

- De Reu, K., W. Messens, M. Heyndrickx, T. B. Rodenburg, M. Uyttendaele, and L. Herman. 2008. Bacterial contamination of table eggs and the influence of housing systems. Worlds Poult. Sci. J. 64:5–19.
- De Souza, P. M., and A. Fernández. 2011. Effects of UV-C on physicochemical quality attributes and Salmonella enteritidis inactivation in liquid egg products. Food Control. 22:1385–1392.
- Favier, G. I., M. E. Escudero, and A. M. de Guzmán. 2001. Effect of chlorine, sodium chloride, trisodium phosphate, and ultraviolet radiation on the reduction of Yersinia enterocolitica and mesophilic aerobic bacteria from eggshell surface. J. Food Prot. 64:1621–1623.
- Forsyth, J. E., P. Zhou, Q. Mao, S. S. Asato, J. S. Meschke, and M. C. Dodd. 2013. Enhanced inactivation of Bacillus subtilis spores during solar photolysis of free available chlorine. Enviro. Sci. Technol. 47:12976–12984.
- Goerzen, P. R., and T. A. Scott. 1995. Ultraviolet light sanitation for broiler hatching eggs. Poult. Sci. 74:83.
- Gomez-Lopez, V. M., P. Ragaert, J. Debevere, and F. Devlieghere. 2007. Pulsed light for food decontamination: a review. Trends Food Sci. Technol. 18:464–473.
- Guerrero-Beltrn, J. A., and G. V. Barbosa-C novas. 2004. Advantages and limitations on processing foods by UV light. Food Sci. Technol. Inter. 10:137–147.
- Hao, X. X., B. M. Li, C. Y. Wang, and W. Cao. 2013. Application of slightly acidic electrolyzed water for inactivating microbes in a layer breeding house. Poult. Sci. 92:2560–2566.
- Herman, L. 2008. Bacterial contamination of table eggs and the influence of housing systems. World Poult. Sci. J. 64:5–19.
- Holck, A. L., K. H. Liland, S. M. Drømtorp, M. Carlehög, and A. McLeod. 2017. Comparison of UV-C and Pulsed UV Light Treatments for Reduction of Salmonella, Listeria monocytogenes, and Enterohemorrhagic Escherichia coli on Eggs. J. Food Prot. 81:6– 16.
- Huang, Y. R., Y. C. Hung, S. Y. Hsu, Y. W. Huang, and D. F. Hwang. 2008. Application of electrolyzed water in the food industry. Food Control. 19:329–345.
- Khadre, M. A., A. E. Yousef, and J. G. Kim. 2001. Microbiological aspects of ozone applications in food: a review. J. Food Sci. 66:1242–1252.
- Kuo, F. L., J. B. Carey, and S. C. Ricke. 1997. UV irradiation of shell eggs: effect on populations of aerobes, molds, and inoculated Salmonella typhimurium. J. Food Prot. 60:639–643.
- Li, G. Q., Z. Y. Huo, Q. Y. Wu, Y. Lu, and H. Y. Hu. 2018. Synergistic effect of combined UV-LED and chlorine treatment on

Bacillus subtilis spore inactivation. Sci. Total Enviro. 639:1233–1240.

- Mamane-Gravetz, H., K. G. Linden, A. Cabaj, and R. Sommer. 2005. Spectral sensitivity of Bacillus subtilis spores and MS2 coliphage for validation testing of ultraviolet reactors for water disinfection. Enviro. Sci. Technol. 39:7845–7852.
- Martelli, F., and R. H. Davies. 2012. Salmonella serovars isolated from table eggs: an overview. Food Res. Inter. 45:745–754.
- Mun, S., S. H. Cho, T. S. Kim, B. T. Oh, and J. Yoon. 2009. Inactivation of Ascaris eggs in soil by microwave treatment compared to UV and ozone treatment. Chemosphere 77:285–290.
- Ni, L., W. Cao, W. Zheng, H. Chen, and B. Li. 2014. Efficacy of slightly acidic electrolyzed water for reduction of foodborne pathogens and natural microflora on shell eggs. Food Sci. Technol. Res. 20:93–100.
- Ragni, L., A. Berardinelli, L. Vannini, C. Montanari, F. Sirri, M. E. Guerzoni, and A. Guarnier. 2010. Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. J. Food Eng. 100:125–132.
- Rodriguez-Romo, L. A., and A. E. Yousef. 2005. Inactivation of Salmonella enterica serovar Enteritidis on shell eggs by ozone and UV radiation. J. Food Prot. 68:711–717.
- Sun, P., C. Tyree, and C. H. Huang. 2016. Inactivation of Escherichia coli, bacteriophage MS2, and Bacillus spores under UV/H2O2 and UV/peroxydisulfate advanced disinfection conditions. Enviro. Sci. Technol. 50:4448–4458.
- Turtoi, M., and D. Borda. 2014. Decontamination of egg shells using ultraviolet light treatment. World Poult. Sci. J. 70:265–278.
- Ukuku, D. O., and D. J. Geveke. 2010. A combined treatment of UV-light and radio frequency electric field for the inactivation

of Escherichia coli K-12 in apple juice. Inter. J. Food Microbiol. 138:50–55.

- Wells, J. B., C. D. Coufal, H. M. Parker, and C. D. McDaniel. 2010. Disinfection of eggshells using ultraviolet light and hydrogen peroxide independently and in combination. Poult. Sci. 89:2499– 2505.
- Yang, Y., D. J. Geveke, C. D. Brunkhorst, J. E. Sites, N. J. Geveke, and E. D Tilman. 2019. Optimization of the radio frequency power, time and cooling water temperature for pasteurization of Salmonella Typhimurium in shell eggs. J. Food Eng. 247:130– 135.
- Yüceer, M., M. S. Aday, and C. Caner. 2016. Ozone treatment of shell eggs to preserve functional quality and enhance shelf life during storage. J. Sci. Food Agric. 96:2755–2763.
- Zang, Y. T., B. M. Li, Sh. Bing, and W. Cao. 2015. Modeling disinfection of plastic poultry transport cages inoculated with Salmonella enteritids by slightly acidic electrolyzed water using response surface methodology. Poult. Sci. 94:2059–2065.
- Zang, Y. T., B. M. Li, Z. X. Shi, X. W. Sheng, H. X. Wu, and D. Q. Shu. 2017. Inactivation efficiency of slightly acidic electrolyzed water against microbes on facility surfaces in a disinfection channel. Inter. J. Agr. Biol. Eng. 10:23–30.
- Zang, Y. T., S. Bing, Y. J. Li, D. Q. Shu, A. M. Huang, H. X. Wu, and H. D. Wu. 2019. Efficacy of slightly acidic electrolyzed water on the microbial safety and shelf life of shelled eggs. Poult. Sci. pez373, https://doi.org/10.3382/ps/pez373.
- Zyara, A. M., E. Torvinen, A. M. Veijalainen, and H. Heinonen-Tanski. 2016. The effect of chlorine and combined chlorine/UV treatment on coliphages in drinking water disinfection. J. Water Health. 14:640–649.