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

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**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, λ = 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} 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*

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**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 chemical 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} 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 CO₂ escaping from eggshell pores. These findings indicate that SAEW is an alternative disinfectant in the control of *S. enteritidis*...
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 (Oezener 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$^2$ 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$_2$O$_2$, 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 mg/L of free chlorine with UV irradiation at 125 mJ/cm$^2$ resulted in an additional 1.8-log reduction in UV$_{254}$+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$_2$O$_2$ 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$_2$O$_2$ and UV light treatment for 8 min. Some studies also reported that UV+Cl$_2$ may disinfect more efficiently than UV+H$_2$O$_2$ due to the hydroxyl radical (•OH) production from photolysis of hypochlorous acid/hypochlorite ions (HOCI/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 × g 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$_{10}$ 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 µg/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-Wiggins, 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 ± 0.01 | 645.5 ± 3.0    |
|                                  | 20               | 6.44 ± 0.03 | 664.2 ± 3.0    |
|                                  | 30               | 6.36 ± 0.01 | 689.9 ± 6.0    |

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

\(^1\)Available chlorine concentration.

\(^2\)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, and then air-dried under a biosafety hood with sterile deionized water to completely remove the contaminant culture mixtures into 200 mL of sterile neutralizing buffer solution, and shaken vigorously for 1 min. After treatment, the egg sample was placed into a sterile plastic bag containing 50 mL of sterile neutralizing buffer solution, and shaken vigorously for 1 min. The viable bacterial population in the washed treatment, the egg sample was placed into a sterile plastic bag containing 50 mL of sterile neutralizing buffer solution, and shaken vigorously for 1 min. The viable bacterial population in the washed treatment, the egg sample was plated in triplicate on TSA 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 Biotechnology 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.

| Treatment    | ACC\(^3\) (mg/L) | 1 min | 2 min | 3 min | 4 min |
|--------------|------------------|-------|-------|-------|-------|
| Control      | 0                | 5.63 ± 0.21\(^a\) | 5.02 ± 0.13\(^a\) | 4.41 ± 0.13\(^a\) | 3.90 ± 0.12\(^a\) |
| SAEW         | 10               | 4.50 ± 0.12\(^b\) | 3.39 ± 0.12\(^b\) | 2.17 ± 0.13\(^b\) | 1.12 ± 0.11\(^b\) |
|              | 20               | 3.95 ± 0.12\(^b\) | 2.53 ± 0.15\(^b\) | 1.06 ± 0.11\(^b\) | ND\(^2\)         |
|              | 30               | 3.66 ± 0.14\(^b\) | 1.59 ± 0.02\(^b\) | ND              | ND              |
| UV           | 0                | 4.71 ± 0.22\(^b\) | 3.96 ± 0.15\(^b\) | 3.09 ± 0.13\(^c\) | 2.02 ± 0.12\(^c\) |
| SAEW + UV    | 10               | 3.71 ± 0.02\(^d\) | 2.79 ± 0.08\(^d\) | 1.51 ± 0.05\(^d\) | 0.47 ± 0.12\(^d\) |
|              | 20               | 3.16 ± 0.07\(^d\) | 1.15 ± 0.09\(^d\) | 0.16 ± 0.08\(^d\) | ND              |
|              | 30               | 2.51 ± 0.14\(^d\) | 0.81 ± 0.08\(^d\) | ND              | ND              |

The data are expressed as the means ± standard deviations.

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

\(^3\)Available chlorine concentration.

\(^2\)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.

| Treatment          | ACC1 (mg/L) | Surviving population of *S. enteritidis* on eggs (log CFU/g) |
|--------------------|-------------|----------------------------------------------------------|
|                    |             | 1 min | 2 min | 3 min | 4 min |
| Control            | 0           | 5.93 ± 0.05\(^a\) | 5.82 ± 0.03\(^a\) | 5.72 ± 0.06\(^a\) | 5.63 ± 0.03\(^a\) |
| SAEW               | 10          | 5.80 ± 0.11\(^b\) | 5.62 ± 0.11\(^b\) | 5.54 ± 0.03\(^b\) | 5.42 ± 0.08\(^b\) |
|                    | 20          | 5.45 ± 0.15\(^b\) | 5.29 ± 0.13\(^b\) | 5.11 ± 0.09\(^b\) | 4.95 ± 0.11\(^b\) |
|                    | 30          | 5.38 ± 0.14\(^b\) | 5.12 ± 0.02\(^b\) | 4.81 ± 0.13\(^b\) | 4.36 ± 0.04\(^b\) |
| UV                 | 0           | 5.91 ± 0.21\(^a\) | 5.82 ± 0.18\(^a\) | 5.79 ± 0.14\(^a\) | 5.68 ± 0.17\(^a\) |
| SAEW + UV          | 10          | 5.71 ± 0.09\(^f\) | 5.59 ± 0.12\(^f\) | 5.31 ± 0.15\(^f\) | 5.07 ± 0.14\(^f\) |
|                    | 20          | 5.36 ± 0.09\(^f\) | 5.09 ± 0.07\(^f\) | 4.76 ± 0.12\(^f\) | 4.27 ± 0.15\(^f\) |
|                    | 30          | 5.01 ± 0.11\(^f\) | 4.59 ± 0.09\(^f\) | 4.03 ± 0.16\(^f\) | 3.51 ± 0.09\(^f\) |

The data are expressed as the means ± 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 \((P < 0.05)\). \(^1\)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-SEAW (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 ± 0.11 log\(_{10}\) 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\(_{10}\) 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\(^2\) 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 ± 0.11 log\(_{10}\) 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...
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 4-min 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. enteritidis 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 $\text{H}_2\text{O}_2$ and UV ($\text{H}_2\text{O}_2$+UV) is more effective at reducing eggshell bacterial counts than $\text{H}_2\text{O}_2$ or UV independently. Al-Ajeeli et al. (2016) also reported that eggshell sanitization with the $\text{H}_2\text{O}_2$+UV treatment produced the greatest reduction in eggshell-contaminating 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 ± 0.13 log$_{10}$ 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 $\cdot\text{OH}$, 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 yield $\cdot\text{OH}$, 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 $\cdot\text{OH}$ showed the highest disinfection efficacy. As can be seen from Figure 2, the primary $\cdot\text{OH}$ 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, $\cdot\text{OH}$ can accelerate HClO and ClO$^-$ 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$_3$), which results from the photolysis of OCl$^-$ by UV wavelengths. Forsyth et al. (2013) asserted that O$_3$ 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$_3$ 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 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.

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**CONFLICT OF INTEREST STATEMENT**

The authors declare that there are no conflicts of interest.

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