Effect of sequential treatments with sodium dodecyl sulfate and citric acid or hydrogen peroxide on the reduction of some foodborne pathogens on eggshell

Maktabi, S. 1*; Zarei, M. 1 and Rashnavady, R. 2

1Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; 2Graduated from Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Correspondence: S. Maktabi, Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran. E-mail: s.maktabi@scu.ac.ir

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Summary

The aim of this study was to investigate the effect of sodium dodecyl sulfate (SDS), citric acid, and hydrogen peroxide (H2O2), alone or in combination, on reducing the population of four foodborne pathogens, including Escherichia coli, Listeria monocytogenes, Salmonella typhimurium, and Staphylococcus aureus on eggshells. In each series of tests, eight fresh eggs were inoculated with each bacterial strain by being immersed in a bacterial suspension and exposed to SDS (1.5%), H2O2 (0.5%), citric acid (1%), or sequential treatments with SDS + citric acid and SDS + H2O2. Viable cell counts were made and the bacterial concentrations results compared to pre-treatment levels. Results showed that all washing solutions except citric acid significantly (P<0.05) reduced the concentration of all tested bacteria (~2-4 log reductions). The sensitivity of monocytogenes alone or in combination, on reducing the population of four foodborne pathogens, including Escherichia coli, Listeria monocytogenes, Salmonella typhimurium, and Staphylococcus aureus on eggshells. Example decontamination procedures have been investigated against foodborne pathogens such as Salmonella, Listeria and Escherichia coli on eggshells. Example decontamination procedures include electrolyzed water (Cao et al., 2009), UV irradiation (De Reu et al., 2006), hydrogen peroxide (H2O2) (Padron, 1995), and combinations of ozone and UV irradiation (Rodriguez-Romo and Yousef, 2005).

Sodium dodecyl sulfate (SDS) is an anionic surfactant used to disrupt membranes and denature proteins (Woo et al., 2000). Sodium dodecyl sulfate also

is a common ingredient in cosmetics, washing detergents, and personal-care products, and is used in the laboratory environment as a denaturing agent in gel electrophoresis and other protein solubilization techniques. It is considered by the United Nations Environment Program to be “of no concern with respect to human health” (Morales-delaNuez et al., 2011). Use of SDS as a disinfectant in foodstuffs, equipment, and surfaces associated with food industry has received more attention in recent years. To date, the efficacy of SDS alone, or in combination with other materials, in reducing bacterial contamination has been studied in beef (Stelzleni et al., 2013), chicken breast meat (Lu and Wu, 2012), and blueberries (Li and Wu, 2013).

Under standard doses, the use of citric acid as a flavoring in foodstuffs is common and harmless. The combination of citric acid with other disinfectants has a synergistic effect on reduction of microorganisms (Park et al., 2009). Hydrogen peroxide is another inexpensive chemical substance with strong bactericidal properties, and has been used to reduce microbial populations in different foods (Lin et al., 2002; Ukuku et al., 2005).

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Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, and Listeria monocytogenes are among the most common foodborne pathogens throughout the globe. These bacteria are associated with faeces and soil
and, as such, often contaminate eggshells. The aim of this study was to evaluate the capacity of SDS, alone or in combination with citric acid or H$_2$O$_2$, in reducing bacterial concentrations on eggshells experimentally inoculated with four foodborne pathogens: *E. coli*, *S. aureus*, *S. typhimurium*, and *L. monocytogenes* at ambient temperature (25°C).

**Materials and Methods**

**Preparation of bacterium suspension for inoculation**

Single, isolated colonies were transferred from agar plates to inoculation tubes containing 5 ml trypticase soy broth (TSB) and incubated for 20 h at 37°C with shaking (10 RPM). An aliquot (1.5 ml) was transferred to 60 ml TSB and incubated for an additional 20 h under the same conditions. The bacterial suspension was centrifuged (4000 RPM for 7 min), the supernatant was discarded, and the pellet was resuspended in 10 ml phosphate-buffered saline (PBS). For enumeration of bacterial populations, cultures were serially diluted and aliquots (0.1 ml) were transferred to nutrient agar (TSA) plates. The results were presented as CFU/ml rinsate.

**Preparation and inoculation of eggs**

In each series of tests, eight medium-sized fresh eggs (total of 96 eggs) were obtained from local markets. Eggs were prepared using a previously described protocol (Rodriguez-Romo and Youssef, 2005). Briefly, all eggs were washed under tap water and then were placed in 70% ethanol for 30 min. Sanitized eggs were rinsed thoroughly with sterile distilled water, transferred to a reticular plastic tray, and aseptically dried under laminar flow for approximately 30 min before inoculation. The concentrated bacterial suspension was added to 500 ml of sterile PBS to reach concentrations outlined in Table 1. Eggs were placed in the different bacterial suspensions for 30 min. Subsequently, the eggs were put under a laminar flow for 1 h to dry at ambient temperature (Upadhyaya et al., 2013).

**Table 1: List of the actual bacterial concentrations**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Actual bacterial concentration (Log CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>9 ± 0.2</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>8.1 ± 0.5</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>8.5 ± 0.1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>8.7 ± 0.1</td>
</tr>
</tbody>
</table>

**Eggs treatments**

Each egg was placed in individual sterile stomacher plastic bags containing 200 ml disinfectant treatment (SDS [1.5%], H$_2$O$_2$ [0.5%], citric acid [1.0%]) or PBS (as control 1) and shaken gently for 5 min at ambient temperature (25°C). To examine the combined effects of the solutions (SDS [1.5%] + citric acid [1.0%] or SDS [1.5%] + H$_2$O$_2$ [0.5%]), inoculated eggs were immersed in the first solution as described above and then aseptically transferred into another sterile stomacher bag containing the second solution and further incubated for 5 min with shaking (Park et al., 2005). Concurrently, individual eggs were also immersed two times in PBS as control 2, and another inoculated egg receiving no disinfectant treatment (PBS or solution) served as an additional negative control.

**Enumeration of bacteria**

After treatment, each egg was transferred to a sterile plastic bag containing 100 ml of PBS and was gently rubbed by hand for 1 min (Park et al., 2005). Eggs were removed, the rinsate was serially diluted and bacterial survival was assessed by viable counts on trypticase soy agar (TSA) plates. Results were presented as CFU/ml rinsate.

**Statistical analysis**

All experiments were performed in triplicate. Final bacterial concentrations in treated eggs were compared to bacterial concentrations in eggs immersed once in PBS (control 1), eggs immersed twice (control 2), and inoculated eggs receiving no treatments. Results were analyzed using a one-way analysis of variance (ANOVA) and the least significant difference test (LSD) using SPSS (version 16; SPSS Inc., Chicago, USA). Results were considered significantly different at P<0.05.

**Results**

**Effect of various treatments on *E. coli***

Changes in the viable count of the bacterium after each treatment are shown in Fig. 1A. In eggs inoculated with *E. coli*, treatment with SDS, H$_2$O$_2$, or citric acid reduced bacterial concentrations by 1, 1.3 and -0.1 Log CFU/ml, respectively. Treatment with SDS and H$_2$O$_2$ resulted in significantly greater (P<0.05) reductions in bacterial concentrations compared to treatment with PBS alone. Treatment with SDS and citric acid or SDS and H$_2$O$_2$ significantly reduced bacterial concentrations, but the differences were not significant when compared to eggs washed twice with PBS alone or eggs treated with SDS or H$_2$O$_2$ alone.

**Effect of various treatments on *Salmonella typhimurium***

Changes in the viable count of the bacterium after each treatment are shown in Fig. 1B. *Salmonella typhimurium* concentrations in egg treated with SDS, H$_2$O$_2$, and citric acid were 2, 2.1 and 0.4 Log CFU/ml lower than *S. typhimurium* concentrations in eggs washed with PBS alone indicating a significant (P<0.05). Additionally, the combination of SDS and citric acid or SDS and H$_2$O$_2$ treatments was more effective in reducing bacterial concentrations (P<0.05) compared to eggs washed either once or twice with PBS.
with SDS and citric acid or SDS and H$_2$O$_2$ reduced bacterial concentrations to undetectable levels. These results indicate that SDS may have a greater antibacterial effect on *Listeria* compared to other bacterial species tested here.

**Effect of various treatments on Staphylococcus aureus**

*Staphylococcus aureus* appeared more sensitive to H$_2$O$_2$ than other solutions tested. As detailed in Fig. 1D, *S. aureus* concentrations in H$_2$O$_2$ treated eggs were significantly reduced (~3 Log CFU/ml; P<0.05) compared to *S. aureus* concentrations in eggs washed either once or twice with PBS alone. Treatment with SDS was also effective compared to treatment with PBS alone; however, *S. aureus* concentrations were not reduced in eggs treated with citric acid. There was no additive effects with the various treatments that were used in combination.

**Discussion**

Contamination of eggshells can reduce shelf-life and safety of eggs and their byproducts. Therefore, application of appropriate antimicrobial agents for decontamination of egg surfaces can play an important role in achieving public health goals. In this study, three different disinfectants, alone and in combination, were used to evaluate the effectiveness of sanitizers in reducing bacterial concentrations on eggshells experimentally inoculated with four different foodborne pathogens.

According to the results of this study, sequential immersion of eggs in SDS and either citric acid or H$_2$O$_2$ resulted in the largest reductions in *E. coli* on eggshells compared to other tested treatments. Similar results were observed on eggshells inoculated with *S. typhimurium*. Both bacteria were not sensitive to citric acid alone, but notable reductions in target bacteria were observed when citric acid was used in combination with SDS. These similar responses to treatments by *E. coli* and *S. typhimurium* could be due to similarity of the bacteria as both pathogens are gram-negative with similar phenotypic (e.g., cell membranes) and genetic makeup.

Other groups have shown similar effects of SDS, H$_2$O$_2$, and citric acid, or similar compounds in reducing *Salmonella* spp. or *E. coli* in other food types. For example, treatment of alfalfa seeds with levulinic acid and SDS for 5 min resulted in 3 Log reductions of *E. coli* O157: H$^-$ and *S. typhimurium* (Zaho et al., 2010). Other groups, however, have reported reduced efficacy of SDS as a bactericide. Lu and Wu (2012) treated chicken breasts with thymol-based washing solutions with and without SDS. Both solutions achieved approximately 2.2 log reductions of *Salmonella* on chicken breasts. The authors mentioned that the combination of thymol and acetic acid had great potential to be a natural alternative to chlorine-based washing solution for reducing *Salmonella* contamination in chicken breast meat, and the addition of SDS did not result in an additive

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**Fig. 1:** Effect of different treatments on reducing. (A) *Escherichia coli*, (B) *Salmonella typhimurium*, (C) *Listeria monocytogenes*, and (D) *Staphylococcus aureus* on experimentally inoculated eggshells. Bars with different subscripts are considered statistically different at P<0.05

**Effect of various treatments on *Listeria monocytogenes***

As is shown in Fig. 1C, SDS had a potentially greater impact in reducing *Listeria* on contaminated eggs. Treatment with SDS or H$_2$O$_2$ significantly (P<0.05) reduced *Listeria* concentrations by 3.2 and 1.4 Log CFU/ml, respectively, compared to control eggs. Additionally, treatment of *Listeria* contaminated eggs
bactericidal effect. In contrast, Li and Wu (2013) evaluated *Salmonella* inactivation on blueberries washed with SDS in combination with chlorine, lactic acid, acetic acid, citric acid, and/or H$_2$O$_2$. Their results showed that the use of acetic acid or H$_2$O$_2$ in combination with SDS may have practical potential as an alternative to the use of chlorine-based washing solution for blueberries (Li and Wu, 2013). In another study, Stelvani et al. (2013) examined the effect of vinegar and SDS with levulinic acid on *S. typhimurium* and shelf-life and sensory characteristics of ground beef. SDS plus levulonic acid resulted in the largest reductions of *Salmonella*. However, beef samples treated with liquid buffered vinegar and powdered buffered vinegar had the least psychrotrophic growth (Stelzleni et al., 2013). Sodium dodecyl sulfate has also been used to enhance the lethality of organic acids against *S. enterica* inoculated on chicken skin. Results showed that combining organic acids, especially lactic or acetic acid, with SDS might be suitable for application by chicken processors to effectively decontaminate chicken carcasses or cuts (Zaki et al., 2015).

Compared to other pathogens, SDS was most effective in reducing *L. monocytogenes* on eggshells, reducing bacterial concentrations to undetectable levels. *Listeria monocytogenes* was resistant to treatment with citric acid alone and it could be due to the inherent resistance of the bacterium to acidic conditions (Koutsoumanis et al., 2003). Various groups have examined the sensitivity of *L. monocytogenes* to SDS (Maktabi, 2003; Byelashov et al., 2008; Kennedy et al., 2011). A combination of SDS with citric acid or H$_2$O$_2$, however, resulted in significant reductions in bacterial concentrations. These results suggest that both sanitizers may contribute to the enhanced effectiveness of the sequential treatments, probably by mutual reinforcement. The U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) enforces a zero-tolerance rule for *L. monocytogenes* in ready-to-eat meats (Byelashov et al., 2008). Thus, effective and alternative means to reduce *L. monocytogenes*, such as those described here, would be advantageous.

In our studies, *S. aureus* showed more sensitivity to H$_2$O$_2$ compared with SDS. Sequential treatments by SDS and H$_2$O$_2$ or SDS and citric acid did not provide additional significant reduction in the viability of *S. aureus* on eggshells. Sensitivity of the *S. aureus* to H$_2$O$_2$ has been reported before. Sander and Wilson (1999) observed that H$_2$O$_2$ (3%) caused significant reductions in the number of *S. aureus* on eggs placed in incubators. The authors did, however, report that the eggs lost a great amount of their moisture during the incubation period, but hatchability was not affected. Additionally, the use of H$_2$O$_2$ as a hatchery sanitizer did not affect broiler livability, body weight, or feed conversion. In 2004, it was reported that H$_2$O$_2$ vapor decontamination effectively reduced methicillin-resistant *S. aureus* (MRSA) from rooms, furniture, and equipment (French et al., 2004).

The results of our study showed that SDS, H$_2$O$_2$, and citric acid, either alone or in combination, each show promise as potential disinfectant egg washes. The efficacy of each treatment was dependent on the targeted pathogen (e.g., *L. monocytogenes* was highly sensitive to SDS *in vitro* and on the eggshell). Our results extend beyond eggshells as SDS may be useful for decontamination of other materials and surfaces in the food industry.

### Acknowledgements

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