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

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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 (H_2O_2), 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%), H_2O_2 (0.5%), citric acid (1%), or sequential treatments with SDS + citric acid and SDS + H_2O_2. 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 *S. typhimurium* and *E. coli* to SDS and H_2O_2 was similar (~2.5 log reduction). *Listeria monocytogenes* (4.1 Log reduction) and *S. aureus* (4.3 Log reduction) were more sensitive to SDS and H_2O_2, respectively. The combination of SDS and citric acid or H_2O_2 in comparison to SDS alone, generally did not produce significant additive reductions in the viability of the bacteria on eggshells. These data suggest that SDS potentially could be used alone or in combination with citric acid or H_2O_2 as an effective and inexpensive method to reduce bacteria, such as *L. monocytogenes*, on eggshells. Additionally, application of SDS may be useful for bacterial decontamination of other materials and surfaces in food industries.

Key words: Decontamination, Eggshells, Foodborne pathogens, Sodium dodecyl sulfate

Introduction

Eggs are highly nutrient dense foods. They contain many vitamins and trace nutrients that are essential for health. Intact eggs, however, can be contaminated by various pathogens during laying (vertical transmission) (Gantois *et al*., 2009) or processing, transportation, and storage (horizontal transmission) (Davies and Breslin, 2003; Messens *et al*., 2007). The most common sources of microbial contamination of eggshells are soil, faeces, nesting particles, and hands of workers. Contamination can adversely affect the shelf life and safety of eggs. Washing of eggs with an appropriate detergent can reduce the microbial load at the eggshells surface (Gole *et al*., 2014).

Previously, various chemical and physical sanitation 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 (H_2O_2) (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).

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_2O_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. This concentrated suspension was used for eggs inoculation as described below.

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-Romoand 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

Strain	Actual bacterial concentration (Log CFU/ml)
Escherichia coli	9 ± 0.2
Listeria monocytogenes	8.1 ± 0.5
Salmonella typhimurium	8.5 ± 0.1
Staphylococcus aureus	8.7 ± 0.1

Eggs treatments

Each egg was placed in individual sterile stomacher plastic bags containing 200 ml disinfectant treatment (SDS [1.5%], H_2O_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_2O_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. The 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_2O_2, or citric acid reduced bacterial concentrations by 1, 1.3 and -0.1 Log CFU/ml, respectively. Treatment with SDS and H_2O_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_2O_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_2O_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_2O_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_2O_2 treatments was more effective in reducing bacterial concentrations ($P<0.05$) compared to eggs washed either once or twice with PBS.

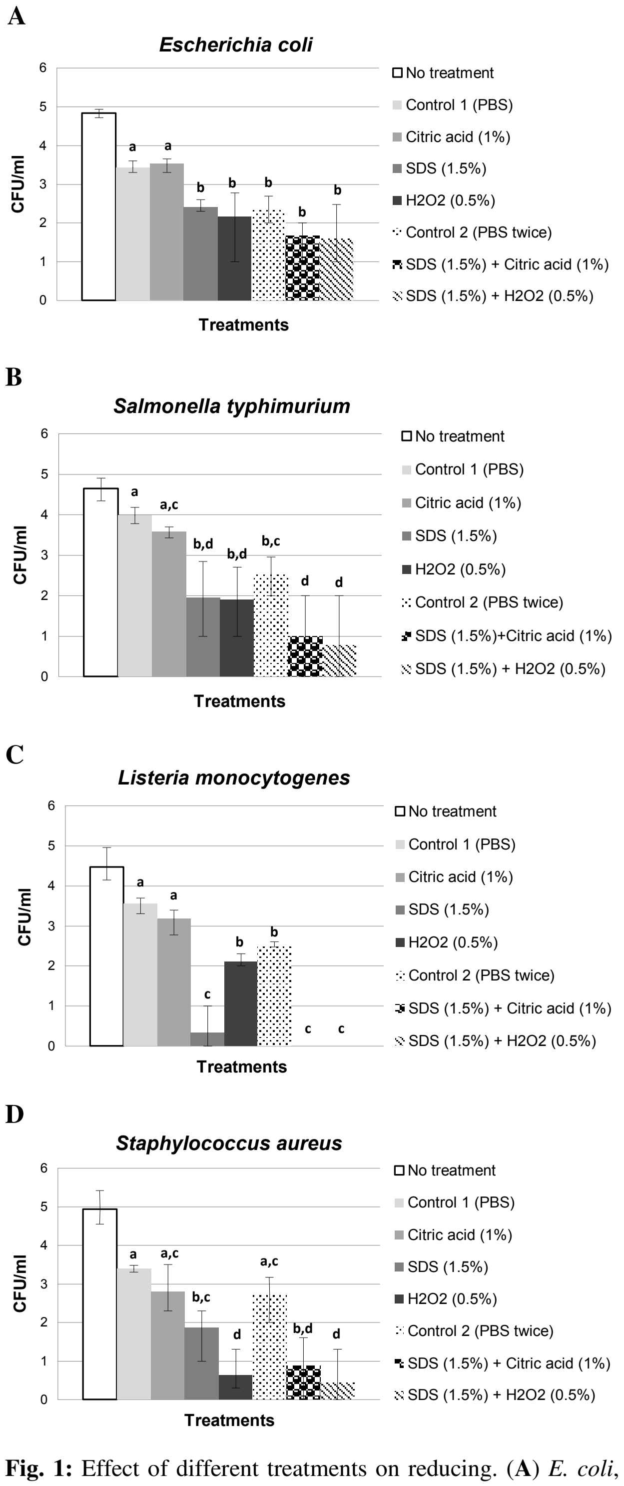

Fig. 1: Effect of different treatments on reducing. (**A**) *E. coli*, (**B**) *S. typhimurium*, (**C**) *L. monocytogenes*, and (**D**) *S. aureus* on experimentally inoculated on eggshells. Bars with different subscripts are considered statistically different at P<0.05

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_2O_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 with SDS and citric acid or SDS and H_2O_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_2O_2 than other solutions tested. As detailed in Fig. 1D, *S. aureus* concentrations in H_2O_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_2O_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_2O_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: H7 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 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_2O_2. Their results showed that the use of acetic acid or H_2O_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_2O_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_2O_2 compared with SDS. Sequential treatments by SDS and H_2O_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_2O_2 has been reported before. Sander and Wilson (1999) observed that H_2O_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_2O_2 as a hatchery sanitizer did not affect broiler livability, body weight, or feed conversion. In 2004, it was reported that H_2O_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_2O_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.

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