Advanced oxidation process sanitization of eggshell surfaces

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ABSTRACT The microbial quality of eggs entering the hatchery represents an important critical control point for biosecurity and pathogen reduction programs in integrated poultry production. The development of safe and effective interventions to reduce microbial contamination on the surface of eggs will be important to improve the overall productivity and microbial food safety of poultry and poultry products. The hydrogen peroxide (H_2O_2) and ultraviolet (UV) light advanced oxidation process is a potentially important alternative to traditional sanitizers and disinfectants for egg sanitation. The H_2O_2/UV advanced oxidation process was demonstrated previously to be effective in reducing surface microbial contamination on eggs. In this study, we evaluated treatment conditions affecting the efficacy of H_2O_2/UV advanced oxidation in order to identify operational parameters for the practical application of this technology in egg sanitation. The effect of the number of application cycles, UV intensity, duration of UV exposure, and egg rotation on the recovery of total aerobic bacteria from the surface of eggs was evaluated. Of the conditions evaluated, we determined that reduction of total aerobic bacteria from naturally contaminated eggs was optimized when eggs were sanitized using 2 repeated application cycles with 5 s exposure to 14 mW cm^{-2} UV light, and that rotation of the eggs between application cycles was unnecessary. Additionally, using these optimized conditions, the H_2O_2/UV process reduced *Salmonella* by greater than 5 $\log_{10}$ cfu egg^{-1} on the surface of experimentally contaminated eggs. This study demonstrates the potential for practical application of the H_2O_2/UV advanced oxidation process in egg sanitation and its effectiveness in reducing *Salmonella* on eggshell surfaces.

Key words: advanced oxidation process, hydrogen peroxide, ultraviolet light, eggshell, *Salmonella*

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INTRODUCTION

Hatchery sanitation is an important component of biosecurity and pathogen reduction programs in integrated poultry operations. Surface microbial contamination of eggs arriving from breeder farms represents a significant challenge to maintaining hygiene within the hatchery. The outer eggshell is exposed to many contaminants that can harbor a wide range of microorganisms (Mine, et al., 2002). Contamination of the eggshell surface by microorganisms is the first step in transshell invasion and subsequent infection of the developing embryo (Bruce and Drysdale, 1991). Excessive microbial contamination of eggshells has been demonstrated to reduce hatchability, chick quality, and growth performance of broiler chickens (Scott and Swetnam, 1993; Kuo, et al., 1996). In addition to microorganisms that can reduce the productivity of poultry operations, *Salmonella* and other human foodborne pathogens have also been demonstrated to penetrate the shell and contaminate the developing embryo (Cox, et al., 2000). Thus, reduction of surface microbial contamination on eggs entering the hatchery represents a critical control point (Cox, et al., 1998) for which the development of effective interventions is expected to improve productivity and microbial food safety in commercial poultry production.

Currently, broiler hatching eggs are not routinely sanitized prior to incubation. Although management practices that promote eggshell cleanliness in order to reduce the number of visibly contaminated eggs have been demonstrated to increase hatchability (Brake, 1985; Anthony, et al., 1992), visibly clean eggs may still harbor a large microbial load (Cox, et al., 1994; Berrang, et al., 1997). Historically, fumigation with formaldehyde gas has been used to sanitize eggs prior to incubation (Proudfoot and Stewart, 1970; Williams and Gordon, 1970). However, formaldehyde fumigation of hatching eggs has been discontinued in the United States due its potential adverse health effects and enforcement of regulations by the Environmental Protection Agency (Toxic Substances Control Act, 1976) and Occupational Safety and Health Administration (Department of Labor, 1987). The use of chemical sanitizers applied as dips, sprays, and foams as alternatives to formaldehyde fumigation of hatching eggs has been evaluated previously (Brake and Sheldon, 1990; Scott and Swetnam,

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1993; Cox, et al., 1998; Russell, 2003; Higgins, et al., 2005). Although many chemical sanitizers have been demonstrated to be effective in reducing the microbial load on the eggshell surface, any potential interaction with the cuticle (Brake and Sheldon, 1990; Buhr, et al., 1994) and negative effects on hatchability (Brake and Sheldon, 1990; Scott, et al., 1993) should also be considered.

Hydrogen peroxide (**H_2O_2**) (Sheldon and Brake, 1991; Padron, 1995; Sander and Wilson, 1999) and ultraviolet (**UV**) light irradiation (Kuo, et al., 1997; Chavez, et al., 2002; Coufal, et al., 2003) have been demonstrated as being effective in sanitizing eggshell surfaces with minimal negative effects on the cuticle and hatchability. The use of H_2O_2 in combination with UV irradiation as an advanced oxidation process (**AOP**) represents a potentially important alternative to the use of conventional sanitizers and disinfectants in egg sanitation. AOPs are aqueous phase oxidation methods based on the in situ generation of highly reactive oxygen species (**ROS**) (Comninellis, et al., 2008). Absorption of UV light by H_2O_2 results in the photolytic generation of hydroxyl radicals (Legrini, et al., 1993). The H_2O_2/UV process is one of the mostly widely used AOPs (Bustillo-Lecompte and Mehrvar, 2015) and has been demonstrated to effectively inactivate vegetative bacteria, bacterial spores, and viruses (Bayliss and Waites, 1982; Mamane, et al., 2007; Ikai, et al., 2010). Proof of principal for the use of H_2O_2/UV advanced oxidation in egg sanitation has been demonstrated previously (Wells, et al., 2010). Although the H_2O_2/UV AOP was found to be effective in reducing microbial contamination on eggshell surfaces, the UV exposure times described previously were far longer than would be practical for use in commercial production. In this study, we evaluated various treatment parameters affecting the combined H_2O_2 and UV sanitization of eggshells in order to maximize effectiveness while reducing the time required for disinfection of eggshell surfaces. Additionally, we investigated the potential of H_2O_2/UV sanitization to reduce surface *Salmonella* contamination of eggshells.

MATERIALS AND METHODS

Eggs

Visibly clean, unwashed eggs were collected at the Texas A&M University Poultry Science Teaching, Research, and Extension Center (Texas A&M University, College Station, TX) immediately prior to use in this study.

H_2O_2/UV AOP Treatment of Eggs

H_2O_2/UV AOP treatment of eggs was performed as described previously with minor modifications (Wells, et al., 2010). UV irradiation of eggs was performed using a custom-built UV chamber modified from Coufal et al. (2003) with 16 germicidal UV-C lamps (254 nm, General Electric Company, Cleveland, OH). Unless indicated otherwise, eggs were treated using custom-built metal wire 32-egg flats that were designed to keep the eggs stationary on the flat and to minimize the area of contact between the egg and the wire. Plastic commercial 42-egg incubator flats (Jamesway, Cambridge, Ontario) also were used when indicated. H_2O_2 was applied to eggs by spraying a fine mist of 3% (wt/vol) aqueous H_2O_2 using a hand-held manual sprayer. A minimal volume of H_2O_2 ($\sim$0.75 mL egg^{-1}) was applied in order to limit any mechanical washing effects from the spray. After being sprayed with H_2O_2, eggs were transferred immediately into the chamber by pushing egg flats manually on metal rails. Unless otherwise indicated, eggs were treated using a UV light intensity of 14.0 mW cm^{-2} and rotated aseptically 180° between application cycles using metal tongs that were disinfected using 70% (wt/vol) aqueous EtOH. When indicated, UV light intensity was varied using either 4, 8, or 16 UV lamps and determined using a UV radiometer (UVP, Inc. Upland, CA) positioned at egg level on a flat within the UV chamber.

Experimental Salmonella Contamination of Eggs

When indicated, eggs were experimentally contaminated using a primary poultry isolate of *Salmonella* Typhimurium (Byrd, et al., 1998) resistant to novobiocin (**NO**) and nalidixic acid **(NA)** to facilitate differentiation of intentionally inoculated *Salmonella* from any microbiota present naturally. *Salmonella* was cultured aerobically at 37 °C using Tryptic Soy broth (**TSB**, Difco, Detroit, MI) supplemented with 25 μg mL^{-1} NO (Calbiochem, La Jolla, CA) and 20 μg mL^{-1} NA (Fisher, Fair Lawn, NJ), harvested by centrifugation, then washed and re-suspended using sterile phosphate buffered saline (PBS; pH 7.2) to 10^9 cfu mL^{-1}. Eggs were massaged for one min in WhirlPak bags (Nasco, Fort Atkinson, WI) containing 10 mL *Salmonella* suspension, removed from the bags, and allowed to dry for 30 min prior to being assigned randomly to treatment groups. *Salmonella* was enumerated from the suspension using Xylose Lysine Tergitol-4 agar (**XLT-4**, Difco) supplemented with 25 μg mL^{-1} NO and 20 μg mL^{-1} NA.

Bacterial Enumeration

Bacteria were enumerated from the surface of eggshells as described previously with minor modification (Coufal, et al., 2003). Briefly, each egg was massaged for one min in an individual WhirPak bag containing 25 mL sterile PBS, and the resulting rinsate was diluted serially using PBS. When indicated, bacteria present in the eggshell pores and membranes were enumerated using the shell and membrane crush method

(Musgrove, et al., 2005) after the first rinse. Briefly, rinsed eggs were transferred aseptically to a second WhirlPak bag for an additional rinse. Eggs were then removed, broken aseptically, and the egg contents discarded. Each broken eggshell was then rinsed with sterile deionized water to remove any remaining material from the interior of the eggshell and pulverized thoroughly in sterile PBS using a sterile glass rod. Total aerobic bacteria were enumerated from eggshell rinsate using Trypticase Soy agar (**TSA**, Difco), and *Salmonella* were enumerated from eggshell rinsate and crushed eggshells using XLT-4 supplemented with 25 μg mL^{-1} NO and 20 μg mL^{-1} NA using the spread plate technique. All plates were incubated at 37°C for 48 h. Eggs from which total aerobes or *Salmonella* were not recovered were assigned the lower limit of detection for the assay. Bacterial counts were $\log_{10}$ transformed and analyzed using ANOVA. When appropriate, results from multiple independent assays were analyzed with each replicate assay as a block. Significantly different means ($P \leq 0.05$) were determined using Duncan's multiple range test or Student's t test as appropriate.

RESULTS

Application Cycles and UV Intensity

The effect of multiple application cycles and UV intensity on microbial inactivation was evaluated for eggs treated using plastic and wire flats. Eggs were sanitized using one, 2, or 4 application cycles of H_2O_2/UV advanced oxidation with one min of UV exposure per cycle (Figure 1A). Fewer total aerobic bacteria were recovered from eggs treated with 2 application cycles as compared to those treated with a single cycle on both plastic and wire egg flats. However, additional application cycles did not further reduce the total aerobic bacteria. Microbial inactivation was greater with wire flats than with plastic flats when eggs were treated with a single application cycle, but no difference was observed when additional application cycles were used.

Eggs were sanitized using 2 application cycles with one min exposure to UV with an intensity of either 1.3, 8.0, or 14.0 mW cm^{-2} (Figure 1B). On plastic egg flats, microbial inactivation was greatest for eggs sanitized using UV with an intensity of 14.0 mW cm^{-2}. Decreasing UV intensity to 8.0 mW cm^{-2} did not significantly reduce the observed reduction in surface microbial contamination on sanitized eggs. On wire egg flats, UV intensity did not significantly affect the total aerobic bacteria recovered from sanitized eggs. At each intensity level, the type of egg flat was not observed to significantly affect the degree of microbial inactivation.

UV Exposure Time and Rotation

The effect of reducing the duration of UV exposure during each of 2 H_2O_2/UV advanced oxidation application cycles and rotation of eggs between each cycle was evaluated. Eggs were sanitized using 2 application cycles with 15, 30, 45, or 60 s UV exposure per cycle (Figure 2A). No significant difference in total aerobic bacteria recovered from sanitized eggs was observed regardless of the duration of UV exposure. Eggs were sanitized using 2 application cycles with either 5 or 60 s UV exposure per cycle with or without rotation between each cycle (Figure 2B). Rotation of eggs between application cycles and reduction of UV exposure time to 5 s per cycle were not observed to significantly affect the degree of microbial inactivation relative to untreated control eggs.

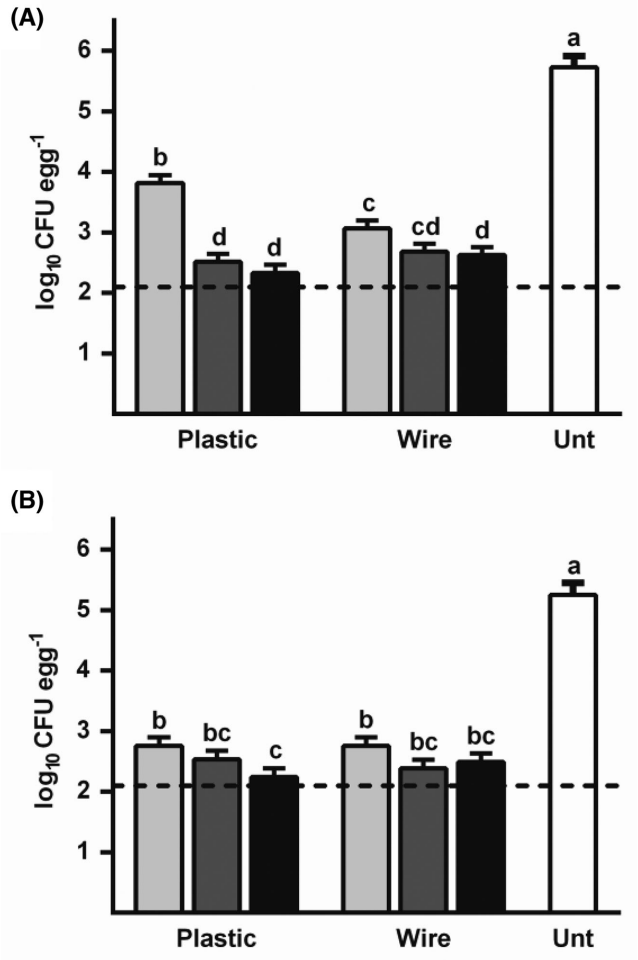

Figure 1. Increasing treatment cycles and UV intensity increased effectiveness of H_2O_2/UV sanitization of egg surfaces. **(A)** Total aerobic bacteria were enumerated from eggs sanitized using one (light gray), 2 (dark gray), or 4 (black) application cycles of H_2O_2/UV advanced oxidation on plastic or wire egg flats and untreated control eggs (white). The mean ± SEM $\log_{10}$ cfu egg^{-1} total aerobic bacteria from 12 treated eggs per treatment and 6 untreated control eggs from 2 independent assays are shown. **(B)** Total aerobic bacteria were enumerated from eggs sanitized using 2 application cycles with exposure to UV with 1.3 (light gray), 8.0 (dark gray) or 14.0 (black) mW cm^{-2} intensity on plastic or wire egg flats and untreated control eggs (white). The mean ± SEM $\log_{10}$ cfu egg^{-1} total aerobic bacteria from 16 treated eggs per treatment and 8 untreated control eggs from 2 independent assays are shown. Dashed line indicates the limit of detection, 2.1 $\log_{10}$ cfu egg^{-1}. $^{a–d}$Means with different letters are significantly different ($P \leq 0.05$).

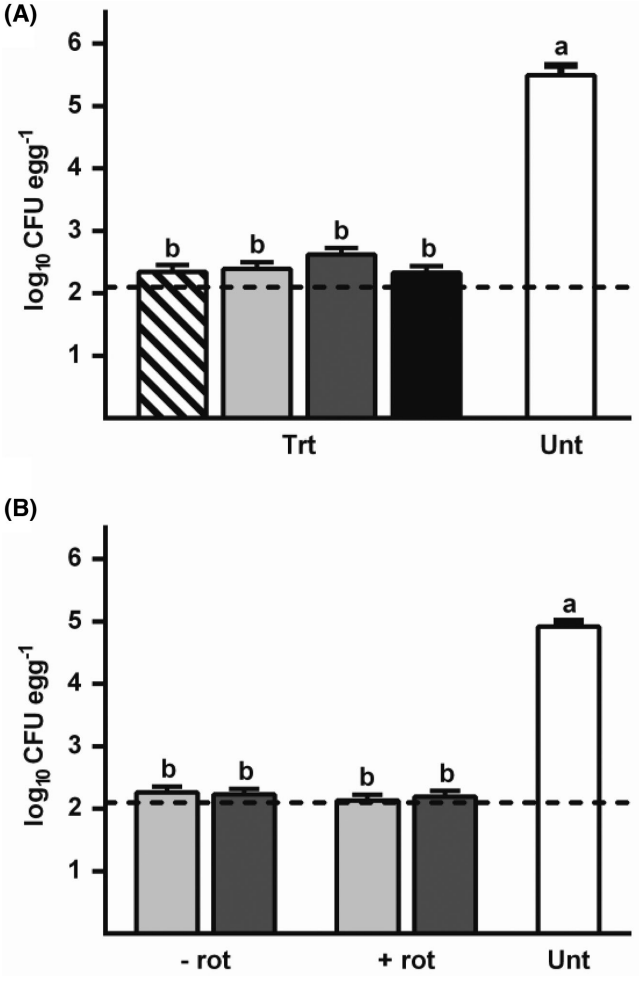

Figure 2. Egg rotation and reduced UV exposure times did not affect efficacy of H_2O_2/UV sanitization of egg surfaces. **(A)** Total aerobic bacteria were enumerated from untreated control eggs (white) or eggs sanitized using 2 application cycles of H_2O_2/UV advanced oxidation with UV exposure times of 15 s (stripes), 30 s (light gray), 45 s (dark gray), and 60 s (black) per application cycle. **(B)** Total aerobic bacteria were enumerated from untreated control eggs (white) and eggs sanitized using 2 application cycles without rotation (− rot) or with rotation (+ rot) and 5 s (light gray) or 60 s (dark gray) of UV exposure. The mean ± SEM $\log_{10}$ cfu egg^{-1} total aerobes from 8 untreated control eggs and 16 treated eggs per treatment from 2 independent assays are shown. Dashed line indicates the limit of detection, 2.1 $\log_{10}$ cfu egg^{-1}. $^{a–b}$Means with different letters are significantly different ($P \leq 0.05$).

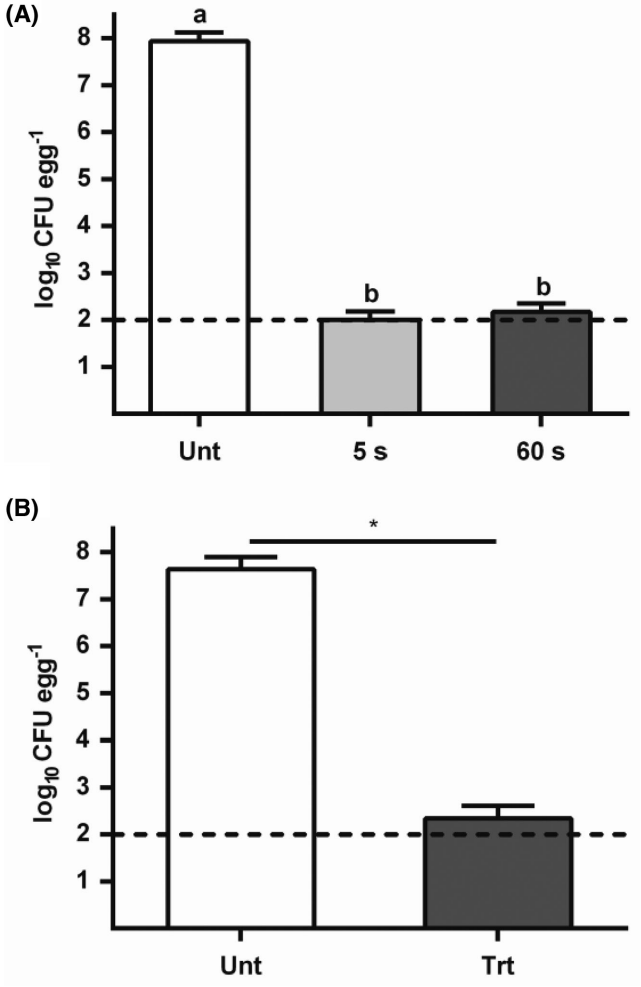

Figure 3. H_2O_2/UV sanitization reduces *Salmonella* contamination on eggshell surfaces. **(A)** *Salmonella* were enumerated from experimentally contaminated eggs that were untreated or sanitized using with 2 application cycles of the H_2O_2/UV AOP with 5 s or 60 s UV exposure per application cycle. The mean ± SEM $\log_{10}$ cfu egg^{-1} *Salmonella* from 6 independent eggs per treatment are shown. $^{a–b}$Means with different letters are significantly different ($P < 0.05$). **(B)** *Salmonella* were enumerated from experimentally contaminated eggs that were untreated or sanitized using 2 application cycles with 5 s UV exposure per cycle. The mean ± SEM $\log_{10}$ cfu egg^{-1} *Salmonella* from 16 independent eggs per treatment are shown. Dashed line indicates the limit of detection, 2 $\log_{10}$ cfu egg^{-1}. * indicates $P < 0.001$.

Disinfection of Salmonella from Eggshell Surfaces

The effectiveness of the H_2O_2/UV AOP in reducing *Salmonella* on experimentally contaminated eggshell surfaces was evaluated using the single shell rinse method. Eggs were experimentally contaminated with *Salmonella* Typhimurium and sanitized using 2 application cycles of H_2O_2/UV advanced oxidation with either 5 or 60 s UV exposure per cycle (Figure 3A). The H_2O_2/UV AOP reduced *Salmonella* almost 6 $\log_{10}$ cfu egg^{-1} as compared to unsanitized eggs, regardless of the duration of UV exposure. An additional assay was performed in which experimentally contaminated eggs were sanitized using 2 application cycles with 5 s UV exposure per cycle (Figure 3B). In this additional assay, sanitizing eggs using the H_2O_2/UV AOP reduced *Salmonella* 5.25 $\log_{10}$ cfu egg^{-1} ($P < 0.001$) as compared to unsanitized eggs.

In order to evaluate the effect of the H_2O_2/UV AOP on *Salmonella* present in the eggshell pores and shell membranes, a secondary shell surface rinse was combined with the shell and membrane crush method (Figure 4). Sanitizing eggs using the H_2O_2/UV AOP reduced *Salmonella* 5.1 $\log_{10}$ cfu egg^{-1} ($P < 0.001$) as compared to unsanitized eggs. A second shell rinse recovered 5.2 $\log_{10}$ cfu egg^{-1} *Salmonella* from unsanitized eggs, whereas the *Salmonella* counts on sanitized eggs was unchanged from the first rinse. Recovery of *Salmonella* from crushed eggshells was approximately 6 $\log_{10}$ cfu egg^{-1} for both sanitized and unsanitized eggs.

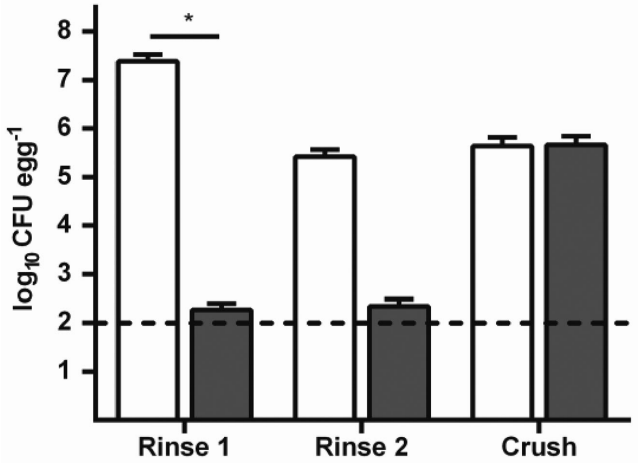

Figure 4. H_2O_2/UV sanitization did not reduce *Salmonella* present in the pores and membrane of eggshells. *Salmonella* were enumerated from the first rinse (Rinse 1), second rinse (Rinse 2), and crushed shells (Crush) from experimentally contaminated eggs that were untreated (white) or sanitized (dark gray) using 2 application cycles with 5 s UV exposure per cycle. The mean ± SEM $\log_{10}$ cfu egg^{-1} *Salmonella* from 16 independent eggs per treatment from 2 independent assays are shown. Dashed line indicates the limit of detection, 2 $\log_{10}$ cfu egg^{-1}. * indicates $P < 0.001$.

DISCUSSION

Hydrogen peroxide (H_2O_2) and ultraviolet (UV) irradiation are commonly used to sanitize microbially contaminated surfaces. The application of H_2O_2 (Sheldon and Brake, 1991; Padron, 1995; Sander and Wilson, 1999) and UV irradiation (Kuo, et al., 1997; Chavez, et al., 2002; Coufal, et al., 2003) individually has been demonstrated to be effective in reducing surface microbial contamination on eggshells. The rapid inactivation of bacteria by ROS generated during UV photolysis of H_2O_2 (Bayliss and Waites, 1982) suggests the H_2O_2/UV AOP to be an effective method for sanitizing eggshell surfaces. The effectiveness of H_2O_2/UV advanced oxidation has been demonstrated previously (Wells, et al., 2010). However, the treatment conditions evaluated were not practical for use in commercial production. In this study, we evaluated treatment conditions including the total number of H_2O_2/UV AOP application cycles, UV intensity, egg rotation, and duration of UV exposure to identify the operational parameters that would be practical for the application of this technology for sanitizing eggs in commercial poultry production.

Wells et al. (2010) previously evaluated various combinations of H_2O_2 concentration and UV exposure times on surface total aerobic bacteria and reported a maximum reduction of approximately 3 $\log_{10}$ cfu when eggs were treated with 1.5% H_2O_2 and 8 min UV exposure. The authors did not detect a significant difference in total aerobic counts when UV exposure times were reduced to 4 min and 2 min as compared to 8 min. The authors also reported that varying H_2O_2 concentration between 1.5 and 3.0% did not affect the degree of microbial inactivation when the duration of UV exposure was less than 8 min.

In this current study, the arrangement of lamps within the chamber was reconfigured to eliminate dead zones and increase exposure of eggshell surfaces to UV. This modification increased observed UV intensity at the egg level from 11 to 14 mW cm^{-2}, and in preliminary assays, allowed the duration of UV exposure to be reduced to one min without a significant reduction in effectiveness (data not shown). In addition to the custom-built wire egg flats that were used previously (Wells, et al., 2010), plastic commercial egg flats also were used in this study to evaluate potential integration of this technology in pre-existing processes in the poultry industry. The similar effectiveness with commercial plastic egg flats as compared to custom-built flats observed in this study suggests that, in principal, an egg sanitizing apparatus using the H_2O_2/UV AOP could be readily added to pre-existing facilities.

The increase in microbial inactivation observed with longer durations of UV exposure alone has been well demonstrated (Berrang, et al., 1995; Kuo, et al., 1997; Chavez, et al., 2002; Coufal, et al., 2003; Wells, et al., 2010). However, UV exposure of several min is not only commercially impractical, but also prolonged exposure to the heat generated by UV lamps is undesirable. Although application of H_2O_2 prior to UV exposure has been demonstrated to decrease the UV exposure times required for microbial inactivation (Wells, et al., 2010), the overall effectiveness was still limited by the duration of UV exposure. Additionally, total microbial inactivation by H_2O_2/UV advanced oxidation has been reported to increase with UV intensity but is limited by the oxidant concentration to an upper limit of approximately 3% H_2O_2 (Bayliss and Waites, 1982; Bounty, et al., 2012). The H_2O_2/UV AOP is dependent on UV photolytic production of ROS from H_2O_2 (Anzai, et al., 2003). Because ROS are short lived, highly reactive, and rapidly quenched as they oxidize bacterial membrane lipids, DNA, proteins, and other essential cellular components of microbes present on the eggshell surface (Fridovich, 1978), the quantity of oxidant applied to the egg is also likely to be limiting. Thus, repeated cycles of H_2O_2/UV application would allow additional oxidant to be applied to the eggs and be expected to improve microbial inactivation and allow the total duration of UV exposure to be reduced.

During this study, the duration of UV exposure was reduced from 60 to 5 s without a significant decrease in effectiveness of H_2O_2/UV advanced oxidation in reducing microbial contamination on eggshell surfaces. The photolytic reaction propagates rapidly once initiated by UV exposure (Legrini, et al., 1993). Rate constants for the propagation of hydroxyl radical formation from H_2O_2 have been reported to be on the order of 10^7 to 10^9 M^{-1} s^{-1} (Liao and Gurol, 1995), suggesting that the photolysis of the approximately 0.75 mL of 3% H_2O_2 (~1.0 M) applied to the eggs in this study would reach completion in less than 100 ns.

Rotation of eggs during UV irradiation has been demonstrated previously to increase the effectiveness of UV disinfection of eggshell surfaces (Kuo, et al., 1997; Chavez, et al., 2002), likely due to the more complete exposure of the total surface area of the eggs. In this study, there was no difference in the degree of microbial inactivation when rotated eggs were compared to eggs that were not rotated. The rearrangement of lamps within the UV chamber likely increased the total surface area of each egg exposed to UV. Additionally, because of the very rapid propagation of the photolytic reaction (Liao and Gurol, 1995), hydroxyl radicals are expected to be present at areas of the eggshell surface not directly exposed to UV.

We demonstrated the effectiveness of the H_2O_2/UV AOP in reducing *Salmonella* on the surface of experimentally contaminated eggs. Although 5 $\log_{10}$ cfu egg^{-1} fewer *Salmonella* were recovered from the surface of sanitized eggs as compared to the unsanitized eggs, recovery of *Salmonella* from the crushed eggshells was similar for both sanitized and unsanitized eggs. This suggests that the H_2O_2/UV advanced oxidation process is effective only at reducing the number of microorganisms present on the surface of the egg and is not likely to affect any microorganisms that have penetrated into the eggshell pores or the egg contents.

The number of bacteria recovered from the second rinse of unsanitized eggs suggests the egg rinse method does not remove all viable microorganisms present on the surface. Indeed, microbial counts remained high for untreated eggs in preliminary assays in which microorganisms were enumerated from up to 5 repeated shell rinses (data not shown). Because the number of *Salmonella* recovered in the second rinse and shell crush from unsanitized eggs was similar, it is impossible to determine whether the *Salmonella* recovered from the crushed shells of unsanitized eggs were present in the pores and membranes of the eggs or were adherent to the exterior surface of the shell. For sanitized eggs, however, because more *Salmonella* were recovered from the shell crush as compared to the second shell surface rinse, it is likely that the *Salmonella* that were recovered in the shell crush were present in the eggshell pores and membranes. This suggests that *Salmonella* was able penetrate the shell surface of experimentally contaminated eggs to a depth inside the shell structure that was not affected by the H_2O_2/UV AOP.

Although *Salmonella* present on the surface of the egg is likely to penetrate the shell if given enough contact time, the degree or incidence of shell penetration may be reduced if the eggs were sanitized prior to penetration. In this study, eggs were submerged in a suspension of culture to maximize artificial contamination by *Salmonella*. The presence of excess moisture, particularly when there is a temperature differential between the egg and the liquid, has been demonstrated to increase bacterial penetration of eggshells (Berrang, et al., 1999). Thus, submersion of the eggs in a chilled suspension likely contributed to the observed penetration of *Salmonella* through the eggshell pores. Additional studies using a non-submersive inoculation method will be required to determine whether surface sanitization can reduce penetration of *Salmonella* into eggs.

In this study, we evaluated the effect of various treatment conditions in order to identify operational parameters for the practical application of the H_2O_2/UV advanced oxidation process for sanitizing eggshell surfaces and demonstrated the effectiveness of this technology in reducing the incidence of *Salmonella* on the surface of eggshells. However, additional studies will be required to determine whether this technology is able to reduce the incidence of *Salmonella* downstream in integrated broiler and turkey production systems. Additionally, further studies will be required to evaluate the effects on hatchability, chick quality, and growth performance. The results of this study will be used to inform the design and construction of a mechanized egg sanitizing apparatus that can be readily integrated into existing commercial production processes. The development of this and other advanced oxidation technologies is expected to have significant impact in multiple sectors of the commercial poultry industry and has the potential to improve the overall animal health, productivity, and microbial food safety of poultry and poultry products.

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