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An ASABE Meeting Presentation

Paper Number: 095974

Decontamination of Shell-Eggs with Pulsed UV-Light

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**Written for presentation at the
2009 ASABE Annual International Meeting
Sponsored by ASABE
Grand Sierra Resort and Casino
Reno, Nevada
June 21 – June 24, 2009**

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Abstract. Currently, most methods used by the food industry to decontaminate shell-eggs involve washing the egg surface with various chemical solutions. In this study, the effectiveness of pulsed UV-light was evaluated for the decontamination of shell-eggs. Samples inoculated with *Salmonella Enteritidis* on top surface on the equator were treated with pulsed UV-light for 1, 3, 5, 10, 15, 20, and 30 seconds at 9.5 and 14.5 cm from the UV-strobe in a chamber. An input voltage of 3,800 V was used to generate a 1.27 J/cm²/pulse of radiant energy at 1.5 cm below the lamp surface, producing a polychromatic radiation in the wavelength range of 100 to 1100 nm, with 54% of the energy being in the UV-light region. Log reductions in microbial population were determined after treatments. A maximum log₁₀ reduction of 5.3 (CFU/cm²) was obtained after 20-s treatment at 9.5 cm without any visual damage to the egg. The temperature and total energy absorbed at each treatment condition was determined using a K-type thermocouple and a radiometer, respectively. Temperature and energy increased with higher treatment time and shorter distance from the UV-lamp. A maximum temperature increase of 10.5 ± 1.6°C and a maximum energy of 35.3 ± 0.1 J/cm² were observed after 30-s treatment at 9.5 cm. This study demonstrated that pulsed UV-light has potential to decontaminate shell-egg surfaces.

Keywords. shell-egg, pulsed ultraviolet light, decontamination, *Salmonella* Enteritidis

Introduction

Salmonellosis associated with eggs is a significant public health problem in the United States and some of the European countries (CDC, 2005). *Salmonella* Enteritidis is the main causative pathogen of salmonellosis related to eggs. *Salmonella* is either horizontally or vertically transferred to eggs. Horizontal transmission is the contamination of egg from another contaminated source while vertical transmission is the contamination of egg from infected hen during formation of ovaries. About 80% of human outbreaks were related to the consumption of eggs or egg containing foods from *Salmonella* Enteritidis outbreaks during the period from 1985 to 1999 (Gast, 2006). Other microorganisms associated with shell-egg surfaces are *Yersinia enterocolitica*, *Escherichia coli* O157:H7, and *Campylobacter jejuni* (Erdogru, 2004; USDA, 2001a).

Eggs naturally have four layers of protection which prevent bacteria from reaching the yolk: the shell, inner and outer membranes between the shell and the albumen, the albumen (eggwhite), and the vitelline (yolk) membrane. Furthermore, the cuticle, a membrane-like substance covers the egg-shell on the outside. The cuticle seals the pores of the egg-shell and prevents bacteria from penetrating into the egg. However, the cuticle can easily be damaged during the washing process. Some egg processors cover the egg surfaces with an extra thin layer of edible oil after they are washed to replace the damaged cuticle (FSIS & FDA, 1998).

Generally, eggs are washed in a detergent bath on conveyor rollers while being cleaned with brushes. The USDA mandates the egg washing practice for all USDA-grade eggs. The Food Safety Inspection Service (FSIS) and the Food and Drug Administration (FDA) are both responsible for egg safety (CFR, 2000). These agencies and the egg industry have been investigating alternative decontamination techniques which could serve the public, minimize costs and benefit both the public and the industry.

Different techniques have been studied and evaluated for the decontamination of egg surfaces. One of the most effective techniques is the use of noxious chemicals or fumigation (Davies and Breslin, 2003). Chlorine, iodine, hydrogen peroxide, and ozonated water are among the disinfectants used

for the decontamination of eggs surfaces. However, these agents are only partially effective. Treatment of eggs with hot water at 80-100 °C is another effective method (Davies and Breslin, 2003), though, may result in cooking of the egg white or cracking of the egg-shell.

Bialka et al. (2004) studied the efficacy of electrolyzed oxidizing water to decontaminate *Escherichia coli* K12 and *Salmonella* Enteritidis loads on shell-eggs surfaces. For typical detergent-sanitizer treatments, 1.7 and 2.0 log reduction were observed for *S. Enteritidis* and *E.coli* K12, respectively, while 2.1 and 2.3 log reductions were observed for *S. Enteritidis* and *E.coli* K12 after treatment with EO water.

Higgins et al. (2005) studied the disinfection of table eggs by ionized reactive oxygen species using Binary Ionization Technology (BIT). Table eggs were treated with BIT spray for 5 s. The log reduction obtained was 7.77. They also found that this treatment did not have a significant effect on the hatchability of eggs.

Ultraviolet light has also been studied for the decontamination of egg surfaces. In the study of De Reu et al. (2006), the effect of UV light on the microbial load of shell egg surfaces was evaluated. The eggs were treated on a double roller conveyor belt with standard UV light (254 nm) at an intensity of 10 mW/cm². *Escherichia coli* and *Staphylococcus aureus* were used for inoculum. Log reductions were 3-4 log CFU obtained for both microorganisms by UV treatment for 4.7 and 18.8 s, respectively. The natural bacterial load of uninoculated shell-eggs was reduced from 4.47 to 3.57 log by standard UV treatment of 4.7 s. The UV treatment did not affect the internal bacterial contamination of shell eggs.

Pulsed UV-light is a novel decontamination technology, which offers effective inactivation of pathogens on the food surfaces in very short periods of time. In 1999, pulsed light treatment of food was approved by FDA (Federal Register, 1999). Basically, a pulsed UV-light lamp produces a continual broadband spectrum from the deep UV to infrared. This continual spectrum is especially rich and efficient in the UV range below 400 nm, which is germicidal.

In this study, the effectiveness of pulsed UV-light as a novel intervention method was evaluated on the *Salmonella* Enteritidis population on shell-eggs. The temperature profile of the samples during treatments and energy absorbed at each treatment condition were also determined.

Materials and Methods

Microorganism

Salmonella Enteritidis phage type 8 (PT8) was obtained from the *Salmonella* Center at the University of Pennsylvania. The culture was maintained on tryptic soy agar (TSA; Difco, Detroit, MI) supplemented with 0.6% yeast extract (TSAYE; Difco, Detroit, MI).

To eliminate any other microorganisms that may be present, antibiotic-resistant *Salmonella* Enteritidis cultures were prepared as described by Catalano and Knabel (1994). Briefly, *S. Enteritidis* PT8 was grown in 10 ml of tryptic soy broth (TSB; Difco, Detroit, MI) supplemented with 0.6% yeast extract (TSBYE) for 24 h at 37°C. Then a loopful portion was streaked onto slants of tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) and the slants were incubated for 24 h at 37°C. After the incubation, colonies were picked and streaked onto TSAYE plates with 100 µg/ml nalidixic acid (TSAYE-N; Fisher Scientific Co., Fair Lawn, NJ), and incubated at 37°C for 24 h. Then, the surviving cells were picked and plated onto TSAYE-N and incubated for 24 h at 37°C. At the end of the fifth subsequent plating, the isolated colonies were maintained in TSBYE containing 100 µg/ml nalidixic acid (TSBYE-N). One ml of this culture was inoculated into 10 ml TSBYE-N and incubated for 24 h at

37°C. Then, a loopful portion was streaked onto TSAYE plates containing 100 µg/ml of both nalidixic acid and streptomycin sulfate (TSAYE-NS; Sigma-Aldrich, St. Louis, MO), and incubated at 37°C for 24 h. After the incubation, the surviving cells were picked and plated onto TSAYE-NS and incubated at 37°C for 24 h. At the end of the fifth subsequent plating, the isolated colonies (spontaneous mutants) were isolated and named as *S. Enteritidis* PT8NSR (nalidixic acid and streptomycin sulfate resistant). This antibiotic-resistant strain was stored at -80°C in 20% glycerol solution and maintained on TSAYE-NS slant at 4°C, which was sub-cultured biweekly to maintain viability.

Preparation of inoculum

Salmonella Enteritidis PT8NSR was grown in 100 ml tryptic soy broth with 0.6% yeast extract (TSBYE) for 24 h at 37°C, and then centrifuged for 30 min at 3,300 x g and 10°C. At the end of the centrifugation, the supernatant was discarded. Cells were then washed with sterile 0.1% peptone water and centrifuged for 30 min at 3,300 x g and 10°C. After discarding the supernatant, cells were re-suspended in 10 ml of 0.1% peptone water, which yielded approximately 10⁹ CFU/ml.

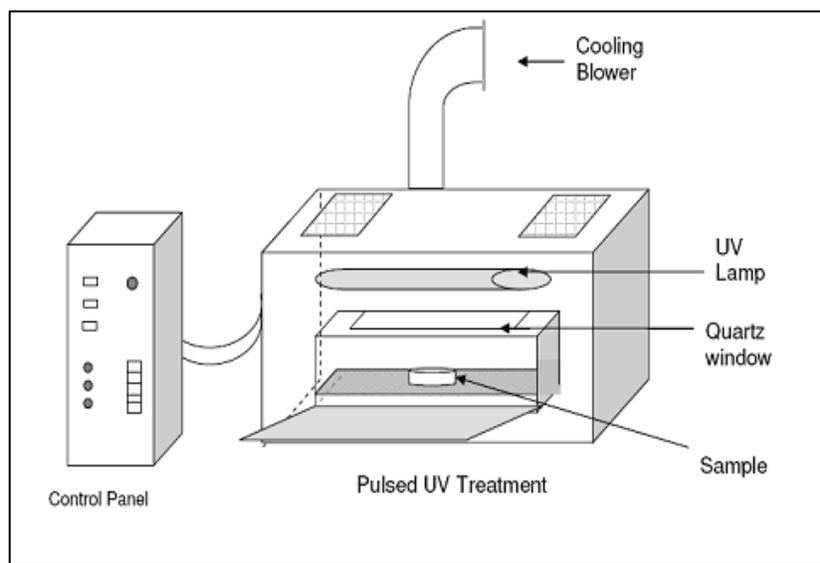
Inoculation of shell-eggs

Eggs from W-36 Hyline hens that are 32 weeks old were obtained from the Pennsylvania State University Poultry Education and Research Center (PERC). Eggs were held at room temperature for 2 h before an experiment. An area of 2 x 1 cm² on top surface on the equator of each egg was inoculated with 0.1 ml of prepared inoculum solution of *S. Enteritidis* PT8NSR to obtain pathogen levels of approximately 5-6 log₁₀ CFU on the surface. The samples were kept under laminar flow in a biological hood for 30 minutes before treatments to facilitate the attachment of microbial cells to sample surfaces.

Only the top surfaces were inoculated, because the pulsed UV-light chamber was equipped with one lamp on the top (Figure 1). In order to expose all the surfaces, samples had to be rotated, which would make the execution of the trials harder. Therefore, samples were exposed to pulsed UV-light only on the top surface without any rotation.

Pulsed UV-light system

Pulsed UV-light was applied to shell-eggs using the SteriPulse-XL®3000 Pulsed UV-Light System (XENON Corporation, Wilmington, MA) (Figure 1). The system included a laboratory test chamber (0.64 m x 0.15 m x 0.19 m) and several shelf settings, which allowed treatment of eggs at varying distances from the lamp. The UV lamp pulsed three times per second. Each pulse's duration was 360 µs. An input voltage of 3,800 V was used to generate 1.27 J/cm²/pulse of radiant energy at 1.5 cm below the lamp surface. With this energy, a polychromatic radiation was produced in the wavelength range of 100 to 1100 nm, with energy being in region of 54% of the the UV-light (Krishnamurthy et al., 2007).



range of 100 to 1100 nm, with energy being in region of 54% of the the UV-light (Krishnamurthy et al., 2007).

Figure 1. Schematic diagram of the pulsed UV-light treatment system
(Bialka, 2007)

Treatment with pulsed UV-light

Each inoculated sample was placed on the middle of the shelf in the pulsed UV-light chamber (Figure 1). The number of pulses (duration) and the distance between the sample and the quartz window were selected to be the system variables. Accordingly, the samples were treated for 1, 3, 5, 10, 15, 20, and 30 seconds at 9.5 and 14.5 cm from the UV-strobe in the pulsed UV-light chamber.

Energy and temperature monitoring

During the pulsed UV-light treatments, temperatures of shell-egg samples were monitored using a type K thermocouple (Omegaette HH306, Omega Eng. Inc., Stamford, CT) for up to 30 s by inserting the thermocouple into a depth of 2-3 mm through a 1.45-mm hole drilled from the top surface of the samples. The amount of energy received at each distance from the UV-strobe in the pulsed UV-light chamber was measured by Nova Laser Power energy monitor (Ophir Optronics Ltd., Wilmington, MA), which averaged the energy level over 9 pulses. The amount of energy was calculated and expressed in J/cm² for each treatment condition.

Microbiological analysis

Each treated or untreated sample was transferred to a plastic bag with 25 ml of 0.1% peptone water rinse solution, and shaken for 1 minute. The peptone water in the bag corresponding to each egg was then serially diluted in 0.1% peptone water (Difco, Detroit, MI). The dilutions were spiral-plated onto plates of TSAYE-NS agar by using an autoplater (Autoplate 4000, Spiral Biotech, Norwood, MA). The plates were then incubated at 37°C for 24 h. Finally, *S. Enteritidis* PT8NSR colonies were counted using an autounter (Q-Count, Version 2.1, Spiral Biotech, Norwood, MA). The counts were expressed as CFU/cm² of sample surface. Typical *Salmonella* colonies were confirmed serologically using a *Salmonella* agglutination latex assay (Remel, Lenexa, KS) and gram-staining.

To ensure detection of low levels of pathogens after treatments, samples that demonstrated zero plate counts were enriched. Enrichments were done by transferring 1 mL of the peptone water rinse solution to 9 mL of TSBYE or TT Broth Base Hajna (Difco, Detroit, MI). After incubating the TSBYE broth at 37°C for 24 h, and the TT Broth Base Hajna at 45°C for 48 h; a loop of each enrichment broth was streaked onto xylose lysine desoxycholate (XLD) agar, and incubated for 24 h at 37°C (Bialka et al., 2004). *Salmonella* colonies were confirmed serologically using the *Salmonella*

agglutination latex assay. The minimum detection limit of 1.3 log₁₀ (CFU/ml rinse solution) was subtracted from the initial log₁₀ (CFU/ml rinse solution) number when plates with zero colonies were observed.

Statistical analysis

Each treatment was replicated in triplicate. Samples which were not treated in the pulsed UV-light chamber were used as control. The microbial reductions were analyzed by using ANOVA – General Linear Model using MINITAB (Version 14) (MINITAB Inc, State College, PA). The significant differences in mean values were determined using Tukey’s method at the 95% confidence interval.

Results and Discussion

The effectiveness of pulsed UV-light was evaluated by determining the log₁₀ reduction of *Salmonella* Enteritidis PT8NSR population on the shell-egg surface. Total energy and temperature increase of samples were determined for each treatment condition by a radiometer and a K-type thermocouple, respectively.

Log reductions

The log reductions of *Salmonella* Enteritidis PT8NSR inoculated on the 2x1 cm area on top surface of shell-egg samples were determined at treatment times of 1, 3, 5, 10, 15, 20, and 30 seconds at 9.5 and 14.5 cm from the UV-strobe in the pulsed UV-light chamber.

Log reductions of *S. Enteritidis* PT8NSR after treatments are given in Table 1. At a treatment distance of 9.5 cm from the UV-strobe, the reduction was between 2.0 and 5.3 CFU/cm² and the visual appearance of samples (before/after cracking) did not show any difference after treatments. Treatments for 1 s and 3 s were not significantly different (p>0.05). Treatments for 3, 5, and 10-s were not significantly different (p>0.05), while 10-s treatment was not significantly different (p>0.05) from 15-s treatment. The treatments for 20 and 30-s were significantly different (p<0.05) from other treatments.

At 14.5 cm, the reduction ranged from 1.3 to 5.3 CFU/cm² and the visual appearance of samples (before/after cracking) did not change after treatments. Treatments for 1 and 3 s were significantly different (p<0.05), while 3-s and 5-s treatments were not significantly different (p>0.05). Treatment for 5 s was not significantly different (p>0.05) from 10-s treatment, while 10-s treatment was not significantly different (p>0.05) from 15-s treatment. Treatments for 20 and 30 s were significantly different (p<0.05) from other treatments.

Table 1. Log reductions of *Salmonella* Enteritidis PT8NSR on shell-eggs¹.

Treatment Time (s)	Log Reduction (CFU/cm ²)	
	at 9.5 cm	at 14.5 cm
1		
3		
5		
10		
15		
20		
30		

1	2.0 ± 0.3A	1.3 ± 0.3A
3	2.8 ± 0.1AB	2.3 ± 0.4B
5	3.2 ± 0.2B	2.7 ± 0.3 BC
10	3.5± 0.5BC	3.2 ± 0.1CD
15	4.0±0.3C	3.4±0.1D
20	≥5.3±0.3D ^{2,3}	≥5.3±0.0E ^{2,4}
30	≥5.3±0.3D ^{2,3}	≥5.3±0.0E ^{2,4}

¹Within the same column, values not followed by the same letter are significantly different (p<0.05).

² Plates with zero colonies were observed. The minimum detection limit was subtracted from the initial log₁₀ population to determine the log₁₀ reduction.

³The enrichments were negative.

⁴The enrichments were positive.

The treatments at 9.5 and 14.5 cm were not significantly different (p>0.05) regardless of the treatment times. Based on these findings, the optimum treatment condition was identified as 20-s treatment at 9.5 cm, which was the treatment with the shortest treatment time that resulted in negative enrichments. Accordingly, the optimum treatment of samples yielded ca. 5.3 log₁₀ reduction (CFU/cm²), which corresponds to about 99.999% reduction.

Energy and temperature measurements

When the distance between the UV-strobe and top surface of egg samples was 9.5 cm, the total amount of energy obtained at the level of the top surface of egg samples ranged from 1.2 to 35.3 J/cm² after the 1-s and 30-s treatment, respectively. The initial temperature of samples was 16.7±0.4 °C, and the temperature difference between the treated and untreated (initial) samples ranged from 0.5 to 10.5 °C after 1-s and 30-s treatment, respectively (Table 2).

When the distance between the UV-strobe and top surface of egg samples was 14.5 cm, the total amount of energy obtained at the level of the top surface of egg samples ranged from 0.8 to 24.8 J/cm² after 1-s and 30-s treatment, respectively. The initial temperature of samples was 17.6±0.1 °C. The temperature increase of samples ranged from 0.2 to 7.8 °C after 1-s and 30-s treatment, respectively.

Table 2. Energy and temperature changes during pulsed UV-light treatments of shell-eggs.

Treatment Time (s)	Total Energy (J/cm ²)
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	9.5-cm Distance	14.5-cm Distance
1	1.2±0.0	0.8±0.0
3	3.5±0.0	2.5±0.0
5	5.9±0.0	4.1±0.0
10	11.8±0.0	8.3±0.0
15	17.7±0.1	12.4±0.1
20	23.6±0.1	16.5±0.1
30	35.3±0.1	24.8±0.1
Temperature increase (T-T _{initial}) (°C)		
1	0.5±0.4	0.2±0.0
3	1.9±0.9	0.9±0.4
5	3.4±1.3	2.0±0.8
10	5.7±1.1	3.5±0.7
15	6.9±0.9	4.5±0.6
20	8.1±0.6	5.6±0.7
30	10.5±1.2	7.8±1.1

Total energy absorbed by the radiometer and temperature change of samples increased with longer treatment time and shorter distance from the UV-strobe. The instantaneous energy (J/pulse/cm²) was accumulated over time, resulting in a higher total energy (J/cm²) absorbed by the radiometer at the same distance from the UV-strobe. On the contrary, total energy received (J/cm²) decreased with longer distance from the UV-strobe for the same treatment time, which is the expected case according to the inverse square law (VanOsdell and Foarde, 2002).

Temperature change increased with longer treatment time at the same distance from the UV-strobe, which is most likely due to the generation of more heat by the UV-strobe during longer treatment times. On the other hand, temperature change decreased with longer distance from the UV-strobe for the same treatment time.

Conclusions

The findings of this study indicate that pulsed UV-light was effective in reducing the population of *Salmonella* Enteritidis PT8NSR on the surfaces of shell-eggs. The optimum treatment was 20-s at 9.5 cm from the UV-strobe, which resulted in ca. 5.3 log₁₀ CFU/cm² reduction or 99.999% reduction without any visual damage on the samples.

Energy and temperature of samples increased with longer treatment time and closer distance the UV-lamp. The maximum energy (35.3±0.1 J/cm²) was observed after 30 s treatment at 9.5 cm. The energy level obtained at the optimum condition was 23.6±0.1 J/cm². A maximum temperature increase of 10.5 ±1.2°C was observed after 30 s treatment at 9.5 cm. The temperature change observed at the optimum condition was 8.1±0.6°C.

In the future, the effect of pulsed UV-light on the quality of shell-eggs including albumen height, egg-shell strength, and presence of cuticle will be evaluated.

Acknowledgments

The pulsed UV-light system was obtained with funds from the NASA Food Technology Commercial Space Center equipment grant. We would like to thank Xenon Corporation for providing the pulsed UV-light system, and the Poultry Education and Research Center (PERC) at The Pennsylvania State University for providing the shell-eggs.

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