# STUDY OF THE UV RADIATION EFFECT ON THE INACTIVATION OF SALMONELLA ENTERICA SEROVAR ENTERITIDIS ON SHELL EGGS

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## Abstract

*The presence of Salmonella enterica serovar Enteritidis in shell eggs has serious public health implications. Several treatments have been developed to control Salmonella on eggs with mixed results. Currently, there is a need for time-saving, economical, and effective egg sanitization treatments. In this study, shell eggs externally contaminated with Salmonella ($8.0 \times 10^5$ to $4.0 \times 10^6$ CFU/g of eggshell) were exposed to UV radiation at 100 to 2500 $\mu W/cm^2$ for 0 to 5 minutes. Eggs that were (1) non-contaminated and untreated, (2) contaminated and untreated, and (3) contaminated and treated with air were used as controls. Results indicated that treating shell eggs with UV significantly ($P < 0,05$) reduced Salmonella on shell eggs. For example, contaminated eggs treated with UV (1500 to 2500 $\mu W / cm^2$) at 22 to 25°C for 5 minutes produced 4,3-log microbial reduction or more, when compared with contaminated untreated controls. Salmonella was effectively inactivated on shell eggs in a short time and at low temperature with the use of UV radiation.*
***Keywords:*** *Salmonella, Enteritidis, UV, eggshell.*

## Introduction

Presence of *Salmonella enterica* subsp. *enterica* serovar Enteritidis in shell eggs constitutes a public health hazard and poses a considerable economic impact on the egg industry. It is estimated that, in the United States, *Salmonella* transmission through contaminated shell eggs or egg products results in 700,000 cases of salmonellosis and costs $1.1 billion annually.

Chemical and physical sanitation procedures have been tested against *Salmonella* spp. on shell eggs with variable success. Some of these decontamination procedures include treatments with boiling water, chlorine and iodine, hydrogen peroxide, pulsed light, gas plasma, and electrolyzed oxidative water. Currently, there is a need for efficient low-temperature

treatments that inactivate large populations of *Salmonella* Enteritidis on shell eggs without affecting the quality of the product. Promising nonthermal technologies for decontaminating shell eggs include UV radiation and ozone. Irradiation with UV radiation inactivated microorganisms on surfaces, in air, and in liquids. Furthermore, UV radiation effectively reduced aerobic bacteria, molds, and *Salmonella* spp. on the surface of shell eggs. Equipment for UV radiation is relatively inexpensive and easy to use. Treatment with UV results in small amounts of heat if low-pressure UV lamps are used for a short time, and the process induces lethal effects to most types of microorganisms.

Therefore, the objective of this study was to determine, the efficiency of use of UV radiation at low temperature on the surface of shell eggs in order to eliminate *Salmonella* Enteritidis.

## Experimental

**Bacterial cultures and growth conditions.** *Salmonella* Enteritidis was obtained from the LSVSJ Buzau. Loops of the stock culture were transferred to brain heart infusion broth and incubated at 37°C for 24 h. Aliquots of the grown cultures (0.1% inoculum) were subsequently transferred in duplicate to 150 ml of MacConkey broth and incubated at 37°C for 24 h in a shaker under mild agitation. Aliquots (50 ml) of the resulting *Salmonella* Enteritidis cultures were centrifuged in duplicate at 3,000 X *g* for 10 min. Cell pellets were resuspended in 20 ml of sterile phosphate buffer (0,1 M, pH 7,0) at 22 to 25°C and mixed by a vortex mixer for approximately 10 s. The resulting cell suspension was centrifuged again and resuspended as previously described. This concentrated suspension was used to prepare 200 ml of working cell suspension in phosphate buffer as a diluent. The working suspension, poured into a 400-ml sterile beaker, had an optical density at 600 nm of approximately 0,4, and the cell density was 2,7 X $10^7$ to 1,0 X $10^8$ CFU/ml.

Inoculation of shell eggs with *Salmonella* Enteritidis.

Fresh, unfertilized shell eggs (53 ± 3 g per egg) were used. Shell eggs were refrigerated at 4°C and used within 3 weeks of laying. Selected refrigerated eggs were kept at 22°C for approximately 2 h, washed with tap water (22 to 25°C), and gently scrubbed with a plastic brush. Washed eggs were rinsed with distilled deionized water (22 to 25°C), and subsequently submerged in ethanol (70%, vol/vol) for 30 min. Sanitized shell eggs were transferred to sterile carton trays and aseptically dried at ambient

temperature for approximately 40 min before inoculation. Dried, sanitized shell eggs were dipped for approximately 10 s into a stirred *Salmonella* Enteritidis cell suspension prepared as described previously. Contaminated shell eggs were transferred to sterile carton trays and permitted to dry for approximately 30 min before treatments. *Salmonella* Enteritidis count on the externally contaminated shell eggs was 8,0 X $10^5$ to 4,0 X $10^6$ CFU/g of eggshell. Sanitized, noncontaminated shell eggs, dipped into sterile deionized water at 22 to 25°C, were used as negative controls.

**Treatment with UV.** Shell eggs, contaminated as previously described, were placed under a shortwave UV lamp (254 nm, 15 Watt) mounted on two cast-iron supports that allowed the adjustment of UV intensity by increasing or decreasing the vertical distance between the lamp and the treated eggs. Protective UV-absorbing face shields were used during experiments, and all safety precautions were observed when operating the UV lamp. Contaminated shell eggs, prepared as previously described, were aseptically transferred to the base of sterile glass petri plates, placed under the UV lamp, and irradiated at a previously calibrated vertical distance to achieve 100 $\mu W/cm^2$ or 1,500 to 2,500 $\mu W/cm^2$ light intensity for 5 min or less. Before each experiment, the UV lamp was turned on for approximately 15 min to achieve stable irradiation intensity. During UV treatments, shell eggs were constantly rotated manually with sterile metallic tongs to allow uniform surface exposure. Intensity of UV radiation was monitored with a radiometer probe and measured in a digital radiometer. Treated shell eggs were aseptically transferred to sterile carton trays and placed in the dark until analyzed.

Contaminated, untreated shell eggs were used as controls. *Salmonella* sp. was enumerated on the shells of treated eggs as described later.

**Enumeration of *Salmonella*.** Surface-contaminated eggs contain *Salmonella* in higher numbers in the eggshell pores and membranes than in egg contents. The pathogen can remain trapped inside the pores without invading the egg interior. Therefore, thoroughly homogenized eggshells and their membranes were analyzed in this study to assess external egg decontamination. Ten untreated eggs were cracked, their shells were separated and weighed, and the average weight of an eggshell was determined (7 ± 1 g). The individually treated and control shell eggs were aseptically placed with metallic tongs inside the upper part of a sterile polyethylene stomacher bag (18 by 30 cm). Each egg was manually held from outside the bag and carefully cracked by knocking on the outside of the bag with the blunt end of a knife blade. Egg contents (yolk and albumen)

were recovered into the stomacher bag. Two eggshells per experimental condition, separated from egg contents, were aseptically recovered from the upper part of the stomacher bag with sterile tongs, placed into blender jars, and used for enumeration of *Salmonella.* Sample preparation of eggshells was performed as described previously with modifications. Briefly, shells of two eggs treated under the same conditions were aseptically placed into a sterile 500-ml glass blender jar. Chilled, sterile peptone water (0,1%, 126 ml) was mixed with shells in a blender at high speed for 1 min. Homogenized shell debris was permitted to settle for approximately 1 min. Serial dilutions of the supernatant were made in peptone water, and 1-ml aliquots were mixed with plate count agar with the pour-plating technique. Plates were incubated at 37°C for 48 h, and colonies were counted. Detection limit of the procedure was 10 CFU/g of eggshell. Selected colonies were confirmed for *Salmonella* by streaking samples onto xylose lysine desoxycholate agar. Plates were incubated at 37°C for 24 h, and characteristic colony morphology of *Salmonella* spp. was observed.

## Results and Discussion

Inactivation of *Salmonella* Enteritidis by UV radiation.

Treatment of *Salmonella*-contaminated shell eggs with UV radiation (100 $\mu W/cm^2$) for 2 and 4 min significantly decreased *Salmonella* Enteritidis population by 2,6 and 2,0 log units, respectively, compared with the untreated controls (Table 1). The UV treatment resulted in a nonlinear inactivation trend (Table 1). Lack of efficacy with extended treatment might be caused by the limited penetrability of UV radiation in shells and to the shielding effect of the shell's porous surface that could limit direct exposure of entrapped bacteria to the radiation.

**Table 1**: Inactivation of Salmonella Enteritidis on shell eggs by UV radiation at 100 $\mu W/cm^2$

| Treatment | Treatment time (min) | Count (log CFU/g of eggshell) | Log reduction |
|---|---|---|---|
| Untreated (control) | 0 | 5.8 ± 0.1 | NA |
| UV treated (254 nm) | 2 | 3.2 ± 0.1 | 2,6 |
| | 4 | 3.8 ± 0.1 | 2,0 |

In another experiment, *Salmonella*-contaminated shell eggs were treated with higher UV radiation intensity (1,500 to 2,500 $\mu W/cm^2$) for up to 5 min. This treatment resulted in significant microbial reductions; UV

treatments for 1, 3, and 5 min decreased *Salmonella* populations by 3,4; 3,0 and 4,3 log units, respectively, compared with the untreated controls. However, no significant difference was observed when reductions in *Salmonella* populations after 1, 3, and 5 min of irradiation were compared. Researchers of a previous study also reported a 4,6-log inactivation of *Salmonella* on shell eggs after a treatment with UV radiation of lower intensity than that used in this study.

UV radiation inactivates microorganisms by inducing cross-linking between pyrimidine nucleotide bases in the DNA; this subsequently results in inhibition of DNA transcription and replication mechanisms and leads eventually to microbial cell death. In addition, UV radiation affects cell membrane integrity, induces protein modifications, and inhibits oxidative phosphorylation. The ability of some microbial cells to recover after UV radiation by enzymatic repair mechanisms could be a possible limitation to the use of UV treatments in food products.

## Conclusions

UV radiation effectively reduced *Salmonella* on shell eggs by 4,3 log units in 5 min and that makes the use of UV radiations a solution for decontaminating the shelleggs. This method, combined other, is capable of guarantying the safe of egg domestic consumption.

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## References

Berrang, M. E., N. A. Cox, J. S. Bailey, and L. C. Blankenship. 1991. Methods for inoculation and recovery of *Salmonella* from chicken eggs. *Poultry Sci.* 70:2267–2270.

Bintsis, T., E. Litopoulou-Tzanetaki, and R. K. Robinson. 2000. Existing and potential applications of ultraviolet light in the food industry - a critical review. *J. Sci. Food Agric.* 80:637–645.

Blatchley, E. R., III, and M. M. Peel. 2001. Disinfection by ultraviolet irradiation, p. 823–851. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation. Lippincott Williams Wilkins, Philadelphia.

Board, R. G., J. C. Ayres, A. A. Kraft, and R. H. Forsythe. 1964. The microbial contamination of eggshells and egg packing materials. *Poultry Sci.* 43:584–595.

Davies, R. H., and M. Breslin. 2003. Investigations into possible alternative decontamination methods for *Salmonella enteritidis* on the surface of table eggs. *J. Vet. Med.* 50:38–41.

Dunn, J. 1996. Pulsed light and pulsed electric field for foods and eggs. *Poultry Sci.* 75:1133–1136.

Hammack, T. S., P. S. Sherrod, V. R. Bruce, G. A. June, F. B. Satchell, and W. H. Andrews.1993. Growth of *Salmonella enteritidis* in grade A eggs during prolonged storage. *Poultry Sci.* 72:373–377.

Himathongkham, S., H. Riemann, and R. Ernst. 1999. Efficacy of disinfection of shell eggs externally contaminated with *Salmonella enteritidis.* Implications for egg testing. *Int. J. Food Microbiol.* 49: 161–167.

Juneja, V. K., and J. S. Novak. 2003. Adaptation of foodborne pathogens to stress from exposure to physical intervention strategies, p. 31–53. *In* A. E. Yousef and V. K. Juneja (ed.), Microbial stress adaptation and food safety. CRC Press, Boca Raton, Fla.

Knape, K. D., J. B. Carey, R. P. Burgess, Y. M. Kwon, and S. C. Ricke. 1999. Comparison of chlorine with an iodine-based compound on eggshell surface microbial populations in a commercial egg washer. *J. Food Saf.* 19:185–194.

Latala, A., and Z. Dobrzanski. 1989. Effect of ultraviolet radiation on the microbiological contamination of the shells of eggs for human consumption. *Med. Wet.* 45:420–422.

Russell, S. M. 2003. The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on the surface of eggs. *Poultry Sci.* 82:158–162.

Shama, G. 2000. Ultraviolet light, p. 2208–2214. *In* R. K. Robinson, C. Batt, and P. Patel (ed.), Encyclopedia of food microbiology. Academic Press, London.