

Inactivation of *Salmonella enterica* Serovar Enteritidis on Shell Eggs by Ozone and UV Radiation

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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* (8.0×10^5 to 4.0×10^6 CFU/g of eggshell) were treated with gaseous ozone (O_3) at 0 to 15 lb/in² gauge for 0 to 20 min. In other experiments, contaminated shell eggs were exposed to UV radiation at 100 to 2,500 $\mu\text{W}/\text{cm}^2$ for 0 to 5 min. Treatment combination included exposing contaminated eggs to UV (1,500 to 2,500 $\mu\text{W}/\text{cm}^2$) for 1 min, followed by ozone at 5 lb/in² gauge for 1 min. Eggs that were (i) noncontaminated and untreated, (ii) contaminated and untreated, and (iii) contaminated and treated with air were used as controls. Results indicated that treating shell eggs with ozone or UV, separately or in combination, significantly ($P < 0.05$) reduced *Salmonella* on shell eggs. For example, contaminated eggs treated with ozone at 4 to 8°C and 15 lb/in² gauge for 10 min or with UV (1,500 to 2,500 $\mu\text{W}/\text{cm}^2$) at 22 to 25°C for 5 min produced 5.9- or 4.3-log microbial reductions or more, respectively, when compared with contaminated untreated controls. Combinations including UV followed by ozone treatment resulted in synergistic inactivation of *Salmonella* by 4.6 log units or more in about 2 min of total treatment time. *Salmonella* was effectively inactivated on shell eggs in a short time and at low temperature with the use of a combination of UV radiation and ozone.

Presence of *Salmonella enterica* subsp. *enterica* serovar Enteritidis in shell eggs constitutes a public health hazard and poses a considerable economic impact on the poultry and 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 (11).

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 (13), chlorine and iodine (19), hydrogen peroxide (26), pulsed light (10), gas plasma (8), and electrolyzed oxidative water (27). 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 non-thermal technologies for decontaminating shell eggs include UV radiation and ozone (20, 23). Irradiation with UV radiation inactivated microorganisms on surfaces, in air, and in liquids (5, 28). Furthermore, UV radiation effectively reduced aerobic bacteria, molds, and *Salmonella* spp. on the surface of shell eggs (23, 24). 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 (5, 6).

Ozone (O_3) is a strong antimicrobial agent that has been studied extensively for its potential food industry applications (17, 18). The U.S. Food and Drug Administration has recently approved the use of ozone as an antimicrobial agent in food (31). Ozone decomposes spontaneously to a nontoxic product (i.e., oxygen), and it can be used effectively at low temperatures (1, 20). Ozone has been tested for disinfection of poultry facilities and products such as hatcheries, hatching eggs, poultry-processing chiller water, and poultry carcasses (3, 15, 18). In addition, ozone inactivates poultry-associated pathogens that routinely contaminate the surface of shell eggs, setters, and hatchers (15, 20, 32). A combination of ozone and UV radiation is effective against pathogenic microorganisms, including *Salmonella* spp. when present in poultry-processing chiller water (9). Therefore, considering the advantages of ozone and UV radiation, the objective of this study was to develop a sanitation procedure, at low temperature, to eliminate *Salmonella* Enteritidis on the surface of shell eggs with the use of gaseous ozone under mild pressure, UV radiation, and their combination.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. *Salmonella* Enteritidis was obtained from the culture collection of the Department of Microbiology at The Ohio State University (Columbus, Ohio). Loops of the stock culture were transferred to brain heart infusion broth (Difco, Becton Dickinson, Sparks, Md.) and incubated at 37°C for 24 h. Aliquots of the grown cultures (0.1% inoculum) were subsequently transferred in duplicate to 150 ml

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of MacConkey broth (Difco, Becton Dickinson) and incubated at 37°C for 24 h in an orbital shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) under mild agitation. Aliquots (50 ml) of the resulting *Salmonella* Enteritidis cultures were centrifuged in duplicate (Sorval RC-5B, Dupont Instruments, Bannoruck, Ill.) at $3,000 \times g$ 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 (Fisher Scientific Industries, Inc., Bohemia, N.Y.) 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 (OD_{600}) of approximately 0.4, and the cell density was 2.7×10^7 to 1.0×10^8 CFU/ml.

Inoculation of shell eggs with *Salmonella* Enteritidis.

Fresh, unfertilized shell eggs (53 ± 3 g per egg) were obtained from the poultry farm of The Ohio State University. 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 as described by Hammack et al. (12). 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 8.0×10^5 to 4.0×10^6 CFU/g of eggshell. Sanitized, noncontaminated shell eggs, dipped into sterile deionized water at 22 to 25°C, were used as negative controls.

Ozone generation. Gaseous ozone was produced in an electrochemical ozone generator (model LT 1, Lynntech, Inc., College Station, Tex.). The generator produced 12 to 14% ozone in oxygen (wt/wt, O_3/O_2 mix) at a gas mix flow rate of 1.45 liters/min. Experimental setup was kept inside a chemical fume hood, and all safety precautions were followed during experiments. Excess ozone was destroyed in a heated catalyst (Lynntech).

Ozone treatment. Ozone treatment setup is shown schematically in Figure 1. Eggs were treated with ozone in a gasket-sealed stainless steel vessel (4,000 ml, 21.6 cm diameter by 15.5 cm height; Alloy Products Corp., Waukesha, Wis.) adapted with a 30-lb/in² pressure gauge (Ashcroft, Dresser Inc., Stratford, Conn.). Before every experiment, the treatment vessel was refrigerated at 4°C overnight; subsequently, the vessel was immersed in ice to maintain its temperature at 4 to 8°C while the experiment was executed. Temperature inside the vessel was measured before ozone treatments with a glass thermometer (Ertco, Ever Ready Thermometer Co., West Paterson, N.J.). Sets of two eggs, inoculated as described previously, were placed within the treatment vessel. Gaseous ozone, generated as previously described, was delivered into the cold vessel (1.45 liters/min, O_3/O_2 mix) at atmospheric pressure for 8 min or less in a continuous treatment system. In a different experiment, shell eggs were treated in a batch mode with gaseous ozone at 15 lb/in² gauge or less for 20 min or less. Come-up time to achieve target pressure was approximately 2 min. After treatments, pressure was slowly released from the vessel in 2 to 3 min. Contaminated nontreated shell eggs

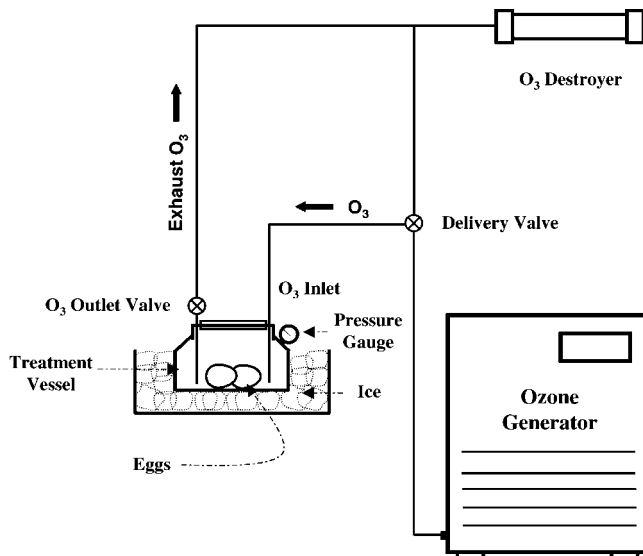


FIGURE 1. Experimental setup to treat shell eggs with gaseous ozone under pressure at low temperature.

were used as controls. Additional controls included shell eggs treated with compressed air (Medipure, Praxair, Inc. Danbury, Conn.) under the conditions described previously. Sets of treated, separated eggshells were tested for enumeration of *Salmonella* as described later.

Treatment with UV. Shell eggs, contaminated as previously described, were placed under a shortwave UV lamp (254 nm, 15 Watt, G15T8 General Electric, Co., Cleveland, Ohio) 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 $\mu\text{W}/\text{cm}^2$ or 1,500 to 2,500 $\mu\text{W}/\text{cm}^2$ 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 254-nm radiometer probe (model UVX-25, Ultraviolet Products, Inc., San Gabriel, Calif.) and measured in a digital radiometer (UVX-digital radiometer, Ultraviolet Products). 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.

Combination of treatments. Externally contaminated shell eggs were treated with UV radiation (254 nm, 1,500 to 2,500 $\mu\text{W}/\text{cm}^2$ intensity) at 22 to 25°C for 1 min. Immediately after UV treatment, shell eggs were aseptically transferred to the ozone treatment vessel, and gaseous ozone was applied at 5 lb/in² gauge and 4 to 8°C for 1 min. Come-up time required to achieve target treatment pressure was 30 to 40 s. After treatments, pressure inside the vessel was released in approximately 30 s. Treated and control shell eggs were analyzed for *Salmonella* count 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 (26). 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; Fisherbrand, Labplas, Inc., Quebec, Canada). 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 (7) with modifications. Briefly, shells of two eggs treated under the same conditions were aseptically placed into a sterile 500-ml glass blender jar (Fisher Scientific). Chilled, sterile peptone water (0.1%, 126 ml; Difco, Becton Dickinson) was mixed with shells in a blender (Waring model WPB05, Dynamics Corporation of America, New Hartford, Conn.) 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 (Difco, Becton Dickinson) 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 (Difco, Becton Dickinson). Plates were incubated at 37°C for 24 h, and characteristic colony morphology of *Salmonella* spp. was observed.

Statistical analyses. Experiments were performed in duplicate with four shell eggs per experimental condition. Statistical estimations included analysis of variance (ANOVA) and comparisons of means by Tukey ($\alpha = 0.05$). Data were analyzed in JMP IN (version 4.0.4 software, SAS Institute, Inc., Cary, N.C.).

RESULTS AND DISCUSSION

Inactivation of *Salmonella* Enteritidis by ozone.

Ozone treatment at atmospheric pressure for 3 min significantly ($P < 0.05$) reduced *Salmonella* Enteritidis on eggshell by 3.1 log units compared with the untreated control (Table 1). However, increasing treatment time to 5 and 8 min resulted in significantly ($P < 0.05$) higher microbial counts when compared with the 3-min treatment. In spite of this trend, ozone treatments for 5 and 8 min significantly ($P < 0.05$) reduced *Salmonella* population on eggshell by 2.3 and 2.6 log units, respectively, compared with the untreated control (Table 1). Ozone treatment, in general, rapidly decreased the *Salmonella* count on eggshell in 3 min, but longer treatment time (up to 8 min) did not cause additional inactivation. A similar inactivation pattern was observed previously when *Escherichia coli* O157:H7 was treated with aqueous ozone in a batch mode (1). Koidis et al. (20) reported that *Salmonella* Enteritidis was inactivated on surface-contaminated shell eggs during treatment with

TABLE 1. Inactivation of *Salmonella* Enteritidis on shell eggs when treated with gaseous ozone at atmospheric pressure and 4 to 8°C for up to 8 min

Treatment ^a	Treatment time (min)	Count (log CFU/g of eggshell) ^b	Log reduction ^c
Contaminated untreated (control)	0	6.3 ± 0.2 A	NA
	3	5.2 ± 0.1 B	1.1
Air-treated	5	5.3 ± 0.1 B	1.0
	8	5.4 ± 0.1 B	0.9
Ozone-treated	3	3.2 ± 0.3 C	3.1
	5	4.0 ± 0.1 D	2.3
	8	3.7 ± 0.1 D	2.6

^a Air treatment was at atmospheric pressure and ozone treatment with 12 to 14% (wt/wt) O₃/O₂ mix at atmospheric pressure.

^b Mean ± standard deviation (SD) obtained in experiments performed in duplicate with four eggs per experimental condition; means within columns not followed by the same letter are significantly different ($P < 0.05$).

^c Log count on the untreated minus log count on the treated. NA, not applicable.

aqueous ozone. Hunt and Mariñas (14) suggested that cellular debris from killed organisms could increase ozone demand, leaving less ozone available to maintain the high initial inactivation rate. A previous study indicated that calcium carbonate, the main mineral component of the eggshell, was almost inert to ozone (2). However, organic matter and proteins embedded in the eggshell matrix could consume ozone and reduce its availability for microbial inactivation (20).

Significant ($P < 0.05$) apparent microbial reduction by 0.9 to 1.1 log units was observed in eggs treated with air for 3 to 8 min compared with the untreated control (Table 1). Airflow might have helped bacteria penetrate into pores and crevices of eggshell, which would make it difficult to recover these embedded microorganisms by the sample preparation and counting technique used in this study (4, 7). Contaminated untreated shell eggs kept at 22°C for 8 min were used as additional controls; no significant ($P > 0.05$) difference in microbial counts was observed on these shell eggs compared with initial counts (data not shown).

Previously, Padron (26) indicated that the use of pressure increased penetration of sanitizers through eggshells and enhanced their effectiveness. In this study, pressurized ozone was applied at low temperature to *Salmonella*-contaminated shell eggs. Treatments were performed in a batch system with gaseous ozone at 15 lb/in² gauge. Application of pressurized ozone for up to 20 min resulted in nonlinear inactivation of the microorganism (Fig. 2), a trend similar to that observed when ozone was applied at atmospheric pressure (Table 1). Populations of *Salmonella* Enteritidis decreased significantly ($P < 0.05$) on shell eggs treated with pressurized ozone; the 10-min treatment inactivated 4.5 and 5.9 log units or more, and the 20-min treatment inactivated 3.7 and 5.7 log units or more compared with air-treated and untreated controls, respectively (Fig. 2). Application of pressurized air resulted in an apparent microbial

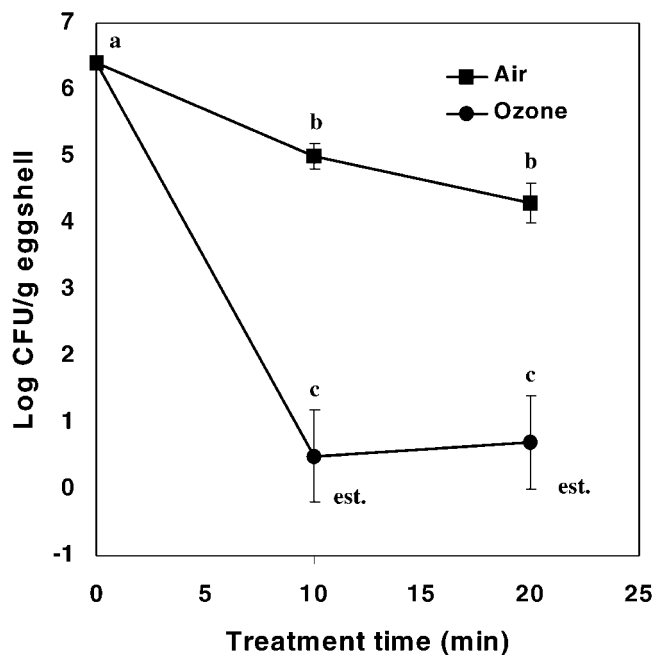


FIGURE 2. Counts of *Salmonella Enteritidis* on shell eggs treated with 12 to 14% gaseous ozone in oxygen (wt/wt, 15 lb/in² gauge, 4 to 8°C). Come-up time to achieve target pressure in the treatment vessel was 2 min. Points represent averages of microbial counts in experiments performed in duplicate with four shell eggs per experimental condition. Error bars indicate \pm SD. Different letters indicate significant ($P < 0.05$) differences among treatments. est., estimated count.

reduction by 1.4 and 2.0 log units after 10 and 20 min, respectively, compared with the untreated control (Fig. 2). This apparent microbial reduction might have resulted from increased penetration of cells into pores and crevices of eggshells and the difficulty in recovering these embedded bacteria by the counting procedure (4). Nonetheless, ozone treatment under pressure at low temperature proved to be effective in reducing *Salmonella* Enteritidis on shell eggs by 4.5 log units or more in 10 min regardless of the air effect (Fig. 2). Padron (26) observed that application of hydrogen peroxide under pressure increased the effectiveness of this sanitizer against *Salmonella* Typhimurium on the surface of shell eggs. Conversely, pressure did not increase the effectiveness of aqueous ozone in decontaminating other food products (29). Previous studies suggest that ozone inactivates microorganisms by reacting with the unsaturated lipids in the cell envelope of gram-negative bacteria, leading to changes in membrane permeability and subsequent lysis and death (18, 20). In addition, ozone might affect intracellular proteins and damage microbial genetic material (22).

Limited effectiveness of ozone against *Salmonella* on shell eggs in a previous study (20) could be the result of its use in an aqueous phase and at low concentrations. Moreover, it has been demonstrated that ozone stability is higher in the gaseous than the aqueous phase (18). Additionally, ozone in the gaseous phase is stable at low temperatures (21). Sanitizers under pressure have enhanced antimicrobial efficacy on shell eggs (26). Therefore, use of

TABLE 2. Inactivation of *Salmonella Enteritidis* on shell eggs by UV radiation at 100 μ W/cm²

Treatment	Treatment time (min)	Count (log CFU/g of eggshell) ^a	Log reduction ^b
Untreated (control)	0	5.8 \pm 0.1 A	NA
UV treated (254 nm)	2	3.2 \pm 0.1 B	2.6
	4	3.8 \pm 0.1 B	2.0

^a Mean \pm SD obtained in experiments performed in duplicate with four shell eggs per experimental condition; means within columns not followed by the same letter are significantly different ($P < 0.05$).

^b Log count on the untreated minus log count on UV treated. NA, not applicable.

gaseous ozone at low temperature and mild pressure could result in a higher number of ozone molecules available for microbial inactivation and increased effectiveness when used as a shell egg sanitizer. Results in this study show that pressurized gaseous ozone effectively inactivated *Salmonella* on shell eggs in 10 min (Fig. 2); however, this treatment time could be unacceptably long in an industrial setting.

Inactivation of *Salmonella* Enteritidis by UV radiation. Treatment of *Salmonella*-contaminated shell eggs with UV radiation (100 μ W/cm²) for 2 and 4 min significantly ($P < 0.05$) decreased *Salmonella* Enteritidis population by 2.6 and 2.0 log units, respectively, compared with the untreated controls (Table 2). The UV treatment resulted in a nonlinear inactivation trend, similar to that observed in the previously reported gaseous ozone experiment (Table 1 and Fig. 2). 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 (6, 23).

In another experiment, *Salmonella*-contaminated shell eggs were treated with higher UV radiation intensity (1,500 to 2,500 μ W/cm²) for up to 5 min. This treatment resulted in significant ($P < 0.05$) 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 (Fig. 3). However, no significant ($P > 0.05$) difference was observed when reductions in *Salmonella* populations after 1, 3, and 5 min of irradiation were compared (Fig. 3). Researchers of a previous study (23) 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 (6). In addition, UV radiation affects cell membrane integrity, induces protein modifications, and inhibits oxidative phosphorylation (5, 23). The ability of some microbial cells to recover after UV radiation by en-

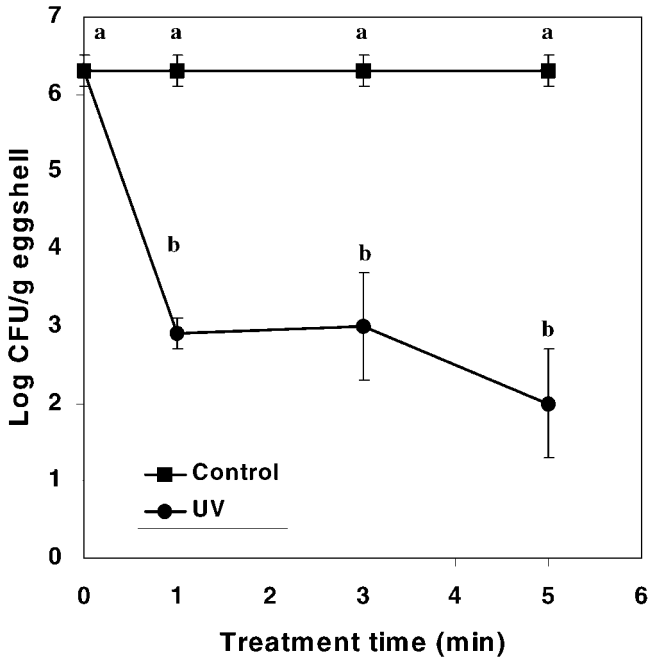


FIGURE 3. Counts of *Salmonella Enteritidis* on shell eggs treated with UV radiation (254 nm, 1,500 to 2,500 $\mu\text{W}/\text{cm}^2$) at 22 to 25°C. Points represent averages of microbial counts in experiments performed in duplicate with four shell eggs per experimental condition. Error bars indicate $\pm\text{SD}$. Different letters indicate significant ($P < 0.05$) differences among treatments.

zymatic repair mechanisms could be a possible limitation to the use of UV treatments in food products (28). However, Kuo et al. (23) observed that *Salmonella* Typhimurium treated by UV radiation on shell eggs did not recover after subsequent incubation under either dark or light conditions. Furthermore, Shama (28) suggested that UV-resistant mutants have only been generated under laboratory conditions and that concerns about possible occurrence of such mutants when UV treatments are applied in commercial facilities could be unsubstantiated. Although UV radiation effectively reduced *Salmonella* on shell eggs by 4.3 log units in 5 min (Fig. 3), process lethality was less than that observed when contaminated eggs were treated with pressurized gaseous ozone (Fig. 2).

Inactivation of *Salmonella Enteritidis* by combined treatments. According to the results just described, gaseous ozone applied under mild pressure effectively eliminates *Salmonella Enteritidis* on shell eggs (Fig. 2). In addition, UV radiation inactivates external microbial contamination on shell eggs within a short time (Fig. 3). Despite these results, the antimicrobial effectiveness of ozone, applied alone, might diminish when the sanitizer is used in foods because of their high organic content (25). Irradiation with UV, though effective against microorganisms, could not achieve product sterilization, and its use alone is not recommended to sanitize foods (16). Previous studies suggest that ozone, applied in combination with other treatments, has enhanced antimicrobial activity compared with the use of the sanitizer alone (9, 25, 30). Synergism is defined as the capability of combined treatments to produce

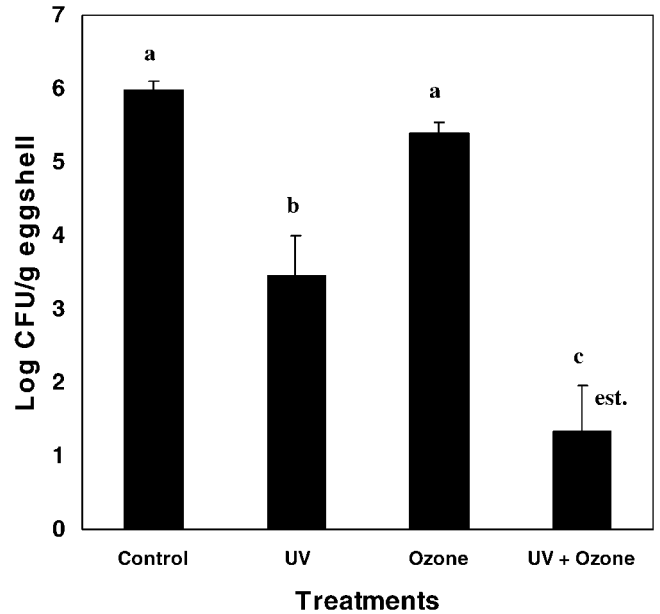


FIGURE 4. Counts of *Salmonella Enteritidis* on shell eggs treated with UV radiation (254 nm at 1,500 to 2,500 $\mu\text{W}/\text{cm}^2$, 22 to 25°C) for 1 min, gaseous ozone (12 to 14%, wt/wt at 5 lb/in² gauge, 4 to 8°C) for 1 min, or their combination. Bars represent averages of microbial counts in experiments performed in duplicate with four shell eggs per experimental condition. Error bars indicate $\pm\text{SD}$. Different letters indicate significant ($P < 0.05$) differences among treatments. est., estimated counts.

a greater antimicrobial efficacy than that attributed to the sum of the treatments applied individually (28). Treating food with combined factors is becoming a popular preservation strategy that is based on the ability of selected hurdles to act additively or synergistically against targeted pathogens (30).

Salmonella-contaminated shell eggs were treated with UV radiation (254 nm, 1,500 to 2,500 $\mu\text{W}/\text{cm}^2$) for 1 min, gaseous ozone (5 lb/in² gauge) at low temperature for 1 min, or their combination. Populations of *Salmonella Enteritidis* decreased significantly ($P < 0.05$) on shell eggs after treatment with UV radiation alone; the associated reduction was 2.5 log units compared with the untreated control (Fig. 4). A decrease in *Salmonella* populations on contaminated eggs treated with gaseous ozone under pressure for this short treatment time (1 min) was not significant ($P > 0.05$; Fig. 4). However, treatment of shell eggs with UV radiation followed by gaseous ozone under pressure decreased *Salmonella* count significantly ($P < 0.05$). *Salmonella* populations on eggshell decreased 4.6, 4.1, and 2.1 log units or more by the combined treatment compared with untreated, ozone-treated, and UV-treated eggs, respectively (Fig. 4). Moreover, microbial reduction after the combination treatment was an estimate determined by a plate count technique with a detection limit of 10 CFU/g of eggshell. A previous study reported enhanced effectiveness when ozone, hydrogen peroxide, and UV radiation were applied simultaneously (9). Simultaneous application of these sanitizing factors could cause photolysis of ozone and hydrogen peroxide by UV radiation to generate hydroxyl radicals, which are highly reactive with organic matter and microbial

cells. These treatment combinations are suitable for inactivating microorganisms in water, but not in solid foods (9, 18). In this study, use of UV radiation followed by a gaseous ozone treatment produced a strong synergistic antimicrobial action against *Salmonella* Enteritidis on shell eggs (Fig. 4). The combined treatment inactivated 4.6 log salmonellae or more on shell eggs in approximately 2 min of total treatment time. In comparison, a similar degree of inactivation required 5 min of UV irradiation and 10 min of gaseous ozone treatment when these sanitizing agents were applied separately (Figs. 2 and 3).

The use of UV radiation and ozone in sequence should have minimized the possibility of microbial reactivation that can occur when UV radiation is used alone (28). In addition, cells surviving sublethal UV radiation doses might synthesize stress proteins and become more resistant to subsequent lethal treatments (5, 6, 16). To prevent recovery and possible stress adaptation of microorganisms after UV radiation treatments, shell eggs were immediately treated with gaseous ozone. Increased penetrability of gaseous ozone under pressure could inactivate microorganisms previously injured by UV radiation and those shielded by the porous surface of the eggshells. Use of this treatment combination in reverse order was not attempted in this study. However, Novak and Yuan (25) observed that vegetative cells of *Clostridium perfringens* previously exposed to aqueous ozone on meat were highly susceptible to subsequent heat treatments.

Results of this study suggest that shell eggs externally contaminated with *Salmonella* Enteritidis could be effectively sanitized with gaseous ozone under mild pressure, UV radiation, and their combination. Furthermore, elimination of microorganisms could be achieved at low temperature, in a short period of time, and under relatively dry conditions. Therefore, use of combined treatments including UV radiation and ozone to eliminate *Salmonella* Enteritidis on shell eggs should be considered for possible applications in the egg industry. In addition, further studies must be considered to determine the effect of these treatments on egg quality.

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