

## Research Paper

# Comparison of UV-C and Pulsed UV Light Treatments for Reduction of *Salmonella*, *Listeria monocytogenes*, and Enterohemorrhagic *Escherichia coli* on Eggs

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## ABSTRACT

Ten percent of all strong-evidence foodborne outbreaks in the European Union are caused by *Salmonella* related to eggs and egg products. UV light may be used to decontaminate egg surfaces and reduce the risk of human salmonellosis infections. The efficiency of continuous UV-C (254 nm) and pulsed UV light for reducing the viability of *Salmonella* Enteritidis, *Listeria monocytogenes*, and enterohemorrhagic *Escherichia coli* on eggs was thoroughly compared. Bacterial cells were exposed to UV-C light at fluences from 0.05 to 3.0 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, for 5 to 300 s) and pulsed UV light at fluences from 1.25 to 18.0 J/cm<sup>2</sup>, resulting in reductions ranging from 1.6 to 3.8 log, depending on conditions used. Using UV-C light, it was possible to achieve higher reductions at lower fluences compared with pulsed UV light. When *Salmonella* was stacked on a small area or shielded in feces, the pulsed UV light seemed to have a higher penetration capacity and gave higher bacterial reductions. Microscopy imaging and attempts to contaminate the interior of the eggs with *Salmonella* through the eggshell demonstrated that the integrity of the eggshell was maintained after UV light treatments. Only minor sensory changes were reported by panelists when the highest UV doses were used. UV-C and pulsed UV light treatments appear to be useful decontamination technologies that can be implemented in continuous processing.

Key words: Egg; *Escherichia coli*; *Listeria monocytogenes*; *Salmonella* Enteritidis; UV light

Although producers strive to keep foods safe, food poisoning outbreaks still occur. In 2015, there were 95,000 registered cases of *Salmonella* in the European Union (EU), with a prevalence of 21 cases per 100,000 individuals. *Salmonella* in eggs was associated with the highest number of reported food outbreaks and accounted for 10% of all strong-evidence outbreaks in the EU (19). Foodborne outbreaks are registered in the EU's Rapid Alert System for Food and Feed (RASFF) and the Early Warning and Response System (EWRS) databases for enhanced awareness during the outbreaks. The prevalence of *Salmonella* on eggshells is approximately 0.03% on single samples and 0.5% on batch samples (18). Contaminated eggshells are more important than internal *Salmonella*, for which the prevalence is only approximately 0.005%. Not all eggs from *Salmonella*-infected flocks are necessarily contaminated (often less than 3% of the eggs). Because of the high number of food poisoning cases, egg safety is prioritized in a coordinated approach from the EU.

Eggs can become contaminated with bacteria from the hen's intestinal tract, feces, infested nests, or from the surrounding environment, including air and conveyor belts

during production (12, 50). The bacteria may penetrate the shell, contaminate internal contents of the egg, and cause damage or death to the embryo. The number of bacteria on eggs vary, with 4 to 5 log CFU per egg depending on the hygienic conditions (30). *Salmonella* infectious dose is 10<sup>2</sup> to 10<sup>3</sup> bacteria but can be as low as 15 to 20 bacteria (7). Implementation of control strategies for egg production (4) has successfully led to reduced incidences of food poisoning (5).

Eggs have traditionally been sanitized either by washing in water (29) or by immersing in disinfectant solutions. Washing of grade A table eggs is generally not allowed in the EU (3). The major disadvantage of egg washing is the potential damage to the physical barriers, such as the cuticle, which may favor trans-shell contamination with bacteria (20). Sanitation of hatching eggs is required to reduce problems with decreased hatchability, poor chick quality and growth (47), and cross-contamination during hatching (11). Commonly used disinfectants for hatching eggs involve formaldehyde fumigation or spraying with solutions containing chlorine-based or quaternary ammonium compounds (17). A number of other decontamination strategies for eggs have been examined, including use of *N*-halamine compounds, electrolyzed oxidative water, ozone, ionized air, sodium hydroxide, and hydrogen peroxide (14, 50, 55).

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Eggs will be downgraded in the EU if any forms of disinfections are used (55).

In recent years, the use of UV light as a surface decontamination method has been met with increasing interest (50). Regulations in conjunction with using conventional continuous UV-C light and pulsed UV light in the United States are given by the U.S. Food and Drug Administration (FDA) (51). UV-C light can be employed in the EU; however, in Germany the use is limited to water, fruit and vegetable products, and stored hard cheeses (2). UV-C light, primarily at 254 nm, provides effective inactivation of microorganisms by damaging nucleic acids creating nucleotide dimers, thus leaving the microorganisms unable to perform vital cellular functions.

When a mixture of *Salmonella* Typhimurium and four other serovars was subjected to UV-C light at 9 mW/cm<sup>2</sup> at a fluence of 0.14 J/cm<sup>2</sup>, 3- to 4-log reduction was observed (22). Coufal et al. (13) obtained a 4-log reduction of a strain of *Salmonella* Typhimurium on hatching eggs after a 3.4-J/cm<sup>2</sup> exposure. In contrast, only 1-log reduction of *Salmonella* was obtained after a 4-J/cm<sup>2</sup> UV-C treatment when eggs were stored for 2 days prior to UV-C treatment (48). When a strain of *Escherichia coli* and a *Listeria monocytogenes* strain were treated with UV-C light at 0.18 J/cm<sup>2</sup>, a 4-log reduction was obtained (15). Total aerobic plate counts were reduced by 1 to 3 log when subjected to UV-C at approximately 0.4 J/cm<sup>2</sup> (12). For a review, see Turtoi and Borda (50). Other articles also describe the use of UV-C light on eggs in combination with other methods, such as ozone treatment (45) and H<sub>2</sub>O<sub>2</sub> (1).

High-intensity pulsed UV light up to 12 J/cm<sup>2</sup> has been approved by the FDA as a means for controlling surface microorganisms on food products. The UV energy spectrum of pulsed UV light is caused by bremsstrahlung and covers the whole spectrum from UV (200 nm) into the infrared region (1,100 nm). In addition to creating nucleotide dimers, pulsed UV light has been suggested to cause cell death by induction of cell membrane damage (49) and rupture of the bacteria by overheating caused by absorption of all UV light from the flash lamp (52). Also, disturbances caused by high-energy pulses have been suggested to contribute to cell damage (33).

Varying results have been obtained when using pulsed UV light for decontamination of *Salmonella* on eggs. Dunn (16) obtained a >7.9-log reduction when a strain of *Salmonella* Enteritidis was exposed to a fluence of 4 J/cm<sup>2</sup>. In contrast, Hierro et al. (27) observed only a 1-log reduction using the same fluence, and 2 J/cm<sup>2</sup> giving only a 0.14-log reduction. When *Salmonella* was subjected to 1.2 to 35.3 J/cm<sup>2</sup>, reductions ranged from 2 to 7.7 log, the high value reached after a 30-s treatment using 90 pulses (31). A sigmoidal model for the inactivation of *Salmonella* on eggs has been developed (32). Factors affecting the efficacy of pulsed UV light for pathogens have been investigated by treating bacteria on petri dishes under different conditions (21).

The effectiveness of UV-C and pulsed UV light for decontamination depends on the length of time a microorganism is exposed, the intensity and wavelength of the illumination, the microorganism's ability to withstand the

UV exposure, properties of the food surface, the penetration of the UV light, and the presence of particles shielding the microorganisms. Varying results have previously been reported. In the present study, the efficiency of UV-C and pulsed UV light against bacteria on eggs under different conditions using the same sets of cells treated identically before various UV light treatments was compared. To our knowledge, a thorough comparison of the two methods for reducing bacteria on eggs has previously not been published. By using several strains of each species of *Salmonella* Enteritidis, *L. monocytogenes*, and enterohemorrhagic *E. coli* (EHEC), the aim was to obtain results representative for the species. The importance of presence of dry matter, presence of feces, and concentration of cells on efficiency of UV light treatment of eggs, which have previously not been studied, was also investigated. Moreover, the effects of different treatments on eggshell integrity was investigated and the sensory changes of the eggshell and egg content as a result of the UV exposures were evaluated. All these elements are important for practical implementation of UV technology in the industry.

## MATERIALS AND METHODS

**Bacterial strains, media, and growth conditions.** Three strains of *Salmonella* Enteritidis, four strains of EHEC, and four strains of *L. monocytogenes* were used (Table 1). The strains were maintained at -80°C in growth medium supplemented with 20% glycerol (v/v). Rifampin-resistant (Rif<sup>R</sup>) derivatives were prepared by growing strains in liquid media containing 200 µg/mL rifampin (Rif; Sigma-Aldrich, St. Louis, MO) as described by Heir et al. (26). Growth of the wild types and the Rif<sup>R</sup> strains were identical in their respective media and growth conditions when tested using a Bioscreen C instrument (Growth Curves Oy, Helsinki, Finland). The strains were cultured separately in tryptic soy broth (Oxoid, Hampshire, UK) with 200 µg/mL Rif (16 h incubation, 37°C) to 6 × 10<sup>8</sup> CFU/mL; thereafter, the different strains of the same species were mixed in equal amounts before the decontamination experiments.

**UV treatment of eggs inoculated with bacterial cells.** Fresh eggs were purchased from local retail stores. The surfaces of eggs were contaminated with approximately 10<sup>7</sup> CFU bacteria in 15 µL of broth by spreading with the pipette tip on approximately 8 cm<sup>2</sup>, unless otherwise stated. In some experiments, eggs were contaminated with 10<sup>7</sup> CFU of bacteria mixed with chicken feces per egg, where 10, 20, and 40 µL of contaminated feces were spread on a 4-cm<sup>2</sup> area, giving average thicknesses of about 0.025, 0.05, and 0.1 mm, respectively. Contaminated eggs were left at room temperature to dry for approximately 1 h prior to UV light treatment. In the continuous UV-C light experiments, samples were treated in a custom-made aluminum chamber (1.0 by 0.5 by 0.6 m<sup>3</sup>) equipped with two UV-C lamps (2 × 95 W; UV-C Kompaktleuchte, Bära GmbH, Leichlingen, Germany) in the ceiling. The UV-C light was emitted essentially at 253.7 nm, and the intensity was measured using a UVX Radiometer (Ultra-Violet Products Ltd., Cambridge, UK) with a UV-C sensor (model UVX-25, Ultra-Violet Products Ltd.). Samples were exposed at a power intensity of 10 mW/cm<sup>2</sup>, which is close to a maximum when using commercial lamps, and exposure times were 5, 10, 30, 60, or 300 s, giving fluences of 0.05, 0.1, 0.3, 0.6, and 3.0 J/cm<sup>2</sup>, respectively. For pulsed UV light treatments, the instrument XeMaticA-SA1L (SteriBeam Systems GmbH, Kehl-Kork am Rhein, Germany) was

TABLE 1. Strains used in this study

Species	Strain/serotype	Source	MF no. Rif <sup>Ra</sup>
<i>Salmonella</i> Enteritidis	1049-1-99	Norwegian Veterinary Institute, Norway	3817
	61-358-1	DTU Vet, National Veterinary Institute, Denmark	3818
		ATCC 13076 <sup>b</sup>	3824
<i>L. monocytogenes</i>	2230/92	Meat product, caused food poisoning in Norway in 1992 (38)	3508
	167	Knife in meat factory (10)	3509
	187	Meat product (10)	3510
	EGD-e	(23)	3571
EHEC	O103:H25	Rif mutant of MF2522, from dry fermented sausage, linked to outbreak in Norway in 2006 (46)	3572
	O157:H7	ATCC 43895	3574
	O111:H <sup>-</sup>	Semidry fermented sausage, outbreak in Australia in 1995 (42)	3576
	O145	Rif mutant of MF2493, human clinical strain, sporadic (37)	5554

<sup>a</sup> Reference number of strains after mutating to Rif<sup>R</sup>. Strains with MF numbers are from Nofima's strain collection.

<sup>b</sup> ATCC, American Type Culture Collection, Manassas, VA.

used. The instrument was equipped with a xenon flash lamp (19 cm), which was water cooled, with an aluminum reflector (with opening 10 by 20 cm), and emitted light of 200 to 1,100 nm with up to 45% of the energy being in the UV-light region with maximal emission at 260 nm for high-energy pulses (SteriBeam Systems GmbH). Eggs were illuminated at 6.5 cm distance barely beneath the opening of the reflector. At this distance, the fluence could be calculated according to the manufacturer's specifications as the total discharge energy of the lamp divided by the opening area of the reflector. The fluence of each pulse was adjusted to 1.25 J/cm<sup>2</sup> (low) or 3.6 J/cm<sup>2</sup> (high) by adjusting the discharge voltage. The samples were exposed with single pulses, either once to the low pulse, or one, three, or five times to the high pulse (3.6, 10.8, or 18.0 J/cm<sup>2</sup>), respectively. After illumination, the treated areas were swabbed to collect surviving bacteria. Temperatures were measured using a Raynger MX infrared thermometer (Raytek Corporation, Santa Cruz, CA). The experiments were performed in a biosafety level 3 pilot plant.

**Analysis of cells.** After UV light treatments, the eggs were swabbed, and the collected surviving bacteria were plated onto tryptic soy agar (TSA; Oxoid) with 200 µg/mL Rif using an automated plate spreader (Whitley Automatic Spiral Plater, Don Whitley Scientific Ltd., West Yorkshire, UK) and incubated overnight at 37°C. The number of colonies were determined using an automatic plate reader. Because Rif<sup>R</sup> strains were used, the background flora on the eggs were negligible.

**Effect of UV-C light intensity.** The effect of the UV-C light intensity on reduction of *Salmonella* was determined by exposing contaminated eggs to pairwise similar fluences. Treatments were 10 mW/cm<sup>2</sup> for 5 s and 2 mW/cm<sup>2</sup> for 25 s (fluence of 0.05 J/cm<sup>2</sup> in both cases), and 10 mW/cm<sup>2</sup> for 30 s and 2 mW/cm<sup>2</sup> for 150 s (fluence 0.3 J/cm<sup>2</sup> in both cases).

**Photoreactivation.** Eggs were contaminated with 10<sup>6</sup> CFU *Salmonella* and exposed to UV-C light at a fluence of 0.05 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 5 s) or pulse UV light at 1.25 J/cm<sup>2</sup>. For detection of photoreactivation, the treated eggs were thereafter either subjected to low or a high UV-A dose as follows: eggs were subjected to low intensity UV-A light for 3 h at room temperature in a dark room using a TW6W Black light Blue lamp (emission spectrum in the 300 to 400 nm range with maximum at 365 nm; Heraeus Noblelight, Cambridge, UK) at approximately 0.01 mW/cm<sup>2</sup>, or

high UV-A dose by using a Black Light Euroline UV-A lamp at approximately 0.9 mW/cm<sup>2</sup> (35 W; Steinigke Showtechnic GmbH, Waldbüttelbrunn, Germany) at a distance of 40 cm. Appropriate controls (exposed to the same UV-C or pulsed UV light doses) were stored in the dark for 3 h. The eggs were, thereafter, swabbed and cells were plated on TSA with 200 µg/mL Rif and incubated at 37°C overnight. For testing whether photoreactivation could be detected under optimal conditions, eggs were exposed to UV-C and pulsed UV light as described above, before they were immediately swabbed to collect bacteria. The collected bacteria were plated on TSA with 200 µg/mL Rif and subjected to the UV-A treatments as described above while on the petri dishes (with the lids removed) rather than on dry eggs, before incubation at 37°C overnight.

**Testing for *Salmonella* eggshell transfer after UV light treatment.** Eggs were illuminated with 3.0 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 5 min) UV-C light or 10.8 J/cm<sup>2</sup> pulsed UV light. Testing for bacterial transfer, the illuminated area was thereafter contaminated with 10<sup>7</sup> CFU *Salmonella* and incubated for 24 h at room temperature in a closed box. Nonilluminated controls contaminated on the outside were kept in the same box, and as positive controls, two eggs were contaminated in the egg white. After incubation, the pointy end of the egg was flame sterilized for 10 s, a hole was made using a sterile scalpel, and the egg content was transferred to a stomacher bag, essentially as described by Himathongkham et al. (28). The content was homogenized for 2 min, and 25 g was removed for qualitative detection of *Salmonella* according to Nordic Committee on Food Analysis, International Organization for Standardization (NMKL ISO) method 6579:2002 (39).

**Scanning electron microscopy.** Eggs were exposed to UV-C light or pulsed UV light. Eggshell samples were thereafter coated with gold-palladium by using a Polaron Sputter Coater (SC 7640, Leica Microsystems, Wetzlar, Germany). The coated samples were examined and photographed with a Zeiss EVO-50-EP scanning electron microscope (Zeiss Microscopy GmbH, Jena, Germany) at an accelerating voltage of 20 kV in the secondary emission mode.

**Sensory evaluation.** Ten well-trained sensory assessors were selected and educated according to recommendations in ISO 8586:2012 ("General Guidelines for the Selection, Training and Monitoring of Selected Assessors, and Expert Sensory Assessors"), and ISO 13299:2003 ("General Guidance for Establishing a

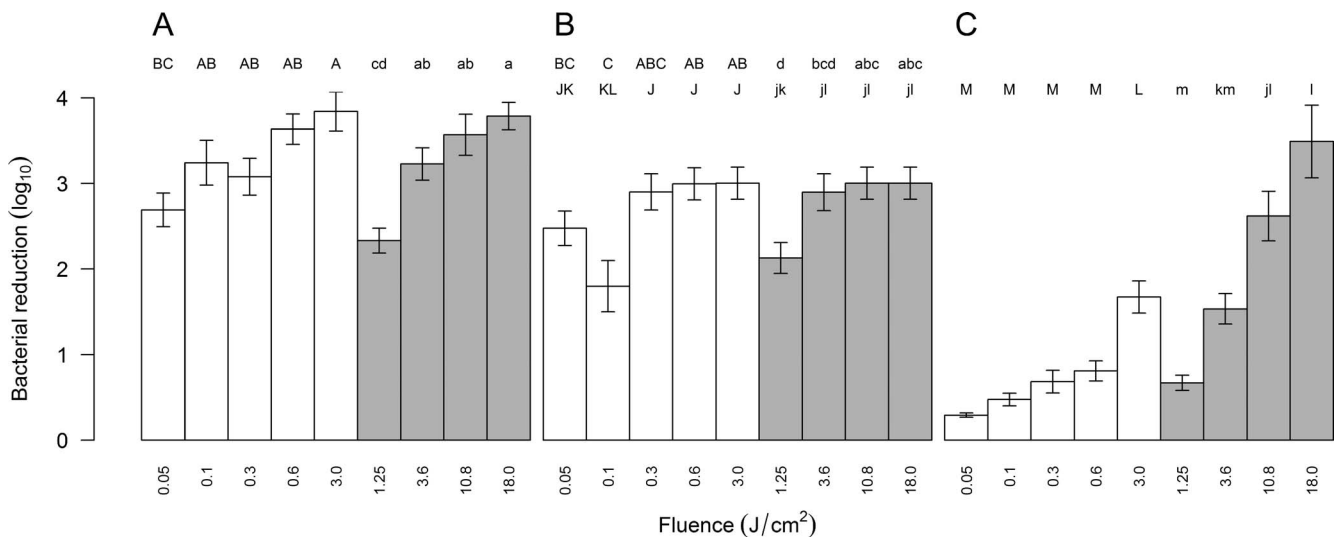


FIGURE 1. Reduction of *Salmonella* on clean eggs by UV-C (white bars) and pulsed UV (gray bars) light treatments. (A) *Salmonella* cells from overnight cultures were mixed and applied directly onto 8 cm<sup>2</sup> of the egg surface. (B) *Salmonella* cells from overnight cultures were washed twice in distilled water before being applied onto 8 cm<sup>2</sup> of the egg surface. (C) Washed *Salmonella* cells were applied onto 3 mm<sup>2</sup> of the egg surface. The UV fluences are indicated and correspond to 10 mW/cm<sup>2</sup> for 5, 10, 30, 60, and 300 s for UV-C light. Samples with upper- and lowercase letters were analyzed separately by ANOVA, and samples containing the same letter were not considered different. Letters A to D and a to d were used to compare treatments of unwashed and washed cells, whereas J to M and j to m were used to compare treatments of washed cells on large and small areas.

Sensory Profile”). Panelists were given treated intact eggs for examination; eggs were then broken and the interior was examined. The samples were untreated control, eggs treated with UV-C light at 0.1 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 10 s), eggs treated with UV-C light at 0.6 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 60 s), eggs exposed to pulsed UV light at low intensity (1.25 J/cm<sup>2</sup>), and eggs exposed to pulsed UV light three times at high intensity (10.8 J/cm<sup>2</sup>). The samples were served at room temperature on white dishes identified by random three-digit numbers. Each panelist’s results were recorded at individual speed on a 15-cm nonstructured continuous scale, with the left side of the scale corresponding to the lowest intensity and the right side of the scale corresponding to the highest intensity. The computer transformed the responses into numbers between 1 (low intensity) and 9 (high intensity). Samples were served in two replicates and in randomized order, following a balanced block experimental design. Sixteen sensory attributes were evaluated in the descriptive sensory analysis of the raw eggs: odor of the eggshell (chalk, sunburn, burnt, metallic, sulfur, hen house, cloying), odor of broken egg (sourness, sunburnt, burnt, metallic, sulfur, sweetish, cloying), and appearance of egg content (transparency of egg white and clarity of egg yolk).

**Statistical analysis.** Three parallels of UV light-treated samples and three or six untreated controls were used in each experiment. The experiments were repeated three times on different days. Analysis of variance (ANOVA) was used to determine statistically significant effects on the bacterial reduction by the treatments. All analyses were performed in R (44). To identify the sensory attributes that discriminated between samples, ANOVA, using a two-way model with interactions and with the assessor and interaction effects considered random, was performed on the descriptive sensory data from the trained panel. The statistical software used in sensory analysis was EyeOpenR (Logic8 BV, Utrecht, The Netherlands).

## RESULTS

**Reduction of *Salmonella* on clean eggs.** *Salmonella* Enteritidis strains in growth medium were mixed and applied to the surface of clean eggs. After drying, the eggs were subjected to various continuous UV-C and pulsed UV light treatments, resulting in bacterial reductions between 2.3 and 3.8 log, depending on the UV dose (Fig. 1 and Supplemental Material Table S1). Some increase in reduction could be obtained by increasing the UV dose, but this strategy appeared limited, as was also indicated from Weibull models constructed from the reduction data (Supplemental Material Table S2 and Fig. S1). For the UV-C light treatments, increasing the dose 60-fold gave only a 1.1-log increase in reduction. By comparing the UV-C light results using ANOVA, the shortest treatment at 0.05 J/cm<sup>2</sup> was considered statistically different from the treatment of the longest duration and 3.0 J/cm<sup>2</sup>. Likewise, some increase in reduction could be observed from low intensity (1.25 J/cm<sup>2</sup>) to high intensity (3.6 J/cm<sup>2</sup>) for the pulsed UV light exposures; however, statistically, no additional increase was achieved by further increasing the fluence to 10.8 and 18.0 J/cm<sup>2</sup>. In control experiments in which *Salmonella* was spread on agar plates and subjected to the same treatments, a 5- to 6-log reduction was obtained even with the mildest UV treatments.

Coinciding results were obtained with cells washed twice in distilled water and thereafter spread on a similar area. The reduction was 2.5 and 2.1 log after UV-C light treatment at 0.05 J/cm<sup>2</sup> and pulsed UV light treatment at 1.25 J/cm<sup>2</sup>, respectively. Increasing the UV doses further enhanced the reduction to approximately 3 log for both UV methods. When comparing the UV reduction results from

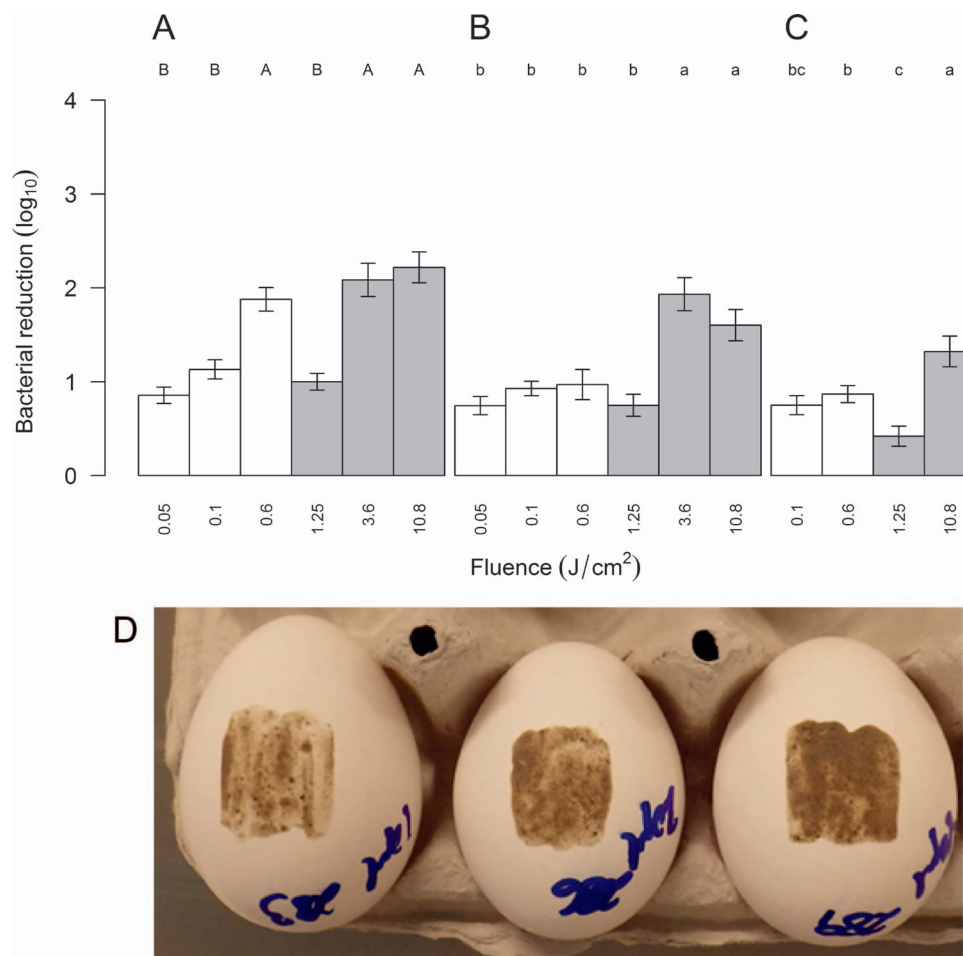


FIGURE 2. Reduction of *Salmonella* on eggs with chicken feces by UV-C (white bars) and pulsed UV (gray bars) light treatments. *Salmonella* was mixed with chicken feces and applied onto 4 cm<sup>2</sup> of the surface of eggs in layers of different thicknesses of 0.025 mm (A), 0.05 mm (B), and 0.1 mm (C), as shown on eggs from left to right, respectively, in (D). The UV fluences are indicated and correspond to 10 mW/cm<sup>2</sup> for 5, 10, and 60 s for UV-C light. Samples with upper- and lowercase letters were analyzed separately by ANOVA, and samples containing the same letter were not considered different.

washed and unwashed cells by ANOVA, no significant differences were found. However, when the same amount of washed bacteria was applied to a small area, a significantly lower reduction was achieved after UV-C light treatment, with only 0.8-log reduction after a 0.6-J/cm<sup>2</sup> treatment, increasing to 1.7 log after treatment at 3.0 J/cm<sup>2</sup>. For pulsed UV light, the reduction increased with increasing UV fluence from 0.7 log at 1.25 J/cm<sup>2</sup> to 3.5 log at 18 J/cm<sup>2</sup>. The temperature increase of the eggs during UV treatments was negligible, transiently at most a few degrees centigrade on the exposed surface.

**Reduction of *Salmonella* on dirty eggs.** A mixture of *Salmonella* strains and chicken feces was applied to eggs, which were then subjected to UV-C and pulsed UV light treatments (Fig. 2). Reductions of bacteria in feces were lower in comparison with the corresponding treatments of bacteria in growth medium or water. Between 0.8- and 2.2-log reduction was observed, depending on the UV dose and the thickness of the feces layer. For the UV-C light, a 0.9-log reduction was obtained after the 0.05-J/cm<sup>2</sup> treatment in the thinnest feces layer, increasing to 1.9 log after the 0.6-J/cm<sup>2</sup>

treatment. For the thicker layers, reductions remained at 0.8 to 1.0 log regardless of treatment. Pulsed UV light exposure at low fluence (1.25 J/cm<sup>2</sup>) gave reductions of 0.4 to 1.0 log, depending on the feces thicknesses. Clearly, an increased reduction for all feces layers was observed when the fluence was increased, with reductions ranging from 1.3 to 2.2 log, indicating a higher penetration of the pulsed UV light.

**Reductions of *L. monocytogenes* and EHEC.** Strains of *L. monocytogenes* and EHEC in growth medium were applied to the surface of clean eggshells, as described above for *Salmonella*. After drying, the eggs were subjected to UV-C light and pulsed UV light treatments (Fig. 3). Reduction of *L. monocytogenes* was between 1.8 and 3.7 log, depending on the UV dose. By comparing the UV-C light results using ANOVA within *L. monocytogenes*, only the 0.05- and the 3.0-J/cm<sup>2</sup> treatments were considered statistically different, indicating that increasing the UV dose will give minor increase in reductions, as was also apparent from the Weibull models constructed from the reduction data (Fig. S2). Similarly, for the pulsed UV light treatment, only the 1.25-J/cm<sup>2</sup> treatment was considered statistically

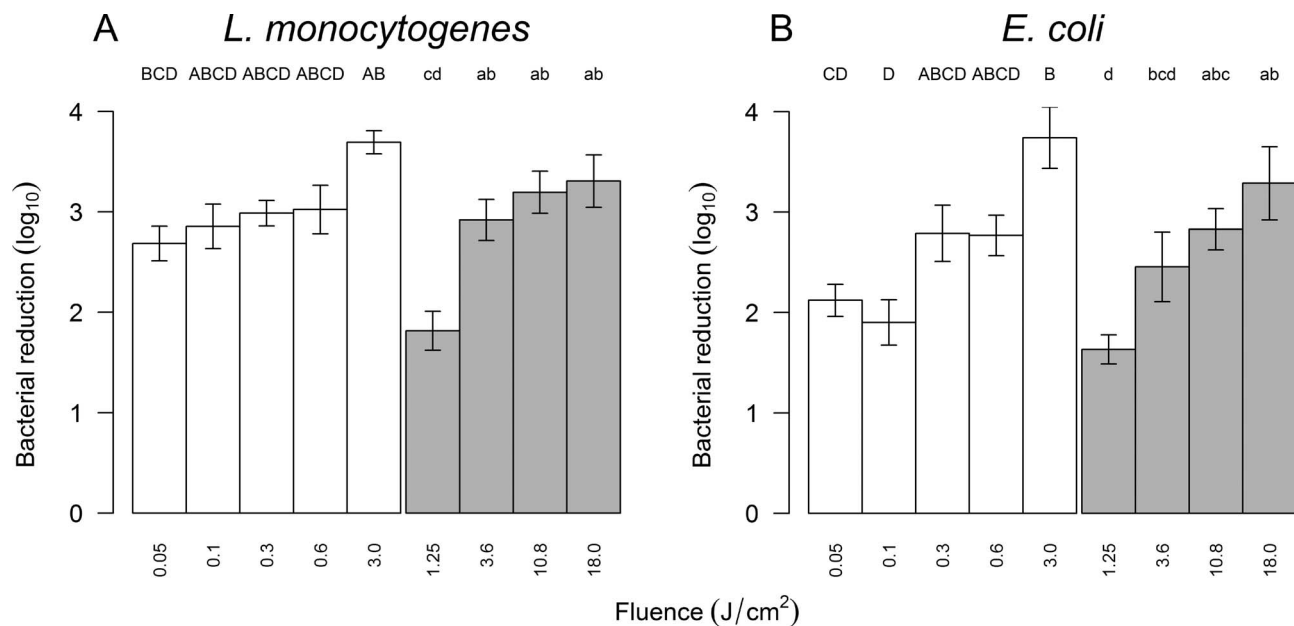


FIGURE 3. Reduction of (A) *L. monocytogenes* and (B) EHEC on clean eggs by UV-C (white bars) and pulsed UV (gray bars) light treatments. Cells from overnight cultures were mixed and applied onto 8 cm<sup>2</sup> of the egg surface. The UV light fluences are indicated and correspond to 10 mW/cm<sup>2</sup> for 5, 10, 30, 60, and 300 s for UV-C light. Samples with upper- and lowercase letters were analyzed separately by ANOVA, and samples containing the same letter were not considered different. ANOVA was performed with *L. monocytogenes*, EHEC, and *Salmonella Enteritidis* (results from Fig. 1A) as levels in a factor of a joint analysis.

different from the other pulsed UV light treatments, and increasing the fluence beyond 3.6 J/cm<sup>2</sup> gave little increase in reduction. Similar results were obtained with the EHEC strains tested. Reductions ranged from 1.6 to 3.7 log, depending on the treatment. For the UV-C light, testing for EHEC separately, only the treatment at 3.0 J/cm<sup>2</sup> was statistically different from the other treatments. For pulsed UV light, the 1.25 J/cm<sup>2</sup> treatment gave a lower reduction than the higher fluence treatments.

When comparing similar treatments for *Salmonella*, *L. monocytogenes*, and EHEC using ANOVA, the different bacterial species showed very similar sensitivities against both continuous UV-C light and pulsed UV light. In control experiments in which the same *L. monocytogenes* and EHEC strains were spread on agar plates and subjected to the same treatments, generally between 5- and 6-log reductions were obtained even after the mildest UV treatments, similar to the results for *Salmonella*.

**Effect of UV-C light intensity.** Treatments at 10 mW/cm<sup>2</sup> for 5 s and at 2 mW/cm<sup>2</sup> for 25 s, giving a fluence of 0.05 J/cm<sup>2</sup> in both cases, resulted in 2.7- and 3.2-log reductions, respectively. No statistical difference was detected by ANOVA between the two treatments. For treatments at 10 mW/cm<sup>2</sup> for 30 s and at 2 mW/cm<sup>2</sup> for 150 s (both 0.3 J/cm<sup>2</sup>), which gave reductions of 3.2 and 4.1 log, respectively, a statistical difference was observed with higher reduction at the lower intensity and longer exposure time.

**Testing for photoreactivation.** Photoreactivation experiments were carried out to determine whether cells exposed to UV-C or pulsed UV light were dead or could be

rescued by induction of UV-A light inducible repair systems. *Salmonella* on eggs that had been treated with UV-C light at fluence 0.05 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup> for 5 s) or pulsed UV light at 1.25 J/cm<sup>2</sup> showed no photoreactivation when they were later exposed to two different fluences of UV-A light for 3 h (results not shown). For *Salmonella* cells on eggs that were treated similarly with UV-C or pulsed UV light but that were transferred to petri dishes to provide optimal opportunities for photoreactivation before the dishes were exposed to the same two intensities of UV-A light, there was also not increased survival by photoreactivation; thus, the cells seemed to be killed and not damaged when exposed to UV-C and pulsed UV light at the fluences tested.

**Eggshell integrity.** The integrity of the eggshells after UV light treatments was examined by exposing eggs to UV-C light at 3.0 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 5 min) or pulsed UV light at 10.8 J/cm<sup>2</sup>. The eggs were subsequently contaminated with *Salmonella* and left for 24 h before the egg content was examined for *Salmonella* passing through the shell (Table 2). No *Salmonella* was detected inside the eggs, indicating that the UV treatments do not change the barrier properties of the eggshell. To further investigate this, eggs exposed to UV-C light at 0.6 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 60 s) and pulsed UV light at 10.8 J/cm<sup>2</sup> were examined by scanning electron microscopy (Fig. 4). No changes were visible in the proteinaceous cuticle after the UV treatments compared with the untreated controls.

**Sensory evaluation.** Ten trained assessors evaluated eggs exposed to UV-C light at 0.01 and 0.6 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 10 and 60 s, respectively), and pulsed UV light at 1.25 and 10.8 J/cm<sup>2</sup>. Treated intact eggs at room temperature

TABLE 2. Testing for increased permeability after UV-C and pulsed UV light treatments of eggs

Treatment	<i>Salmonella</i> positive/total <sup>a</sup>
Continuous UV-C, 3.0 J/cm <sup>2</sup>	0/13
Pulsed UV, 10.8 J/cm <sup>2</sup>	0/12
No UV, control <sup>b</sup>	0/13
Contaminated control <sup>c</sup>	2/2

<sup>a</sup> Qualitative determination of *Salmonella* according to NMKL ISO 6579:2002.

<sup>b</sup> Eggs contaminated with *Salmonella* on the outside.

<sup>c</sup> Eggs deliberately contaminated with *Salmonella* in the egg white.

were examined for odor (Fig. 5). The eggs were then broken, and the egg contents were examined for odor and transparency. Generally, small differences were registered for intact eggs treated with UV light. Most notably, treatment with the highest fluence of pulsed UV light gave a higher intensity of sunburnt odor, sulfur odor, and henhouse odor compared with the untreated controls. Sunburnt odor is associated with that of sunburnt human skin. Some increase in this attribute was also found for the egg content after exposure to the highest doses of both UV-C light and pulsed UV light. However, a sensory intensity value of 2 is considered very low.

## DISCUSSION

To avoid possible changes in sensory perception, it is desirable to maximize the reduction of bacteria without treating the eggs more than necessary. The fluence treatment levels for UV-C light were selected from low levels suitable for practical use, at which limited kill of bacteria was observed, up to fluences comparable to those of the pulsed UV light. Pulsed UV light was tested at fluences from 1.25 J/cm<sup>2</sup> up to and above the limit value of 12 J/cm<sup>2</sup> determined by FDA. The fluences of the two methods are not directly comparable because the different wavelengths in the UV spectrum have different germicidal effectiveness (9). For bacteria spread over a large area on the egg, the germicidal effect of UV-C light with a fluence of 0.05 J/cm<sup>2</sup> was comparable to a fluence of 1.25 J/cm<sup>2</sup> for the pulsed UV light (Fig. 1). The higher germicidal effect at lower fluence for the UV-C light is likely explained by most of the energy being emitted at 254 nm, at which relative germicidal effect is close to the maximum (9). The germicidal effect also seemed to be influenced by intensity-time combinations, as seen in the difference in reduction at pairwise similar fluences. Limited dose-response effects in the ranges tested are likely caused by shading effects of the irregular surface structure of the eggs (53). The efficacy of using UV light for decontamination of foods is often lower than when tested on clean surfaces (25). UV light does not penetrate well through organic matter, such as protein and other organic matrices, and the penetration into opaque matter seems to depend on the type of matter. Medium components did not shield cells from UV exposure; this agrees well with results obtained when bacteria dissolved in fetal calf serum were plated on petri dishes, where no differences in levels of inactivation were observed (21). Pulsed UV light has been reported to

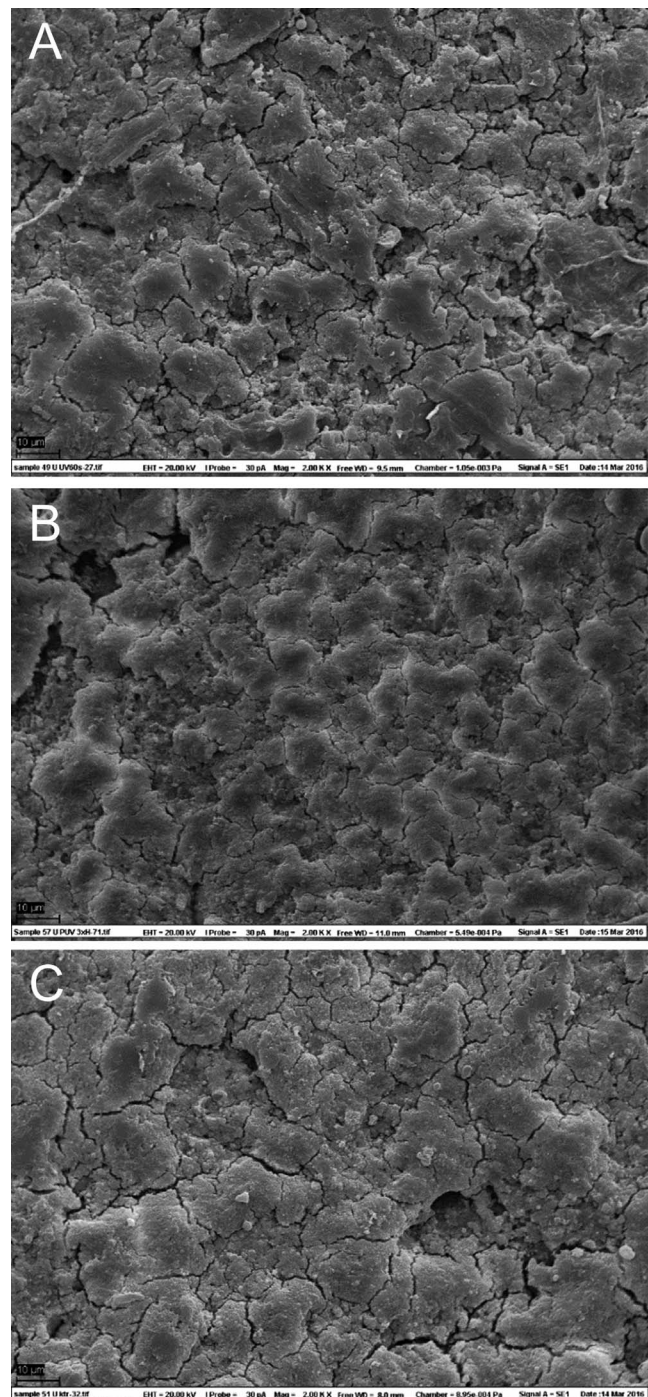


FIGURE 4. Scanning electron microscopy of eggshell surfaces after UV light treatments. (A) UV-C light treatment at fluence 0.6 J/cm<sup>2</sup> (10 mW/cm<sup>2</sup>, 60 s); (B) pulsed UV light treatment, at fluence 10.8 J/cm<sup>2</sup>; (C) untreated control.

penetrate opaque denatured whey protein isolate down to at least 10 mm (8). However, impaired germicidal effect was evident when bacteria were shielded by fecal matter (Fig. 2), and a higher penetration of pulsed UV light compared with UV-C light was evident. Bacteria stacked on top of each other can also create a shadowing effect, as has been reported for colonies of *L. monocytogenes* growing on petri dishes, where the upper cells of a colony appeared to protect the lower cells against pulsed UV light (24). Such a

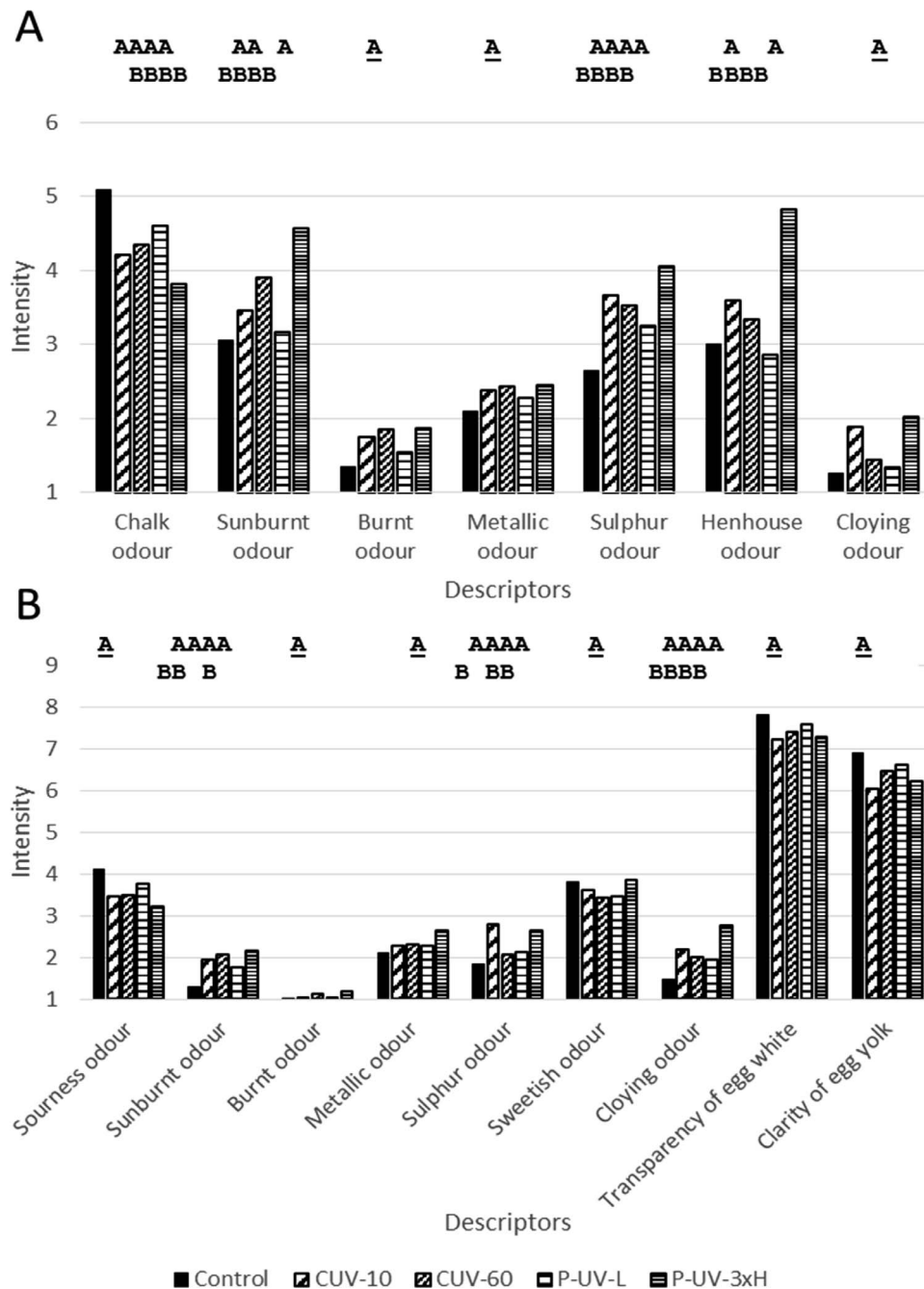


FIGURE 5. Sensory analysis of (A) intact eggs and (B) egg content. Eggs were exposed to UV-C light at fluences 0.1 J/cm<sup>2</sup> (CUV-10, 10 mW/cm<sup>2</sup>, 10 s) and 0.6 J/cm<sup>2</sup> (CUV-60, 10 mW/cm<sup>2</sup>, 60 s) and to pulsed UV light at fluences 1.25 J/cm<sup>2</sup> (P-UV-L) and 10.8 J/cm<sup>2</sup> (P-UV-3xH). The intensities of different odors of intact eggs were registered. The eggs were then opened and the contents of the same eggs were evaluated. 1, low intensity; 9, high intensity. The letters above the columns indicate grouping according to ANOVA and Tukey multiple comparison test. Samples with the same letter are considered to be equal for the specific property. Underlined A indicates that all intensities belong to the same group for that specific property.

shadowing effect was clearly observed when bacteria were applied on small spots on the eggs (Fig. 1C). The highest intensities of pulsed UV light appeared to partly overcome this shadowing effect. This increased reduction may, in part, also be caused by local overheating of bacteria due to absorption of UV light of many different wavelengths (33, 52).

In accordance with our findings, very high *Salmonella* reductions have been achieved on smooth surfaces. Gomez-Lopez et al. (24) reported a >6.85-log reduction on petri

dishes after pulsed UV light treatment, and Paskeviciute et al. (41) observed a 6.5- to 7-log reduction. Our results on eggs were in good agreement with several previous reports. When eggs were treated with UV-C light at fluences 0.15, 0.45, and 0.75 J/cm<sup>2</sup> (2.5 mW/cm<sup>2</sup> for 1, 3, and 5 min, respectively), reductions from 3.0 to 4.3 log were obtained (45). These resulting reductions were not statistically different from each other, which reflects the often relatively large variation that is probably due to differences of the egg surface. Gao et al. (22) obtained a 2.5- to 4-log reduction of



*Salmonella* on eggs after a treatment at 0.09 J/cm<sup>2</sup> (9 mW/cm<sup>2</sup>, 10 s) depending on contamination titer. When eggs were treated at fluences 1.5 and 3.1 J/cm<sup>2</sup> (1.72 mW/cm<sup>2</sup>, 15 and 30 min, respectively), a 2-log reduction was observed, regardless of treatment time (34). Pertaining to pulsed UV light, large variations in germicidal effect have previously been presented. Dunn (16) reported a >7.97-log reduction of *Salmonella* on unprocessed eggs, using a fluence of 4 J/cm<sup>2</sup>. Varying degrees of reduction of a *Salmonella* Enteritidis strain were obtained, from 1.3 log after 1 s treatment to >5.3 log after a 20-s treatment at fluence 23.6 J/cm<sup>2</sup> (31). A strain of *Salmonella* Typhimurium was shown to be reduced by up to 5 log at fluence 2.1 J/cm<sup>2</sup> (35).

Approximately 30% of the eggs produced in the EU are processed. *L. monocytogenes* and *E. coli* are considered microbiological hazards in the egg processing industry (6). Different bacteria have been shown to vary in their sensitivity to pulsed UV light, with *L. monocytogenes* showing higher resistance (21, 24, 36). In the present investigation, however, no differences in sensitivity to either UV-C nor pulsed UV light on eggs among the three mixes of *Salmonella*, *L. monocytogenes*, and EHEC strains could be seen.

Cells commonly have UV-A inducible repair systems to correct damages in their DNA. Photoreactivation studies could indicate whether bacteria are killed or only damaged after UV light treatment. Hierro et al. (27) reported photoreactivation of a *Salmonella* Enteritidis strain on Noble agar petri dishes after a pulsed UV light treatment of up to 0.525 J/cm<sup>2</sup>. A 10-fold lower fluence for the UV-C treatment was chosen in the present work, and no photoreactivation was detected under the conditions tested, indicating that *Salmonella* were killed even with the mildest treatments. Photoreactivation of *E. coli* on petri dishes after UV-C treatment has been described previously (40, 43, 54). For *L. monocytogenes* on agar plates, subjected to pulsed UV light and subsequently illuminated with fluorescent light, photoreaction has also been observed (24). On the other hand, no photoreactivation was detected when a *Salmonella* Typhimurium strain was exposed to UV-C light on eggs and thereafter was treated with fluorescent light for 1 h (34). Likewise, neither a strain of *Salmonella* Typhimurium nor a *L. monocytogenes* strain showed photoreactivation after pulsed UV light treatment on agar plates followed by UV-A treatment at 365 nm for 1 to 3 h (41). It is, however, difficult to directly compare our results and the previously published results because different light sources have been used and often the fluences are not given. The general impression is that photoreactivation may occur only after mild UV light treatment.

The cuticle is an important natural physical defense involved in protection of egg contents from invading organisms. Based on scanning electron microscopy images of the eggshell (Fig. 4) and the lack of transfer of *Salmonella* after UV light treatments (Table 2), our results strongly indicate that the integrity of the eggshell was maintained. Similar results have been reported when a *Salmonella* strain on eggs was treated with 10.5 J/cm<sup>2</sup> pulsed UV light and the egg content was later examined for *Salmonella* (35). A very low number of *Salmonella* cells survived the UV light

treatment, giving a greatly reduced probability of transfer compared with the situation in our experiments, in which a high dose of *Salmonella* (10<sup>7</sup> CFU) was applied to the eggs after pulsed UV light treatment.

Considering the low penetration of UV light into organic matter, limited influence of UV light on the egg content would be expected after illumination of intact eggs. Because changes in odor were small, changes in taste is expected to be insignificant compared with changes occurring when processing eggs (frying, cooking, used in baking, etc.). A small change in sunburnt odor observed after using the highest UV doses of both UV-C and pulsed UV light implies an upper limit of treatment of eggs in cases that require that there be no sensory changes. A triangle test performed to detect possible changes in egg white and yolk odor after pulsed UV light treatment of eggs did not reveal any differences (35). Keklik et al. (31) found no changes in albumen height, Haugh unit, or the eggshell strength when comparing pulsed UV light-treated eggs with control eggs. Coloring eggs with a cuticle-sensitive dye revealed no visible changes after pulsed UV light treatment (31), and changes in rheological properties have been reported to be insignificant (35). Together, these results indicate that sensory changes are small or negligible after both UV-C and pulsed UV light treatments.

In conclusion, both continuous UV-C and pulsed UV light efficiently reduce bacterial levels on the egg surface. The reductions are dependent on the treatment conditions used and on whether the bacteria are directly exposed to the UV light. The UV-C light treatments appear to give higher reductions at lower fluences, whereas the pulsed UV light shows higher penetration and gives higher reductions for shielded bacteria. The methods can easily be implemented in production and can contribute to reducing the risk of human salmonellosis and other infections.

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## SUPPLEMENTAL MATERIAL

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