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The effect of a commercial UV disinfection system on the bacterial load of shell eggs

K. De Reu[1], K. Grijspeerdt[1], L. Herman[1], M. Heyndrickx[1], M. Uyttendaele[2], J. Debevere[2], F.F. Putirulan[3] and N.M. Bolder[3]

1 Department of Animal Product Quality and Transformation Technology, Ministry of the Flemish Community, Agricultural Research Centre, Melle, Belgium
2 Laboratory of Food Microbiology and Food Preservation, Department of Food Technology and Nutrition, University of Ghent, Ghent, Belgium
3 Animal Sciences Group, Wageningen UR, Division of Infectious Diseases, AB Lelystad, the Netherlands

Abstract

Aims: To study the effect of UV irradiation on the bacterial load of shell eggs and of a roller conveyor belt.

Methods and Results: The natural bacterial load on the eggshell of clean eggs was significantly reduced by a standard UV treatment of 4·7 s; from 4·47 to 3·57 log CFU per eggshell. For very dirty eggs no significant reduction was observed. Eggs inoculated with *Escherichia coli* and *Staphylococcus aureus* (4·74 and 4·64 log CFU per eggshell respectively) passed the conveyor belt and were exposed to UV for 4·7 and 18·8 s. The reduction of both inoculated bacteria on the eggshell was comparable and significant for both exposure times (3 and 4 log CFU per eggshell). *Escherichia coli* was reduced but still detectable on the conveyor rollers. The internal bacterial contamination of eggs filled up with diluent containing *E. coli* or *S. aureus* was not influenced by UV irradiation.

Conclusions: There is a significant lethal effect of UV irradiation on the bacterial contamination of clean eggshells and recent shell contamination, contamination of rollers can be controlled and the internal contamination of eggs is not reduced.

Significance and Impact of the Study: The penetration of UV into organic material appears to be poor and UV disinfection can be used as an alternative for egg washing.

Introduction

De Reu *et al.* (2006) reported a correlation between bacterial eggshell contamination and internal egg infection. Disinfection of the eggshell surface is therefore an important tool to prevent egg spoilage and egg-related illnesses. The cuticle is an important physical barrier for egg invading organisms (De Reu *et al.* 2005). It obstructs bacterial invasion by closing the pores resulting in a reduced permeability of the shell (Fromm and Margolf 1958). Egg-washing chemicals can damage the cuticle layer (Kim and Slavik 1996), change the microstructure of eggshells or leave chemical residues on shell surfaces (Kim and Slavik 1996; Wang and Slavik 1998;

Favier *et al.* 2001). Ultraviolet irradiation could be a more favourable alternative for decontamination of the eggshell (Kuo *et al.* 1997a). Previous studies, using pilot UV irradiation systems, have shown UV irradiation to be effective in reducing the bacterial load on the surface of visibly clean eggs (Kuo *et al.* 1997a,b; Chavez *et al.* 2002; Coufal *et al.* 2003). Gao *et al.* (1997) studied, also with a pilot system, the effectiveness of UV irradiation on different types of egg belt conveyor materials. The effect of UV irradiation on dirty (faeces) eggs and on internal egg decontamination has not been published to our knowledge.

The aim of this study was to compare the effect of a commercial irradiation system, linked to a commercial

roller system, on the elimination of aerobic bacteria on clean eggs and dirty eggs, to study the effect on recent surface contamination (eggshell and rollers) and to check the influence of UV irradiation on the contamination of the egg content.

Materials and methods

Egg samples

Clean eggs were collected from a commercial conventional housing system, with Isabrown laying hens, on the day of lay. Very dirty eggs (eggs with visible faecal contamination) were collected from a commercial aviary housing system, with Bovans Goldline laying hens, on the day of lay.

Ultraviolet irradiation

A commercial UV-C disinfection system having a wavelength of 253·7 nm with an intensity of 10 mW cm^{-2} was used (UV-disinfection system; MOBA, Barneveld, the Netherlands). The UV-disinfection system was linked to a MOBA plastic double roller conveyor belt. Two different speeds of the conveyor belt were used; one with a maximum speed of 10 000 eggs h^{-1} per row and another with a moderate speed of 2500 eggs h^{-1} per row. The roller system was operated at two different speeds of 0·2167 and 0·0542 m s^{-1}. As the UV-C disinfection system had a length of 102 cm, the exposure time for one egg was 4·7 and 18·8 s.

Inoculation of eggs

Escherichia coli (ATCC 25922) and *Staphylococcus aureus* (ATCC 6535) were used to inoculate the eggshell of clean eggs. Inoculation was performed by immersing the whole egg for 1 min in phosphate-buffered saline (PBS; Oxoid, Hampshire, UK) which contained 10^5–10^6 CFU ml^{-1} of the selected bacterium and was allowed to dry at ambient temperature. This resulted in an average eggshell contamination with 5·5 × 10^4 CFU *E. coli* per eggshell or 4·6 × 10^4 CFU *S. aureus* per eggshell.

Escherichia coli (ATCC 11775) and *S. aureus* (ATCC 6535) were also used to inoculate the egg content. The egg contents (egg white and egg yolk) were drained after cutting a hole of *c.* 1 cm^2 with a rotary tool (Dremel; S-B Power Tool Company, Chicago, IL, USA) and a pair of tweezers (De Reu *et al.* 2006). The inner part of the shell was rinsed with sterile 1/4 Ringer solution (Oxoid) to remove the albumen adhering to the membranes and after that the egg was filled up with 1/4 Ringer solution containing 1·0 × 10^3 CFU *E. coli* ml^{-1} or 6·1 × 10^2 CFU

S. aureus ml^{-1}. After filling up the eggs, the hole was closed with silicone.

Determination of the contamination of eggshell, conveyor rollers and internal egg fluid

The total aerobic mesophilic bacteria of uninoculated clean and uninoculated dirty eggs was determined by washing the individual eggs in a plastic bag with 10 ml of diluent and by rubbing the eggshell through the bag to detach the bacteria. The diluent was subsequently plated by a spiral-plater on Nutrient Agar (Oxoid). Plates were incubated at 30°C for 72 h (De Reu *et al.* 2005).

The *E. coli* or *S. aureus* count on eggshells was determined by washing the egg with 10 ml of diluent as described before. The diluent was subsequently plated on McConkey No. 3 agar (Oxoid) for *E. coli* and Baird-Parker medium with Rabbit Plasma Fibrinogen (Oxoid) for *S. aureus*. Plates were incubated at 37°C for 24 and 48 h respectively.

Individual rollers of the conveyor belt were swabbed with plain cotton swabs, soaked in Buffered Peptone Water (BPW; Oxoid). Swabs were immediately streaked on McConkey No. 3 agar and enriched for 24 h at 30°C in BPW, followed by streaking the enrichment on McConkey No. 3 agar. The selective plates were incubated at 37°C for 24 h.

After aseptic removal of the silicone, the internal egg *E. coli* or *S. aureus* count was determined by sampling 1 ml from the internal fluid with a sterile pipette through the hole and plating on Violet Red Bile Lactose Agar (Oxoid) for *E. coli* and Baird-Parker medium with Rabbit Plasma Fibrinogen (Oxoid) for *S. aureus*. Plates were incubated at 37°C for 24 and 48 h respectively.

Decontamination experiments

In the first test cycle 80 clean and 80 dirty eggs were sampled, where both were not inoculated. The next day 40 eggs from both categories were irradiated at an exposure time of 4·7 s; the remaining 40 eggs from each category were used as control group. The total aerobic bacterial count was determined the day after the irradiation.

In the second test cycle 15 clean eggs were inoculated with a culture of *E. coli* bacteria and 15 clean eggs with *S. aureus* bacteria. After drying at ambient conditions, 10 inoculated eggs of both groups were passed on the conveyor belt, of them five eggs were UV irradiated for 4·7 s and the other five eggs for 18·8 s. After the test with *E. coli* the individual rollers of the conveyor belt were swabbed. The remaining five eggs of both groups were

used as control group. The *E. coli* and *S. aureus* contamination was determined on the same day.

To study the influence of UV irradiation on internal bacterial egg contamination, the egg content of 40 clean eggs was removed; 20 eggs were filled up with 1/4 Ringer-solution containing *E. coli* and the other 20 eggs with 1/4 Ringer-solution containing *S. aureus*. From each set of filled up eggs, 10 eggs were irradiated with UV for 4·7 s and the remaining 10 eggs were used as control group. Microbiological analyses were performed on the same day.

Species identification

Species identification of the major natural contamination on the eggshell was carried out using 16S rDNA sequencing (Scheldeman *et al.* 2004). Identification of the species was performed on colonies picked up from the Nutrient Agar plates used for the determination of the total aerobic mesophilic bacteria of the non-UV treated clean eggs.

Statistical analysis

Analysis of variance was performed on the log-transformed counts. The homogeneity of the variances among groups was assessed using Bartlett's chi-squared test, and the homoscedasticity was verified with a mean *vs* SD plot. All these analysis were carried out in Statistica 7 (Statsoft; Tulsa, OK, USA).

Results

The natural bacterial load (total aerobic bacteria) on the eggshell of uninoculated clean eggs was significantly reduced ($P < 0·001$) by UV treatment; from 4·47 to 3·57 log CFU per eggshell (Fig. 1). For the uninoculated dirty eggs a non-significant ($P > 0·05$) reduction from 6·17 to 5·99 log CFU per eggshell was observed (Fig. 1). Sequencing showed that the major natural contamination on the eggshell was *Staphylococcus linens* and *Staphylococcus equorum* on both types of eggs.

The reduction of *E. coli* surface contamination after inoculation was significantly ($P < 0·001$) for both exposure times respectively. A reduction of 3 log (4·7 s UV) and 4 log (18·8 s UV) occurred, compared with the control group having an average contamination of $5·5 \times 10^4$ CFU *E. coli* per eggshell. For *S. aureus* comparable results were obtained; significant ($P < 0·001$) reductions of 3 log (4·7 s UV) and 4 log (18·8 s UV) occurred, compared with an initial eggshell contamination of $4·3 \times 10^4$ CFU *S. aureus* per eggshell.

No *E. coli* could be isolated from the plastic rollers surface by direct plating of the swab after passing the UV device three times at both conveyor speeds. However, after enrichment of the swabs taken after three and even eight times passing the device, *E. coli* was still detectable.

UV treatment did not significantly influence the internal egg contamination. For *E. coli*, UV-treated eggs contained on an average 4·07 log CFU ml^{-1} compared with 4·37 log CFU ml^{-1} for non-treated eggs ($P < 0·05$), for
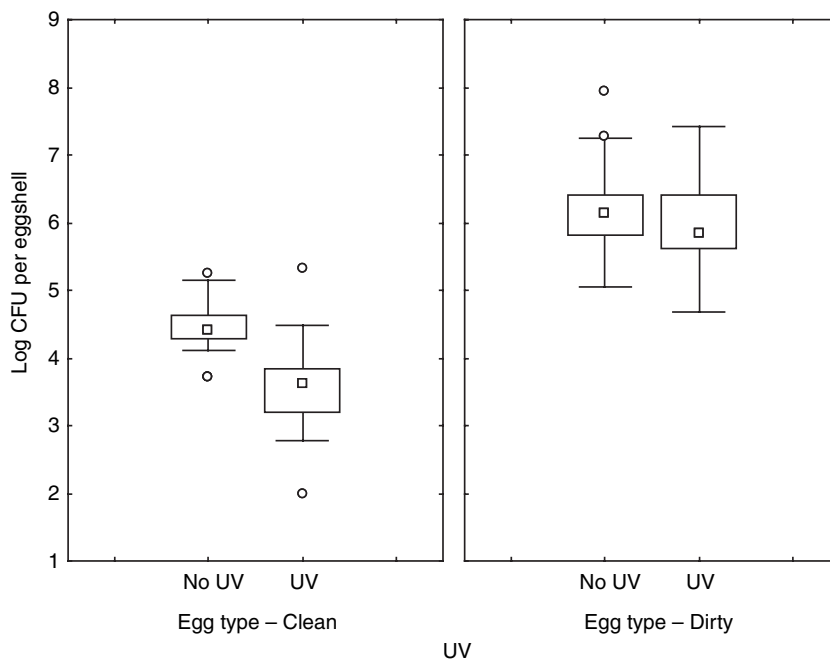

Figure 1 Influence of UV disinfection (10 mW cm^{-2}, 4·7 s) on the natural bacterial load (total aerobic bacteria) of uninoculated clean and dirty eggshells. □, Median; ▭, 25–75%; ⊥, Nonoutlier range; ○, Outliers.
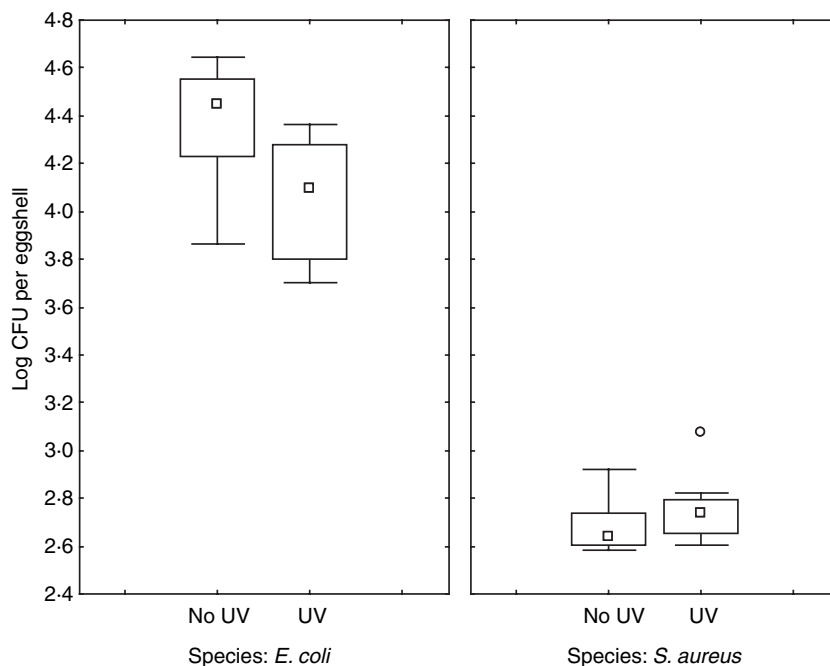
Figure 2 Influence of UV disinfection (10 mW cm^{-2}, 4·7 s) on the internal contamination of eggs. □, Median; ▭, 25–75%; ⊥, Nonoutlier range; ○, Outliers.

S. aureus the count in UV treated eggs was even higher compared with non-treated eggs, 2·75 *vs* 2·64 log CFU ml^{-1} ($P = 0·14$) (Fig. 2). To determine the effect of repeated UV-treatments, two eggs filled up with *E. coli* (4·37 log CFU ml^{-1}) were irradiated three times subsequently for 4·7 s and afterwards still contained 4·08 and 4·36 log CFU *E. coli* ml^{-1}.

Discussion

Our data showed no significant reduction of the natural bacterial load on very dirty uninoculated eggs compared with a significant reduction on visible clean uninoculated eggs. Possibly the top faeces particles on the shell of the dirty eggs formed a protective layer for the bacteria against the UV treatment. The penetration of UV into the organic material appears to be poor, only the outer surface layer was apparently exposed. Stermer *et al.* (1987) also found that the bactericidal effect of UV light was less effective on rough meat surfaces because bacteria were partly shielded from the radiation. Kuo *et al.* (1997a) evaluated different UV (254 nm) treatment times (0, 15 and 30 min) at an intensity of 620 μW cm^{-2} and different intensities (620, 1350 and 1720 μW cm^{-2}) at a treatment time of 15 min. For all UV treatments a 2 log reduction of CFU of aerobic bacteria per eggshell was observed. The visibly clean eggshell surfaces initially contained 5·0 log CFU aerobic bacteria per eggshell. Favier *et al.* (2001) found a reduction of 1·6 log on uninoculated clean eggs after an UV exposure for >25 min (254 nm; 4573 μW cm^{-2}). In one of the

experiments of Chavez *et al.* (2002), visibly clean eggs were exposed to UV treatment (254 nm; 7·35 mW cm^{-2}) for 0, 15, 30 and 60 s. Exposure of eggshells to UV for 30 and 60 s resulted in 0·8–2 and 2–3 log reduction of the aerobic plate count per eggshell respectively. Coufal *et al.* (2003), using an UV cabinet (254 nm, 4 min and 4–14 mW cm^{-2}), found a 1·3 log reduction. In our experiment, using a 253·7 nm to 10 mW cm^{-2} UV treatment, a reduction of 0·9 log was found after 4·7 s of UV treatment. Gao *et al.* (1997) arrived at the conclusion that the exposure time was more important than the UV intensity.

The significant reduction of the surface contamination after eggshell inoculation was also found by other researchers. Kuo *et al.* (1997a) found a significant reduction of *Salmonella* serovar Typhimurium inoculated on eggshell surfaces (2·5 × 10^6 CFU per eggshell); 1 min of irradiation (254 nm; 620 μW cm^{-2}) decreased the population with approximately 3 log cycles. Coufal *et al.* (2003) found a 4 log reduction for *S.* Typhimurium and 4–5 log reduction for *E. coli* (254 nm, 4 min and 4–14 mW cm^{-2}). The latter is comparable with our 4 log reduction for the inoculated *E. coli* bacteria (18·8 s UV). Favier *et al.* (2000) found UV irradiation was more effective on groups of eggs with low *Yersinia enterocolitica* inoculum (2·4 × 10^4 CFU per eggshell) than on those groups with high inoculum (2·2 × 10^7 CFU per eggshell). A decrease in 4·39 and 1·43 log cycles was observed after 40 min of 4573 μW cm^{-2} UV exposure respectively.

Gao *et al.* (1997) demonstrated that *Salmonella* was easier to eliminate from plastic belt than from other

materials tested; fibre belt was most difficult, eggshell and metal were within median range. In our study the contamination of the rollers with *E. coli*, a less dangerous substitute for *Salmonella*, was not completely eradicated.

Although *E. coli*, *S. aureus* (inoculated eggs) and *S. linens* or *S. equorum* (major flora on clean eggs) have a comparable amount of energy needed to be deactivated by UV (6600 μJ for *E. coli* and 5720–6600 μJ for *Staphylococcus* sp. respectively) (Srikanth 1995); our study showed that the UV decontamination was clearly more effective on *E. coli* and *S. aureus* inoculated eggs compared with naturally contaminated clean eggs. The freshness of the inoculum (which might lead to a higher susceptibility of the bacteria), the more protected position (shielded) of the natural flora on the eggshell or the presence of organisms that are only partly or effectively not deactivated by the UV system on clean eggs might explain this difference. No determination of the initial composition of all the microflora of the eggshells was performed.

Although the effect of UV treatment on internal *E. coli* contamination was less significant, these results show a limited relevance for practice. Both organisms used for the internal egg contamination (*E. coli* and *S. aureus*) need the same UV deactivation energy; 6600 μJ (Srikanth 1995). Our results show that UV cannot penetrate the eggshell. Gao *et al.* (1997), using a UV sensor placed beneath a piece of eggshell, confirmed that UV penetration could not be detected on the other side of an eggshell.

We can conclude that there is a significant lethal effect of the commercial UV disinfection system on bacterial contamination of visibly clean eggshells and recent shell contamination, that contamination of rollers can be controlled but not completely eradicated, and that the internal contamination of eggs was not reduced by the UV irradiation used.

Acknowledgements

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