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Meat Technology — Special Issue 64/2

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 637.412.065

ID: 126656777

https://doi.org/10.18485/meattech.2023.64.2.76

Original scientific paper

Reduction of eggshell microbial load of table eggs by ultra-violet treatment

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ARTICLE INFO

Keywords:
Table eggs
Ultraviolet irradiation
Decontamination
Bacteria
Molds and yeast

ABSTRACT

Table eggs *are an excellent animal-based food with an important role in human diet. However, eggs are often associated with* foodborne illnesses. The aim of this study was to evaluate the commercially available ultraviolet irradiation system incorporated in an egg grading and packing machine, and to compare the number of naturally present microorganisms on eggshells and the number of microorganisms on eggshells after ultra-violet treatment. The study showed reductions of the total number of aerobic microorganisms, *Enterobacteriaceae*, *Escherichia coli*, and the number of yeasts and molds on the eggshells, while the pathogenic bacteria, including *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella* spp., as well as the number of coagulase-positive staphylococci, were under the limit of quantification on the shells of both not ultra-violet treated and ultra-violet treated table eggs. In conclusion, the 7 s ultra-violet treatment was effective in reducing the bacteria, molds and yeast on the eggshells, which could contribute to better health of consumers.

1. Introduction

The table eggs are considered as a complete food in the human diet providing a well-balanced source of nutrients. They consist of a protective eggshell, egg white, and egg yolk contained within thin membranes. The presence of microorganisms on the eggshell can affect the safety of table eggs. Two sources of contamination of the contents of intact table eggs can be distinguished. In the case of horizontal transmission, microorganisms penetrate through the eggshell, while vertical transmission refers to the transovarial route and the presence of microorganisms in the reproductive organs (*Gautron et al*., 2022). Penetration of microorganisms through the pores of the eggshell can cause the spoilage of table eggs, while the presence of foodborne pathogens can cause severe food poisoning (*Eke et al*., 2013). Therefore, disinfection of the eggshell surface is an important tool to prevent egg spoilage and egg-related illnesses. However, the washing and cleaning of Grade A eggs in Serbia, before and after sorting, is prohibited (*Official Gazette of the Republic of Serbia*, 2019), because such practices can cause damage to the eggshell. Such damage may cause increase chances of bacterial cross-contamination (*Musgrove*, 2011). Contaminated table eggs are a significant public human health hazard worldwide (*Abebe et al*., 2020).

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Paper received Jun 30th 2023. Paper accepted July 18th 2023.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia

Ultraviolet radiation (UV) could be a more favorable alternative for eggshell decontamination. Previous studies show that UV radiation is effective in reducing the bacterial load on the surface of visibly clean eggs (*De Reu et al*., 2006;).

The aim of this study was to evaluate UV treatment of a commercially available egg grading and packing machine, comparing the number of naturally present microorganisms on eggshells and the number of microorganisms on eggshells after UV treatment.

2. Materials and methods

2.1. Egg samples

The table eggs originated from a local farm and were produced by 32-week-old Isa Brown laying hens reared in enriched cages. All eggs were collected the day of lay. Average weight of eggs was 58.20 ± 1.68 g, which falls within the classification of "medium" eggs (*Official Gazette of the Republic of Serbia*, 2019). A total of 60 visibly clean table eggs were collected in 3 successive trials. For each trial, 20 table eggs were tested: 10 samples of table eggs collected before the UV treatment and 10 samples of table eggs collected after the UV treatment. Each table egg was sampled in a sterile Whirl-Pak bag, and transported to the laboratory in cold storage conditions within 2 h.

2.2. Ultra-violet treatment

The UV treatment was conducted by a commercial UV-C disinfection system, incorporated in an egg grading and packing machine (MOBA Omnia 125, Barneveld, the Netherlands), having a wavelength of 253.7 nm with an intensity of 10 mW cm^2. The speed of the conveyor belt was 0.142 m/s. As the UV-C disinfection system had a length of 100 cm, the exposure time for each egg was 7 s.

2.3. Microbial analysis of table eggs

At the laboratory, 18 mL of saline peptone solution was added to each bag with the table egg. The surface of each table egg was gently hand massaged through the bag for 5 minutes to detach the bacteria. The rinse solution was diluted, and volumes of 1.0 mL of suitable dilutions were further used. To quantify the total number of aerobic microorganisms, *Enterobacteriaceae,* and *Escherichia coli*, pour plate methods were used. Volumes of 1.0 mL of suitable dilutions were plated in plate count agar (PCA), violet red bile glucose (VRBG) agar and tryptone bile agar (TBX). The PCA plates were incubated aerobically at 30°C for 72 h (*ISO*, 2013), VRBG plates were incubated at 37°C for 24 h (ISO, 2017a), and TBX plates were incubated at 44°C for 24 h (*ISO*, 2008). Enumeration of coagulase-positive staphylococci (*ISO*, 2021), *Listeria monocytogenes* (*ISO*, 2017b), and *Campylobacter* spp. (*ISO*, 2017c) were carried out using the spread plate technique. Briefly, 1.0 mL of initial dilution was spread on three plates of Baird-Parker agar, agar Listeria according to Ottaviani and Agosti (ALOA), and modified charcoal cefoperazone deoxycholate (mCCD) agar, respectively. Both Baird-Parker and ALOA agar plates were incubated at 37 °C for 24–48 h, while mCCD agar plates were incubated in a microaerobic atmosphere at 41.5 °C for 48 h. *Salmonella* was determined by direct isolation on selective agar, xylose lysine deoxycholate agar (XLD), incubated at 37°C for 24 h. Enumeration of yeasts and molds was performed on Dichloran 18% glycerol agar (DG-18), incubated at 25°C for 5 days. All microbial media, except for mCCD agar which was purchased from Oxoid, used in the study were purchased from Biokar Diagnostics (Beauvais, France). To estimate the microbial load recovered from egg surfaces, the colony counts were calculated and transformed to log_{10} colony-forming units per egg (log_{10} cfu/eggshell). The lowest detection level for this procedure was 1.26 log_{10} cfu/eggshell.

2.4. Statistical analysis

The experiments were replicated three times. Descriptive statistics and t-test at a 0.05 level of significance were used to analyze the data.

3. Results and discussion

The visibly clean eggshell surfaces of table eggs were initially contaminated with microorganisms up to 5.57 log_{10} cfu/eggshell (Table 1). The total numbers of aerobic microorganisms, *Enterobacteriaceae*, *Escherichia coli*, and yeasts and molds on the eggshells were significantly ($p<0.05$) reduced after exposure to UV disinfection for 7 s. A decrease of 0.85 log_{10} cfu/eggshell of the total number of aerobic microorganisms was registered in our study. Similar results were obtained by *De Reu et al*. (2006) and *Pasquali et al.* (2014), when the natural bacterial load on eggshell was reduced by 0.9 log_{10}

Table 1. Microbial reduction on the eggshells of table eggs after UV treatment.

Microorganism	Before UV treatment	After UV treatment
Total number of aerobic microorganisms	5.57±0.83[a]	4.72±0.74[b]
Enterobacteriaceae	1.44±0.49[a]	<1.26±0.00[b]
Escherichia coli	1.43±0.41[a]	<1.26±0.00[b]
Coagulase-positive staphylococci	<1.26±0.00	<1.26±0.00
Listeria monocytogenes	<1.26±0.00	<1.26±0.00
Campylobacter spp.	<1.26±0.00	<1.26±0.00
Salmonella spp.	<1.26±0.00	<1.26±0.00
Yeasts and molds	2.34±0.53[a]	1.57±0.44[b]

Results are presented as mean (log10 cfu/eggshell) ± standard deviation. Values within the same row marked with different letters (a, b) in superscript indicate statistically significant differences (P < 0.05).

cfu/eggshell after 4.7 s of UV treatment, and by 1.04 log_{10} cfu/eggshell after exposure to UV disinfection for 7 s, respectively.

Bacteria from the *Enterobacteriaceae* family were similar in numbers to *Escherichia coli*. This may be attributed to the fact that this bacterium is a normal inhabitant of intestinal tracts of hens. Number of *Enterobacteriaceae* and *Escherichia coli* were reduced by >0.18 log_{10} cfu/eggshell and >0.17 log_{10} cfu/eggshell, respectively. The number of yeasts and molds was reduced by 0.77 log_{10} cfu/eggshell. The UV treatment was effective in this study. Other researchers also found the UV treatment as significantly effective in eggshell decontamination (*Chavez et al.*, 2002; *Turtoi and Borda*, 2014; *Clímaco et al*., 2018).

The number of pathogenic bacteria, *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella* spp., as well as the number of coagulase-positive staphylococci, were under the limit of quantification (<1.26 log_{10} cfu/eggshell) on the eggshells of both not UV treated and UV treated table eggs. A similar finding was reported in Iran (*Safaei et al*., 2011). However, these pathogenic bacteria have been isolated from eggshell previously (*De Reu et al*., 2005).

Generally, the population of microorganisms on eggs can vary (*Musgrove*, 2011), and usually represents the farm hygiene. Moreover, a variety of methods have been developed for the recovery of microorganisms from eggshells. Different methods, including surface rinsing, shaking crushed shells with glass beads, blending eggshells and membranes, and surface swabbing, aggravate comparison of results gained in different studies. In this study, we massaged the egg by hand for 5 min in saline peptone solution, while other studies used different times for massaging. This could also have impact in the number of detached microorganisms.

Although table eggs are estimated to be microbiologically sterile at oviposition, cross-contamination occurs at the moment of lay. Sources of contamination are feces, dust, caging and nesting materials, hands, transport belting and other matrices (*Musgrove*, 2011). In a previous study, among the microorganisms isolated from egg samples were *Staphylococcus* spp., *Streptococcus* spp., *Salmonella* spp., *Shigella* spp., *Proteus*, *Klebsiella*, *Citrobacter*, *Corynebacterium*, *Bacillus* spp., *Escherichia coli*, and fungi of the genus *Aspergillus* (*Salihu et al*., 2015). The microorganisms on eggshell could cause severe health problems like, diarrhea, nausea and abdominal pain.

The UV-C treatment involves short UV waves ranging from 200 to 280 nm. The UV light is lethal to most microorganisms, causing damage of nucleic acids (DNA and RNA). The damage does not kill the microorganisms, but it prevents cell replication. Therefore, an adequate UV treatment must provide a sufficiently high level of disruption to ensure that the nucleic acid is damaged beyond the repairable stage (*Turtoi and Borda*, 2014).

4. Conclusion

The results of this study showed that the commercially available UV disinfection system was effective in reducing different microorganisms on the shells of table eggs. Data show that UV light can significantly reduce microbial populations on eggshells, allowing fewer bacteria to penetrate and contaminate the interior of the egg. This decontamination would result in fewer bacteria that could cause spoilage of table eggs and foodborne illnesses. Future studies remain to investigate the ideal length of UV treatment against the natural eggshell microbiota.

Disclosure statement: No potential conflict of interest was reported by the authors.

Funding: This study was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

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