THE USE DIFFERENT CHEMICALS AND UV RADIATION FOR THE REDUCTION OF MICROORGANISM IN THE EGGSHELL AND THEIR COMPARISON

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ABSTRACT

The present paper evaluates the effect of different chemicals on microorganisms in the eggshells and eggs' quality. 120 chicken eggs were randomly collected from a poultry farm in Kosovo and divided for treatment purposes into the following gropus: Group 1 undergoing the 13.33 g/m^3 formaldehyde fumigation, Group 2 undergoing the ozone fumigation 5-10 ppm, Group 3 undergoing the 6.36 mW/cm^2 light UV-C radiation, Group 4 undergoing the spraying with 1.56% hydrogen peroxide, Group 5 undergoing the spraying with water (wet control) and Group 6 not undergoing any disinfection procedure (dry control). Samples were divided into 6 groups of 10 eggs per each procedure; before and after the disinfection process to evaluate the presence of *Salmonella, Enterobacteriaceae* and total bacteria in the eggshell. Results reported that only the eggs undergoing formaldehyde and UV treatments showed a significant reduction in the total number of aerobic mesophilic bacteria in the eggshell, when compared to those of the dry control group. The treatments did not affect neither the thickness of the eggshell nor its resistance. The UV treatment effectively reduces microbial load in the eggshells without affecting their quality.
***Keywords*:** ozone, hydrogen peroxide, bacterial count, UV light, chicken eggs

1. INTRODUCTION

Approximately 1.140 billion eggs are annually produced and, 700 billion/year are consumed worldwide (Mantouanelli *et al*., 2001; Rehault-Godbert 2019). They are less expensive and a source of dietary protein (Mantouanelli *et al*., 2001). In addition, they are considered functional food due to their high nutritional content (Mantouanelli *et al*., 2001; Bradley and King, 2016). However, eggs might be a source of foodborne illness caused by *Salmonella* spp. In general, there are two possible routes of transmission: i) horizontal, from penetration through the eggshell from the colonized intestine or from feces contaminated during or after issues eggs, and ii) vertical, from

the use of UV-C light radiation, (Wells *et al*., 2011) has been effective in reducing the number of bacteria in egg shells. Moreover, since these methods do not emit toxic waste after their use, (Braun *et al*., 2011) they can also be characterized as environmentally friendly.

In 1982, ozone was generally recognized as safe (GRAS-Government Receipt Accounting System) by the Food and Drug Administration (FDA), and in 2001 the direct use of ozone was recognized in food products including fish, red meat, and chicken and used it in the food industry (Mielcke and Ried 2004). Ozone, which is a strong oxidizer, is effective against Gram-positive and Gram-negative bacteria, yeasts, fungi and viruses. Since ozone leaves no material in food products, it does not make a difference in the taste and color of the product (Okayama *et al*., 2002).

Ultraviolet (UV) light is the electromagnetic radiation found in the electromagnetic spectrum between X-rays and visible light and includes wavelengths between 200 and 400 nm. UV light is subcategorized into UV-A (400-315 nm), UV-B (315-280 nm) and UV-C (280-100 nm) based on the respective wavelength range (Iso 2007).

The main mechanism of microbial inactivation by UV radiation is the dimerization of DNA bases. The formation of these dimers within bacterial DNA prevents DNA duplication, eventually leading to a reduction in the bacterial population. These bases have a maximum UV absorption velocity at a wavelength of 260 nm, which corresponds to the bactericidal peak effectiveness of UV radiation which varies between 260 and 270 nm. Pyrimidine bases are 10 times more reactive than purine bases at a wavelength of 254 nm, the predominant wavelength of which is irradiated by UV-killing micro-lamps.

Thymine pyrimidine requires the least amount of energy to form a dimer, consequently, the complex thymine dimer is the predominant photoproduct of UV254 radiation. UV-C includes the wavelength of 254 nm within its range 200-290 nm and is therefore often referred to as UV germicide.

UV radiation does not expose eggs to toxic chemicals or by-products and is safe for the environment (Coufal *et al*., 2003). Except it is a low-heat process, UV cannot reach the developing embryo or cause DNA damage as UV does not penetrate the eggshell (De Reu *et al*., 2006).

Hydrogen peroxide (H_2O_2) is a very strong oxidizer that forms free radicals exerting a destructive effect on cell membranes. As a result, it has found wide application as a biocidal (Linley *et al*., 2012). When O_3 is exposed to UV the net reaction results in the formation of hydrogen peroxide (H_2O_2) and any hydroxyl radicals formed when O_3 reacts with UV are unable to escape this solvent cage. Although the advanced oxidation process (AOP) O_3/UV is an effective disinfectant, the bactericidal properties are the result of

the production of hydrogen peroxide instead of the hydroxyl radicals formed by the initial O_3 molecule.

The net photolysis of H_2O_2 yields two hydroxyl radicals, per quantum of absorbed radiation, which can continue to form peroxyl radicals leading to secondary oxidation reactions (Wells *et al*., 2011). The H_2O_2/UV photolytic reaction is one of the most widely used AOPs, and has been demonstrated to effectively inactivate vegetative bacteria, bacterial spores and viruses (Ikai *et al*., 2010). After treatment of eggs, H_2O_2 evaporates easily without leaving chemical waste and poses minimal safety issues for workers or embryo development (Keita *et al*., 2016). The bactericidal effects of H_2O_2 increase after UV photolysis (Ikai *et al*., 2010).

Other benefits of this system include the commercial availability of H_2O_2, its endless water solubility, and lower health risk than O_3 for workers. These benefits together with its effectiveness as a cleaner make the H_2O_2/UV AOP system an attractive method of eggs disinfection.

The present investigation aims to compare the results obtained from the use different chemicals and UV radiation for the reduction of microorganism in the eggshell of the eggs collected from a farm in Kosovo, to supply the highest quality and safe products to the customer.

2. MATERIALS AND METHODS

In the present investigation the 120 eggs have been randomly collected from a poultry farm in Kosovo and underwent two procedural phases: i) eggs collection and disinfection in the farm and, ii) microbiological investigation at the Laboratory of microbiology, the Faculty of Medicine, Prishtina, Kosovo. Eggs were collected from the nests using disposable latex gloves to avoid any sort of contamination from the collector's hands. Once collected, the eggs were placed in the litter and packed in previously disinfected plastic boxes. and distributed in disinfection treatments. The 120 eggs randomly selected underwent four different disinfection procedures (ozone fumigation, formaldehyde fumigation, type C ultraviolet irradiation and hydrogen peroxide spraying) and two control procedures (water spraying and no disinfection procedure) with subsequent microbiological investigation of the eggshell. The forthcoming paragraphs provide information about the disinfection procedures and control treatments.

For the egg procedure without disinfection, the eggs were kept in the same room where the other treatments were performed. Thermometer and thermohygrometer were used to measure room temperature and humidity, respectively, which varied from 27.5 to 30.4^{o}C and from 49 to 55%.

The water spraying process consisted of the collected eggs only sprayed with water. Water spraying was carried out to investigate how egg wetting affected the variables studied.

The water temperature went up to 26ºC in all disinfection processes, and was measured in the same way as in other spraying procedures. The spraying time varied from 8 to 11 min.

For ozone fumigation, eggs were disinfected with ozone gas at a concentration of 5-10 ppm for 20 min, as recommended by the ozone supply company. Each collected egg was placed in an exposed plastic box, inside a $3m^3$ fumigation room for disinfection purposes. The relative humidity of the air was adjusted to 70% inside the room, as suggested in (Braun 2011), and was measured through a thermo hygrometer (Boeco Germany). After fumigation, the product was consumed and the eggs were sent for bacterial count.

For formaldehyde fumigation, a concentration of 13.33 g m^{-3} formaldehyde was used to disinfect eggs as recommended in (Cadirci 2009). The egg box used for this procedure was plastic and was placed in the fumigation chamber which was the same room used to disinfect the eggs with ozone. Therefore, 1 hour (fumigation time plus the interval for the total waste disposal of the previously applied product). The relative humidity of the air inside the room was also adjusted to 70%, as recommended in (Cadirci 2009), and measured as during ozone fumigation process.

For the C ultraviolet radiation, disposable latex gloves were used and the eggs placed one by one in aluminum trays with a capacity of 10 eggs and then placed inside an enclosed room where the UV-C lamp -30W at a distance of 80 cm and 254 nm- provided an average light intensity of 6.36 mW cm^{-2}, as adapted by (Gottselig *et al.*, 2016). Egg compartments, designed to prevent eggs from touching each other and, consequently, to allow greater exposure to UV-C light, were placed in the center of the room to allow radiation from the bulb located over the entire length of the tray. In order to obtain significant reductions in the number of eggshell microbes, the disinfection time was 60s as in (Chavez 2002).

The temperature inside the room which ranged from 28.6 to 31.3°C throughout the disinfection period was measured by a probe equipped with a digital thermometer.

For hydrogen peroxide spraying, a solution of 1.56% hydrogen peroxide with 650 ppm active product was used to disinfect the eggs, as recommended by the manufacturer. Once collected and separated, 10 ml of disinfectant solution was sprayed on 10 eggs at the same time using a hand sprayer. A thermometer was employed to measure the temperature of the solution which ranged from 26 to 29ºC. For a complete spraying, the boxes of the eggs were placed on a horizontal surface and 5 ml of solution was dispersed on one side

of the eggs, and 5 ml were dispersed on the other side. An average of 1 mL of hydrogen peroxide was sprayed over each egg. Disinfection procedure lasted 9 to 12 min.

After each collection, shortly before and 1 hour after disinfection, 10 eggs undergoing each treatment were randomly selected to count the microbes in the eggshell. The eggs, collected with disposable handles, were placed in groups in autoclaved bags, which were properly identified according to each treatment and then refrigerated at 4ºC. The samples were transported to the laboratory for microbiological investigation purposes carried out 24 hours after cooling. Each bag was opened, and the eggs were transferred to another autoclaved bag, to which 250 ml of buffer phosphate (PBS) solution was added. The eggs were massaged for 5 min to remove bacterial cells from their eggshell surfaces. Then, a 1.0 ml sample of PBS was taken from each bag, and plaque was placed on the agar, in order to obtain the count of *Salmonella*, *Enterobacteriaceae* and total other bacteria.

The plates were incubated at 37°C for 24 to 48 hours and, subsequently, bacterial colonies were counted and recorded. The microbial count was expressed as $\log_{10}$ CFU 1.0 mL^{-1} batch of eggs.

3. RESULTS AND DISCUSSION

The present paper evaluates the effectiveness of the chemicals used to disinfect the chicken eggs. *Salmonella* contamination is considered an important hygienic issue, especially on small-scale farms that are not controlled by an authorized agency or when a Risk Analysis Critical Control Plan (HACCP 2001) is unavailable.

First, the bacterial count was carried out on the undisinfected eggs, and the results are in the Figure 1 depicted. Total number of aerobic mesophilic bacteria resulted to be of 3.8 and 4.56 $\log_{10}$ CFU/eggs, of which 1.37$\log_{10}$ CFU/eggs were *Enterobacteriaceae*. *Salmonella* resulted to be of 2.55 log10 CFU/eggs. These eggs were then treated with chemicals to investigate impact on *Salmonella enterica*, *Enterobacteriaceae* and total aerobic mesophilic bacteria from the egg shell.

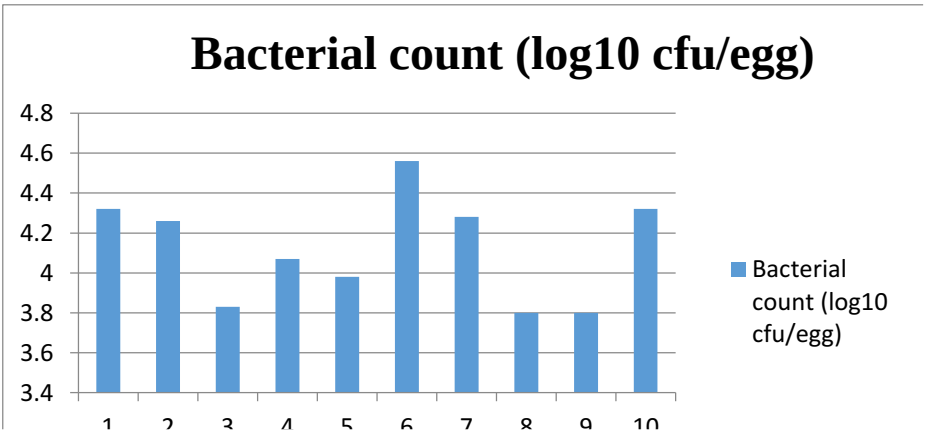

Fig. 1: Count of total bacteria in samples taken at a farm in Kosovo.

Among the various disinfection procedures, only eggs subjected to formaldehyde and UV treatments showed a significant reduction in the total mesophilic aerobic bacteria count (where here we are focused on eliminating *Enterobacteriaceae*) and *Salmonella* to relative to the control group (Figure 1). In this case, formaldehyde-treated and UV-treated eggs presented the lowest count of total aerobic mesophilic bacteria (*Enterobacteriacea*) and *Salmonela*.

Eggshell contamination with total mesophilic aerophilic bacteria, prior to each disinfection procedure, ranged from 3.8 and 4.56 $\log_{10}$ CFU/egg, where from them, 1.37$\log_{10}$ CFU/egg were *Enterobacteriacea*, confirming the results reported in (Coufal 2003) for eggs collected from nests. However, values between 4.0 and 7.0 $\log_{10}$ CFU for sterile eggs were also reported in (Wells et al., 2011; Zeweil *et al*., 2014) proving that values in the initial contamination of the eggshell vary greatly between studies. Despite the microbial challenge that eggs underwent prior to disinfection, formaldehyde and UV treatments were effective in reducing egg shell contamination by total mesophilic aerobic bacteria at 1.56 $\log_{10}$ CFU/egg (1.01 $\log_{10}$ CFU/egg *Enterobacteriacea*), and 1.52 $\log_{10}$ CFU/egg total mesophilic aerobic bacteria (1.02 $\log_{10}$ CFU/egg *Enterobacteriacea*). After treatment with formaldehyde a slight decrease of *Salmonella* was observed at 1.28 $\log_{10}$ CFU/egg, while after UV treatment we had a decrease to 1.26 $\log_{10}$ CFU/egg.

Compared to formaldehyde fumigation, achieving the same pattern of microbial reduction with UV light was impossible, because as Coufal *et al*., (2003) said this might be due to the difficulty of UV light reaching the entire surface of the egg, making it impossible for bacteria to be exposed to radiation and, therefore, causing a greater reduction in the number of eggshell

microbes. Other possible explanations might be the insufficient light intensity and exposure time to meet or exceed the reduced values due to formaldehyde smoking.

Although low, the total number of mesophilic aerobic bacteria for egg groups disinfected with ozone was 2.65 (1.08 $\log_{10}$ CFU/egg *Enterobacteriaceae*) and with hydrogen peroxide 2.55 (1.06 $\log_{10}$ CFU/egg *Enterobacteriaceae*). After ozone treatment, a slight decrease of *Salmonella* was observed at 2.11 $\log_{10}$ CFU/egg, while after treatment with hydrogen peroxide, we had a decrease to 1.98 $\log_{10}$ CFU/egg (Table 1).

It was proved that disinfectants were ineffective in reducing the number of microbes in the egg shells probably due to the long disinfection time which is industrially unfit.

Table 1. Total aerobic count of aerobic mesophilic, *Enterobacteriaceae* and *Salmonella* before and after disinfection of the chicken eggshells of a farm in Kosovo, using ozone, formaldehyde, UV-C light, hydrogen peroxide, water spraying and control dry.

Treatment	*Salmonella enterica* ($\log_{10}$ cfu/egg)		*Enterobacteriaceae* ($\log_{10}$ cfu/egg)		*Total aerobic mesofile bacteria* ($\log_{10}$ cfu/egg)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Dry control (without disinfection)	2.55	2.55	1.37	1.37	4.12	4.12
Spraying with water	2.55	2.55	1.37	1.37	4.12	4.12
Ozone	2.55	2.11	1.37	1.08	4.12	2.65
Formaldehyde	2.55	1.28	1.37	1.01	4.12	1.56
UV-C light	2.55	1.26	1.37	1.02	4.12	1.52
Hydrogen peroxide	2.55	1.98	1.37	1.06	4.12	2.55

Even when the manufacturer's recommendations were followed, noticing the microbial content reduction in the egg shells was irrelevant. The presence of *Salmonella* and total aerophilic mesophilic bacteria reduce Cox *et al*., (2007) and Wells *et al*., (2011) reported that the presence of *Salmonella* and total aerophilic mesophilic bacteria reduce diminishes when concentration of these products increase.

In the present investigation, very low counts of *Enterobacteriaceae* were found in all the groups, even before disinfection, as reported in (Musgrove). The rapid penetration of this group of bacteria through the pores of the eggs after laying and the lack of colonization of *Enterobacteriaceae* in the eggshell might be the source of the presence at low levels of these microorganisms.

4. CONCLUSIONS

The following conclusions could be drawn: i) only UV light irradiation and formaldehyde fumigation proved to be effective in reducing the bacterial count of *Salmonella*, *Enterobacteriaceae* and *mesophilic* bacteria in the eggshells. Formaldehyde after being detected long time ago that it has side effects on the embryo and adverse effects in the health of farm workers, alternative methods of disinfection are required, so formaldehyde is not preferable, ii) UV light could be recommended as an effective alternative procedure for large-scale disinfection of eggs, iii) other disinfectants such as ozone and hydrogen peroxide degrade longer times and higher concentration than that used in the work, leading to high reductions in Salmonella, Enterobacteriacea and total aerobic mesophilic bacteria.

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