Comparison of the use of different disinfection agents as antimicrobial preparations

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Abstract

Salmonella infections in egg contents may be related to external contamination of the eggshell. And the purpose of this study was to discover the effects of applying two antimicrobial drugs (ozone and chlorine) on chicken eggshell in a poultry farm in Kosovo. Ozone and chlorine in the form of sodium-NaHClO hypochlorite were applied to the eggshell at different concentrations and at different times to prove at what concentration and at what time each of these two disinfectants has the highest effect. During egg processing, samples were taken from 10 different points on the production line, 5 points on the equipment surface and 5 samples from the hands of workers for microbiological analysis. A high amount of total bacteria was observed in all samples, but after the application of disinfectants we saw a decrease of these bacteria. It is assumed that workers hands and equipment may be a source of secondary pollution. Ozone can also be used in lower concentration and safer than chlorine.

Keywords: eggs for consumption, salmonella, antimicrobial, ozone, chlorine.

Introduction

Eggs are one of the most consumed foods; approximately 1.140 billion eggs are produced annually and 700 billion/year are consumed worldwide. [22,17] They are less expensive and are a source of dietary protein (0.30$ / g protein, [17]), and from a nutritional point of view, they are considered a functional food due to their high nutritional content. [17,2] However eggs are one of the main food means of pathogens; for example, Salmonella is a potential food safety hazard in eggs. In general, there are 2 possible routes of transmission: 1) horizontal, from penetration through the eggshell from colonized intestine either from feces contaminated during or after egg and 2) vertical, from direct contamination of egg yolk, albumin, egg shell membranes or egg shell before oviposition. [17] Salmonella is the second leading cause of foodborne illness in poultry production, after Campylobacter, [5] with a mortality rate of less than 1%. About 2 million diseases/year occur due to Salmonella contaminated eggs in the United States, while in Europe, the range is 7,400 cases/year. [11]
The calcareous skin surrounding the egg is porous and permeable to bacteria. The cuticle is a protein film covering the egg shell that provides a natural barrier to help prevent internal bacterial contamination, [20] however, defects in the shell or thinning of the cuticle can lead to invasion of the egg shell by bacteria in surface [15]. Salmonella can easily penetrate the egg cuticle and contaminate the internal contents. [33,30]
In egg shells, the total number of aerobic mesophilic bacteria can reach 3.75 to 7.07 $\log_{10}$ colony forming units (CFU) per egg. Therefore, reducing the microbial load of the egg shell through disinfection procedures would improve the quality of the egg

to be incubated and reduce the incidence of bacterial infections in newborn embryos and hens.

Currently, most egg processors in the United States use chemical sanitation systems to decontaminate egg shell surfaces prior to packaging. Chlorine is approved for use by the U.S. Department of Agriculture-Agricultural Market Service [28] in egg cleaning solutions at levels of 50 to 200 ppm chlorine available. [28]

Favier [7,8] reported that 100.0 mg/L available chlorine applied through washing for 10 min at 25°C reduced the number of Yersinia enterocolitica inoculated in egg shells by 2.9 to 3.1 $\log_{10}$cfu/egg.

The efficiency of chlorine has been reported to be neutralized by the presence of high organic load and suspended solids in the bathing waters. [13,14]

Emphasis is placed on HACCP-based programs for identifying and preventing potential microbiological hazards that may arise from raw material, processing stages, product, and food plants. [9,17]

Chemicals such as chlorine and chlorine compounds, [6] ozone, [3,32] organic acids, [1] trisodium phosphate [25] are being widely used for decontamination purposes.

In 1982, ozone was generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), and in 2001 the direct use of ozone in food products including fish, red meat, and chicken was recognized and used his in the food industry. [16] 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. [19]

This study was conducted in order to compare the use of ozone and chlorine as antimicrobial preparations in disinfection of eggshells for consumption on a farm in Kosovo, to supply the highest quality and safest products to the consumer.

Materials and Methods

Sampling procedure

During the experiment, 150 chicken eggs were used for microbiological evaluation. The eggs were bought from a commercial poultry farm in Kosovo, which uses brown chickens raised on the floor.

For microbiological evaluation, 150 eggs were divided into 3 groups: 1) eggs without disinfection; 2) contaminated eggs that are then treated with ozone (0,2,4,6 and 8ppm) at 0, 5, 10 and 15 minutes; 3) eggs contaminated and then disinfected with 50, 100, 150 and 200 ppm sodium hypochlorite (NaHClO). This test was performed at 5 sampling points.

As illustrated in Figure 1, in the process line, samples were taken at 5 locations at different points from egg release to packaging. Eggs were collected using disposable latex gloves, in order to avoid contamination between the collector's hands and the eggs; eggs laid in the litter or that were too dirty were discarded. Then, the eggs were packed in previously disinfected plastic housing. A total of 150 eggs were randomly selected and distributed in disinfection treatments.

5 samples were also taken from 5 different people working on the farm. Samples from staff hands were taken as follows: workers were left to wear sterile latex gloves and 20 ml of 0.1% sterile pepton water was carefully collected in gloves. The hands on the

gloves were fully massaged and the gloves were carefully removed, tied at the top and transferred to the laboratory.

Enumeration of aerobic mesophilic bacteria was performed on agar plates from dilutions prepared by the diffuse plate method. Colonies formed after 48 hours incubation at 30°C under aerobic conditions were counted. [27]

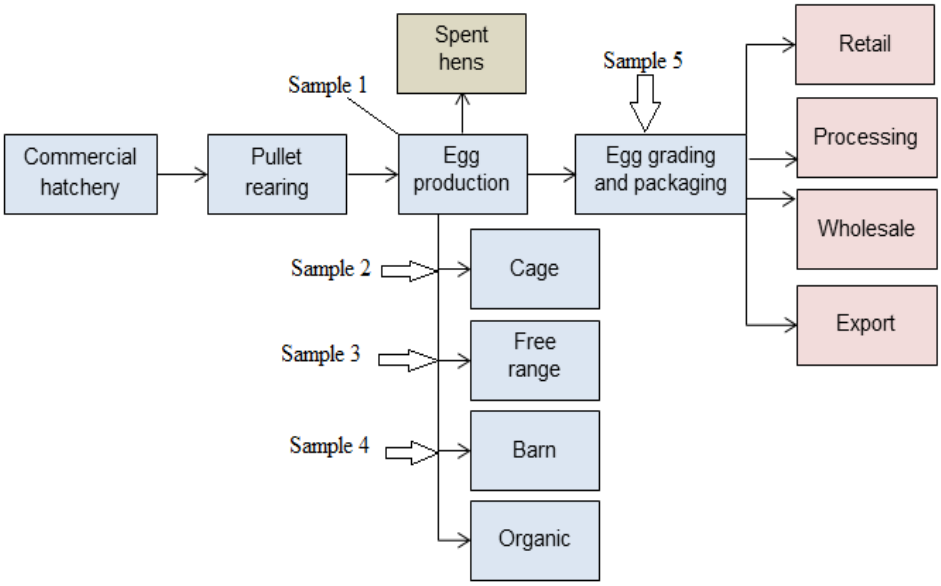

Figure 1. Egg production diagram, and assignment of sampling points

Realization of the work

For the egg procedure without disinfection, the egg rows were kept in the same room where the other treatments were performed, but the eggs did not undergo any disinfection procedure. Room temperature and humidity were recorded, from 26.7 to 30.5°C and from 49 to 53%, respectively.

For the second experiment each of the samples taken in Fig.1 should go through the disinfection procedures and control treatments described below. For ozone treatment, eggs were disinfected with ozone in concentrations ranging from 0.2, 4.6 and 8ppm for 0.5, 10 and 15 minutes, according to the recommendations of the company that supplied us with ozone. Each row of collected eggs was placed in an exposed plastic box, inside a disinfection room. This experiment was done to determine which concentration and at what time the maximum reduction of bacteria in the egg shells will be achieved.

In the third experiment each of the samples taken had to go through chlorine disinfection procedures (in the form of Na-NaHClO Hypochlorite) at different concentrations of 50,100,150 and 200ppm for 0.5,10 and 15 minutes. Control eggs were placed directly into sterile Whirl-Pak bags and bacterial counting was done.

Also 5 samples were taken from 5 different people working on the farm, and the

total bacteria in these samples were counted where the largest number were: Staphylococcus / Micrococcus, E.coli and aerobic mesophilic bacteria.

Results and discussion

The purpose of this study was to evaluate the effectiveness of ozone and chlorine in Salmonella pollution and in the quality of 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, [12]) is not available.

We first performed the bacterial count on the eggs without disinfection, and the results are presented in the figure 2. We have resulted in a total number of bacteria of 3.83 and 4.32 $\log_{10}$ cfu / eggs Salmonella. These eggs are then treated with ozone and chlorine to see how effective these two products are in eliminating Salmonella enterica from the egg shell.

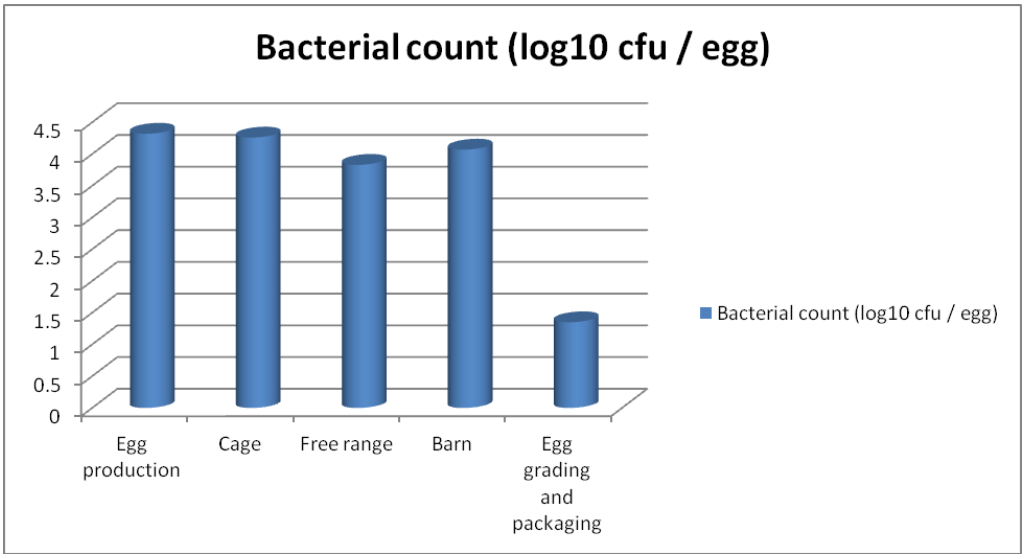

Figure 2. Bacterial counting at different sampling points

Despite the microbial amounts that eggs had before disinfection, treatments with O_3 and Cl were effective in reducing egg shell contamination by Salmonella enterica bacteria at 2.5 and 1.0 $\log_{10}$ cfu / egg, respectively.

Specifically in relation to ozone disinfection, Whistler & Sheldon (1989) [31] observed a significant reduction of 2.5 $\log_{10}$ cfu/egg in the count of these microorganisms in egg shells, which was also observed in the present study. This disinfection also relates to the time spent disinfecting the eggshell, as the authors adopted a considerably long disinfection period of about 2 hours, which may be impractical on an industrial scale. Therefore even increasing the concentration of these products can lead to a significant reduction of total aerophilic mesophilic bacteria, as reported by other authors. [25,4,30]

After conducting the experiment we proved that a concentration of 4ppm O_3, in a time of 5 minutes gave us the best result in disinfecting the eggshell of 2.5$\log_{10}$

cfu / egg, without damaging it (figure 3). Higher concentrations of O_3, gave a better disinfection, but negatively affected the quality of the eggshell.

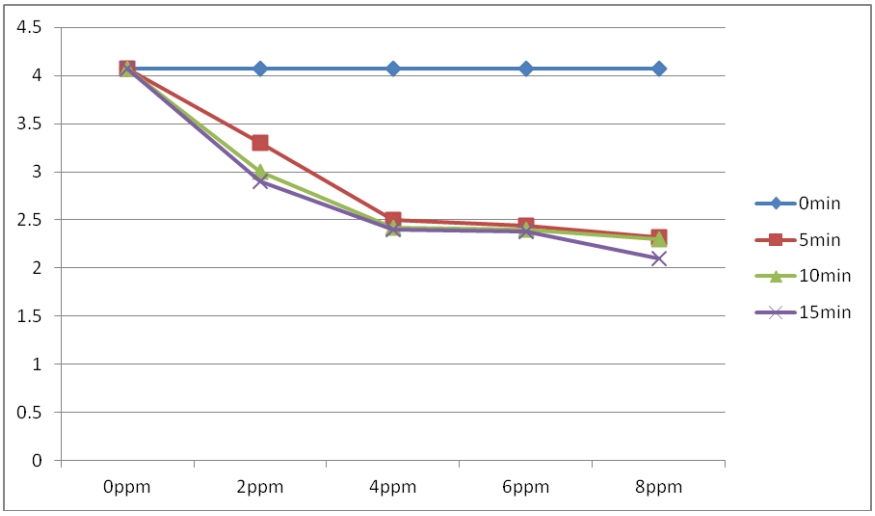

Figure 3. Use of ozone (ppm) during disinfection of eggs by chickens on a farm in Kosovo

After chlorine treatment of salmonella-contaminated eggs, the number of Salmonella decreased significantly compared to that of the positive control and ozone groups. Sodium hypochlorite at 100 ppm for 10 minutes resulted in microbiologically pure eggs and did not destroy the egg shell surfaces, which protected the eggs from future bacterial recontamination. Prolonged contact time (2 to 10 min) reduced the minimum chemical concentrations needed to inactivate the bacteria.

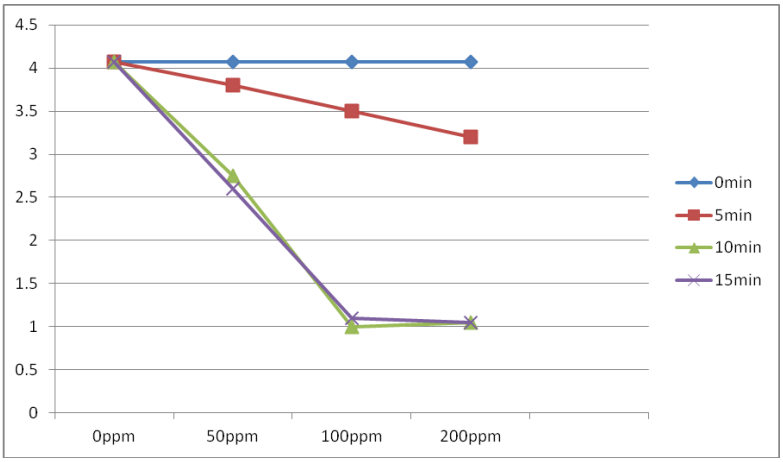

Figure 4. Concentration of Cl (in the form of Na hypochlorite- ppm) during disinfection of eggs from chickens on a farm in Kosovo

The results of the microbiological analysis of the samples taken from the hands of the

personnel are given in Table 1. In the hands of the personnel, the total aerobic bacteria were found as 10^2cfu/cm2, the number of Staphylococcus/Micrococcus was found as 10cfu/cm2. No E.coli was detected in 3 of the personnel and was in the order of 10 cfu/cm2 in two of the personnel.

Personnel	Staphylococcus/ Micrococcus	E.coli	Aerobic mesofilic bacteria
Personnel 1	3.66x101	2.65x101	1.33x102
Personnel 2	2.5 x101	1.55x101	1.69x102
Personnel 3	4.3 x101	Not found	2.32x102
Personnel 4	3.6x101	Not found	1.69x102
Personnel 5	1.6x101	Not found	2.10x102

Table 1. Analysis (cfu / cm2) of sample results (n = 5) obtained by staff hands during egg production processes

Conclusions

Güzel-Seydim et al (2004) [10] have studied the effects of ozone on reducing the bacterial population using different food ingredients. The effects of ozone on various microorganisms were compared with chlorine, and it was found that ozone can kill E. coli 125 times faster than chlorine products. It was found that ozone is 51 times more effective on bacterial cell membranes compared to chlorine. Ozone has also been reported to have a broad bactericidal effect including Gram-negative and Gram-positive bacteria. [23]

Cleaning and disinfection are critical processing steps to introduce secondary contamination caused by equipment. [28]

As seen in Table 3, samples were taken among groups of insignificant personnel. However, when examining the overall microbiological loads of the personnel hands, it is seen that they may be a source of contamination of aerobic mesophilic bacteria, Staphylococcus / Micrococcus and also, E. coli was detected in the hands of both personnel.

After analyzing the samples taken from the packaging section, it was determined that this part does not have a microbiological risk.

As a result of the study, it was found that ozone could be used to disinfect egg shells at lower levels, more safely and effectively compared to chlorine, however among the controls examined during the egg production stages was seen that equipment that is in hand contact with personnel is a source of secondary contamination and especially of processes that require more manual operations, and microbiological quality may be adversely affected. The microbiological increase in the samples taken from all these points we have taken may be related to this side effect and also to the fact that the company is working on its capacity and the general rules of hygiene are not being observed.

For food safety, in order to reduce microbiological threats to poultry farms, the initial risks of microbiological contamination must be reduced and, no matter how effective the disinfection is, the necessary hygiene rules must be followed during operation, storage, transportation and sale and HACCP-based systems should be implemented.

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