Effect of shell disinfection with AQUALYTE (NEUTRAL ANOLYTE) on inactivation of Salmonella enteritidis and quality of edible eggs

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Abstract. The results of studies, conducted by a number of scientists, prove that chicken eggs can cause food poisoning, as well as poultry. To prevent foreign microflora ingress into egg products, scientists have developed a number of methods aimed at the pathogenic microflora inactivation on the surface of the table eggs shells; nevertheless, the search for new methods is an urgent task. The article describes the results of the studies on the effect of disinfecting the shells of table eggs using AQUALYTE NEUTRAL ANOLYTE (NA) on the eggs quality. NA disinfecting activity was studied using cambric test objects. For the purposes of studying NA disinfecting efficiency, an experimental contamination of the surface of the table eggs shells with a test culture of Salmonella enteritidis was made. It was established that disinfection of the table eggs shells with 100% (2 min exposure) and 50% (5 min exposure) NA solutions provides Salmonella inactivation on the shell, and it does not affect the veterinary and sanitary parameters of eggs.

1. Introduction

Egg production provides the population with complete animal protein. Eggs laid by healthy hens are free of any microorganisms. The sterility of eggs is determined by the physical structure of an egg and by the protein chemical composition. However, contamination of the egg shell surface cannot be completely eliminated due to the natural origin of this product [1, 2]. The results of studies, conducted by a number of scientists, prove that eggs, just as poultry meat, which is the most pathogens-contaminated product, can be the reason of poisoning more often than it is commonly believed. The main cause of microbial contamination, including Salmonella, in the finished egg products is contaminated egg shells, although a small percentage of all eggs may be contaminated with Salmonella [3, 4].

More often, eggs are contaminated exogenously – through the shell, especially if it is contaminated with excrements, blood and other organic substances, as well as when microorganisms (mold, yeast, cocci microflora, bacteria, bacilli, actinomycetes, viruses, etc.) precipitates from the air. If the eggs are stored in rooms with high humidity – 90-100% and temperature above 16°C, microbes grows on the shell takes place. The microbes destruct mucin in the poresand penetrate the contents of the egg, causing its spoilage [5, 6].
In this regard, in the process of eggs production there is a possibility of pathogenic and opportunistic microflora ingress into the finished product. The most common pathogenic bacteria on the shell surface are salmonella, which can cause food poisoning in consumers. Washing eggs with drinking water reduces the shell microbial contamination to some extent, but it cannot ensure absolute cleaning of Salmonella and other undesirable microflora [4, 7, 8]. Egg shell disinfection is required to increase the microbiological safety of fresh chicken eggs used for human consumption, egg production or incubation [5, 9, 10].

To prevent the ingress of foreign microflora into egg products, scientists develop the ways to inactivate pathogenic microflora on the surface of the shell of food eggs. Currently, a number of physical methods have been proposed for the shell surface disinfection, namely, ultraviolet (UV) radiation [1, 5] and chemical methods – the use of ozone, hydrogen peroxide [1, 5], natural white vinegar [10], chlorine-based disinfectants [8, 11, 12], quaternary ammonium compounds [4], organic acids [4, 13], acidic electrolyzed water [13], etc. However, the search for new ways to inactivate pathogenic bacteria on the surface of the shell of food eggs using modern disinfectants is still an urgent task.

In this regard, the disinfectant AQUALYTE (NEUTRAL ANOLYTE) (hereinafter referred to as NA) is of interest. Active substances of NA are represented by a mixture of highly active metastable (electrochemically activated) oxidants, the concentration of which in equivalent to active chlorine is not less than 0.5 g/L (0.05%) with a total content of dissolved substances (mineralization) of ≤ 0.9 g/L and the pH of 6.0-8.0. The metastable mixture of oxidants is represented by chlorine-oxygen and hydroperoxide compounds: hypochlorous acid (50...95%), chlorine dioxide (1...7%), hydrogen peroxide (3...8%), other peroxide and superoxide compounds (1...5%) [14].

The NA has antimicrobial activity against gram-negative and gram-positive bacteria, viruses, and pathogenic fungi, although it does not require rinsing from surfaces or decontamination after use. The NA is low-hazardous substance when administered into the stomach [15], applied to the skin or inhaled. The agent does not irritate the respiratory organs, mucous membranes of the eyes and skin.

The aim of this work was to determine the disinfecting effect of the test preparation when it is treated with the shell of edible eggs.

2. Materials and methods
The studies were carried out at the All-Russian Scientific Research Institute of the Poultry Industry (VNIIPP), a branch of the Federal State Budgetary Scientific Institution of the Federal Scientific Center «All-Russian Research and Technology Institute of Poultry Farming of the Russian Academy of Sciences» within the framework of State Order No. AAAA-A20-120010690002-3.

NA bactericidal activity was studied in experiments with cambric test objects contaminated with a test culture – S. enteritidis, strain 5765 (hereinafter referred to as S. enteritidis) as per R 4.2.2643-10 [16].

When studying the agent disinfecting efficacy, the shell of food eggs was contaminated with S. enteritidis under laboratory conditions. The bacterial suspension was prepared from daily agar cultures. The eggs were preliminarily cleaned with brushes, washed with tap water, then flamed and immersed in the salmonella-containing suspension. After contamination, the eggs were dried at a room temperature and immersed in test solutions for 2 and 5 min.

NA working solutions were prepared using sterile tap water on the day of the experiment. The moment of immersing the eggs was considered as the experiment start. The eggs that were not exposed to the agent, immersed in a container with sterile tap water for a time equal to the test eggs exposure, were used as control specimens. After the exposure, the eggs were removed from the NA solution and immersed twice in a sterile container with 400 mL of sterile tap water.

Sampling and microbiological studies were carried out in accordance with the requirements of GOST 32149-2013 [17]. The eggs quality indicators were evaluated according to GOST 31654-2012 [18].

In a microbiological study of the contents of the eggs, the surface of the eggshell was washed for 1.5 min with a 0.5% warm 30±2°C solution of caustic soda. After washing, the egg was rinsed with tap water prior to draining and immersing in 70% ethanol and flaming. At the sharp end of the egg, a hole about 1 cm in diameter was made with a sterile scalpel and settled down. The contents of one egg or
several eggs were poured into a wide-mouth Erlenmeyer flask and mixed using sterile beads or chopsticks. The resulting homogenate was immediately used for research. 10 cm³ of the egg mass (sample) was transferred using a sterile pipette into a flask containing 90 cm³ of physiological sodium chloride solution, and thus a 1:10 dilution (dilution 1) was obtained. After stirring, 1 cm³ of dilution 1 was transferred by means of a pipette into a test tube containing 9 cm³ of saline, and thus a 1:100 dilution (dilution 2) was obtained. The required amount of other dilutions was obtained by a similar approach.

In the microbiological study of the eggshell surface, 10 cm³ of sterile tap water or sterile saline NaCl solution was poured into a sterile dish or plastic bag, and the egg was immersed prior to shaking for 5 min. Then, the dishes were preliminarily closed with a sterile lid; the top of the bag was squeezed sufficiently to prevent liquid spillage (bag’s size was 15×25 cm). Then, the egg was removed, the wash was used for the study.

To determine the number of mesophilic aerobic and facultative anaerobic microorganisms (QMAFA\text{anM}), the initial and a series of 10-fold dilutions were prepared to such an extent that the estimated QMAFA\text{anM} in 1 cm³ of washout could be determined. Inoculations were carried out by the submerged agar method simultaneously in two Petri dishes (parallel determinations) of 1 cm³ of the corresponding serial dilutions. In each Petri dish with inoculum no later than 15 min later, 18±2 cm³ agar melted and cooled to a temperature of 45±1°C meat-peptone agar was added and the inoculum was evenly distributed throughout the nutrient medium. Petri dishes with inoculations were placed on a horizontal surface until the nutrient medium solidified completely. After solidification of the medium, the inoculation dishes, turned upside down, were cultured in a thermostat at 30±1°C for 72±3 h. To calculate the QMAFA\text{anM}, all grown colonies were taken into account in dilutions, the number of colonies in which was not more than 300.

After that we studied the impact of NA shell disinfection on the microbiological parameters of egg contents, their weight and pH during storage.

The control batch of eggs was not treated with a product or was hed with water; experimental eggs were treated with 50% (with an exposure of 5 min) and 100% (with an exposure of 2 min) NA solutions. After the specified exposure, the eggs were washed with sterile tap water, dried at room temperature and stored at 4±2°C for 15 days in a refrigerator. Eggs were examined for 5, 10 and 15 days of storage. We measured the weight, pH, determined the microbiological parameters: the presence of Salmonella (Salm.) / 25 g, the number of bacteria of the group of E. coli (BCGC) / 0.01 g and QMAFA\text{anM}, CFU/g.

When Salmonella was detected for preliminary nonselective, peptone-buffered medium was inoculated with the test sample at a ratio of 1:10, the inoculations were incubated at 37±1°C for 18±2 h were subcultured in 10.0±0.1 cm³ of Rappaport-Vassiliadis medium with soy (RVS-broth) and 1.0 cm³ of the culture were subcultured in 10.0±0.1 cm³ in tetrathionate broth (Müller-Kaufmann). Inoculations in RVS broth were incubated at 41.5±1.0°C for 24±3 h, in tetrathionate broth (Müller-Kaufmann) they were incubated at 37±1°C for 24±3 h. To isolate a pure culture after incubation on selective media, inoculum was inoculated on XLD-arap and bismuth-sulfite agar. For the identification of Salmonella, the API 20EAPI 20E (BioMerieux, France) test kits were used. Confirmation of the belonging of the isolated cultures to bacteria of the genus Salmonella was carried out by serological identification using the agglutination reaction.

When bacteria of the group of Escherichia coli (coliform bacteria) (BGKP) were detected, 1±0.1 g of the sample was transferred into a test tube containing 10 cm³ of an enrichment selective medium (McConkey broth). Tubes with inoculations were incubated at 37±1°C for 24±2 h. crystal surface violet neutral red bile lactose agar (VRBL-agar). The inoculations were incubated at 37±1°C for 24±2 h and the growth of typical and atypical colonies was noted. For additional confirmation, at least five typical and atypical colonies were selected from Petri dishes with inoculations. Each selected colony is subcultured onto the surface of a nutrient agar slant. The inoculations were incubated at 37±1°C for 24±2 h. The belonging of the grown bacteria to coliform bacteria was determined in relation to the Gram stain, the absence of oxidase, and the fermentation of lactose.

To detect the quality indicators of the eggs [18], the purity of the shell of the selected eggs was checked visually in bright diffused light. The state of the air chamber and its height, the state and position
of the yolk and the integrity of the shell were determined by translucent eggs on an ovoscope by turning them. The height of the air chamber is measured with a gauge while the eggs are translucent with an ovoscope.

To determine the pH of the egg’s content, the samples were thoroughly mixed, poured into clean numbered bottles, 15-18 g each. The pH was determined directly in weighing bottles immediately after breaking the evaluated eggs, without dilution, each sample 3 times, after which the average value was calculated. Before measuring the pH of the protein and yolk, the instrument was adjusted to standard buffer solutions with pH 8.5-9.0 and 4.9 g-5.8, respectively. To determine the pH of the egg content, we used a pH-211 Microprocessor pH Meter (HANNA Instruments, Germany).

3. Results and discussion

At the beginning of the research, we studied the agents disinfecting activity using cambric test objects contaminated with S. enteritidis. The research results are given in table 1.

Table 1. Bactericidal properties of NA against S. enteritidis.

| Exposure time, min | Solution concentration, % | 20 | 50 | 100 |
|-------------------|---------------------------|----|----|------|
|                   | Control                   | Detected | Detected | Not detected |
| 2                 | 5                          | Detected | Not detected | Not detected |
| 5                 |                            | Detected | Not detected | Not detected |

It was established that the use of 20% (2 and 5 min exposure) and 50% solutions (exposure period of 2 min) NA solutions provides no bactericidal effect against S. enteritidis.

S. enteritidis inactivation on cambric test objects was ensured by 50% (5 min exposure) and 100% (exposure period of 2 and 5 min) NA solutions.

The disinfecting efficacy of NA in the process of the egg shell disinfection was studied in further studies. For this purpose, the eggshells were previously experimentally contaminated with the bacterial suspension in meat-peptone broth containing \((8.24 \pm 0.36) \times 10^8\) microbial bodies/mL of S. enteritidis.

After contamination, the eggshell contained \((2.45 \pm 0.12) \times 10^4\) CFU/cm² of S. enteritidis. 50% (exposure period of 5 min) and 100% (exposure period of 2 min) NA solutions inactivate S. enteritidis on the egg shell surface (table 2).

Table 2. Disinfecting efficacy of NA solutions (n=10).

| Exposure time, min | Test culture growth | Solution concentration, % | 20 | 50 | 100 |
|-------------------|---------------------|---------------------------|----|----|------|
|                   | Control             |                           | +  | ±  | –    |
| 2                 | \((2.45\pm0.12)\times10^4\) |                           | +  | ±  | –    |
| 5                 |                      |                           | –  | –  | –    |

According to the results of microbiological studies (table 3), QMAFA\(_{AnM}\) of the eggs’ contents in the control and experimental groups at laying and after 15 days of storage did not exceed 10 CFU/g. Coliforms and Salmonella were observed in the eggs’ contents neither in the control group nor in the experimental groups.

The data on the eggs weight and pH change after treatment with 100% NA solutions are given in table 4. The weight loss in eggs during 15 days storage amounted to 2.51±0.11 g in eggs of the control batch, and 2.56±0.11 g in eggs after the shell surface treatment with 100% (by agent concentration) NA solutions. pH of the eggs in the control batch amounted to 6.9±0.14 at the beginning of the storage period, and 7.1±0.11 after 15 days of storage, pH of the eggs in the experimental group amounted of 6.9±0.13 and 7.1±0.12 units, respectively.
Table 3. The impact of the eggshell treatment with NA on the microbiological parameters of the eggs contents.

| Storage period, days | Control QMAFANM | Coliforms/ Salm. | 5 min exposure by 50% solution QMAFANM | Coliforms/ Salm. | 2 min exposure by 100% solution QMAFANM | Coliforms/ Salm. |
|----------------------|-----------------|-----------------|-----------------------------------------|-----------------|-----------------------------------------|-----------------|
| 0                    | < 10 N/d / N/da | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  |
| 5                    | < 10 N/d / N/d  | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  |
| 10                   | < 10 N/d / N/d  | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  |
| 15                   | < 10 N/d / N/d  | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  | < 10 N/d / N/d                            | < 10 N/d / N/d  |

a N/d – not detected.

Table 4. The impact of the eggshell treatment with NA solutions with 2 min exposure on the eggs weight and pH.

| Egg groups | Parameter | Storage period, days |
|------------|-----------|----------------------|
|            | 5         | 10                   | 15                   |
| Control    | Weight, g before storage 69.90±0.15 | 69.80±0.19 | 69.89±0.23 |
|           | after storage 69.33±0.23 | 68.22±0.37 | 67.38±0.42 |
|           | drying loss, g 0.57±0.02 | 1.58±0.07 | 2.51±0.11 |
|           | pH before storage 6.9±0.14 | 7.0±0.12 | 7.1±0.11 |
|           | after storage 6.99±0.16 | 68.96±0.19 | 67.83±0.23 |
| Experimental | weight, g before storage 69.32±0.23 | 67.35±0.37 | 66.17±0.42 |
|           | drying loss, g 0.67±0.03 | 1.61±0.08 | 2.56±0.11 |
|           | pH before storage 6.9±0.13 | 6.9±0.13 | 7.0±0.11 |
|           | after storage 6.9±0.13 | 7.0±0.11 | 7.1±0.12 |

The qualitative indicators of eggs after disinfection with NA solutions are given in table 5. As you can see from the data in the table, there were no significant differences in the state of the air chamber and its height, the state and position of the yolk, the density and color of the white in the control batch and in the eggs treated with 50% and 100% NA solutions before and after storing.

Before storing, the eggshells were clean, without discoloration, the eggs had a fresh smell (faint lime smell), which did not change during storage both in the control batch and in the batch of eggs with the shell disinfected using NA solutions. Thus, the use of electrochemically activated oxidants is possible for disinfection of the shell of edible eggs, ensures the inactivation of Salmonella on the shell and does not affect the quality of the eggs.

The results obtained agree with a study of the antimicrobial properties of electrochemically activated oxidants [19], where the authors evaluated the effectiveness of slightly acidic electrolyzed water (SAEW) at various temperatures (4, 20 and 45°C) to inactivate S. enteritidis and it on the surface of eggs in shell. The bactericidal activity of SAEW, sodium hypochlorite solution (NaClO) and acidic electrolyzed water (AEW) for inactivation of S. enteritidis was also compared. SAEW with the pH of 6.0-6.5 was used by electrolysis of dilute hydrochloric acid (2.4 mM) in a chamber without a membrane. Studies have shown that although the pH value of SAEW was significantly higher than that of AEW (pH=2.6-2.7), SAEW had a relatively potent bactericidal activity at the same available chlorine concentrations. The effectiveness of SAEW in inactivating pure cultures of S. enteritidis increased with the available chlorine concentration and treatment time at three different temperatures. S. enteritidis decreased to less than 1.0 log CFU/mL with 2 mg/L chlorine available and 100% inactivation (8.2 log CFU/mL reduction) led to the use of SAEW with more than 4 mg/L chlorine available at 4, 20 and 45°C after 2 min of treatment, while no decrease was observed in the control samples. In addition, SAEW was also effective in inactivating S. enteritidis grafted onto the surface of in-shell eggs. A 6.5 log CFU/g
reduction in S. enteritidis on in-shell eggs was achieved by SAEW containing 15 mg/L available chlorine over 3 min, but only a 0.9-1.2 log CFU/g reduction for controls. Survival of S. enteritidis was not restored by flushing SAEW waste after treatment. The researchers concluded that SAEW could be a promising disinfectant for the treatment of eggs in shell without contamination of the environment [19].

Table 5. Qualitative indicators of chicken eggs during storage after disinfection with NA solutions.

| Egg groups               | Qualitative indicators                        | Air chamber state and height | Yolk state and position | White density and color |
|--------------------------|------------------------------------------------|-----------------------------|-------------------------|-------------------------|
| Before storage           |                                                |                             |                         |                         |
| Control                  | Stable, slightly visible, contours are not visible, takes central position and does not move | Permanent, height NMT 4 mm | The same                | Dense, light, transparent |
| Disinfection - 50% NA    |                                                | Stable, slightly visible, small deviation from the center | The same                | The same                |
| Disinfection - 100% NA   |                                                | Stable, slightly visible, contours are not visible, takes central position and does not move | The same                | The same                |
| After 15 days storage    |                                                |                             |                         |                         |
| Control                  |                                                | Stable, slightly visible, small deviation from the center | The same                | The same                |
| Disinfection - 50% NA    |                                                | Stable, slightly visible, small deviation from the center | The same                | The same                |
| Disinfection - 100% NA   |                                                | Stable, slightly visible, small deviation from the center | The same                | The same                |

According to in vitro studies of the electrolysis of oxidizing (EO) water [20], eggs were soaked in alkaline EO water followed by soaking in acidic EO water at various temperatures and times. The treated eggs showed a population reduction. A decrease in Log10 of 1.7 and 2.0 for S. enteritidis and E. coli K12, respectively, was observed for typical commercial detergents-disinfectants, while a decrease in log10 ≥ 2.1 and ≥ 2.3 for S. enteritidis and E. coli K12, respectively, were achieved using EO water purification. For a pilot study, both EO water fractions were compared with a detergent-disinfectant using E. coli K12. Decreases in Log10 > or = 2.98 and > or = 2.91 were found using EO water treatment and detergent-disinfectant, respectively. The effect of 2 treatments on egg quality was investigated. EO water and detergent disinfectant did not significantly affect protein height or egg shell strength; however, there was a significant effect on the presence of the cuticle. It was concluded that EO water can be used as a disinfectant for disinfecting eggs [20].

4. Conclusion
The conducted studies have studied the effect of disinfection of the shell with NA solutions on the safety and quality of food eggs. It has been established that the use of 100% for disinfection of the shell of edible eggs with mechanized (exposure 2 min) and 50% (by means) with manual (exposure 5 min) methods of disinfection of NA solutions based on highly active metastable (electrochemically activated) oxidants ensures inactivation of S. enteritidis on its surface and does not affect the quality indicators of eggs. Thus, the use of 100% (at an exposure of 2 min) and 50% (at an exposure of 5 min) NA solutions is effective in disinfection of egg shells.

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