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Quality and safety of chicken eggs after washing and disinfection with a chlorine-containing agent

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Abstract. Contamination of food-grade chicken eggs with pathogenic and conditionally pathogenic microorganisms in the process of their production, storage, and sale causes a high risk of toxicoinfections in humans. One of the most effective ways to reduce microbial load is to wash and disinfect the surface of eggshells with active chlorine-based products. The purpose of the study was to determine the quality of washing and disinfection of food-grade chicken eggs during storage in a chilled form. In the experiment, the quality and microbiological indicators of food-grade chicken eggs were determined after washing and disinfection on the 1st, 60th, and 80th days of storage at a temperature of 4°C. The microbiological parameters of eggs were studied using MALDI TOF technology, and the quality was studied using digital ovoscopy. Washing and disinfection with a preparation based on active chlorine at a concentration of 0.5% freed eggshells from colonies of MAFAnM and mould fungi. Storage of washed and disinfected eggs in a chilled form until the 60th and 80th days contributed to an increase in the QMAFAnM by 2.45 lg CFU/cm² and at 5.68 lg CFU/cm² accordingly. The quantity of mould fungi on the shell surface of washed and disinfected eggs on the 60th day of storage reached 4.37 lg CFU/cm², and on the 80th day, their number was equal to the QMAFAnM. The quantity of MAFAnM and mould fungi in the yolks of food-grade eggs had a strong direct dependence on their quantity on the shell and on their shelf life in chilled form. Washing and disinfection of food-grade chicken eggs with a chlorine-containing preparation did not affect the loss of weight, albumen index, yolk colour, strength and thickness of shell, but reduced the HU index to 78.5-79.5 units, which, combined with microbiological indicators, allowed them to be stored in a chilled form up to 80 days. The results obtained should be factored in when choosing the product, washing and disinfection mode, and shelf life of food-grade eggs, considering the species composition of microorganisms characteristic of a particular poultry farm

Keywords: shell; yolk; QMAFAnM; mould fungi; ovoscopy; microbial contamination

Introduction

During the production of food-grade eggs, there is a risk of contamination of the shell with droppings, dust, and microorganisms that pose a danger to human health. Chicken eggs are a source of dietary protein, lipids, polyunsaturated fatty acids, vitamins, minerals and carotenoids, which are necessary for the human body (Shevchenko *et al.*, 2020; 2021). As noted by S. Khan *et al.* (2024), the suitability of eggs for consumption is determined by the level of safety, which is affected by both the conditions of keeping and feeding an industrial herd of chickens, and the sanitary and hygienic conditions of egg production, collection, processing, and storage. Although eggshells are a natural barrier to many species of opportunistic and pathogenic microorganisms, they may not only accumulate on the surface, but also enter the egg (Mahmoud *et al.*, 2023).

According to J. An & H. Lee (2023), contamination of eggshells and food contents is an important public health issue. Eggshells can be contaminated both vertically and horizontally, including infectious diseases of chickens and contamination that can enter eggs from the environment (Borysevich & Lisova, 2020; Khatun *et al.*, 2022). Thus, microbial contamination of eggs depends on the conditions of keeping chickens, storing, distributing, processing, or cooking food. According to K. Petrovič *et al.* (2024), the spread of toxicoinfections through eggs and egg products occupy the first place in the list of human toxicoinfections. Micro-organisms can enter eggs during laying, harvesting, packaging, storage, and transportation. *Salmonella* is one of the most dangerous toxicoinfections of food origin, which is often

found in food-grade eggs and affects the human digestive system in EU countries (Chan *et al.*, 2023). Contact of eggs with chicken droppings can also cause infection, which can get inside the egg and cause it to spoil. Spoilage of eggs is one of the main reasons that determine their shelf life, so in order to reduce the contamination of the surface of eggshells with microorganisms and mitigate the risk of human infection with dangerous toxicoinfections, eggs are subjected to certain treatments, since infected eggshells cause more than 75% of the 1.2 million cases of salmonellosis and infectious diseases that occur in different segments of the population every year.

Washing and disinfecting eggs can reduce the number of viable *Salmonella* bacteria on the shell. In the United States, eggs are usually washed using chemical disinfectants such as quaternary ammonium salts or chlorine compounds. Recently, a new non-thermal disinfection technique has been developed for washing eggshells using cold plasma-activated water, which allows inactivating *Klebsiella michiganensis* by reducing the number of colonies $>5.28 \log$ CFU/egg. It is known that chemicals designed to wash eggs can remain on the shell and damage the cuticle, which increases the risk of penetration and contamination of the eggs with *Salmonella* and other bacteria (Medina-Gudiño *et al.*, 2020). Washing food-grade eggs is prohibited in some countries due to the risk of pathogenic microorganisms entering through the shell and cross-contamination. An alternative approach to the inactivation of *Salmonella* in eggs is pasteurisation, which is widely used in the United States. Pasteurisation of eggshells is carried out using infrared radiation, hot water, or hot air. However, such heat treatment causes protein denaturation and the formation of a gel mesh of protein around the inner surface of the shell, which obviously affects the rheological properties of yolk and albumen proteins, leads to a deterioration in texture properties and, as a result, a decrease in attractiveness for consumers.

Several non-thermal technologies, such as various types of packaging, the use of ultraviolet irradiation, ozonation, and chilled storage, have been used to extend the shelf life of eggs (Sokołowicz *et al.*, 2023). UV radiation is widely used for disinfection of surfaces, but it has disadvantages, the main of which is the low ability to penetrate through dirt on the surface of the shell, under which there may be microorganisms.

Washing improves the appearance of eggs, but does not benefit their storage, as it leads to the opening of pores in the shell. Therefore, the washing strategy is usually used before breaking eggs in the food industry. As noted by S. Sokovnin (2021), toxic waste is generated when washing eggs, in particular, wastewater with residues of disinfectants and detergents, such as formaldehyde or phenolic compounds. Given the large list of methods and means of washing and disinfecting eggs used in the food industry, it can be concluded that there are no universal ones that would meet the requirements of hygiene, ecology, quality, and safety. A reliable and promising method of processing food-grade chicken eggs is the use of complex products that simultaneously have washing and disinfecting properties. They have a milder effect on the structure of the internal components of eggs compared to pasteurisation. Disinfection is applied to soft treatments in which eggshells are disinfected using certain chemicals (such as chlorine) or new technologies. Therefore, the purpose of these studies was to determine the microbiological parameters and quality of chicken eggs after washing and disinfection with a chlorine-containing preparation for storage in a chilled form.

Materials and Methods

For the study, food-grade chicken eggs obtained from chickens of the industrial flock of the High-Line W36 cross at Ovostar LLC, Kyiv Oblast, were used. The study was conducted during September-December 2024. Chicken eggs were selected on the 1st day of laying and

divided into two batches: Batch 1 was subjected to ovoscopy and microbiological studies of the shell and yolks were performed. Batch 2 was

washed and disinfected and placed in egg pads for storage in a refrigerator at a temperature of 4°C (Table 1).

Table 1. Scheme of the experiment to study the quality of washing and disinfection of food-grade chicken eggs

Characteristics of eggs	Shelf life, days	Storage conditions
Eggs before washing and disinfecting	1	temperature 4°C, relative humidity 80-85%
Eggs after washing and disinfection	1	
	60	
	80	

Source: developed by the authors

Washing and disinfection of chicken eggs was carried out using a 0.5% solution of detergent with disinfectant effect Blanidas-C Gen Deo (Blanidas LLC, Kyiv, Ukraine), which contained sodium hypochlorite and sodium hydroxide, with a mass fraction of active chlorine of 5.6%. Eggs were washed using a Unifortes B.V. line (Unifortes, Netherlands), which is designed to wash 36,000 eggs per hour. The washing line was equipped with a UNI-EW350.6 + drying module, which provided egg drying. Egg washing consisted of the following steps: washing with a detergent and disinfectant solution, rinsing eggs from the remains of detergents and disinfectants, and venting the eggs to remove moisture residues from the shell surface. After drying, the process of packing eggs in egg pads and storing them in the egg warehouse was carried out.

Ovoscopy and microbiological studies were performed on the first day before and after washing and disinfection, and on the 60th and 80th days after washing and disinfection of eggs. For ovoscopy, 15 eggs were used for each study, and 5 samples were prepared for microbiological analysis. Each sample for microbiological studies was formed from 5 eggs. The experiment determined the weight of eggs, albumen index, yolk colour (16-point YolkFan™ colour scheme), HU units, shell strength and thickness using the DET 6000 digital egg tester (NABEL Co., Ltd., Japan). Among the main

indicators that help to determine their quality are the HU unit, yolk colour, shell thickness and strength, albumen index, and egg weight. HU is a common indicator used in poultry farming to measure the quality of egg white. It measures the index of thick albumen surrounding the yolk.

Microbiological studies of eggs were carried out at the Expert Centre “Biolights” LLC, Ternopil (accredited according to ISO/IEC17025). To determine the QMAFAnM (quantity of mesophilic aerobic and facultative anaerobic microorganisms) and mould fungi, egg yolks were used and eggshell washes were prepared. To do this, consecutive tenfold dilutions were made in sterile saline solution. The number of microorganisms on the shell surface and in egg yolks was determined in colony-forming units (CFU), the results were expressed in lg CFU/cm² and lg CFU/g, respectively. The QMAFAnM was determined using the Plate count agar M091 medium (HiMedia, India), mould fungi – Sabouraud Agar (HiMedia, India).

To determine the species composition of microorganisms, the samples were inoculated on differential diagnostic media and plated by the method of sectoral cultivation (Baird-Parker Agar (HiMedia, India), XLD Endo Agar (HiMedia, India), Pseudomonas Agar (HiMedia, India), Enterococcus Agar (HiMedia, India), Bacillus Cereus Agar (HiMedia, India)) and grown for 24 ± 1 or 48 ± 1 h at 37 ± 1 or 30 ± 1°C (according to the incubation requirements of the culture

medium). After incubation, the grown microbial colonies were identified using the MALDI TOF method. This method was based on the detector's determination of the flight time of ionised ribosomal proteins of various microorganisms, followed by the conversion of this information into a molecular mass spectrum, which was compared with spectra from a unique database (Singhal *et al.*, 2015). For microbiological studies, a Bruker Daltonics mass spectrometer, Maldi ToF microflex (Bruker Daltonics, Germany) was used. Identification of isolated cultures of microorganisms from eggs was performed using MBT Compass MALDI Biotyper 3.1 Compass 1.4 for FLEX – Volume 1, 2 Software and Manuals (Bruker Daltonik, Bremen, Germany).

The results obtained were processed statistically using univariate analysis of variance

using Microsoft Excel 2016. The dynamics of the number of microbial colonies in food-grade eggs was determined by regression and correlation analysis, the data in the tables were presented as $\bar{x} \pm SD$ (mean \pm standard deviation). The difference between the values of washed and unwashed chicken eggs during the storage period was calculated using the Tukey test, the probability was considered $P < 0.05$ (taking into account the Bonferroni correction).

Results and Discussion

The results of the study showed that there was a small quantity of MAFAnM on the surface of the shell of freshly laid unwashed eggs, and after washing on the first day of storage, no colonies of viable microorganisms were detected (Table 2).

Table 2. Microbial contamination of the surface of the shell of chicken eggs after washing and disinfection during storage in a chilled form, $\bar{x} \pm SD$, $n = 5$, lg CFU/cm²

Characteristics of eggs	Shelf life, days	MAFAnM	Mould fungi
Eggs before washing and disinfecting	1	0.43 \pm 0.14 ^a	< 1 ^a
Eggs after washing and disinfection	1	< 1 ^b	< 1 ^a
	60	2.45 \pm 0.17 ^c	4.37 \pm 0.21 ^b
	80	5.68 \pm 0.42 ^d	5.61 \pm 0.38 ^c

Note: different letters of the upper indexes indicate values that significantly differed in the same column of the table ($p < 0.05$) based on the results of comparison using the Tukey test

Source: developed by the authors

Storage of washed and disinfected eggs up to 60 days in a chilled form contributed to an increase in the QMAFAnM by 2.02 lg CFU/cm² compared to unwashed eggs and at 2.45 lg CFU/cm² compared to washed and disinfected ones on the 1st day of storage. The increase in the shelf life of washed and disinfected eggs to 80 days contributed to an increase in the QMAFAnM colonies on the shell surface by 5.25 lg CFU/cm² compared to unwashed eggs on the 1st day of storage, by 5.68 lg CFU/cm² compared to washed and disinfected eggs on the 1st day of storage, and by 3.23 lg CFU/cm² – compared to washed and disinfected eggs on the 60th day of storage.

Colonies of mould fungi on the shell surface of unwashed and washed and disinfected eggs were not found on the 1st day of storage. On the 60th day of storage of washed and disinfected eggs using chlorine-containing agent Blanidas-C Gen Deo, the quantity of mould fungi on the shell surface reached 4.37 lg CFU/cm², and on the 80th day of storage, the number increased to 5.61 lg CFU/cm². As noted by B. Abdoli *et al.* (2024), this is associated with the specificity of the structure of the eggshell cuticle and the presence of soluble matrix proteins in it, which at a concentration of 0.1 mg/mL can inhibit the growth of certain microorganisms, in

particular, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, for more than 8 hours, and *S. enteritidis* and *E. coli* for 4 hours.

Storage of washed and disinfected food-grade eggs up to 60 days in a chilled form contributed to the growth and reproduction of microorganisms and mould fungi on the surface of the shell, but their total number did not exceed the permissible standard value, which allowed considering such eggs suitable for consumption. On the 80th day of storage on the surface of the shell of washed and disinfected eggs, the quantity of MAFAnM and mould fungi was almost equal, which indicates their spread and intensive reproduction. The shells of unwashed food-grade chicken eggs were contaminated by representatives of genera *Bacillus* and *Staphylococcus*. During the entire storage period, no pathogenic microorganisms such as *Salmonella* spp., *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* were detected. As emphasised by L. Lin *et al.* (2020) and D. Bermudez-Aguirre & B. Niemira (2023), this is particularly important because most of the rules of good hygiene practices in egg poultry farming apply to preventing egg contamination with *Salmonella* spp., *E. coli* and other pathogenic and conditionally pathogenic microorganisms. In research by A. Aygun (2017), various bacteria were isolated and identified from the surface of eggshells and egg contents, including *Salmonella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Campylobacter* spp., *E. coli*, *Clostridium perfringens*, *Listeria monocytogenes*, *Citrobacter freundii*, *Bacillus cereus*. These microorganisms pose a potential risk to human health and can cause food toxicoinfections (Lin *et al.*, 2021; Chousalkar *et al.*, 2021).

A strong direct correlation was found between the QMAFAnM on the shell surface of washed and disinfected chicken eggs and their shelf life in chilled form ($r = 0.937 \pm 0.122$, $P < 0.001$). An increase in the QMAFAnM on the shell surface of washed and disinfected food-grade eggs was characterised by a linear dependence on the shelf life of their chilled form (Fig. 1).

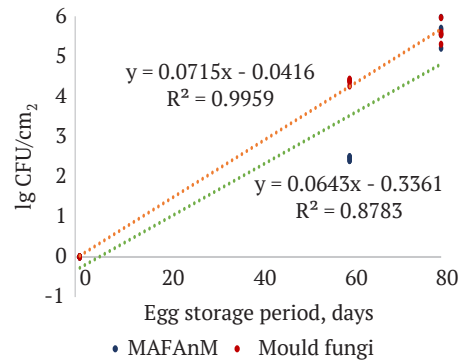


Figure 1. Dependence of the quantity of MAFAnM and mould fungi on the shell surface of washed and disinfected chicken eggs on the shelf life in chilled form, $n = 15$
Source: developed by the authors

Increasing the number of microorganisms on the surface of the eggshell increases the likelihood of their penetration into the internal environment of the egg (Oliveira *et al.*, 2022; 2024). In the yolks of unwashed, as well as washed and disinfected chicken eggs on the 1st day of storage of colonies, MAFAnM were not observed. On the 60th day of storage of washed and disinfected eggs, an increase in the QMAFAnM to 2.82 lg CFU/g was established, and on the 80th day – to 4.57 lg CFU/g, which almost reached the MPL (maximum permissible level) according to DSTU 5028:2008 (2010) (Table 3).

Table 3. Microbial contamination of chicken egg yolks after washing and disinfection during chilled storage, $x \pm SD$, $n = 5$, lg CFU/g

Characteristics of eggs	Shelf life, days	MAFAnM	Mould fungi
Eggs before washing and disinfecting	1	< 1 ^a	< 1 ^a
Eggs after washing and disinfection	1	< 1 ^a	< 1 ^a

Table 3. Continued

Characteristics of eggs	Shelf life, days	MAFAnM	Mould fungi
	60	2.82 ± 0.19 ^b	1.43 ± 0.12 ^b
	80	4.57 ± 0.34 ^c	2.54 ± 0.17 ^c

Note: different letters of the upper indexes indicate values that significantly differed in the same column of the table ($p < 0.05$) based on the results of comparison using the Tukey test

Source: developed by the authors

Mould fungi in the yolks of unwashed and washed and disinfected food-grade chicken eggs were not isolated on the 1st day of storage. On the 60th day of storage, a small quantity of mould fungi appeared in the yolks of washed and disinfected chicken eggs, the number of which increased by 1.11 lg CFU/g on the 80th day of storage compared to the previous period. Correlation analysis showed a strong direct dependence of the QMAFAnM in the yolks of washed and disinfected chicken eggs on their shelf life in chilled form ($r = 0.984 \pm 0.035$, $P < 0.001$). The increase in the quantity of MAFAnM colonies in the yolks of washed and disinfected chicken eggs during the shelf life was characterised by a linear relationship (Fig. 2).

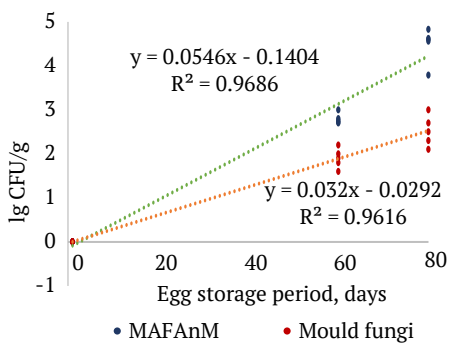


Figure 2. Dependence of the quantity of MAFAnM and mould fungi in the yolks of washed and disinfected food-grade chicken eggs on the shelf life in chilled form, $n = 15$
Source: developed by the authors

The quantity of mould fungi in the yolks of washed and disinfected chicken eggs had a strong direct dependence on their shelf life

($r = 0.981 \pm 0.038$, $P < 0.01$). The regression line showed that there is a direct linear relationship between the number of mould colonies in the yolk of washed and disinfected chicken eggs and their shelf life in chilled form (Fig. 2). Determination of the dependence of the QMAFAnM in yolks on the QMAFAnM on the shell surface of washed and disinfected chicken eggs showed a strong direct correlation ($r = 0.973 \pm 0.053$, $P < 0.001$). Regression analysis confirmed a linear relationship between microbial contamination of the shell surface and egg yolk (Fig. 3).

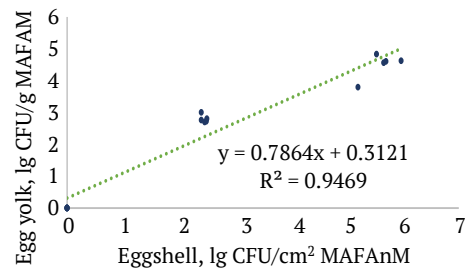


Figure 3. Dependence of the QMAFAnM in yolks on the QMAFAnM on the shell surface of washed and disinfected chicken eggs for storage in chilled form, $n = 15$
Source: developed by the authors

A strong direct relationship was also found between the level of contamination of the yolk and shell of washed and disinfected eggs by mould fungi ($r = 0.976 \pm 0.048$, $P < 0.001$) during their chilled storage. The regression line confirmed the existence of a direct linear relationship between the number of mould colonies on the shell surface and in the yolks of washed and disinfected food-grade chicken eggs when stored chilled (Fig. 4).

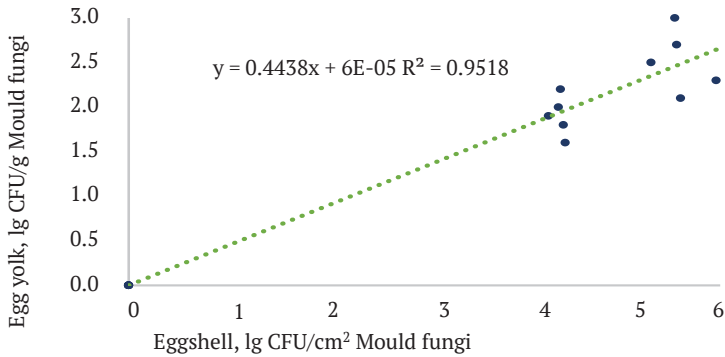


Figure 4. Dependence of the quantity of mould fungi in yolks on the number of quantity fungi on the shell surface of washed and disinfected food-grade chicken eggs for storage in chilled form, n = 15

Source: developed by the authors

Analysis of the species composition of microorganisms in the shells of unwashed chicken eggs showed that the main representatives of microorganisms on the 1st day of storage were the

genus *Bacillus* (family Bacillaceae) which includes *B. mycoides* and *B. pumilus* and gender *Staphylococcus* (family Staphylococcaceae), which includes two species: *S. epidermidis* and *S. xylosus* (Table 4).

Table 4. Microbiological screening with MALDI-TOF identification of chicken eggshells after washing and disinfection for storage in chilled form

Characteristics of eggs	Shelf life, days	Types of dominant microorganisms
Eggs before washing and disinfecting	1	<i>Bacillus mycoides</i> , <i>Bacillus pumilus</i> , <i>Staphylococcus epidermidis</i> *, <i>Staphylococcus xylosus</i> ,
Eggs after washing and disinfection	1	<i>Staphylococcus epidermidis</i> *, <i>Staphylococcus saprophyticus</i> , <i>Lactococcus garvieae</i>
	60	<i>Staphylococcus equorum</i> **
	80	<i>Staphylococcus equorum</i> **

Note: *, ** – identical types of bacteria on the surface of eggshells

Source: developed by the authors

Bacillus mycoides and *B. pumilus* come from the environment, in particular, belong to soil microorganisms that promote plant growth (Di Franco *et al.*, 2002). Thus, these types of bacteria could get into the composition of chicken feed, and from the feed to the surface of the eggshell. After washing and disinfecting food-grade eggs on the 1st day of storage in a chilled form, representatives of microorganisms of two genera were identified on the surface of the shell, in particular the genus *Staphylococcus* which includes *S. epidermidis* and *S. saprophyticus*, and the genus *Lactococcus* (family Streptococcaceae) – *L. garvieae*.

On the 60th and 80th days of storage of washed and disinfected eggs, only one representative of the genus *Staphylococcus* was isolated from the shell surface – *S. equorum*. It is believed that staphylococci make up an important part of the microbiome of the shell and the contents of the egg (albumen and yolk). The results of this study are consistent with the data obtained by D. Stepień-Pyśniak *et al.* (2009), which indicate a relatively high level of contamination of food-grade eggs with staphylococci.

Of the 1,125 bacteriological studies of egg whites, yolks, and shells, staphylococci were

detected in 514 cases in this study. The largest percentage of staphylococci was isolated from the surface of eggshells – 302 strains. 12 types of staphylococci were isolated from the eggs under study, including coagulase-positive strains such as *S. hyicus* and *S. aureus*, and coagulase-negative, in particular, *S. warneri*, *S. lentus*, *S. xylosus* and *S. epidermidis*. Results of the study by S. Hsu et al. (2023) also indicate that in unwashed chicken eggs, 197 samples were positive for *Staphylococcus* spp. In addition to staphylococci, there are a number of other organisms that can contaminate the shell of chicken eggs, in particular, *Alcaligenes*, *Salmonella*, *Pseudomonas*, *Arthrobacter*, *Proteus*, *Escherichia*, *Serratia*, *Bacillus*, *Hafnia*, *Aeromonas*, *Citrobacter* and *Micrococcus* (Legros et al., 2021; Kukhtyn et al., 2024). Mould fungi and yeast were isolated in smaller amounts from eggshells when eggs were stored in high humidity conditions and were represented by five genera

Aspergillus, *Penicillium*, *Cladosporium*, *Mucor*, *Rhizopus*, and yeast by one genus – *Rhodotorula*.

Washing and disinfecting chicken eggs with Blanidas-C Gen Deo did not ensure the release of the shell surface from staphylococci and streptococci, which dominated other microorganisms. Moreover, the species composition of staphylococci changed, and on the 60th and 80th day of storage of washed and disinfected food-grade eggs, the shell surface contained only *S. equorum*. The results obtained are consistent with the data of A. Wilson et al. (2021), who identified *S. equorum* in poultry houses, in particular, it was most common in bedding (33.7%), nests (25.1%) and egg conveyor belts (35.0%) when using a free-range chicken system. Analysis of the species composition of microorganisms in the yolks of chicken eggs showed their significant difference from the shells of both unwashed and washed and disinfected eggs (Table 4, 5).

Table 5. Microbiological screening with identification of MALDI-TOF egg yolks of food-grade eggs after washing and disinfection for storage in chilled form

Characteristics of eggs	Shelf life, days	Types of dominant microorganisms
Eggs before washing and disinfecting	1	<i>Pantoea agglomerans</i> , <i>Pseudomonas koreensis</i>
Eggs after washing and disinfection	1	<i>Staphylococcus epidermidis</i> *
	60	<i>Pseudomonas alcaliphila</i> , <i>Pseudomonas oleovorans</i>
	80	<i>Bacillus subtilis</i> , <i>Staphylococcus epidermidis</i> *

Note: * – identical types of bacteria in egg yolks

Source: developed by the authors

In the yolks of unwashed eggs on the 1st day of storage, two genera were distinguished, which belong to two different families, which included one type of microorganisms each. Genus *Pantoea* (family Erwiniaceae) in the yolks of unwashed washed eggs is represented by *P. agglomerans*, and genus *Pseudomonas* (family Pseudomonadaceae) – *P. koreensis*. *P. agglomerans* is widespread both in animals, soil and water, and in humans and currently, there are a

significant number of contradictory statements about its pathogenicity for humans (Haralampidou et al., 2022; Casale et al., 2023). However, as noted by P. Ezeh et al. (2024), it is quite resistant to a number of antibiotics, which requires further research. *P. koreensis* belongs to newly isolated bacteria and is also a natural component of the soil microbiome (Kho et al., 2023).

After washing food-grade eggs on the 1st day of storage, one type of microorganisms was

isolated in the yolks – *Staphylococcus epidermidis*. On the 60th day of storage of washed and disinfected food-grade eggs, microorganisms belonging to the genus *Pseudomonas*, namely *P. alcaliphila* and *P. oleovorans*, were found in the yolks, which differ in species composition from the yolks of unwashed eggs (Table 5). On the 80th day of storage of washed and disinfected chicken eggs, two types of microorganisms belonging to different families were identified: *Bacillus subtilis* and *Staphylococcus epidermidis*. The species composition of microorganisms in the shells and yolks of unwashed and washed chicken eggs depended on the shelf life of chilled form and differed from each other, with the exception of *S. epidermidis*, which was found both in flushes from the shell and egg yolks.

According to A. McWhorter & K. Chousalkar (2020), bacteria, viruses, and protozoa that get on the surface of the shell can enter the shell and survive in the eggs. If the eggs are not washed, they can become infected, and internal pathogens can multiply and grow during harvesting, sorting, transportation, and storage. Washing eggs did not release the yolks from *S. epidermidis* on the 1st day of storage, which also indicated a possible vertical path of contamination. Increasing the shelf life of washed and disinfected food eggs to 60 days indicates that bacteria of the genus *Pseudomonas* penetrate and multiply into the yolks, in particular *P. alcaliphila* and *P. oleovorans*. There is little information about the origin of these bacteria in the literature, but they are also part of the plant and soil microbiome (Chan *et al.*, 2021; Zeng *et al.*, 2023). In this study, it was found that increasing the shelf life of washed and disinfected eggs to 80 days contributed to reproduction *Bacillus subtilis* and *Staphylococcus epidermidis*. Given that the compound feed of industrial herd chickens included a probiotic, it can be assumed that *Bacillus subtilis* had an alimentary origin and ensured the dominance of beneficial microorganisms in the digestive system of chickens (Tsai *et al.*, 2023;

Tajudeen *et al.*, 2024). Similar data was obtained by S. Hsu *et al.* (2023) when analysing internal fractions of unwashed and washed chicken eggs. The prevalence of *Staphylococcus* spp., *Enterobacteriaceae* and *E. coli* was 1.9%, 1.9%, and 0.5%, respectively, which allowed the researchers to conclude that washed eggs have better microbial safety and quality.

It is possible to get a guaranteed effect from washing food eggs only considering many factors that relate to their quality. During egg storage, certain physical and chemical changes in albumen and yolk occur that affect their quality. Eggshells have a porous structure that provides a constant gas exchange between the internal contents of the egg and the environment. The porous eggshell also ensures the penetration of microorganisms, in particular, bacteria and mould fungi from the external environment. All of these processes contribute to chemical reactions in eggs, which ultimately leads to undesirable changes in albumen and yolk that degrade egg quality (Caner & Yüceer, 2015; Obianwuna *et al.*, 2022).

In fresh eggs, the thick albumen makes up about 60% of the egg's weight, and its pH is 5.6-7.5 and depends on the exchange of gases and moisture with the environment. The duration of storage and temperature are the main factors that determine the rate of spoilage of eggs. As the shelf life of eggs increases, the thick albumen becomes thinner due to the enzymatic activity of ovomucin-lysozyme, disulfide bonds or reactions between α - and β -ovomucins, and an overall increase in the pH of the contents. K. Fikiin *et al.* (2020) suggest that when storing eggs at a temperature of 10°C, the HU index almost did not change for 20 days, but at 30°C this indicator decreased from 85 to 35, which indicates a reduction in their shelf life. Washing and disinfecting chicken eggs with a chlorine-containing agent in this experiment did not affect egg weight, albumen index, yolk colour, shell strength and thickness during 80 days of storage (Table 6).

Table 6. Quality of chicken food-grade eggs after washing and disinfection when stored in a chilled form, $\bar{x} \pm SD$, $n = 15$

Indicator	Characteristics of eggs			
	Eggs before washing and disinfecting	Eggs after washing and disinfection		
	Shelf life, days			
	1	1	60	80
Egg weight, g	58.43 ± 1.88 ^a	57.66 ± 2.07 ^a	57.11 ± 1.54 ^a	56.41 ± 1.87 ^a
Albumen index, mm	7.67 ± 1.16 ^a	7.17 ± 0.79 ^a	6.14 ± 0.88 ^a	6.21 ± 0.43 ^a
Egg yolk colour, points	10.13 ± 0.92 ^a	10.20 ± 0.86 ^a	11.00 ± 0.93 ^a	11.14 ± 1.57 ^a
HU units	88.79 ± 5.61 ^a	85.78 ± 4.29 ^{ab}	78.48 ± 6.17 ^b	79.53 ± 2.59 ^b
Shell strength, kgf	4.96 ± 1.06 ^a	4.53 ± 1.12 ^a	4.52 ± 1.40 ^a	3.89 ± 0.67 ^a
Shell thickness, mm	0.38 ± 0.03 ^a	0.38 ± 0.04 ^a	0.39 ± 0.02 ^a	0.37 ± 0.04 ^a

Note: different letters of the upper indexes indicate values that significantly differed in the same column of the table ($p < 0.05$) based on the results of comparison using Tukey test

Source: developed by the authors

It was found that storing washed and disinfected eggs for 60 days contributed to a decrease in the HU index by 10.31 units, and for 80 days – by 9.26 units compared to unwashed eggs. Moreover, the HU index of washed and disinfected eggs, even before the 80th day of storage, did not reach the critically low value characteristic of spoiled eggs. These data are consistent with the results obtained by K. Drabik *et al.* (2021), who showed that decontamination of eggshells with an aqueous solution of citric acid at concentrations of 10% and 15% for storing eggs at 14°C and 70% humidity after 7, 14, 21, and 28 days reduces weight loss, provides a smaller air chamber, better albumen structure, less intense water diffusion from albumen to yolk.

The quality indicators of washed and disinfected eggs obtained in the study using a chlorine-containing agent also do not contradict the data of G. Akarca *et al.* (2021), where after washing, the eggshells were coated with almond, apricot, or cherry tree resins and stored at 4°C and 22°C for 60 days. In the present study, it was found that air chamber height, weight loss, albumen and yolk pH values increased in all samples during storage, while HU units, albumen and yolk index, on the contrary, decreased. The lowest weight loss (0.54 g) and

air chamber height (2.89 mm), the highest HU index (73.9), albumen index (8.8%) and yolk index (40.4%) were found in cherry resin-coated eggs when stored at 4°C. A similar pattern regarding changes in the quality of washed and disinfected eggs using pulsed light technology was obtained by B. Wang *et al.* (2022), who proved that with increasing storage time, the total value of the HU index and yolk index decreases, and albumen pH and weight loss increase. Thus, washing and disinfecting chicken eggs with a chlorine-containing agent ensures their proper quality and safety and allows them to be stored in a chilled form for up to 80 days.

Conclusions

The surface of the shell of unwashed chicken eggs on the 1st day of storage in chilled form did not contain colonies of mould fungi and was contaminated with a small quantity of MAFAnM microorganisms. The use of a chlorine-containing detergent and disinfection of eggs on the 1st day of storage inactivated MAFAnM colonies on their surface. With the increase in the shelf life of washed and disinfected eggs to 60 and 80 days, there was an increase in the quantity of MAFAnM and mould fungi on the surface of the shell and in the yolks, which was linear. The

following representatives of the family Bacillaceae were isolated in flushes from the shells of unwashed chicken eggs on the 1st day of storage in chilled form: *Bacillus mycoides* and *B. pumilus*; the family Staphylococcaceae: *Staphylococcus epidermidis* and *S. xylosus*. On the surface of the shell of washed and disinfected eggs on the 1st day of storage in chilled form, the basis was made up of microorganisms of the family Staphylococcaceae: *S. epidermidis* and *S. saprophyticus*, as well as family Streptococcaceae – *Lactococcus garvieae*. With an increase in the shelf life of washed and disinfected eggs to 60 and 80 days, the shell was dominated by *Staphylococcus equorum*. The surface of the shell of chicken eggs differs in the species composition of microorganisms from the yolks. The contamination of unwashed food egg yolks on the first day of storage with microorganisms of the family Erwiniaceae was revealed: *Pantoea agglomerans*, and Pseudomonadaceae: *Pseudomonas koreensis*. In the yolks of washed and disinfected food eggs, *Staphylococcus epidermidis* dominated on the 1st day of storage; on the 60th day – *Pseudomonas alcaliphila* and *P. oleovorans*; on the 80th day – *Bacillus subtilis* and

Staphylococcus epidermidis. The main type of microorganisms in the shells and yolks of washed and unwashed eggs for storage in a chilled form was *Staphylococcus epidermidis*. Washing and disinfecting food-grade eggs does not affect their quality, with the exception of the HU index, which decreased on the 60th and 80th days of storage, but was within the limits characteristic of high-quality eggs. The results obtained can be the basis for choosing a means of washing and disinfecting food-grade chicken eggs to reduce their contamination with pathogenic and conditionally pathogenic microorganisms, considering the epidemiological situation in a particular poultry farm. Future research may focus on the quality and safety of egg products made from processed chicken eggs.

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Conflict of Interest

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Якість та безпечність курячих яєць після миття і дезінфекції хлорвмісним засобом

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Анотація. Контамінація курячих харчових яєць патогенними та умовно патогенними мікроорганізмами в процесі їх виробництва, зберігання та реалізації спричиняє високий ризик виникнення токсикоінфекцій у людей. Одними з ефективних засобів зниження мікробного навантаження є миття і дезінфекція поверхні шкаралупи яєць препаратами на основі активного хлору. Метою дослідження було визначити якість миття та дезінфекції харчових курячих яєць під час зберігання в охолодженому вигляді. В досліді визначали якість та мікробіологічні показники харчових курячих яєць після миття та дезінфекції на першу, 60-ту та 80-ту добу зберігання за температури 4°C. Мікробіологічні показники яєць досліджували з використанням технології MALDI TOF, якість – за допомогою цифрової овоскопії. Миття та дезінфекція препаратом на основі активного хлору в концентрації 0,5 % звільняла шкаралупу яєць від колоній МАФАМ і плісневих грибів. Зберігання митих і дезінфікованих яєць в охолодженому вигляді до 60-ї та 80-ї доби сприяло збільшенню кількості МАФАМ на 2,45 lg КУО/см² і на 5,68 lg КУО/см² відповідно. Чисельність плісневих грибів на поверхні шкаралупи митих і дезінфікованих яєць на 60-ту добу зберігання досягала 4,37 lg КУО/см², а на 80-ту добу їх кількість зрівнялася з чисельністю МАФАМ. Чисельність МАФАМ і плісневих грибів в жовтках харчових яєць мала сильну пряму залежність від

їх кількості на шкаралупі і від терміну їх зберігання в охолодженому вигляді. Миття і дезінфекція харчових курячих яєць хлорвмісним препаратом не впливала на втрату маси, висоту білка, колір жовтка, міцність і товщину шкаралупи, але знижувала індекс НУ до 78,5-79,5 одиниці, що в поєднанні з мікробіологічними показниками дозволило зберігати їх до 80-ї доби в охолодженому вигляді. Отримані результати необхідно враховувати при виборі засобу, режиму миття і дезінфекції та терміну зберігання харчових яєць з урахуванням видового складу мікроорганізмів, характерного для конкретної птахофабрики

Ключові слова: шкаралупа; жовток; МАФAM; плісеневі гриби; овоскопія; мікробна контамінація