Research Article

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Nanocomposite coatings for hatching eggs and table eggs

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Abstract: Theoretical and applied aspects of the concept of using biomimetic protective coatings GREEN ARTICLE (ARTIficial cutiCLE) in the poultry industry, namely in the production of table and hatching eggs, are developed. The basic matrix component of the protective coatings GREEN ARTICLE is chitosan, an environmentally friendly, inexpensive, and harmless material. It is experimentally proved that electrochemical and ultrasonic technologies for modifying a solution of chitosan in peroxide compounds (peracetic acid and hydrogen peroxide) with nanoparticles of oxides like titanium, iron, zinc, and metals, such as titanium, copper, and calcite, allow to create protective coatings of double action in accordance with the technologies: (a) extending the shelf life of table eggs, food green article (FGA) and (b) preventing contamination of hatching eggs with pathogenic microflora, increasing the hatchability of eggs, and the quality of chicken, hatching green article (HGA). In the technology of storing table eggs, artificial cuticles based on chitosan FGA are characterized by the following characteristics: increased thickness of 6–10 μm, low gas and moisture permeability, and high biocidal activity against pathogenic bacteria and viruses. It is shown that the use of FGA technology in the production of table eggs can extend the period of transportation and storage of products by 33–35 days at a temperature of 24°C and maintained a grade A (Haugh unit = 71–60) through the entire 35 days period. The technology HGA reduces the rate of contamination of hatching eggs with pathogenic microflora by 99.29–99.7%, while increasing the egg hatching rate by 2.3–11.6% compared to the control, depending on the cross of the poultry and the storage conditions of the hatching eggs. It is important that these technologies have a great prospect for application in countries that develop green technologies for the production of poultry products.

Keywords: table and hatching eggs, chitosan, protective coatings, technology, metal nanoparticles, biocidal activity

1 Introduction

Eggshell quality has always been one of the most important parameters in the poultry industry (food and hatching eggs), and this has become even more important due to the automation of technological systems and the improvement in the crosses of modern poultry [1–8]. An increase in the quantitative indicators of egg production led to a deterioration in the quality of the barrier properties of the protective bionanocomposite calcite structure of the bird eggshell, since the increase in egg production of poultry is associated with the permeability of the calcite layer relative to pathogenic microflora, gases, and water vapor by a negative correlative relationship [9–12].

Negative trends in modern breeding poultry farming require the use of bold new approaches to solve them. Thus, it is proposed to use one of the promising areas of protection of hatching and table eggs, which consists in improving the existing and developing new technologies in poultry farming according to the biomimetic (*biomimetics*, from *bios* – life, *mimesis* – similarity) principle, the basis of which is to simulate the morphological and biochemical parameters of protective bioceramic structures of eggs using natural green components in order to achieve the maximum level of protection [13]. The main difficulty in constructing artificial protective structures similar in morphological and functional parameters to

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the natural cuticle, consisting of glycoproteins, is the selection of organic matter preferably of natural origin, which would easily form a thin-layer film on the surface of the egg and combine the following characteristics: elasticity, the ability to modulate the rate of gas diffusion through it (controlled gas permeability depending on the composition), environmental safety, and would have a powerful biocidal activity against pathogenic bacteria and viruses [14]. Such an organic substance is a well-known substance – chitosan (poly-*N*-acetyl-D-glucosamine) $(C_6H_{12}O_5N)_n$. Chitosan is a derivative of chitin, which is extremely common in nature, non-toxic, environmentally friendly, and cheap material extracted in large industrial volumes from the covers of crustaceans and insects and which has powerful biocidal properties against pathogenic microflora (bacteria and viruses) [15–18]. The protective film of chitosan is formed on the outer surface of the shell by evaporation of a solvent (aqueous solutions of peroxide compounds and organic acids) and is a multicomponent coating for improved activation of restoration and protection of barrier properties of bioceramic structures of the shell and shell membranes, which is characterized by biocidal action as well as the ability to improve the gas exchange of embryos during the incubation period and contribute to the increase in the embryo metabolism processes and improve the quality of young poultry. Scientists' research has built a solid foundation for the research of this topic and the development of a modern, natural, and environmentally safe artificial protective cuticle [18–21].

Thus, the purpose of this work was to develop directions for the physical–chemical modification of chitosan as the main component of green technology for the protection of hatching and table eggs of chickens artificial cuticle GREEN ARTICLE (ARTIficial cutiCLE) [13], by controlled introduction of biologically active nanoparticles (titanium oxides, zinc (Zn), and iron (Fe); metals like copper (Cu) and titanium (Ti); and nanoscale calcite) [22–24] and electrochemical [25–27] and ultrasonic [28–30] treatments into the matrix substance. This will allow to use the same basic matrix substance artificial cuticle chitosan in the technologies for prolonging the storage of table eggs, food green article (FGA) and preventing contamination with pathogenic microflora of hatching eggs, hatching green article (HGA) for opposite purposes: to reduce the gas permeability of the shell and the level of surface contamination with microflora for table eggs and, conversely, to increase this indicator for hatching eggs with the simultaneous ability to maintain high biocidal activity during the specified periods of time.

2 Materials and methods

2.1 Experimental site

The research was conducted at LLC Avis-Ukraine, Kosovshchyna Village, Sumy district, Sumy region, and Sumy National Agrarian University.

2.2 Experimental establishment

Samples of chicken eggs of two chicken crosses *Hisex White* (11,220) and *Hisex Brown* (10,780) were stored for 10 days at a temperature of +15°C and humidity of 70% and a temperature of +12°C and humidity of 80%, respectively, for hatching according to the established method (incubator Universal 55, Fauna, Modified, 55,000 eggs; country of origin, former USSR). The incubator was calibrated using a standard thermometer and hygrometer before incubation. The temperature and humidity were monitored every 3 h during the whole incubation period. The incubation was maintained at a temperature of 37.8 ± 0.01°C and a rate humidity (RH) of around 60% until day 18. From day 19, eggs were transferred to hatching baskets, and a temperature of 37.2 ± 0.1°C and RH of 70% was set for the hatcher. The age of the chicken was 45 weeks.

Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

2.3 Methods of egg treatment and composition of solutions for the preparation of artificial cuticle

Eggs were sprayed with aqueous solutions for egg treatment no earlier than 1 h before incubation in the case of pre-incubation treatment.

Composition of solutions were sprayed for the formation of artificial cuticles on the shell surface of food and hatching eggs.

2.3.1 HGA solution 1

For the formation of HGA based on the composite chitosan-nanoparticles $CaCO_3$–Cu^{2+}, a solution containing a composition of chitosan (soluble in acid) (1% of chitosan solution in 2% of peracetic acid [PAA], pH 3.0), Cu ions of 80.3 mg/g with sorption activity of 0.1–3.4, hydrogen peroxide (H_2O_2) of 0.5–5.5 wt%, calcium carbonate ($CaCO_3$) nanoscale of 0.1–2.2 wt%, EDTA of 0.05–0.1 wt%, trace elements (magnesium [Mg], cobalt [Co], Zn, and Cu) of 0.01–0.1 wt%, and water up to 100 wt% was prepared. Nanoscale $CaCO_3$ was prepared using a method with modifications. Option 1: $CaCO_3$ nanoparticles from $CaCl_2$ and Na_2CO_3 precursors were synthesized by mechanical and chemical treatments without subsequent thermal exposure of the ground powder. The powder mixture was ground once in a planetary ball mill with NaCl as the solvent initially, and with KCl the second time. The level of nanodisperse dispersion of $CaCO_3$ was reached after 10 min of continuous grinding in a planetary ball mill. The particle size ranges from 60 to 100 nm [24]. Option 2: $CaCO_3$ nanoparticles from $CaCl_2$ and Na_2CO_3 precursors were subjected to ultrasound treatment in a solution containing chitosan and other ingredients (22 kHz, 20 min). Just before spraying, the solutions were subjected to electrochemical activation by electrolytic treatment using Cu as the cathode and anode (20 min, temperature 35–40°C).

2.3.2 HGA solution 2

For the formation of HGA based on the composite chitosan-nanoparticles TiO_2 Fe_2O_3, a solution containing a composition of chitosan (soluble in acid) (1% of chitosan solution in 2% of PAA, pH 3.0), Cu ions of 80.3 mg/g with sorption activity of 0.1–3.0, titanium dioxide (TiO_2) in anatase crystal form (particle diameter 0.2–2.0 μm) of 0.1–3.0 wt%, yellow iron oxide pigment (iron oxide(III) [Fe_2O_3]) of 0.1–3.0 wt%, H_2O_2 of 0.5–5.5 wt%, copper sulfate ($CuSO_4$) of 1.0–2.5 wt%, EDTA of 0.1 wt%, trace elements (Mg, Co, and Zn) of 0.1 wt%, and remaining water was prepared.

2.3.3 HGA solution 3

For the formation of HGA based on the composite chitosan-nanoparticles ZnO, a solution containing 3.0% of chitosan solution in 3% of PAA (pH 3.6), zinc oxide (ZnO) of 3.0 wt%, and H_2O_2 of 5.5 wt% was prepared.

2.3.4 FGA solution 4

Chitosan was used to form the FGA in increased amounts (5%) compared to the HGA. Chitosan was dissolved in 4–5% of PAA and this solution was subjected to electrolysis for 30 min, at a temperature of 60–70°C (anode and cathode made of Ti grade W 1-00 [W 1-0]), and constant current (8–15 mA at 60–100 V), before being used for the formation of a protective coating on table eggs by spraying.

2.4 Weight loss

Weight loss (%) of the eggs during storage was calculated by subtracting the final weight of the egg from the initial weight and then dividing by the initial weight and multiplying by 100 as described by Caner and Cansiz [16]. Ten eggs for each treatment type were taken at weekly intervals for determination of weight loss. Each egg was weighed using a sensitive laboratory electronic balance Mettler-Toledo MS-TS.

2.5 Haugh unit (HU) and yolk index (YI)

HUs were calculated for 3 × 10 eggs for each treatment using dial caliper (L 222 mm, 0.1 mm; BAH134160001, Merck) based on the following equation [17]: $HU = 100 \times \log 10 \times (h - 1.7G^{0.37} + 7.6)$, where h is the thickness of the albumen (mm) and G is the mass of the entire egg (g). The parameter h was estimated by averaging three measurements carried out at different points at a distance of 10 mm from the yolk using dial caliper. The eggs were graded as follows: AA, if HU >72; A, if HU = 71–60; B, if HU = 59–31; and C, if HU <30 [17]. The YI was calculated as yolk height divided by yolk width. Yolk height and width were measured without removing the albumen. Five measurements were taken for each of the 10 eggs per treatment type at weeks 0, 1, 3, and 5 for determination of the HU and YI.

2.6 Total solids (dry matter) of albumen and yolk

Total solids (dry matter) (% w/w) in 5 g of egg albumen and yolk were determined using an Abbe refractometer (2WAJ, China) at 20 ± 1.5°C [17].

2.7 Microbial contamination

The number of colony forming unit (CFU) was determined using the formula: $X_2 = ((X_1 \times 100)/78.5) \times 100$, where X_1 is the number of colonies that grew on the Petri dish and 78.5 is the area of the Petri dish.

2.8 Electron microscopy

The study was performed by scanning electron microscopy (scanning electron microscopes and microanalyzer) using electron microscopes REMMA-102 and REM-106I (OJSC "SELMI," former Ukraine).

2.9 Statistical analysis

The data of hatching time indicators and hatching performance were analyzed using the Origin 9.0 (SAS 9.2, SAS Institute Inc., Cary, NC) in relation to chemical treatments and strains of birds. *t* test was used and the significance of difference was set at $p < 0.05$. Statistical significance was defined from the mean values at $p < 0.05$ with a Tukey multiple comparison test.

3 Results

3.1 Artificial cuticle HGA solution 1 options 1 and 2 based on the composite chitosan-nanoparticles $CaCO_3$–Cu^{2+}

The theoretical basis for the design of the composition for the preparation of artificial cuticle was, first, the modern direction in disinfectology, in particular, the combination of different active substances in one preparation in order to enhance the synergistic dependencies of useful properties (biocidal activity) and inhibition of undesirable properties (corrosion activity) and, second, the prospect of using a biomimetic approach in industrial biotechnologies. In our case, achieving an improvement in the hatchability of chicken eggs exposed to elevated temperatures during storage, which is an important risk factor in poultry farming in countries suffering from global warming, is provided by the introduction of a hydrophilic chitosan matrix together with nanoscale particles of $CaCO_3$ into the composition, which also have a powerful adsorption effect against water vapor as well as other organic and inorganic compounds [22,24,31,32]. The need for hydrophilic and water-retaining qualities in the artificial cuticle formed during drying on the surface of the hatching egg of poultry is due to the fact that the natural cuticle is initially hydrated and structured in such a way as to ensure trans-shell delivery of moisture (while bacterial penetration is also possible). In the next period, the natural cuticle becomes dry and prevents further movement of moisture. The cuticle undergoes even greater changes during the storage of hatching eggs at elevated temperatures, that is, an increase in the duration of storage of hatching eggs per day leads to an increase in embryo mortality by about 1% and requires an extension of the incubation duration by 40 min. In general, the quality of chicks after long-term storage of eggs worsens. For long-term storage of hatching eggs of chickens for more than 7 days, the optimal temperature is +12 to 13°C and the humidity index is 75–80%. In the first series of experiments, eggs of chickens of two crosses *Hisex White* (1,440) and *Hisex Brown* (1,440) were incubated. The eggs were stored for 10 days at a temperature of +15°C and humidity of 70%, according to the established method under the conditions of preincubation treatment with solution 1 (options 1 and 2) for the formation of a protective coating on hatching eggs HGA, which in addition to chitosan in concentrations of 0.1–3.4 wt% (depending on the initial quality of eggs), PAA, and water includes additional substances like H_2O_2, $CaCO_3$ nanoscale, water softener, and trace elements (Mg, Co, Zn, and Cu). The acidity index of the solution (pH) does not exceed 3.0. Control is a variant of the experiment, where the classic method of formaldehyde vapor treatment was used.

Data presented in Tables 1 and 2 indicate that the egg hatching rate of *Hisex Brown* cross chickens was 89.7 and 86.9% and that of *Hisex White* cross chickens was 94.5 and 95.6%, which is significantly higher than in the control groups where the classic formaldehyde steam treatment was used and it was 81.2 and 75.3% for *Hisex Brown* cross chickens and 87.1 and 85.8% for *Hisex White* cross chickens.

The study of the biocidal activity of the composition for the destruction of pathogenic microflora on the surface of hatching eggs of chickens showed that the technology of artificial cuticle HGA based on the composite chitosan-nanoparticles $CaCO_3$–Cu^{2+} caused a decrease in

Table 1: Effects of treatment of chicken eggs with HGA solution 1 option 1 based on the composite chitosan-nanoparticles $CaCO_3$–Cu^{2+} for the formation of an artificial cuticle on the shell surface on the incubation results

Treatment method	Eggs per tray	Fertilization egg (%)	Early death (%)	Late death (%)	Hatchability of fertile egg (%)
Hisex Brown					
Control	1,440	84.2	12.8	8.1	81.2
HGA solution 1, option 1	1,440	85.9	6.0*	3.9*	89.7*
Hisex White					
Control	1,400	87.3	7.6	4.2	87.1
HGA solution 1, option 1	1,440	89.0	2.3*	1.7*	94.5*

*$p < 0.05$; Early death, the death from E0 to E10; Late death, the death from E11 to E21; Control – egg treated by fumigation with formaldehyde.

Table 2: Effects of treatment of chicken eggs with HGA solution 1 option 2 based on the composite chitosan-nanoparticles $CaCO_3$–Cu^{2+} for the formation of an artificial cuticle on the shell surface on the incubation results

Treatment method	Eggs per tray	Fertilization egg (%)	Early death (%)	Late death (%)	Hatchability of fertile egg (%)
Hisex Brown					
Control	1,440	85.3	13.7	7.4	75.3
HGA solution 1, option 2	1,440	86.6	7.9*	3.4*	86.9*
Hisex White					
Control	1,440	89.0	8.5	4.5	85.8
HGA solution 1, option 2	1,440	87.6	2.4*	1.5*	95.6*

*$p < 0.05$; Early death, the death from E0 to E10; Late death, the death from E11 to E21; Control – egg treated by fumigation with formaldehyde.

the amount of pathogenic microflora on the surface of eggs of *Hisex Brown* cross chickens during incubation by 99.10–99.78% and *Hisex White* cross chickens by 99.61–99.78% compared to that of the control (Table 3).

Table 3: The level of microbial contamination in hatching eggs of *Hisex Brown* and *Hisex White* chickens under the conditions of using artificial cuticle HGA solution 1 during incubation (mPa, CFU), $\bar{X} \pm S_{\bar{x}}$, $n = 10$

	Treatment method	
	Control	HGA solution 1, option 2
Hisex Brown		
Before treatment	463.09 ± 12.512	
After treatment		
2 h	3.14 ± 0.011	1.02 ± 0.010*
5 days	17.88 ± 1.123	1.18 ± 0.013*
11 days	36.04 ± 4.208	2.03 ± 0.047*
19 days	47.04 ± 4.450	4.36 ± 0.061*
Hisex White		
Before treatment	282.18 ± 22.615	
After treatment		
2 h	2.37 ± 0.055	1.11 ± 0.033*
5 days	12.38 ± 1.131	1.85 ± 0.016*
11 days	51.46 ± 2.216	2.78 ± 0.112*
19 days	132.10 ± 5.132	6.25 ± 0.013*

*$p < 0.05$.

3.2 Artificial cuticle HGA solution 2 based on the composite chitosan-nanoparticles TiO_2 Fe_2O_3

In these series of experiments, a variant of the artificial cuticle HGA solution 2 based on the composite chitosan-nanoparticles TiO_2 Fe_2O_3 was used. The basis for the development was that it was environmentally safe and nontoxic and at the same time the biocidal substance TiO_2 in nano- and ultrafine forms has the following properties: organic pollutants and pathogenic microflora are destroyed by a photocatalytic mechanism on the surface of TiO_2 particles with a size of 50–500 nm under the influence of visible light. At the same time, there are data about the high prospects of the compositions for the destruction of organic pollutants, including pathogenic microflora, based on a variant of the effective oxidation process (advanced oxidation processes), based on a combination of H_2O_2 and tri- or divalent Fe ions Fe(III) and Fe(II). Such processes are caused, in particular, by the well-known Fenton reaction between organic peroxide compounds (PAA and H_2O_2) and transition metal ions, in particular Fe and Cu, which leads to the formation of highly reactive ions: OH, O_2^-, and O_2 molecules that can destroy pathogenic microorganisms by oxidation [33,34].

Table 4: Effects of treatment of chicken eggs with HGA solution 2 based on the composite chitosan-nanoparticles TiO_2 Fe_2O_3 for the formation of an artificial cuticle on the shell surface on the incubation results

Treatment method	Eggs per tray	Fertilization egg (%)	Early death (%)	Late death (%)	Hatchability of fertile egg (%)
Hisex Brown					
Control	1,440	84.2	5.2	3.5	89.9
HGA solution 2	1,440	83.9	4.3	2.2*	92.3
Hisex White					
Control	1,440	87.7	3.4	3.7	92.1
HGA solution 2	1,440	87.2	3.3	1.6*	94.4

*$p < 0.05$; Early death, the death from E0 to E10; Late death, the death from E11 to E21; Control – egg treated by fumigation with formaldehyde.

In view of the above, we have created a composition to protect hatching eggs of chickens from pathogenic microflora based on acid-soluble chitosan as well as PAA, H_2O_2, TiO_2 in nano-and ultrafine states and anatase crystal form, yellow Fe_2O_3 pigment, $CuSO_4$, water softener, trace elements (Mg, Co, and Zn) and water. The results of tests of the effect of this variant of artificial cuticle on the results of egg hatching are presented in Tables 4 and 5.

Table 5: The level of microbial contamination in hatching eggs of chickens under the conditions of using the technology of artificial cuticle HGA solution 2 during incubation (mPa, CFU), $\bar{X} \pm S_{\bar{x}}$, $n = 10$

	Processing method	
	Control	HGA solution 2
Hisex Brown		
Before treatment	257.19 ± 7.65	
After treatment		
2 h	2.16 ± 0.02*	0.04 ± 0.02*
5 days	6.07 ± 1.06*	1.02 ± 0.01*
11 days	13.25 ± 1.12*	2.15 ± 0.02*
19 days	27.44 ± 3.08*	4.15 ± 0.05*
Hisex White		
Before treatment	269.23 ± 10.24	
After treatment		
2 h	2.51 ± 0.01*	0.07 ± 0.01*
5 days	10.63 ± 0.05*	0.15 ± 0.01*
11 days	27.62 ± 3.18*	2.91 ± 0.11*
19 days	32.24 ± 3.19*	5.42 ± 0.01*

*$p < 0.05$.

3.3 Artificial cuticle HGA solution 3 based on the composite chitosan-nanoparticles ZnO

The results of incubation and dynamics of contamination with pathogenic microflora during the incubation of chicken eggs treated before incubation with the composition of artificial cuticle HGA based on the composite chitosan-nanoparticles ZnO (solution 3) are shown in Tables 6 and 7.

The results of incubation for 21 days showed that egg hatching between the control and experimental groups differed by 4.7% (Table 6). In the control group, this indicator was 85.0%, while in the experiment it was 89.7%. Also, the composite chitosan-nanoparticles ZnO had a positive effect on reducing the contamination of pathogenic microflora on the surface of chicken egg shells to 0.3–0.71% of the initial number of bacterial colonies in 19 days (Table 7).

The effectiveness of the action of artificial cuticle in relation to hatching eggs of chickens depends on the initial state of eggs, that is, high-quality eggs of poultry of high-performance crosses, meeting the standards and requirements of pre-incubation storage, first of all, the period and temperature of storage, do not need to increase the rate of hatchability of eggs. At the same time, eggs obtained from poultry of medium and low-performance crosses, with an extended shelf life in conditions of elevated temperatures with the use of

Table 6: Effects of treatment of chicken eggs with HGA solution 3 based on the composite chitosan-nanoparticles ZnO for the formation of an artificial cuticle on the shell surface on the incubation results

Treatment method	Eggs per tray	Fertilization egg (%)	Early death (%)	Late death (%)	Hatchability of fertile egg (%)
Hisex Brown					
Control	770	91.8	9.9	4.0	85.0
HGA solution 3	770	90.6	7.1*	2.4*	89.7*

*$p < 0.05$; Early death, the death from E0 to E10; Late death, the death from E11 to E21; Control – egg treated by fumigation with formaldehyde.

Table 7: Level of microbial contamination in hatching eggs of *Hisex Brown* chickens under the conditions of using artificial cuticle HGA solution 3 during incubation (mPa, CFU), $\overline{X} \pm S_{\overline{x}}$

Experiment	Processing method	
	Control	HGA solution 3
Before treatment	350.41 ± 8.011	
After treatment		
2 h	2.70 ± 0.002	1.08 ± 0.002*
5 days	6.88 ± 1.016	1.31 ± 0.002*
11 days	10.059 ± 1.002	1.75 ± 0.003*
19 days	12.45 ± 2.009	2.21 ± 0.007*

*$p < 0.05$.

technology of artificial cuticle show a significant increase in the rate of hatchability.

3.4 Artificial cuticle FGA

In order to protect table eggs and extend their shelf life and protect against pathogenic microflora of bacterial and viral origin throughout the entire shelf life, a composition has been developed for processing eggs by applying a biocidal protective film that is environmentally safe and non-toxic on the surface of the shell, which includes chitosan in combination with PAA and H_2O_2, subjected to electroactivation in an aqueous solution using Ti electrodes.

Eggs coated with chitosan solution to form the FGA eggs of chicken *Hisex Brown* retained a value of HU of 65.83 after 35 days of storage, compared with 58.12 for the control eggs. Eggs coated with chitosan solution to form the FGA eggs of chicken *Hisex White* retained a value of HU of 60.02 after 35 days of storage, compared with 51.27 for the control eggs (Table 8). This indicated that the coatings of FGA helped seal pores on the eggshell and slow down the escape of carbon dioxide and water during long term storage, maintaining albumen quality. While chicken eggs of the two chicken crosses *Hisex White* and *Hisex Brown* coated with FGA and stored at 24°C maintained a grade A (HU = 71–60) through the entire 35-days period, the uncoated eggs dropped from grade A to B after 21 days of storage.

The yolk index is a measure of egg freshness and is based on the ratio of yolk height to yolk width [17]. During storage at room temperature, the YI value decreased as a result of a progressive weakening of the vitelline membranes and liquefaction of the yolk caused mainly by diffusion of water from the albumen to yolk part. After 35 days of storage, the YI of the uncoated eggs decreased from 0.49 to 0.33, while eggs coated with FGA displayed YI values of 0.40 and 0.39 in *Hisex Brown* cross chickens and *Hisex White* cross chickens, respectively (Table 9).

FGA coatings effectively reduced the mass transfer rate (water and CO_2 loss) from the albumen through the eggshell during long term storage. Consequently, this process inhibits albumen liquefaction and water uptake by the yolk and minimizes a reduction in yolk quality.

The total solid (dry matter) concentration of albumen has also been used as an indicator of egg freshness which is related to thinning or liquefaction of albumen. This liquefaction could occur due to protease enzymes, depolymerisation by hydroxyl ions at increasing pH values, and the interaction of ovomucin-lysozyme complex. Water contained in the albumen permeates the yolk and some nutrients contained in the yolk can permeate the albumen. These osmotic tracks and changes in albumen and yolk concentrations can be measured by the refractometric method. During storage, the dry matter accumulation (DMA) increases due to mixing of the yolk into the

Table 8: Effects of coating FGA solution 4 on Haugh unit (HU) and egg grade during 35 days of storage at 24°C, $n = 10$

	Haugh unit (Egg grade)[a]			
Coating	0 days	7 days	21 days	35 days
Hisex Brown				
Control	83.03(AA) ± 1.012	73.72(AA) ± 0.121	63.17(A) ± 0.723	58.12(B) ± 0.558
FGA solution 4	83.72(AA) ± 0.831*	79.49(AA) ± 0.191*	69.99(A) ± 1.312*	65.83(A) ± 1.449*
Hisex White				
Control	83.53(AA) ± 1.322	71.39(A) ± 1.053	59.14(B) ± 0.714	51.27(B) ± 0.452
FGA solution 4	84.07(AA) ± 0.948*	76.84(AA) ± 1.294*	65.09(A) ± 1.612*	60.02(A) ± 1.271*

*$p < 0.05$.

[a] Egg grades: AA, HU >72; A, HU = 71–60; B, HU = 59–31; C, HU <30. Control – untreated eggs.

Table 9: Effects of coating FGA solution 4 on yolk index during 35 days of storage at 24°C, $n = 10$

	Yolk index			
Coating	**0 days**	**7 days**	**21 days**	**35 days**
Hisex Brown				
Control	0.48 ± 0.05	0.42 ± 0.01	0.36 ± 0.04	0.33 ± 0.04
FGA solution 4	0.49 ± 0.02*	0.46 ± 0.03*	0.43 ± 0.01*	0.40 ± 0.06*
Hisex White				
Control	0.47 ± 0.04	0.41 ± 0.03	0.37 ± 0.03	0.33 ± 0.05
FGA solution 4	0.47 ± 0.03	0.45 ± 0.05*	0.42 ± 0.03*	0.39 ± 0.04*

*$p < 0.05$. Control – untreated eggs.

Table 10: Effects of different coatings on total solids (dry matter) of albumen during 5 weeks of storage at 24°C, $n = 10$

	Albumen total solids (Dry matter) (% w/w)					
Coating	**0 days**	**7 days**	**14 days**	**21 days**	**28 days**	**35 days**
Hisex Brown						
Control	11.44 ± 0.04	12.70 ± 0.12	15.20 ± 0.17	17.34 ± 0.1	18.27 ± 0.15	18.98 ± 0.18
FGA solution 4	11.46 ± 0.05	11.81 ± 0.09	12.16 ± 0.11*	12.71 ± 0.0*	13.40 ± 0.17*	14.95 ± 0.13*
Hisex White						
Control	11.42 ± 0.03	12.63 ± 0.11	14.95 ± 0.13	17.14 ± 0.21	18.07 ± 0.21	18.80 ± 0.1
FGA solution 4	11.43 ± 0.03	11.71 ± 0.15	12.06 ± 0.11*	12.51 ± 0.08*	13.24 ± 0.19*	14.58 ± 0.01*

*$p < 0.05$. Control – untreated eggs.

albumen. Albumen DMA values of uncoated eggs were higher than those of coated eggs (Table 10). The increase in DMA during storage has been attributed to the liquefaction of the yolk and subsequent mixing into the albumen. Liquefaction is a result of an enhanced interaction between lysozyme and ovomucin as the pH increases during storage. The chemical cleavage effect of pH on the *O*-glycoside link between trisaccharides and β-ovomucin has been considered to be responsible for the collapse of the albumen structure. In general, liquefaction would result in an increase in fluidity of egg albumen and is associated with a deterioration in egg quality. Thick albumen is a gel and thin albumen is a fluid. During storage, the gelatinous structure of thick albumen changes its physical and chemical characteristics and gradually breaks down into a clear liquid, losing its consistency. The DMA of the control (uncoated) egg albumen of eggs of chickens *Hisex Brown* and *Hisex White* ranged from 11.44 initially to 18.95 and from 11.44 initially to 18.80, respectively (Table 10). For the coated eggs, albumen

Table 11: Level of microbial contamination in control and table eggs treated with FGA solution 4 during storage, $n = 4$

Method of table eggs treatment	**Sampling period**	**Number of samples, pcs**	***E. coli***	**Staphylococci**	***Salmonels***	**Spore-forming bacteria**
Control	After 14 days	20	In 3 samples	—	—	—
	After 19 days	20	In 4 samples	—	—	—
	After 28 days	20	In 8 samples	—	—	—
	After 33 days	20	In 13 samples	In the 1st sample	—	In 2 samples
FGA solution 4	After 14 days	20	—	—	—	—
	After 19 days	20	—	—	—	—
	After 28 days	20	In the 1st sample	—	—	—
	After 33 days	20	In 2 samples	—	—	In the 1st sample

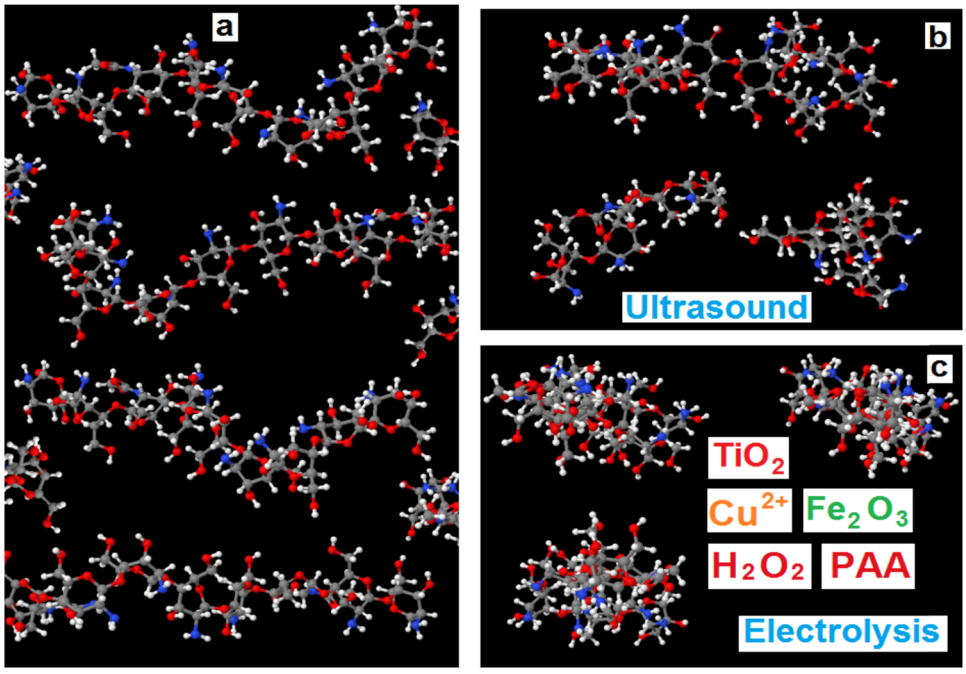

Figure 1: The scheme of the molecular structure and 3D spatial arrangement of chitosan molecules in the artificial cuticle film: (a) native (untreated) chitosan; (b) chitosan subjected to ultrasound irradiation; (c) electrolytic treatment of chitosan in the presence of Cu^{2+} metal nanoparticles, oxides (Fe_2O_3 and TiO_2), and peroxide compounds (peroxide acid and PAA).

DMA values reached 14.95 and 14.58 for eggs of chickens *Hisex Brown* and *Hisex White*, respectively.

As can be seen from Table 11, artificial cuticle based on chitosan FGA allows to prevent contamination of the surface of table eggs with pathogenic microflora during their storage at elevated temperature and moisture levels.

Figure 1 shows a scheme of the molecular structure of native chitosan and the image when subjected to ultrasonic and electrochemical (electrolysis) treatments. During both types of treatment, the length of the polymer chains is significantly reduced, which, in turn, has a direct effect on the gas permeability of films obtained from acidic aqueous solutions applied by spraying on the shell surface. Thus, native chitosan is suitable for processing table eggs, since the films formed from it have a large thickness, low gas permeability, and provide a high level of resistance to pathogenic microflora (bacteria and viruses) (Figures 2, 4, and 5).

On the other hand, the artificial cuticle based on chitosan, designed for hatching eggs, formed from a low-molecular polymer has a sufficient level of gas

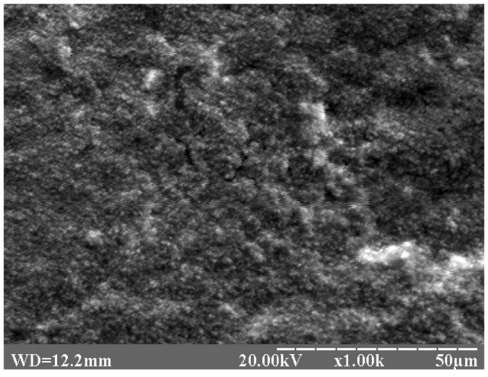

Figure 2: Electron microscopic image of the protective artificial cuticle FGA.

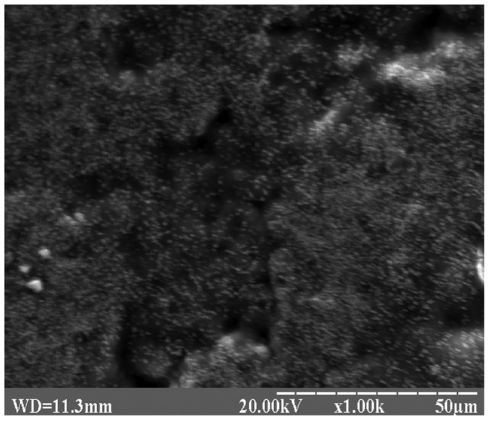

Figure 3: Electron microscopic image of the protective artificial cuticle HGA based on the composite chitosan-nanoparticles $CaCO_3$–Cu^{2+}.

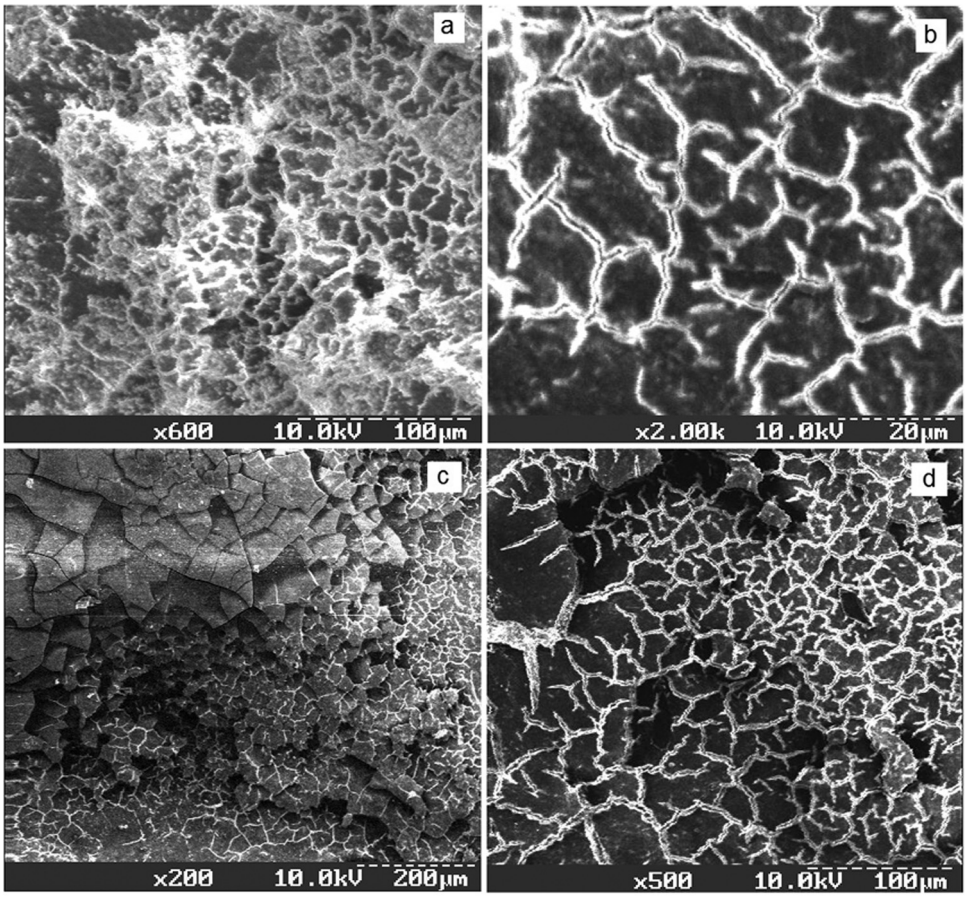

Figure 4: Electron microscopic image of the native cuticle of a chicken egg (a); protective artificial cuticle HGA based on the composite chitosan-nanoparticles TiO_2 Fe_2O_3, HGA solution 2 (b); cuticle treated with formaldehyde (c); protective artificial cuticle HGA based on the composite chitosan-nanoparticles ZnO, HGA solution 3 (d).

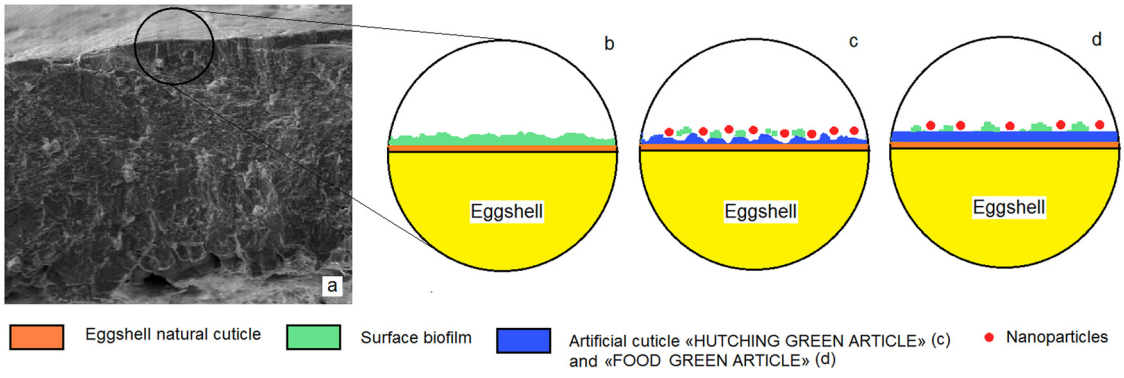

Figure 5: Schematic diagram of the artificial cuticle coating formed on the surface of the table and hatching eggs. (a) and (b) eggshell natural cuticle; (c) HGA; (d) FGA. The chitosan film (blue) of HGA has local thinnings and is much thinner (due to ultrasonic and electrolytic treatment), so it is more gas permeable than the chitosan film of FGA. Lower gas permeability is required to extend the shelf life of eggs.

permeability to provide comfortable conditions for the development of the embryo and at the same time a high level of resistance to pathogenic microflora, due to both the spatial effects (in the case of nanoscale $CaCO_3$) (Figure 3) and activation of catalytic processes of intensive oxidation of organic substances and pathogenic organisms, provided by nanoparticles of metal oxides (TiO_2, Fe_2O_3) (Figure 4b), and ZnO (Figures 4d and 5).

4 Discussion

Modern poultry farming is the most precocious and economical industrial field with a quick payback. According to Global Industry Analysts Inc., by 2015, the global egg market reached 1,154 billion dollars. With the growth of middle-class population and their growing purchasing power, egg consumption per capita is steadily increasing in major Asian countries such as China and India, providing every reason to expand the production of food and hatching eggs of poultry, primarily chickens. A simple increase in the number of eggs is no longer enough today. It is necessary to ensure high-quality and green environmental standards for both the products themselves and egg production technologies [2–7]. In this aspect, the strengthening of all parts of the natural protection of poultry eggs (technologies for keeping and feeding poultry, breeding work, methods of processing products, and their transportation), which has lost its relevance, has become even more important. In particular, one of the key issues is to strengthen the barrier qualities of the shell as a complex and perfect system for protecting the internal contents of the egg [1,3,7,10,20,21]. The main purpose is to resist mechanical influences on the egg, to preserve its integrity. The shell, in addition, successfully counteracts microbial attacks, slows down the dehydration of the egg, and its pores make the egg an open biological system [3]. The strength of the shell is the most important indicator of its quality. Poor quality of the shell, and even more so notches and micro-cracks, devalue the egg. Egg fighting in poultry farming leads to significant economic losses. Eggs with damaged shells can neither be stored nor incubated, and their selling price, as unsorted, is reduced by 1.5–3 times. The main reason for a high egg fighting are either poor feeding of laying hens, more often violations of mineral and vitamin nutrition, or technical errors in the line of egg movement from a laying hen to sale. Many works have been devoted to the problems of improving the calcite layer of the shell, but it has recently been carefully rethought the role and place of the cuticle of the bird egg as the first line of defense [9–11]. The cuticle is an ultrashell film covering the surface of the shell, and is thin and transparent consisting of mainly glycoproteins, polysaccharides, hydroxyapatite crystals, and lipids. The film has a stable structure and dissolves only in hot water (above 40°C). Its thickness on chicken eggs is 0.005–0.01 mm. The cuticle is permeable to gases and impervious to microorganisms [35–42]. Peebles et al. found that when the cuticle is removed from the eggshell, late embryonic mortality of embryos increases [43]. Obviously, this removal increases egg mass loss and promotes the penetration of microorganisms into the egg. Previous studies have linked the degree of cuticle coverage to the ease of microbial penetration into egg contents and highlighted its importance in the food safety chain [44]. The cuticle plays an important role in preventing microbial penetration through the eggshell and making the product safer for consumers. In a newly laid egg, the cuticle becomes fuller and hardens after exposure to the external environment [45]. The cuticle has been shown to be most effective in preventing egg spoilage in the first week after egg laying [46]. Electron microscopy revealed the presence of inner and outer layers of the cuticle [47]. The inner layer (bubble layer) consists of bubbles, each consisting of a core and mantle. The outer cuticle (non-vesicular cuticle) is much more compact and uniform and does not contain any matrix vesicles [48]. In addition to physical barrier properties, recent studies have identified many proteins in the cuticle, some of which are known to be antimicrobial agents [49,50], therefore, this confirms the evidence that the cuticle provides a chemical and mechanical barrier against the penetration of microbes. Moreover, even the spatial 3D structure of the cuticle of eggs of some bird species determines the biocidal properties. Thus, the cuticle, which is a thin layer of spherical micro-formations, can provide both an effective barrier against microbial infection and modulate UV reflectivity. The wide variation in the composition and structure of the cuticle of eggs of different species, the different values of wettability indicators, and the ability to regulate the gas exchange that is inherent in them, emphasize the need for a thorough study of: (1) how the physico-chemical parameters of the cuticle change during the incubation period and (2) the contribution of the eggshell cuticle to ensure optimal conditions for embryo development. Summing up the above, we can conclude that there are promising developments in the field of strengthening the barrier functions of the shell through modification of cuticle layers in order to enhance their ability to both mechanically delay pathogenic microflora and destroy bacteria and viruses due to chemical processes, in particular chemical oxidation of biomolecules and membranes of the latter [51]. This technology is called artificial cuticle and consists in constructing a layer of film on the surface of the egg, similar in functional properties to the natural cuticle. As a matrix, the main substance was chitosan as one of the most promising substances that can be used in compositions applied as working solutions to hard surfaces of both food and hatching eggs. Chitosan is the only positively charged polymer (polycation) of natural

origin, which is obtained in large quantities with a high degree of chemical purity, and, importantly, its cost, especially in the form of a technical crude substance, is quite moderate. The global amount of chitin is estimated at 10 gigatonnes, which, given its constant biosynthesis, makes it an inexhaustible source for the production of chitosan. In the last two decades, there has been a significant increase in interest in the biocidal properties of chitosan, which along with its nontoxicity, biocompatibility, hypoallergenic, and biodegradability allow it to be used in food and green technologies. However, the biocidal properties of chitosan strongly depend on its molecular weight and chemical structure. This is due to the fact that chitosan, which is a copolymer of glucosamine and acetyl glucosamine, is a heterogeneous group of substances that differ in molecular weight, the degree of deacetylation, the location of residual acetylated links along the polymer chain, viscosity, and pK_a values. Due to the wide variation in the molecular mass parameters of polymer samples, chitosan is characterized by significant variations in the manifestation of biocidal properties. The biocidal activity of chitosan depends: (a) on the degree of deacetylation, i.e., on the proportion of amino saccharide residues with a free amino group. An increase in the degree of deacetylation of chitosan leads to an increase in the antibacterial properties of polyamino saccharide; (b) on the degree of protonation of free amino groups of the polymer, since it is positively charged amino groups that largely determine the affinity of chitosan to microbial cells. With an increase in the degree of protonation of chitosan amino groups, the antibacterial properties of the polymer increase. The degree of protonation of the polymer depends on the acidity of the medium – the more acidic the medium, the more positively charged the chitosan polymer is. So, with the increasing acidity of the medium, the biocidal properties of chitosan increase, and with alkalization, they decrease. The biocidal activity also depends on the molecular weight (degree of polymerization) of chitosan [52–54]. Usually, with an increase in the molecular weight of chitosan, its antibacterial properties are enhanced. This is due to its increasing ability to interact with microbial cells with increasing molecular weight. However, there is evidence that indicates an increase in the antibacterial properties of chitosan with a decrease in its molecular weight, which is associated with better solubility of lower molecular forms of the polymer. It is possible to change both the molecular weight and thickness as well as the degrees of gas conductivity and biocidal activity of chitosan artificial cuticle by using electrochemical and ultrasonic technologies and doping films with nanoparticles of metal oxides or $CaCO_3$.

5 Conclusion

Ways of physical–chemical modification of chitosan, as the main component of green technology of artificial cuticle GREEN ARTICLE for the protection of hatching and table eggs of chickens [13], by the controlled introduction of biologically active nanoparticles (titanium oxides, Zn, and Fe; metals like Cu and Ti; and calcite) into the matrix substance by electrochemical and ultrasonic treatments were developed. This made it possible to use the same basic matrix substance chitosan artificial cuticle in the technologies of storage of table eggs using FGA and prevention of contamination with pathogenic microflora of hatching eggs using HGA for opposite purposes: to reduce the gas permeability of the shell for table eggs and, conversely, to increase this indicator for incubation eggs with the simultaneous ability to maintain high biocidal activity during the specified periods of time.

For the first time, it was experimentally proved that the use of the artificial cuticle nanocomposition in poultry provides an increase in the hatchability of *Hisex Brown* chicken eggs in option 1 by 8.5% and in option 2 by 11.6% compared to formaldehyde and the hatchability of *Hisex White* cross chickens increased in option 1 by 7.4% and in option 2 by 9.8% compared to formaldehyde, thereby increasing embryonic viability and reducing the amount of pathogenic microflora on the surface of eggs during incubation by 99.29–99.7% compared to the untreated control. The effectiveness of the action of artificial cuticle in relation to hatching eggs of chickens depends on the initial state of eggs, that is, high-quality eggs of birds of high-performance crosses, meeting the standards and requirements of preincubation storage, first of all, the period and temperature of storage, do not need to increase the rate of hatchability of eggs. At the same time, eggs obtained from poultry of medium and low-performance crosses with an extended shelf life in conditions of elevated temperatures using the artificial cuticle technology show a significant increase in the rate of hatchability.

Abbreviations

CFU	colony forming unit
DMA	dry matter accumulation
FGA	food green article
HGA	hatching green article
HU	Haugh unit
PAA	peracetic acid
RH	rate humidity
YI	yolk index

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Solomon SE. Egg and eggshell quality. London: Wolfe Publ. Ltd; 1991. p. 4–12.
[2] Hester P, Hester P, editors. Egg innovations and strategies for improvements. San Diego, CA: Elsevier Inc; 2017. p. 625.
[3] Bain M. Composition and properties of eggshell. Achiev Sustain Prod Eggs. 2017;1:3–24.
[4] Hincke M. The eggshell: structure, composition and mineralization. Front Biosci. 2012;17:1266–80.
[5] Gautron J, Stapane L, Le Roy N, Nys Y, Rodriguez-Navarro AB, Hincke MT. Avian eggshell biomineralization: an update on its structure, mineralogy and protein tool kit. BMC Mol Cell Biol. 2021;22:11. doi: 10.1186/s12860-021-00350-0.
[6] Nys Y, Gautron J, Garcia-Ruiz JM, Hincke MT. Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. Comptes Rendus Palevol. 2004;3(6–7):549–62. doi: 10.1016/j.crpv.2004.08.002.
[7] Ketta M, Tumova E. Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. Czech J Anim Sci. 2016;61:299–309.
[8] Ar A, Rahn H, Paganelli CV. The avian egg: mass and strength. Condor. 1979;81:331–7.
[9] D'Alba L, Jones DN, Badawy HT, Eliason CM, Shawkey MD. Antimicrobial properties of a nanostructured eggshell from a compostnesting bird. J Exp Biol. 2014;217:1116–21.
[10] Bain MM, McDade K, Burchmore R, Law A, Wilson PW, Schmutz M, et al. Enhancing the egg's natural defence against bacterial penetration by increasing cuticle deposition. Anim Genet. 2013;4:661–8.
[11] Dunn IC, Joseph NT, Bain M, Edmond A, Wilson PW, Milona P, et al. Polymorphisms in eggshell organic matrix genes are associated with eggshell quality measurements in pedigree Rhode Island Red hens. Anim Genet. 2009;40:110–4.
[12] García-Ruiz JM, Rodríguez-Navarro A. Competitive crystal growth: the avian eggshell model. In: Allemand D, Cuif J-P, editors. Biomineralization 93, Musée Oceanographique de Monaco; 1994. p. 85–94
[13] Bordunova O. The nanocuticle technology (Artificial Cuticle: "article") to improve the hatching of chicken eggs in the poultry farms of countries affected by global warming. Procedings 2020 IEEE 10th international conference nanomaterials: applications & properties (NAP). IEEE 01TFC15-1-01TFC15-4; 2020.
[14] Gole VC, Roberts JR, Sexton M, May D, Kiermeier A, Chousalkar KK. Effect of egg washing and correlation between cuticle and egg penetration by various Salmonella strains. Int J Food Microbiology. 2014;182–183:18–25.
[15] Pillai CKS, Paul W, Sharma CP. Chitin and chitosan polymers: chemistry, solubility and fiber formation. Prog Polym Sci. 2009;34:641–78.
[16] Caner C, Cansiz O. Chitosan coating minimises eggshell breakage and improves egg quality. J Sci Food Agric. 2007;88(1):56–61.
[17] Yuceer M, Caner C. Antimicrobial lysozyme–chitosan coatings affect functional properties and shelf life of chicken eggs during storage. J Sci Food Agric. 2014;94:153–62. doi: 10.1002/jsfa.6322.
[18] Kostag M, El Seoud OA. Sustainable biomaterials based on cellulose, chitin and chitosan composites – a review. Carbohydr Polym Technol Appl. 2021;2:100079. doi: 10.1016/j.carpta.2021.100079.
[19] Sahariah P, Másson M. Antimicrobial chitosan and chitosan derivatives: a review of the structure–activity relationship. Biomacromolecules. 2017;18(11):3846–68. doi: 10.1021/acs.biomac.7b01058.
[20] Koseman A, Akdemir F, Şeker I. Effects of coating with chitosan film and storing at different periods of brood broiler eggs on hatching performance. J Anim Plant Sci. 2020;30(5):1123–8. doi: 10.36899/JAPS.2020.5.0128.
[21] Silva TA, Gorup LF, de Araújo RP, Fonseca GG, Martelli SM, de Oliveira KMP, et al. Synergy of biodegradable polymer coatings with quaternary ammonium salts mediating barrier function against bacterial contamination and dehydration of eggs. Food Bioprocess Technol. 2020;13:2065–81. doi: 10.1007/s11947-020-02545-3.
[22] Zhang X, Xiao G, Wang Y, Zhao Y, Su H, Tan T. Preparation of chitosan-TiO_2 composite film with efficient antimicrobial activities under visible light for food packaging applications. Carbohydr Polym. 2017;169:101–7.
[23] ELmezayyen AS, Reicha FM. Preparation of chitosan copper complexes: molecular dynamic studies of chitosan and chitosan copper complexes. Open J Appl Sci. 2015;5:415–27.
[24] Boyjoo Y, Pareek VK, Liu J. Synthesis of micro and nano-sized calcium carbonate particles and their applications. J Mater Chem A. 2014;2:14270–88. doi: 10.1039/C4TA02070G.
[25] Reicha F, Shebl A, Badria F, EL-Asmy A. Electrochemical synthesis, characterization and biological activity of chitosan metal complexes. Int J Basic Appl Chem Sci. 2012;2:7–22.
[26] Halada G. Method of electrochemical formation of noble nanoparticles in a chitosan matrix. WO2011106526A2; 2011-02-24.

[27] Cai Q, Gu Z, Fu T, Liu Y, Song H, Li F. Kinetic study of chitosan degradation by an electrochemical process. Polym Bull. 2011;67(4):571–82.
[28] Hiléia KS, Campiña JM, Sousa MM, Silva F, Gonçalvesa MP. Ultrasound-assisted preparation of size-controlled chitosan nanoparticles: characterization and fabrication of transparent biofilms. Food Hydrocoll. 2013;31(2):227–36. doi: 10.1016/j.foodhyd.2012.10.005.
[29] Yuan D, Meng H, Huang Q, Li C, Fu X. Preparation and characterization of chitosan-based edible active films incorporated with Sargassum pallidum polysaccharides by ultrasound treatment. Int J Biol Macromol. 2021;183:473–80. doi: 10.1016/j.ijbiomac.2021.04.147.
[30] Sheng X, Shu D, Li Y, Zhan Z, Yuan X, Liu S, et al. Combined approach consisting of slightly acidic electrolyzed water and chitosan coating to improve the internal quality of eggs during storage. J Sci Food Agric. 2021;101(6):2355–61. doi: 10.1002/jsfa.10858.
[31] Yu D, Yu Z, Wu Z, Regenstein JM, Wenshui Xia W. Advances in the application of chitosan as a sustainable bioactive material in food preservation. Crit Rev Food Sci Nutr. 2021;5:1–36. doi: 10.1080/10408398.2020.1869920.
[32] Fernandez-Martinez A, Lopez-Martinez H, Wang D. Structural characteristics and the occurrence of polyamorphism in amorphous calcium carbonate. In: Van Driessche AES, Kellermeier M, Benning LG, Gebauer D, editors. New perspectives on mineral nucleation and growth. Springer; 2017. p. 77–92. doi: 10.1007/978-3-319-45669-0.
[33] Tufail A, Price WE, Faisal I, Hai FI. A critical review on advanced oxidation processes for the removal of trace organic contaminants: a voyage from individual to integrated processes. Chemosphere. 2020;260:127460. doi: 10.1016/j.chemosphere.2020.127460.
[34] Amor C, Marchão L, Lucas MS, Peres JA. Application of advanced oxidation processes for the treatment of recalcitrant agro-industrial wastewater: a review. Water. 2019;11(2):205. doi: 10.3390/w11020205.
[35] Rose-Martel M, Du J, Hincke MT. Proteomic analysis provides new insight into the chicken eggshell cuticle. J Proteom. 2012;75(9):2697–706.
[36] Samiullah S, Roberts JR. The eggshell cuticle of the laying hen. J World's Poult Sci J. 2014;70(4):693–708. doi: 10.1017/S0043933914000786.
[37] Dauphin Y, Luguet G, Perez-Huerta A, Salomé M. Biomineralization in modern avian calcified eggshells: similarity versus diversity. Connect Tissue Res. 2018;58(S1):67–73. doi: 10.1080/03008207.2018.1430144.
[38] D'Alba L, Jones N, Badawy T. Antimicrobial properties of a nanostructured eggshell from a compost-nesting bird. J Exp Biol. 2014;217:1116–21.
[39] Athanasiadou D, Jiang W, Goldbaum D, Saleem A, Basu K, Pacella MS, et al. Nanostructure, osteopontin, and mechanical properties of calcitic avian eggshell. Sci Adv. 2018;4(3):3219. doi: 10.1126/sciadv.aar3219.
[40] Soler M, Rodriguez-Navarro AB, Pérez-Contreras T, García-Ruiz JM, Soler JJ. Great spotted cuckoo eggshell microstructure characteristics can make eggs stronger. J Avian Biol. 2019;50(11):jav.02252. doi: 10.1111/jav.02252.
[41] Arzate-Vázquez I, Méndez-Méndeza JV, Flores-Johnson EA, Nicolás-Bermúdezc J, Chanona-Pérezc JJ, Santiago-Cortésc E. Study of the porosity of calcified chicken eggshell using atomic force microscopy and image processing. Micron. 2019;118:50–7. doi: 10.1016/j.micron.2018.12.008.
[42] Bain M, Rodriguez-Navarro A, McDade K, Schmutz M, Preisinger R, Waddington D, et al. The potential of eggshell crystal size and cuticle coverage for genetic selection in laying hens. Abstract from 60th annual meeting of the european association for animal production, Barcelona, Spain; 2009. p. 169
[43] Peebles ED, Brake J, Gildersleeve RP. Effects of eggshell cuticle removal and incubation humidity on embryonic development and hatchability of broilers. Poult Sci. 1987;66(5):834–40. doi: 10.3382/ps.0660834.
[44] Board RG, Halls NA. The cuticle: a barrier to liquid and particle penetration of the shell of the hen's egg. Br Poult Sci. 1973;14:69–97. doi: 10.1080/00071667308415999.
[45] Sparks NH, Board RG. Bacterial penetration of the recently oviposited shell of hens' eggs. Aust Vet J. 1985;62(5):169–70. doi: 10.1111/j.1751-0813.1985.tb07281.x.
[46] Vadehra DV, Baker RC, Naylor HB. Role of cuticle in spoilage of chicken eggs. J Food Sci. 1970;35(1):5–6. doi: 10.1111/j.1365-2621.1970.tb12354.x.
[47] Lunam CA, Ruiz J. Ultrastructural analysis of the eggshell: Contribution of the individual calcified layers and the cuticle to hatchability and egg viability in broiler breeders. Br Poult Sci. 2000;41(5):584–92. doi: 10.1080/713654975.
[48] Fraser AC, Bain MM, Solomon SE. Transmission electron microscopy of the vertical crystal layer and cuticle of the eggshell of the domestic fowl. Br Poult Sci. 1999;40(5):626–31. doi: 10.1080/00071669987016.
[49] Jingwen D. Chicken eggshell membrane and cuticle: Insight from bioinformatics and proteomics. University of Ottawa (Canada). ProQuest Dissertations Publishing; 2013. MR86134.
[50] Solomon SE. The eggshell: strength, structure and function. Br Poult Sci. 2010;51:52–9. doi: 10.1080/00071668.2010.497296.
[51] Wlazlo L, Drabik K, Al-Shammari Karrar IA, Batkowska J, Nowakowicz-Debek B, Gryzińska M. Use of reactive oxygen species (ozone, hydrogen peroxide) for disinfection of hatching eggs. Poult Sci. 2020;99:2478–84. doi: 10.1016/j.psj.2019.12.039.
[52] Wu T, Zivanovic S, Hayes DG, Weiss J. Efficient reduction of chitosan molecular weight by high-intensity ultrasound: underlying mechanism and effect of process parameters. J Agric Food Chem. 2008;56(13):5112–9. doi: 10.1021/jf073136q.
[53] da Silva Pires PG, da Silva Pires PD, Cardinal KM, Bavaresco C. The use of coatings in eggs: A systematic review. Trends Food Sci & Technol. 2020;106:312–21. doi: 10.1016/j.tifs.2020.10.019.
[54] Gabriela da Silva Pires P, Bavaresco C, Daniela da Silva Pires P, Cardinal KM, Rodrigues Leuven AF, Andretta I. Development of an innovative green coating to reduce egg losses. Clean Eng Technol. 2021;2:100065. doi: 10.1016/j.clet.2021.100065.