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The effect of hydrogen peroxide prepared with silver ions on the qualitative traits of table eggs and reducing the dynamics of mycobiota growth

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Abstract

The quality and safety of raw materials and food products are inextricably linked. Table eggs are subjected to special monitoring due to the microbial hazards. So far, bacterial hazards have been monitored on table eggs. However, latest reports have pointed a threat that has not been considered for table eggs, which is microfungi. Microfungi can grow on the surface of eggshells and penetrate inside the eggs. Therefore, it is necessary to improve the microbiological state of the eggshells surface, which will guarantee the safety of egg consumption and also reduce spoilage. Therefore, the aim of the present work was to examine on how egg sanitation with prepared hydrogen peroxide (H_2O_2) containing silver ions affected the growth dynamics of microfungi and the production of mycotoxins during egg storage. The results showed that H_2O_2 with silver ions was effective against microfungi, while simultaneously inhibited production of mycotoxins. The egg sanitation treatment with a solution of H_2O_2 with silver ions reduced the count of microfungi and stopped growing after one week of storage. The effectiveness of lower concentrations of the prepared solution against the microfungi may have been caused by silver ions. There was a small decrease in Haugh unit value of eggs sanitised with H2O2 and silver ions in the final period of storage. The results showed that the treatment of eggs with H_2O_2 with silver ions slowed down the spoilage process and effectively reduced the content of microfungi and mycotoxins.

Introduction

The production of table eggs is increasing worldwide, and this includes the alternative systems, *i.e.* organic and deep-litter production systems (FAOSTAT, 2017). It has been observed that eggs, especially those produced by alternative systems, are likely to be microbiologically contaminated, and may be potentially dangerous to consumers' health (Svobodová and Tůmová, 2014). So far, existing research has been focusing on bacterial contamination (De Reu *et al.,* 2006; Corry, 2007; Gantois *et al.,* 2009). However, latest research has indicated on the risk of the presence of microfungi and their metabolites in table eggs. Studies on the population of microfungi in table eggs have shown that they may penetrate through the shell and subshell membranes and contaminate the egg content (Szablewski *et al.,* 2010, Tomczyk *et al.,* 2018b; 2019). Researchers have found microfungi from the genera *Alternaria, Penicillium, Chaetomium*, and *Fusarium* on the eggshell surface (Tomczyk *et al.,* 2018b). During egg storage, these microfungi grow and penetrate into the egg content, and produce mycotoxins in the egg white. The highest diversity and quantity of microfungi was observed on the shells of eggs laid by hens kept in the deep-litter and free-range systems (Tomczyk *et al.,* 2018b; 2019). Therefore, it is necessary to improve the microbiological state of the eggshell surface to ensure the safety of consumption and to reduce the spoilage of table eggs.

Washing of eggs before being sold may limit the microbial contamination on the eggshell surface (Liu *et al.,* 2016). Other treatments such as ionising radiation (Farkas, 1998), UV radiation (Coufal *et al.,* 2003; Rodriguez-Romo and Yousef, 2005; Szablewski *et al.,* 2010), ozonation (Davies and Breslin, 2003; Rodriguez-Romo and Yousef, 2005), and ultrasound treatment (Cabeza *et al.,* 2005; Sert *et al.,* 2011; 2013; Aygun and Sert 2012; Aygun *et al.,* 2012) may additionally reduce the microbial count, including that of microfungi on the eggshell surface.

Hydrogen peroxide (H_2O_2) can be used as an

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effective alternative agent to sanitise the surface of table eggs. The antimicrobial activity of H_2O_2 is based on the oxidation of proteins. A simultaneous treatment of eggs with H_2O_2 solution and silver ions may increase the effectiveness of sanitation and reduce the exposure time on the surface of eggs. Previously, a preliminary research found that when eggs were treated with H_2O_2 prepared with silver ions, their microbiological contamination level was reduced. The procedure was safe, and the required mechanical properties of the eggshell were maintained (Tomczyk *et al.,* 2018a). To date, the interaction mechanism of the prepared solution under various egg storage conditions on microfungi and the production of mycotoxin has not been investigated. Therefore, the aim of the present work was to investigate the effect of egg sanitation with H_2O_2 containing silver ions on the growth dynamics of microfungi and the production of mycotoxins during egg storage.

Material and methods

Collection of eggs

A total of 290 eggs with an average weight of 53 g, and laid by Hy-Line white hens kept in the free-range system were collected in the 32nd week of laying. An aqueous preparation containing H_2O_2 (1.5%) and silver ions (0.015%) was used for the tests which would not cause an elastic deformation of eggshells (Tomczyk *et al.,* 2018a). The eggs were sanitised by immersion in 3% aqueous solution at 10°C for 5 min. Next, they were dried at 15°C, placed in sterile egg boxes, and separately stored according to different treatments; humidity (35, 65, 95% RH) and temperature (8, 20°C) for 4 w. Eggs were collected at the start of the experiment, and after 7, 14, and 21 d. In each sampling set, 58 eggs were collected. Next, egg white samples were freeze-dried (FreeZone Plus, Labconco, Kansas City, MO), and the shells were dried in a laboratory dryer at 50°C.

Quality evaluation of eggs

The following parameters were taken into consideration to evaluate the quality of the eggs: egg white index, yolk index, Haugh units, and egg white pH. The pH measurements were carried out by Elmetron CP- 551 pH meter (Elmetron, Zabrze, Poland). The measurements were made according to the Polish standard PN-A-86503: 1998.

ERG analysis

The total mycobiota content was measured based on ergosterol concentration (ERG). A modified ERG measurement method described by Perkowski *et al.* (2008) was used in the experiment. This method works by releasing the metabolite from biomaterial by means of microwave-assisted saponification with simultaneous extraction. HPLC with an absorbance detector at a wavelength of $\lambda = 282$ nm and an external standard was used for the ERG analysis. The ERG recovery was 97%, and the detection level was 0.01 mg/kg.

Mycotoxins in egg white

Egg white was analysed for the content of selected mycotoxins. The concentrations of the following mycotoxins were measured: (1) type A trichothecenes: scirpentriol (STO), diacetoxyscirpenol (DAS), HT-2 toxin, T-2 toxin, T-2 tetraol, and T-2 triol; and (2) type B trichothecenes: deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-AcDON), 15-acetyl-deoxynivalenol (15-AcDON), nivalenol (NIV), and fusarenone X (FUS-X). Samples (10 g) were extracted with 100 mL mixture of acetonitrile:water (82:18). The extracts were purified by means of a solid phase extraction using columns filled with a mixture of active carbon (Draco G 60, 100 mesh), celite (Celite 545), and neutral aluminium oxide (70 - 230 mesh) at a weight ratio of 1:1:1. Type B trichothecenes were analysed as trimethylsilyl derivatives by using an external model. They were separated chromatographically and analysed individually by means of a gas chromatograph (Hewlett Packard 6890, Waldbronn, Germany) coupled with a mass detector (Hewlett Packard 5972 A, Waldbronn, Germany). Selected ions (SIM) were analysed in type B trichothecenes namely (1) DON: ions 103 and 512; (2) 3-AcDON: ions 117 and 482; (3) 15-AcDON: ions 193 and 482; (4) FUS-X: ions 103 and 570; and (5) NIV: ions 191 and 600. A full-range scan of masses was analysed (100 - 700 amu) to confirm that the analysed mycotoxins were present in the samples. The mass spectrum was compared with the analogical spectrum referring to the standard. Apart from the qualitative analysis, the mycotoxin concentrations were also measured. The results were processed with the ChemStation program. Type A trichothecenes were analysed as trifluoroacetyl derivatives. The analysis consisted in searching for selected ions (SIM). The following ions were identified in type A trichothecenes: (1) STO: ions 456 and 555; (2) T-2 tetraol: ions 455 and 568; (3) T-2 triol: ions 455 and 569; (4) DAS: ions 402 and 374; (5) HT-2: ions 455 and 327; and (6) T-2: ions 327 and 401. The method of analysis resulted from mycotoxin concentration recovery for STO, T-2 triol, T-2, T-2 tetraol, FUS-X, HT-2, DON, 3-AcDON, 15-ACDON, and NIV were as follows:

82 ± 5.3, 79 ± 5.1, 86 ± 3.8, 88 ± 4.0, 79 ± 3.1, 91 ± 3.3, 84 ± 3.8, 78 ± 4.8, 74 ± 2.2, and 81 ± 3.8%, respectively. The limit of detection was 0.001 mg/kg.

Statistical analysis

The statistical tests were performed using Statistica 13.1 software (StatSoft, Tulsa, OK, USA). The differences were considered significant at $p \leq 0.05$. The one-way analysis of variance (ANOVA) was performed accordingly (dependent variables: egg white index, yolk index, Haugh units, egg white pH, ERG in eggshell and egg white, mycotoxins in egg white; independent variables: storage time, humidity, and temperature). The principal component analysis was used for sample discrimination.

Results

The qualitative traits and the concentration of ERG and mycotoxins in the eggs sanitised with H_2O_2 containing silver ions were analysed to observe whether the sanitation treatment reduced the growth of microfungi and mycotoxin production, thus inhibiting egg spoilage.

Basic changes in egg quality

The analysis of qualitative traits, *i.e.* Haugh unit, egg white index, yolk index, and egg white pH revealed a natural ageing process during the storage of the eggs which were not treated with the H_2O_2 containing silver ions. After 4 w of storage, the qualitative traits including Haugh index decreased by 39 units (Figure 1). When the eggs were sanitised with H_2O_2 containing silver ions, there was a small decrease in the Haugh unit at the final storage period. This means that the egg spoilage process was less dynamic (Figure 2). The average Haugh unit value in the sanitised eggs stored for 4 w decreased by 22 units, regardless of the storage conditions (Figure 1). This effect was significantly more pronounced in the eggs stored under refrigeration (8°C) (Figure 2).

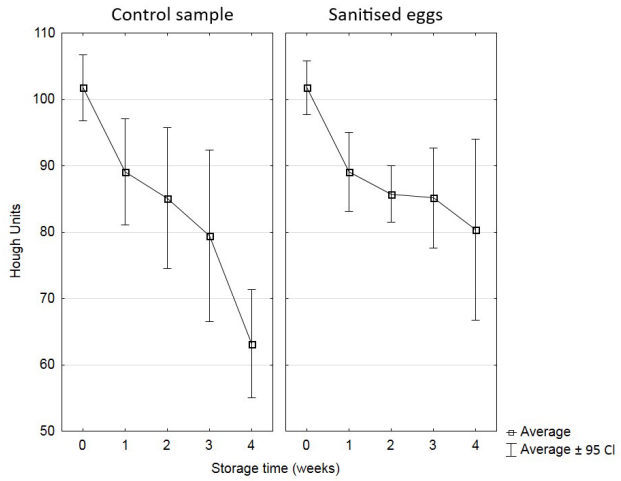

Figure 1. Variation of Haugh unit in sanitised eggs regardless of their storage conditions.

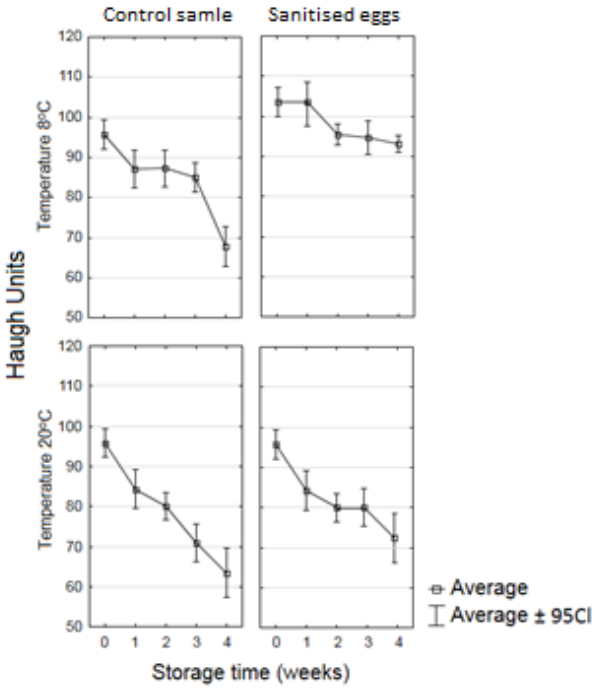

Figure 2. Variation of Haugh unit during the storage of sanitised eggs at two temperature variants.

ERG in eggshells and egg white

The analysis of the ERG content showed mycobiota was neither in the shell nor in the content of freshly laid eggs. After 3 w of storage, the eggs which were not treated with H_2O_2 containing silver ions had a statistically significant dynamic growth of mycobiota on the eggshells surface. The results of measurements of the ergosterol content shows that it is possible to inhibit the growth of mycobiota on the eggshells surface by sanitation, regardless of the egg storage conditions (Figure 3).

There were no microfungi found in the content of non-sanitised eggs immediately after laying. After 2 w of storage, there was an increased production of mycotoxins (Figure 4). Increased humidity and temperature during egg storage significantly affected the microfungal growth dynamics. The microfungal growth in the egg content sanitised with H_2O_2 containing silver ions was reduced regardless of the storage conditions (Figure 5).

Mycotoxins in egg white

There were no type A or B trichothecenes found in the content of non-sanitised eggs

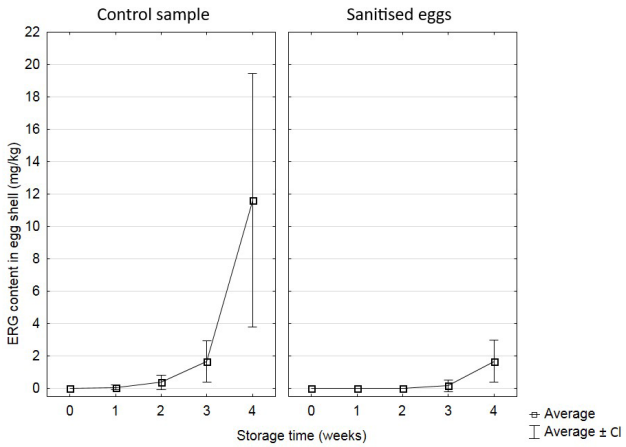

Figure 3. Variation of ERG content in sanitised eggs regardless of their storage conditions.

immediately after they had been laid, and after 1 w of storage, regardless of temperature and humidity (Figure 5). After 2 w of storage, there was intensified mycotoxin production (Figure 3), which depended significantly ($p \leq 0.05$) on humidity during storage. After 4 w of storage at 95% RH, the following concentrations of type A and B trichothecenes namely DON, FUS-X, 3-AcDON, 15-AcDON, NIV, Scirpentriol, T-2 tetraol, T-2 triol, DAS, HT-2, and T-2 were measured at 24, 16, 8, 16, 12, 5, 3, 2, 2, 14, and 21 µg/kg, respectively. The presence of mycotoxins in the sanitised eggs stored at high humidity (95% RH) was detected after 3 w. The storage of the sanitised eggs at low humidity (35% RH) totally inhibited the production of mycotoxins. The concentrations of the abovementioned

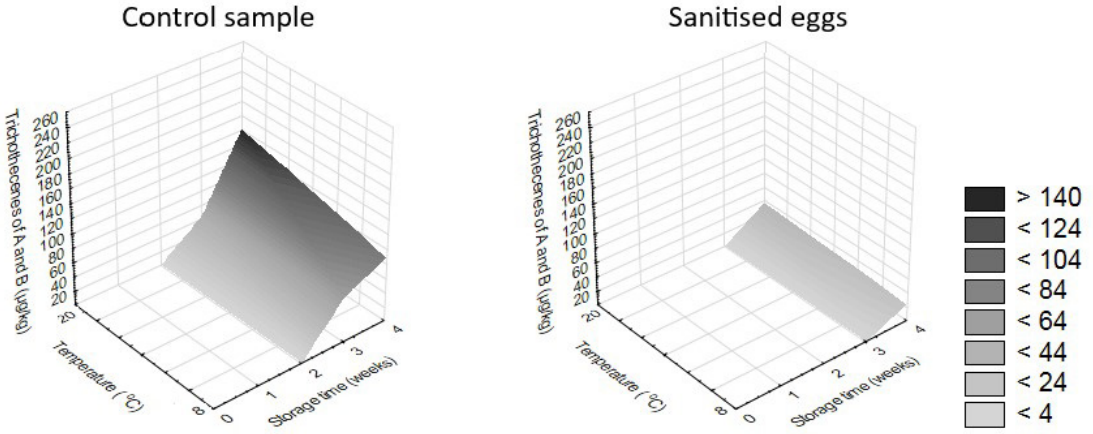

Figure 4. Variation of ERG content in sanitised eggs during their storage at different temperatures.

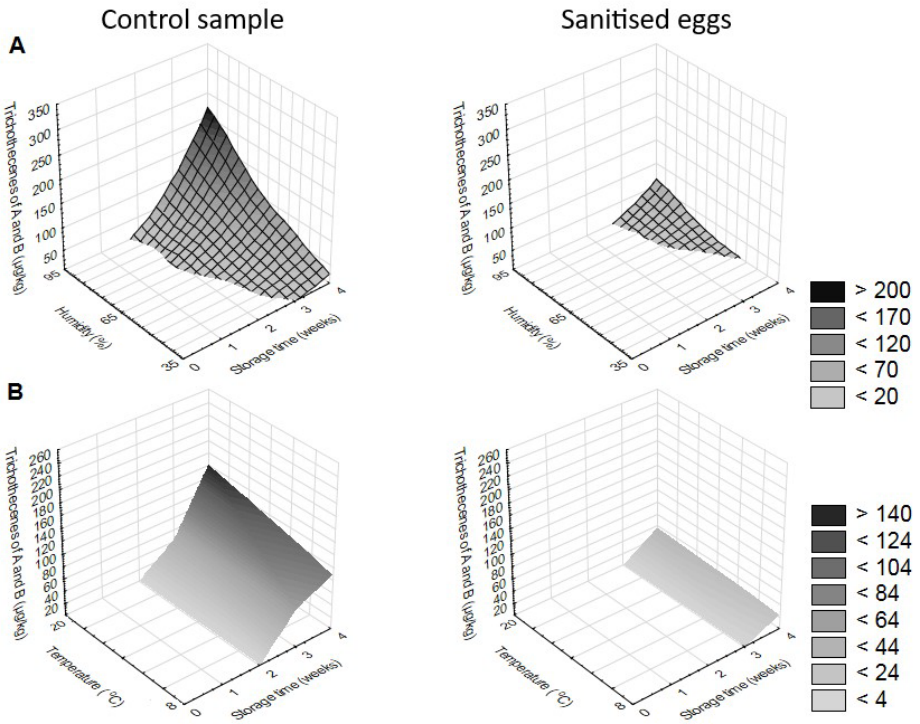

Figure 5. Content variation of type B trichothecenes in the eggs stored at different humidity (A) and temperature (B) levels.

mycotoxins in the sanitised eggs stored for 4 w at 95% RH were as follows: 15, 4, 2, 1, 1, 23, 0, 0, 5, 8, and 13 µg/kg.

Analysis of factors affecting determinants under study

Figure 6 shows the results of principal component analysis (PCA) based on correlation. The PCA was carried out for the sanitised eggs and control samples stored for 4 w at various humidity and temperature levels. The results were used in the PCA. PC1 and PC2 accounted for 65.9% of the total variance. The results of the ERG and type A and B trichothecenes content formed separate clusters from the results of qualitative traits. The loading signs indicate that the concentration of ERG and mycotoxins were negatively correlated with the egg sanitation treatment. This means that the prepared solution limited the growth of microfungi and inhibited the production of mycotoxins. The changes in the qualitative traits, *i.e.* Haugh units, egg white index, and yolk index were arranged relative to PC2, perpendicular to the ERG and mycotoxin loadings. This means that they were not correlated. The changes in the qualitative traits were strongly influenced by the storage temperature.

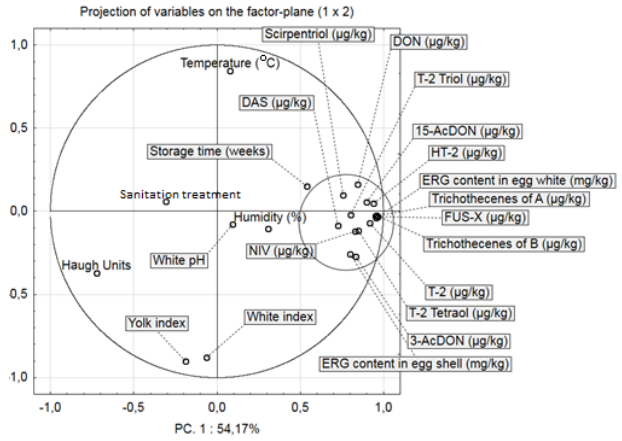

Figure 6. Principal component and loading analysis of qualitative traits of eggs, ERG, and mycotoxin content depending on different storage conditions.

Discussion

The knowledge on the influence of H_2O_2 preparation on the contamination of eggshell surface and the content of eggs laid by hens kept in various systems in the temperate climate with mycobiota and *Fusarium* mycotoxins was limited. The risk of contamination is high in hen housing systems where litter is used (Rohweder *et al.,* 2011; Tomczyk *et al.,* 2018b). Specific storage and distribution conditions, *i.e.* storage time, temperature, and humidity may affect the development dynamics of mycobiota and mycotoxins in table eggs (Tomczyk *et al.,* 2019). There is a demand for modern, inexpensive, and effective antimicrobial agents that are easy to apply and do not have negative effect on the qualitative traits of eggs and consumers' health. A prepared silver-stabilised H_2O_2 can effectively sanitise the surface of eggshells (Tomczyk *et al.,* 2018a). Therefore, the goal of the present work was to examine how H_2O_2 prepared with silver ions affected the qualitative traits of table eggs stored for 4 w at different humidity and temperature levels, and whether it limited the growth of microfungi and *Fusarium* mycotoxins.

Results obtain in the present work confirmed those of the previous work, which showed that fungal biomass may penetrate into the egg content (Figure 7). After 1 w of storage, the ERG concentration in the untreated eggs was 3.88 mg/kg. After 4 w, this increased to 25.17 mg/kg. In consequence, the high ERG content in the eggshells caused rapid penetration of fungal biomass into the content of eggs stored at high humidity. The H_2O_2 preparation with silver ions applied in the experiment at specific humidity and temperature successfully inhibited the microfungal growth on the eggshell surface. This effect was more pronounced in eggs stored at lower temperature and humidity. The effectiveness of the preparation may have been caused by the presence of silver ions, whose antifungal properties have been proved in previous studies (Choi *et al.,* 2008; Zheng *et al.,* 2008; Jo *et al.,* 2009). Hydrogen peroxide may increase the inactivation of microorganisms when the preparation is applied at lower concentration (Sheldon and Brake, 1991).

As the duration of storage of the untreated eggs progressed, natural spoilage occurred, especially at elevated temperature and humidity. Eke *et al.* (2013) and Jones *et al.* (2018) noted similar results of temperature in their studies. Generally, proteolytic bacteria are chiefly responsible for changes in the quality of eggs. However, microfungi also significantly contribute to egg spoilage (Tomczyk *et al.,* 2019). The sanitation treatment with H_2O_2 containing silver ions limited the microfungal growth, thus slowing down the egg spoilage.

The PCA showed a significant correlation between the ERG content and the concentration of mycotoxins. After 2 w of storage, there was an increased production of type A and B trichothecenes in the egg white. A dynamic mycotoxin production was observed at elevated temperature and humidity (Figure 5). The inhibition of microfungal growth on

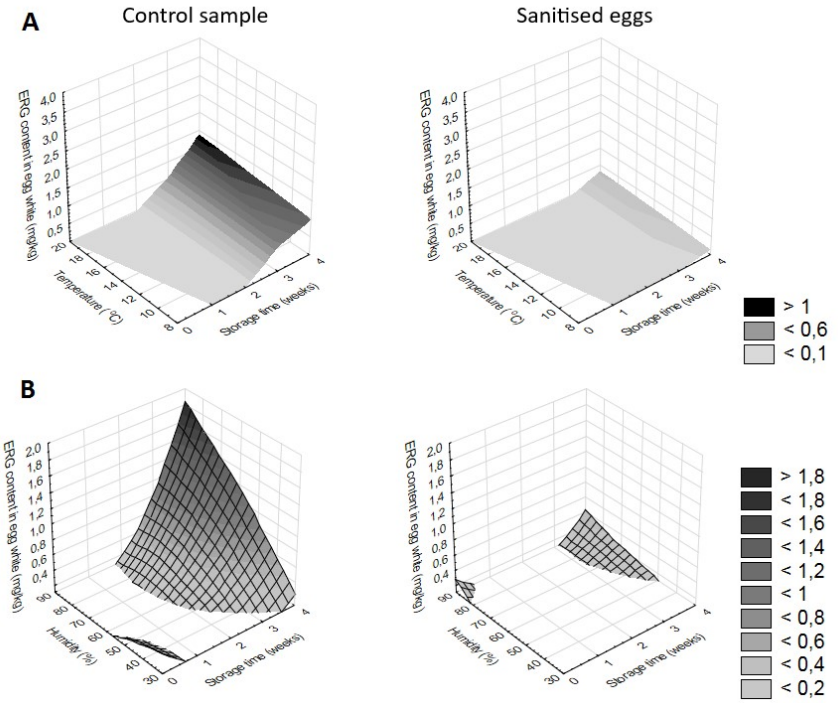

Figure 7. Variation of ERG content in the egg white stored at different humidity levels.

the eggshell surface by H_2O_2 containing silver ions also resulted in trace amounts of mycotoxins in the egg white, which were found only after 3 w of storage.

Conclusions

To conclude, due to the risk of egg contamination with microfungi and their metabolites, it is necessary to search for methods to reduce the initial microfungal load on eggshell surfaces. The results of the microbiological and chemical tests showed that H_2O_2 with silver ions effectively limited the growth of microfungi and the production of mycotoxins. The egg sanitation treatment with H_2O_2 containing silver ions reduced the microfungal growth after 1 w of egg storage. It is likely that the preparation, which was used at much lower concentrations in the present work, was effective against microfungi because it contained silver ions.

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