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EFFECT OF AN OXIDIZING GAS ON THE DENSITY OF SALMONELLA ENTERITIDIS ON THE SURFACE OF THE EGG SHELL FOR DISH

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Abstract: The effect of an oxidizing gas on Salmonella enteritidis inoculated on the surface of the dish egg shell was evaluated. A gas of electrolytic origin was used, at a single concentration and based on its oxidizing-reducing potential value greater than 950 mV. Exposure to this gas was carried out for periods of 1, 5 and 10 minutes. The initial load of S. enteritidis was 5.32 Log CFU/egg. The effect of the treatments on the external and internal quality of the egg was also determined, that is: mechanical resistance and shell thickness, yolk index, pH and Haugh units. All tests were carried out at 4ºC and 30ºC, for time intervals until the end of its useful life. Treatment with oxidant gas was lethal for S. enteritidis, such that this bacteria was not detectable from the first minute of exposure to the treatment. Egg quality parameters were not altered by the effects of the treatments. It is concluded that oxidizing gas is a viable and effective alternative in the sanitary control of dish eggs, particularly in one of the most persistent bacteria of interest in this food.
Keywords: oxidizing gas, plate egg, food safety.

INTRODUCTION

The egg is one of the most important foods for the world population, being considered an essential part of breakfast. The eggshell, composed mainly of calcium carbonate, provides it with a considerable shelf life; However, there are different microorganisms that can affect the egg from the moment it is formed inside the hen until it passes through the cloaca, among them: E. coli and Salmonella, which is why it is vital to eliminate them and prevent it from getting food safety at risk; Therefore, it is important to have strategies for this objective. Currently, some companies use techniques such as ultraviolet light for disinfection, but it is advisable to have different alternatives.

Worldwide, non-typhoid Salmonella represents one of the most frequent causes of foodborne illnesses and the S. enteritidis serotype is closely related to the consumption of eggs and foods that use eggs as an ingredient. (De Knegt et al., 2015). In the United States, it is the second cause of foodborne illness outbreaks and around 20% of illnesses caused by Salmonella are related to poultry and poultry products, such as eggs (Cardoso et al., 2021). Although there are not statistics for all countries, it can be assumed that the behavior is similar in most of them; considering the increase in the consumption of these types of foods and the sanitary conditions that are verified during their handling, until their consumption.

There are a significant number of methods for food sanitization and, in the case of eggs, those that do not moisturize must be considered, that is, those that are dry treatments, due to the deterioration they may cause in the egg shell. Allen et al. (2023), evaluated the bactericidal power of a transcinnamaldehyde nanoemulsion, prepared with tween 80 or GAL, resulting in significant reductions, from 2 to 2.5 log of S. enteritidis in shell eggs. Another method evaluated, by Guo et al. (2023), is that of air activated with sliding arc discharge plasma, this effectively inactivated S. enteritidis on the surface of the eggs. Disinfection of eggshell with H2 O2 + UV was evaluated by Morouj and Al-Ajeeli (2016) and resulted in a reduction of S. enteritidis below the level of detection immediately after treatment.

For the present study, a dry oxidizing gas generator was used, through an electrolytic process that is presumed to be lethal for all microorganisms, given its high oxidation-reducing potential value. Therefore, the exposure of whole eggs inoculated in their shells with S. enteritidis to an atmosphere of this oxidizing gas was proposed, considering as a

process parameter a reducing oxide potential greater than 950 mV for times of 1, 5 and 10 minutes, to determine the lethal effect on the experimental microorganism and variables that determine the quality of the product were also evaluated, such as: resistance to rupture and shell thickness, Haugh units, pH and yolk index; with the purpose of observing if these are altered by the treatments applied. For these quality tests, a maximum storage period of 30 days was contemplated, at temperatures of 4ºC and 30ºC.

MATERIALS AND METHODS

Experimental units and bacterial strain.36 eggs were selected from a local production center based on their uniformity and integrity and taken to the institute's laboratory for microbiological testing. S. enteritidis was obtained from a National Food Safety Research Laboratory, in Culiacán, Mexico. Bacteria stored at -80ºC were plated on XLD agar and incubated at 37ºC for 18 hours. A representative colony was selected and used to inoculate 10 ml of trypticasein soy broth (TSB), which was then incubated at 37ºC for 24 hours. After the incubation period, the culture was centrifuged at 10,000 x g for 10 minutes. The resulting pellet was resuspended in 10 ml of phosphate buffer saline (PBS) and the optical density (OD) was measured at 600 nm. Subsequently, a dilution was carried out based on the concentration relationship previously established for this strain, resulting in a final concentration of 8 log10 CFU/mL.

Preparation and inoculation of eggs. Egg samples were equilibrated at room temperature before testing. Subsequently, they were washed sequentially with tap water and a commercially available chlorine-based disinfectant with a free chlorine concentration of 100 mg/L for 1 minute. The eggs were then rinsed in sterile deionized water to completely remove the disinfectant and allowed to dry in a laminar flow hood. For inoculation, egg samples were individually immersed in the inoculum for 10 minutes and subsequently air-dried under a laminar flow hood for 1 hour at room temperature, allowing the bacteria to adhere.

TREATMENTS

Of the 36 inoculated eggs, the following treatments were used:

- 9 eggs for control
- 9 eggs exposed to oxidizing gas for 1 minute.
- 9 eggs exposed to oxidizing gas for 5 minutes.
- 9 eggs exposed to oxidizing gas for 10 minutes.

BACTERIOLOGICAL ANALYSIS

After treatment, eggs from each exposure time were quickly removed and transferred to sterile plastic bags. For bacteriological analysis, samples were taken from the surface of each egg using a sterile sponge saturated with 9 ml of PBS. The sponge was then placed in a sterile swivel bag and an additional 9 ml of PBS was added to facilitate homogenization of the sample for 15 seconds. Viable counts in the resulting buffer were serially diluted in sterile 0.1% peptone water and 0.1 ml of each dilution was plated on XLD plates. These plates were then incubated at 37°C for 24 hours before counting. Each treatment was evaluated in triplicate.

External and internal quality of the experimental egg. After the treatments, but without microbial inoculation, the eggs were stored at 5ºC and 30ºC and were evaluated periodically to determine the effect of the treatments on the external and internal quality of the egg. The following were evaluated: firmness and thickness of the shell, yolk index, pH and Haugh units. It was stopped being evaluated until four weeks had been completed or its shelf life had ended.

EXPERIMENTAL DESIGN AND DATA ANALYSIS

In this study, a block experimental design was used and the storage temperature and the exposure time to the oxidant gas were considered as crossed factors, and the storage time of the egg was considered as a block. Repeated measurements were carried out over storage time to evaluate changes in egg quality. To analyze the collected data, an analysis of variance (ANOVA) was performed using the STATISTICA v.13 statistical package (TIBCO Software Inc.). Compliance with the assumptions of normality and homogeneity of variance was verified before performing the ANOVA.

RESULTS AND DISCUSSION

Evaluation of the microbiological quality of the egg inoculated with S. enteritidis.

When the inoculated eggs of S. enteritidis were subjected to oxidant gas treatments, such bacteria could not be detected after 1 minute of exposure; On the other hand, the amount of S. enteritidis, present in the control treatment, was 5.32 Log10 CFU/egg. The above exhibits the high impact that an oxidizing environment exerts on aerobic bacteria that, according to this study, are not capable of surviving in atmospheres that register values above 950 mV and for exposure times equal to or greater than 1 minute.

If compared with other studies, such as that of Rathod et al. (2020) using Staphylococcus spp., where combined treatments of UV radiation and hydrogen peroxide have been applied to eggshells and where the best result was a reduction of 1.5 log10, the treatment presented in the study in question results, for much, more effective; That is, the oxidizing gas obtained by an electrolytic generator is lethal for Salmonella.

Cabanillas-Beltrán et al. (2020) also evaluated the combined effect of chitosan and an acidic electrolyzed water solution on the density of E. coli and S. enteritidis inoculated on the egg shell. The result was also of interest, with a reduction of 8 Log10 CFU, particularly on Salmonella. The difference between these treatments, in relation to the one proposed in the present study, is the moistening that is generated; although very low, due to the applied ultrasonic nebulization system. In this study it is a dry gas, since the issue is taken into account that humidification can cause a deterioration of the shell in terms of mechanical resistance and permeability of gases and microorganisms.

In relation to the impact of the experimental treatments on the external and internal quality of the egg, the recorded data establish that there was no effect. That is, the evaluated characteristics did not experience changes in relation to the control samples, nor did they have an effect on the shelf life of this food.

HAUGH UNITS

Significant differences were found in Haugh units depending on storage time, storage temperature and the interaction between these two factors ($p = 0.0817$). It was observed that as storage time and storage temperature increased, Haugh units decreased, indicating lower egg quality. However, the exposure time to the oxidant gas did not have a significant effect on Haugh units ($p = 0.952$).

YOLK INDEX

Significant effects of storage time ($p = 0.00$) and storage temperature ($p = 0.000$) on the bud index were found. However, the exposure time to the oxidant gas did not have a significant effect on the yolk index ($p = 0.244$). No interaction was observed between storage temperature and exposure time ($p = 0.408$). As the storage time increased and also the storage temperature increased, the yolk index decreased, indicating lower yolk quality.

PH

Both storage time and storage temperature had significant effects on the average egg pH ($p < 0.01$). As storage time and storage temperature increased, egg pH increased. Exposure time to oxidant gas did not have a significant effect on egg pH ($p = 0.1647$). A weak interaction was observed between storage temperature and exposure time ($p = 0.099$). The pH increased with exposure time when the storage temperature was 30°C, but there was no significant change in pH when the storage temperature was 5°C.

ENDURANCE

Shell strength was not affected by storage temperature or exposure time to oxidant gas ($p > 0.233$). No interaction was observed between storage time and exposure time to oxidant gas ($p = 0.8539$). However, a significant effect of storage time on shell resistance was found ($p = 0.0276$). As the number of weeks of storage increased, the shell resistance decreased.

In summary, the results indicated that storage time and storage temperature had significant effects on egg quality, as evidenced by Haugh units, yolk index, pH, and shell strength. The exposure time to the oxidant gas did not show significant effects on any of the variables evaluated.

CONCLUSIONS

The oxidizing gas turned out to be lethal in S. enteritidis inoculated on the surface of the dish eggshell, being effective after 1 minute of exposure to an oxidizing atmosphere of 950 mV. The treatments applied as bactericidal did not have an adverse effect on the external and internal quality of the experimental egg.

The results show the effectiveness of the oxidizing gas of electrolytic origin in the reduction of S. enteritidis inoculated on the egg shell for dishes and its effect on the external and internal quality of the egg is null. The above allows us to establish the evaluated alternative as highly recommended for food safety purposes.

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