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Effect of high voltage atmospheric cold plasma on chicken eggs quality during refrigerated storage

Alba E. Illera [a,b,*], Vanessa R. Souza [a], Linyi Tang [a], Nooshin Nikmaram [a], Kevin M. Keener [a]

[a] School of Engineering, University of Guelph, 50 Stone Road East, N1G 2W1, Guelph, ON, Canada
[b] Faculty of Science, University of Burgos, Plaza Misael Bañuelos s/n, 09001, Burgos, Spain

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ABSTRACT

Salmonellosis outbreaks caused by *Salmonella* Enteritidis are usually linked to the consumption of eggs and egg products. To assure egg safety, High Voltage Atmospheric Cold Plasma (HVACP) has demonstrated its efficacy in achieving higher than 10^5 CFU/egg reduction of this bacteria on the egg's surface. Although no quality changes were found immediately after treatment, there was a lack of knowledge on how the quality of HVACP-treated eggs could evolve during their shelf life, and a six-week quality study was performed in this work. Eggs were treated under two sets of conditions, 0.5 min of treatment within 24 h post treatment and 8.5 min of treatment and no post treatment time were the selected ones. No significant differences were found between untreated and treated eggs regarding albumen and yolk pH, Haugh unit, yolk color, vitelline membrane strength or shell strength. The changes detected during the six weeks of storage at 4 °C were attributed to the natural aging of the eggs and not to the effect of HVACP treatments. These results prove HVACP technology as an effective alternative to current techniques to assure both the safety and quality of eggs during their shelf life.

1. Introduction

Eggs are an interesting source of micro and macronutrients for humans, while providing a moderate calorie amount. From a nutritional point of view, eggs contribute to the diet with essential lipids, proteins, vitamins and minerals (Drewnowski, 2010). Furthermore, eggs offer a great versatility in culinary processes, high digestibility and a low cost, making this product a staple food in many countries worldwide (Réhault-Godbert et al., 2019). The actual trend is towards an increase in the consumption of eggs, accompanied with a higher egg production. According to a recent study, in 2020, global egg production achieved 86.67 million tonnes (Shahbandeh, 2022).

However, consumption of eggs and eggs products is also linked to a foodborne pathogen, *Salmonella* spp., and particularly to the serovar *Salmonella* Enteritidis (SE), which accounts with the 70% of the salmonellosis infections in humans, according to the EFSA (European Food Safety Authority) (EFSA, 2021). The most frequent case of egg surface contamination is after the laid, becoming a safety risk for consumers (Whiley & Ross, 2015). Current practices of egg washing and sanitizing provide some reduction in SE shell contamination; however, SE outbreaks from eggs still frequently occur. For example, recently in 2022, a salmonellosis outbreak caused by SE, and linked to eggs and eggs products consumption, affected to five countries in the European Union and United Kingdom, causing 2 deaths and 25 hospitalizations (EFSA, 2022).

Therefore, it is clear that the egg industry plays an essential role in controlling and eliminating the contamination on eggs surface before their commercialization, and there is a need to keep improving the current practices. This study focuses on the use of High Voltage Atmospheric Cold Plasma (HVACP) technology as an alternative decontamination tool. Cold plasma consists of a partially ionized gas composed of electrons, ions and elements in their fundamental and excited states generated after the application of a high voltage electric field into any gas at room temperature and pressure (Sarangapani et al., 2018), producing a range of reactive gas species with a known antimicrobial effect, such as peroxides, nitrates or ozone among others (Misra et al., 2011).

In a previous research, the demonstration and optimization of HVACP treatment was performed on the contaminated surface of eggs (Illera et al., 2022). None of the selected optimized conditions showed significant changes over the most characteristic quality properties of eggs right after HVACP treatment.

Storage time and temperature are the two main parameters affecting

* Corresponding author. School of Engineering, University of Guelph, 50 Stone Road East, N1G 2W1, Guelph, ON, Canada.
E-mail address: aeillera@ubu.es (A.E. Illera).

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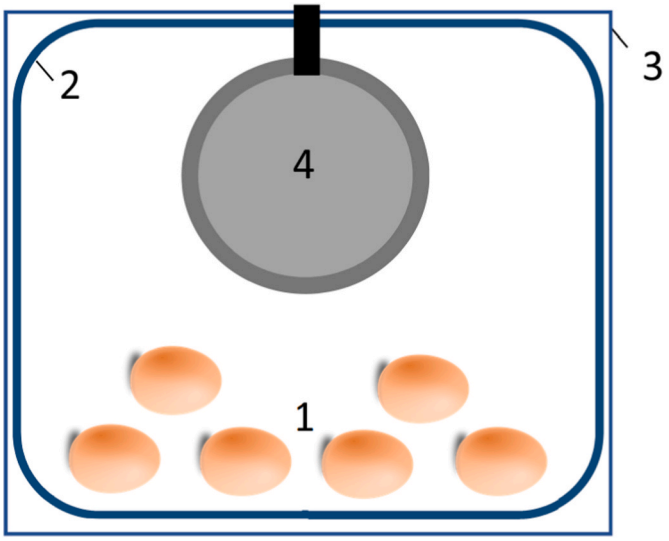

Fig. 1. Simplified view from above of a treatment set, where: 1. Position of the egg for the indirect treatment, 2. ArtBin® box, 3. Packaging film, 4. High voltage electrode.

the quality detriment of eggs, reflecting in changes in egg properties such as albumen height, Haugh Unit (HU), yolk color, yolk and albumen pH, or vitelline membrane strength among others (Lee et al., 2016). It is expected that eggs maintain their optimum properties under appropriate storage conditions during a certain time. Regulations regarding marketing of eggs vary by countries. The USA Department of Agriculture stablishes the sell by date of eggs in 30 days after packing (USDA, 2019), while in the EU, the egg marketing regulations indicates that the best before date can be no more than 28 days after laying (EUR-Lex, 2017).

Egg quality is an essential parameter to consider when thinking on the future commercialization of cold plasma treated eggs, and although it has been proved that HVACP does not alter eggs quality immediately after treatment, it is necessary to study its effect over their shelf life. Therefore, the aim of this work is to study the effect of two different HVACP treatments over the key quality parameters of refrigerated storage of chicken eggs during 6 weeks at 4 °C.

2. Methods

2.1. HVACP set up

HVACP treatments were performed using a dielectric barrier discharge (DBD) set-up. Plasma was generated using a high voltage transformer (Phenix Technologies, Inc., Accident, Maryland, USA) (30-130 kV RMS, 60 Hz) and two circular aluminum electrodes of 15 cm diameter, with two polypropylene layers of 1 mm of thickness acting as dielectric barriers. The upper electrode was connected to the high voltage generator, and the bottom one to the ground, creating plasma in the gap between them. Samples were treated inside a polypropylene ArtBin® box of 5 cm height sealed in high barrier nylon pouches (Fig. 1) with the selected gas inside, which was placed between the electrodes for each treatment. Eggs were placed at a minimum distance of 8 cm to the edge of the electrodes.

Power consumption of each treatment was collected using a power meter (MegaPower™ 70- 020, DigiPart, Irvine, California, USA). Voltage and current were collected in real time using the transformer panel control. Average values of power, voltage and current for T1 were 208.4 ± 4.4 W, 102.7 ± 1.2 kV RMS and 4.6 ± 0.1 mA, and 207.9 ± 4.1 W, 100.5 ± 1.8 kV RMS and 4.7 ± 0.1 mA for T2.

2.2. HVACP treatments

Six different treatment variables were studied and optimized over SE decontamination in a previous study, being treatment time, post treatment time, voltage, gas, gas humidity and the position of eggs in the plasma field (Illera et al., 2022). Post treatment storage refers to the time that the treated eggs remain packaged until the sealed bag is opened for analysis. When talking about the gas, air, and modified air with 65% oxygen where tested, with their relative humidity ranging from 0 to 80%. Regarding eggs position, those received a direct treatment when placed directly between the two electrodes, inside the plasma field. When eggs were out of the electrode discharge area, the eggs were indirectly treated. Results showed that higher voltage, treatment time and gas humidity increased the bacterial reduction of eggs surface, due to an increase in the concentration of bactericidal gas species, such as ozone or peroxide radicals. Also related to this effect, post treatment time increased the bacterial reduction when compared to immediate opening of the packages, since the contact time between those species and the eggs surface was greatly extended. Finally, indirect treatments showed better results due to the contact of the eggs with the long-life species such as ozone, with a higher bactericidal effect than short-life species generated in the direct field (Illera et al., 2022). Optimized HVACP treatment conditions that achieved greater than 10^5 CFU/egg reduction were an indirect treatment using air at 60% relative humidity at 100 kV for 1 min treatment and 6 h post treatment storage or, alternatively, 5 min of treatment and 4 h post treatment storage. Initial load of bacteria corresponded to 10^8 CFU/egg, and only one egg was treated per package.

In the current study, to simulate a more realistic situation, the number of eggs per treatment was increased to six. HVACP treatment conditions were selected based on their effectiveness on reducing the presence of *Salmonella* Enteritidis on the surface of uncracked chicken eggs by a minimum of 10^5 CFU/egg when six eggs are treated at the same time (Fig. 1) with an initial SE load of 10^8 CFU/egg. Fixed variables were voltage (100 kV), gas (air), gas relative humidity (60%), and eggs position during the treatment (indirect), based on previous evidence. Therefore, treatment and post treatment time were the two remaining variables to select the treatment conditions for the performance of the six-week quality study. Time and storage are two important factors to consider in the food industry, and the availability of both can rapidly change during processing periods. Therefore, in order to mimic two opposite situations that could take place in an egg facility, two set of conditions were selected. The first conditions would describe a situation where there is no time or space to store the eggs during a post treatment, so that they would have to be treated and directly delivered out of the facility. The second situation would represent a faster treatment due to a higher volume processing, combined with a post treatment.

Based on the previous results, it was possible to determine which was the minimum treatment time required to assure a 10^5 CFU/egg SE reduction when there is no post treatment storage, and in the same way, what was the required post treatment storage time when a very short treatment was performed. To assure that this reduction was also achieved when treating six eggs, the required microbiological assays were performed in triplicate. After the microbiological analysis, the two sets of conditions selected for the treatment of eggs were: T1) 0.5 min of treatment and 24 h post treatment at 4 °C; T2) 8.5 min of treatment and no post treatment.

2.3. Sample preparation

Three hundred and six eggs were purchased in a local store (Guelph, Ontario, Canada), and stored at 4 °C until treatment. All eggs came from the same batch and provider, and were less than two weeks old from the laid time. Eggs were tested during six weeks after treatments, and an initial testing was also included (week 0), right after the plasma

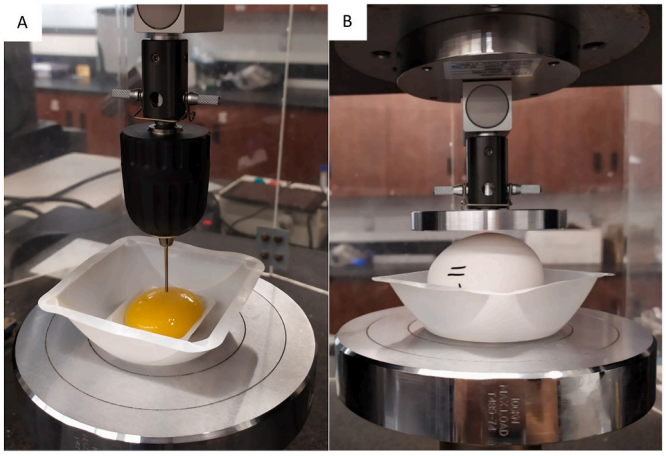

Fig. 2. Experimental set up for A) Vitelline membrane strength and B) Shell strength measurements using Instron texture analyzer.

treatments (T1 and T2). Untreated eggs, used as control (C) were also weekly tested. In each analysis day, the quality parameters from 12 eggs from each set of conditions (T1, T2 and C) were analyzed. Therefore, each analysis day, 12 eggs from 3 sets of eggs were analyzed, making a total of 36 eggs per week, for 7 weeks (initial day + 6 weeks), or a total of 252 eggs required for the study. Extra 54 eggs were included equally in the 3 set of eggs, with 18 extra eggs in each, to overcome possible damages. A total of 102 eggs were treated under T1 conditions, and another 102 under T2 conditions, in sets of 6 eggs/treatment. The remaining 102 eggs were stored as controls. Eggs were randomly selected for treatments or controls, and then randomly chosen from each set for analysis each testing day.

2.4. Six-week egg quality study

The most characteristic quality parameters of eggs were evaluated both in untreated and treated eggs. Eggs were treated under the two sets of conditions previously described and kept at 4 °C up to six weeks. Twelve eggs from each sample group were randomly selected for the quality analysis each week. Eggs were maintained at room temperature (21.0 ± 2.0 °C) for at least 2 h before the quality measurements, with an average inner temperature of 14.5 ± 0.9 °C.

Measured parameters were shell strength, Haugh unit, yolk color, vitelline membrane strength and albumen and yolk pH.

Table 1
Albumen and yolk pH values for control and treated samples during storage.

Week	Albumen pH			Yolk pH		
	Control	T1	T2	Control	T1	T2
0	9.45 ± 0.11^{Aa}	9.46 ± 0.12^{Aa}	9.49 ± 0.06^{ABa}	6.10 ± 0.33^{Ba}	5.94 ± 0.06^{Da}	5.95 ± 0.04^{Da}
1	9.30 ± 0.07^{Ba}	9.34 ± 0.03^{Ba}	9.35 ± 0.06^{Ca}	6.19 ± 0.05^{ABa}	6.12 ± 0.03^{BCb}	6.14 ± 0.04^{Bab}
2	9.32 ± 0.04^{Ba}	9.30 ± 0.04^{Ba}	9.29 ± 0.07^{Ca}	6.15 ± 0.16^{ABa}	6.14 ± 0.03^{BCa}	6.20 ± 0.25^{ABa}
3	9.31 ± 0.08^{Ba}	9.30 ± 0.06^{Ba}	9.29 ± 0.05^{Ca}	6.28 ± 0.25^{ABa}	6.11 ± 0.04^{Cb}	6.12 ± 0.05^{BCab}
4	9.48 ± 0.14^{Aa}	9.45 ± 0.10^{Aa}	9.45 ± 0.09^{Ba}	6.10 ± 0.0^{9Ba}	6.08 ± 0.05^{Ca}	6.06 ± 0.04^{CDa}
5	9.50 ± 0.06^{Aa}	9.51 ± 0.04^{Aa}	9.47 ± 0.06^{ABa}	6.28 ± 0.21^{ABa}	6.18 ± 0.05^{Ba}	6.17 ± 0.03^{ABCa}
6	9.53 ± 0.12^{Aa}	9.51 ± 0.07^{Aa}	9.54 ± 0.07^{Aa}	6.42 ± 0.21^{Aa}	6.27 ± 0.06^{Ab}	6.29 ± 0.05^{Aab}

Means followed by the same capital letter do not differ statistically among each other in the same column (comparison of a single sample during different analysis days) and means followed by the same small letter do not statistically differ among each other in the same row (comparison between samples on the same analysis day) ($p > 0.05$; Tukey test). n = 12 eggs/group.

Shell strength was the first measurement performed. It was measured using an Instron texture analyzer (Model 5969) coupled to a 10-kg load cell whit a 75 mm diameter aluminum compression plate, the experimental set up can be seen in Fig. 2B. Test speed was 0.5 mm/s with a trigger force of 0.1 g, and a fixed distance of 16 mm. Eggs were placed in a horizontal way below the compression plate using a square dish (4.4 cm * 4.4 cm), assuring always the same and centered position of the egg. The egg was then compressed until the shell cracked, and the applied strength was recorded by the equipment software and provided in Newtons (N).

Once the egg shell was cracked, it was transferred to an Egg Analyzer (ORKA Food Technologies LLC, West Bountiful, Utah, USA), which measured egg weight, albumen height, Haugh Unit, and yolk color based on the YolkFan™ color (DSM Co., Heerlen, Limburg, Netherlands).

After the Egg Analyzer measurement, yolk was separated from the albumen using a yolk separator to assure its integrity and transferred to a polypropylene weighting plate for vitelline membrane strength (VMS) measurement. VMS was measured over isolated yolks, with no albumen, using the same texture analyzer and same load cell as in the shell strength analysis, but in this case, coupled to a 1-mm round-tipped probe (Fig. 2A). Punction of the yolk was performed in its middle part, avoiding measurements close to the chalazae area, and the required strength to break the vitelline membrane was automatically reported in Newtons.

After that, the color of the yolk was additionally evaluated using a hand colorimeter (CSM 4, PCE Inst., Palm Beach, Florida, USA) in the CIEL*a*b* scale using a glass petri dish where the isolated yolk had been previously homogenized. Color difference (ΔE) was determined considering the results just after T1 or T2 (Week 0) and the control, and the values after 6 weeks storage:

$$\Delta E = \sqrt{(L_{6W} - L_{0W})^2 + (a^*_{6W} - a^*_{0W})^2 + (b^*_{6W} - b^*_{0W})^2}^{1/2} \quad (1)$$

To complete the quality analysis, the pH measurements of previously separated albumen and yolk were performed using a hand pH meter (Oakton pH Testr® 5, Cole-Parmer, Vernon Hills, Illinois, USA).

2.5. Characterization of the reactive gas species (RGS)

2.5.1. Ozone measurement in the gas

Ozone concentration was measured using an ozone monitor (106-MH, 2B Technologies, Broomfield, Colorado, USA) after each treatment and post-treatment time, with a highest measuring limit of 10,000 ppm of ozone. A small perforation was made in the packaging film of the sample and the gas inside was pumped into the monitor, immediately providing the ozone concentration of the gas in ppm. Ozone concentration was determined as the average of the ozone measurements performed after the 17 treatments (T2) and after the 17 treatments with the additional 24 h post treatment time in the case of T1. In order to know what was the produced ozone after T1 treatment (0.5 min), three additional set of samples were treated during this time and immediately measured. Those eggs were not used for the rest of the study.

2.5.2. Nitrate, nitrite and peroxides measurement in the egg

The presence and quantification of nitrates, nitrites and peroxides was measured in the inside of the eggs after their treatment or post treatment and during their storage. Indicator strips (Bartovation, White Plains, New York, USA) were used for all species measurements, providing different concentration ranges; nitrates (0-500 ppm), nitrites (0-25 ppm) and peroxides (0-100 ppm). To perform the measurements, three random yolks and three random albumens from each sample group were selected once the previously described quality analysis had been completed.

2.6. Statistical analysis

For the egg quality results, data was presented as mean ± standard deviation. The results were evaluated by analysis of variance (ANOVA) and Tukey's post-hoc test with a 95% significance level using SPSS software (SPSS® Statistics version 20, IBM, Chicago, Illinois, USA).

3. Results and discussion

3.1. Six-week quality study

The most important quality parameters in eggs are shown in this section for untreated and treated eggs right after treatments and during the six-week storage study. Eggs were treated under the two sets of optimized conditions, 0.5 min treatment +24 h post treatment (T1) and, 8.5 min treatment + no post treatment (T2) at 100 kV using air at 60% RH.

3.1.1. Albumen and yolk pH

Eggs submitted to HVACP treatments did not show a significant difference in albumen pH (Table 1) after none of the treatments, with an average value of 9.47 ± 0.02 from all samples. Both control and treated eggs did not show a clear trend on pH during storage since a significant decrease occurred during the first 3 weeks of storage to then restore their initial pH and end up the 6 weeks study with a not significant difference when compared to week 0, except for T2, which showed a slightly higher pH after the storage time, but a very similar increase when compared to control and T1. Again, no significant difference was found between the control and the treated samples after 6 weeks, with an average value of 9.53 ± 0.02. Lee et al. (2016) studied the effect of storage time and temperature over the quality parameters of chicken eggs stored during 30 days at 2 °C, and they did not observe a significant increase in their albumen pH, varying from around 7.7 to 8.0 in that time. They determined that albumen pH was not affected by storage time, but by storage temperature, showing that alkalinization was accelerated when storage temperature was 12 or 25 °C (Lee et al., 2016). In the present study, eggs were kept at 4 °C, so a similar behavior can be expected in control eggs. On the other hand, treated eggs were not affected after treatments, and showed the same trend as control eggs. Higher pH in albumen is related to a loss of freshness, since CO_2 escapes through the eggshell pores and alkalinizes the inner egg (Lin et al., 2021), so with these results, it can be concluded that HVACP did not contribute to this effect or its evolution during storage, maintaining egg albumen variations equal to the control eggs.

Regarding yolk pH, a slight, but not significant acidification was observed after both treatments, decreasing from 6.10 ± 0.33 to 5.94 ± 0.06 and 5.95 ± 0.04 after T1 and T2, respectively. However, both control and treated samples showed a trend of increasing pH during storage time, with final values of 6.42 ± 0.21; 6.27 ± 0.06 and 6.29 ± 0.05 for control, T1 and T2, respectively after 6 weeks. All samples experimented a significant increase in their yolk pH after 6 weeks of storage, and the statistics showed a significant difference among control and T1 sample by this time. However, as it can be seen in Table 1, this effect changed from significant to not significant during all the storage period between those samples, not showing a clear trend of changes between the control and the T1 sample. The evolution of yolk pH values was pretty similar between C, T1 and T2, and the researchers consider that it was not conclusive that T1 HVACP treatments affected the yolk pH during storage in a great extent. As explained, an increase in egg pH is caused by the aging process, and this could also be the case of the observed difference between C and T1 after 6 weeks, which could be caused by a similar but still different aging among the selected eggs of each group.

Lee et al. (2016) saw a significant increase in yolk pH after 30 days of storage of untreated eggs, varying from 5.64 to 5.73, and another study obtained a significant increase from 6.01 to 6.28 after 6 weeks of storage at 7 °C (Biladeau & Keener, 2009).

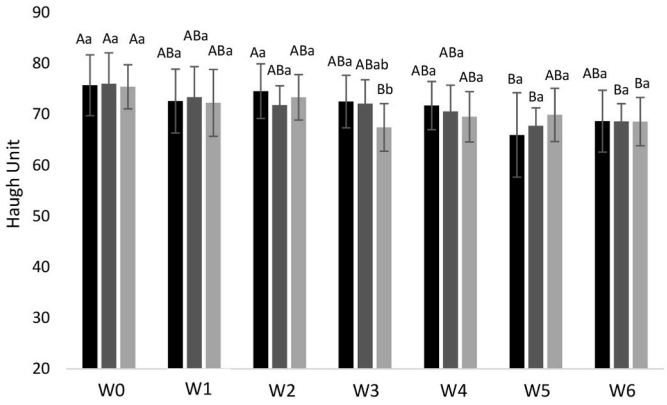

Fig. 3. Haugh unit values for control (black), T1 (dark grey) and T2 (light grey) after treatments and during the storage period.
Means followed by the same capital letter do not differ statistically among each other in the same column color (comparison of a single sample during different analysis days) and means followed by the same small letter do not statistically differ among each other in the same group of three columns (comparison between samples on the same analysis day) ($p > 0.05$; Tukey test). n = 12 eggs/group.

No changes in egg pH after plasma treatments have been found in other studies under a variety of treatment conditions and plasma set ups. Wan et al. (2017) used a similar HVACP set up, and after a 15 min treatment at 85 kV using MA65 as gas, no changes in the yolk or albumen pH were observed. Gavahian et al., (2019) used duck eggs in their study. After 0.5 min of treatment at 12 kV using air with 65% RH, no significant differences were observed in the pH of the egg. Lin et al. (2021) compared untreated commercial washed eggs and eggs treated under a non-thermal argon plasma jet in a conveyor belt. They did not observe a significant difference in albumen pH, being 8.94 ± 0.00 and 9.10 ± 0.01 for untreated and treated eggs, respectively. They also monitored egg quality during the storage time, and after 15 days, they did not find significant differences in albumen pH of treated eggs, being 9.39 ± 0.04. In the case of yolk pH, a significant change was observed after 15 days, changing from 6.10 ± 0.07 to 5.84 ± 0.03. Although it represents a significant difference, the plasma treatment that they applied can not be compared to the ones applied in our study, which was indirect and not direct, with a larger distance to the electrodes, and using the half of power for the treatments, altogether decreasing the energy that the eggs received and mitigating its effect over egg components.

As it can be seen, pH values of egg components vary in a great extent among studies, showing its high natural variability, and overall, HVACP treatment did not negatively affect this parameter.

3.1.2. Haugh unit

Haugh unit (HU) is another indicator of freshness in eggs, as well as an egg albumen protein quality measure. Haugh unit values are calculated using the albumen height and egg weight, and are used for the grading of eggs. According to the USDA - US Standards, Grades, and Weight Classes for Shell Eggs, a firm white has a Haugh unit value of 72 or higher when measured between 7 and 15.5 °C (USDA, 2000). Therefore, as it can be seen in Fig. 3, on the first day, all eggs showed a Haugh unit higher than 72, with an average among samples of 76, grading the eggs as 'AA', and this grade was maintained after both treatments, showing no detrimental effect of plasma over the protein quality of the egg. During the storage time, a clear trend can be seen, where HU of eggs decreases with storage time, reaching an average value among samples of 68.6. All treated samples HU significantly changed after 6 weeks of storage, but they did not show a significant difference among them or with the control. After this time, all eggs were

Table 2
YolkFan™ color values for control and treated samples during storage.

Week	Control	T1	T2
0	6.50 ± 2.58^{Aa}	7.00 ± 1.81^{ABa}	7.33 ± 2.06^{ABa}
1	6.42 ± 2.02^{Aa}	5.83 ± 1.64^{Ba}	6.92 ± 2.15^{ABa}
2	7.25 ± 1.91^{Aab}	7.58 ± 1.98^{ABa}	5.58 ± 1.78^{Bb}
3	7.75 ± 1.42^{Aa}	7.83 ± 2.12^{ABa}	7.92 ± 2.02^{Aa}
4	7.25 ± 2.34^{Aa}	8.58 ± 1.51^{Aa}	8.50 ± 1.62^{Aa}
5	8.17 ± 1.99^{Aa}	7.17 ± 2.08^{ABa}	8.42 ± 1.98^{Aa}
6	8.67 ± 1.87^{Aa}	7.67 ± 2.19^{ABa}	9.17 ± 1.11^{Aa}

Means followed by the same capital letter do not differ statistically among each other in the same column (comparison of a single sample during different analysis days) and means followed by the same small letter do not statistically differ among each other in the same row (comparison between samples on the same analysis day) ($p > 0.05$; Tukey test). n = 12 eggs/group.

graded as 'A', due to values lower than 72, but higher than 59 (USDA, 2000).

The decrease in the HU is a common trend in eggs during their aging since they lose their freshness and part of their quality. Many studies have monitored the changes in the HU of eggs during storage time, all showing significant decreases. After just 10 days, Samli et al., (2005) observed a decrease in the HU from 91.37 to 76.27 (15.1 HU points) in eggs from 50 weeks old hens. After 30 days, Lee et al. (2016) showed a decrease of 6.9 HU points in eggs stored at 2 °C, but of 20.8 and 59.5 HU points decrease when eggs were stored at 12 and 25 °C, respectively. When stored at 7 °C, in a different study, HU in eggs decreased from 82.8 to 68.6 after 6 weeks, and remained not significantly different during 6 weeks more (Biladeau & Keener, 2009). These values provide a wide idea of how variable HU parameter is, and how it strongly depends on hen age, period after laying of the egg and storage temperature. This concludes that although HU decreases during storage, it was caused the aging of eggs, as a natural process, and not because of HVACP treatments effect.

Similar findings were reported in literature regarding the effect of plasma treatments over HU. Moritz et al. (2021) used a semidirect plasma source to treat one egg for 5 min, and no significant changes were reported over HU, where eggs showed an average of 75.57 ± 6.16. In a similar way, eggs treated under MA65 at 85 kV for 15 min, maintained their HU in 87.65 ± 6.15 in Wan et al. (2017) study. Neither Dasan et al. (2018) nor Lin et al. (2021) observed significant differences in their plasma treated eggs. In the latter study, untreated eggs showed a HU of 89.68 ± 2.27, and after non thermal plasma treatment, it did not significantly change, being 91.54 ± 1.78, and after 15 days of storage, HU was 83.08 ± 0.93 (Lin et al., 2021).

Although Haugh unit has been generally accepted as a quality index in eggs, some authors point out its bias, due to its dependence on age and strain of hen, as explained before. Silversides and Budgell (2004), suggested the use of albumen pH to measure eggs freshness since it is not dependent on these factors.

Whereas albumen pH or Haugh Unit are chosen in this study to evaluate the effect of HVACP over the quality and freshness of eggs during storage, both of them indicated that these treatments did not affect them, maintaining the same properties as untreated eggs.

3.1.3. Yolk color

Color plays an essential role in the perception of food and its quality, and yolk color is a key aspect for consumers. Preferences for yolk color greatly varies among countries and cultures, but in a general way, darker yolks are preferred rather than light ones (Beardsworth & Hernandez, 2014).

YolkFan™ color values were directly provided by the Egg Analyzer. This scale gives values from 1 to 16 according to the color density of the yolk, corresponding to a pale-yellow yolk in level 1 and increasing in redness to a dark orange yolk color in level 16.

As it can be seen in Table 2, none of the HVACP treatments applied to eggs significantly modified their color, being their values 6.50 ± 2.58 for the control eggs and 7.00 ± 1.81 and 7.33 ± 2.06 after T1 and T2, respectively. As it can be appreciated in the standard deviation values accompanying the averages, there is a great variability in yolk color among eggs of the same set of samples, including the untreated ones. These variations are so pronounced since the YolkFan Color scale only provides with whole numbers, and variations among them in the scale are very slight, so variations of even five grades in the range have been found among samples of the same group. As a result of this, a clear trend could not be appreciated during the storage time, and after 6 weeks of storage, no significant differences have been found in yolk color for none of the samples groups when compared to the initial ones. Furthermore, the yolk color of control, T1 and T2 eggs remained not significantly different, being 8.67 ± 1.87, 7.67 ± 2.19 and 9.17 ± 1.11 respectively after 6 weeks. Although statistically there is not significant difference due to the mentioned high standard deviation in samples, a trend to maintain darker colors rather than a lightning can be seen. All YolkFan color obtained measurements are in a similar range of the fan, characterized by a light orange.

As mentioned, using the YolkFan color provides with whole numbers and does not give a finest grading. Therefore, yolk color was also measured using a colorimeter. The two key color components determining yolk color are redness (a*), and yellowness (b*). These two values as well as L* are provided in Table 3. The more yellow component

Table 3
CIE L*a*b* color values for control and treated samples during storage.

Week	L*			a*			b*		
	Control	T1	T2	Control	T1	T2	Control	T1	T2
0	57.88 ± 2.08^{ABa}	57.99 ± 0.94^{Ba}	58.13 ± 0.84^{ABCa}	15.17 ± 6.28^{Aa}	16.86 ± 5.09^{Aa}	17.01 ± 6.04^{Aa}	72.58 ± 4.15^{Aa}	73.48 ± 3.23^{Aa}	73.18 ± 3.46^{Aa}
1	58.10 ± 1.35^{ABa}	57.95 ± 0.82^{Ba}	57.91 ± 1.14^{BCa}	17.85 ± 7.45^{Aa}	21.31 ± 7.27^{Aa}	18.34 ± 6.21^{Aa}	73.43 ± 2.58^{Aa}	73.88 ± 1.67^{Aa}	73.42 ± 2.98^{Aa}
2	57.42 ± 1.17^{Ba}	57.46 ± 1.46^{Ba}	57.52 ± 1.38^{BCa}	19.91 ± 5.28^{Aa}	18.80 ± 8.50^{Aa}	17.58 ± 9.53^{Aa}	74.48 ± 1.83^{Aa}	72.36 ± 4.09^{Aab}	70.75 ± 4.58^{Ab}
3	57.75 ± 0.89^{Ba}	57.57 ± 1.65^{Ba}	57.92 ± 1.32^{BCa}	18.55 ± 5.30^{Aa}	19.26 ± 8.92^{Aa}	20.67 ± 8.81^{Aa}	73.43 ± 2.01^{Aa}	72.71 ± 3.73^{Aa}	72.75 ± 3.97^{Aa}
4	57.96 ± 1.12^{ABa}	57.70 ± 1.66^{Ba}	57.34 ± 1.15^{Ca}	17.95 ± 6.45^{Aa}	17.38 ± 5.26^{Aa}	15.43 ± 4.55^{Aa}	72.17 ± 2.98^{Aa}	73.10 ± 2.47^{Aa}	71.26 ± 4.80^{Aa}
5	58.75 ± 1.11^{ABa}	58.54 ± 0.74^{ABa}	58.80 ± 0.75^{ABa}	17.94 ± 8.65^{Aa}	13.75 ± 5.85^{Aa}	19.18 ± 7.41^{Aa}	73.40 ± 2.41^{Aa}	71.70 ± 3.61^{Aa}	73.72 ± 3.30^{Aa}
6	59.40 ± 1.08^{Aa}	59.79 ± 1.05^{Aa}	59.40 ± 0.98^{Aa}	17.09 ± 10.44^{Aa}	17.28 ± 9.01^{Aa}	17.92 ± 6.80^{Aa}	73.57 ± 2.73^{Aa}	73.82 ± 2.66^{Aa}	74.62 ± 3.50^{Aa}

Means followed by the same capital letter do not differ statistically among each other in the same column (comparison of a single sample during different analysis days) and means followed by the same small letter do not statistically differ among each other in the same row (comparison between samples on the same analysis day) ($p > 0.05$; Tukey test). n = 12 eggs/group.

Table 4
Vitelline membrane strength (VMS) values for control and treated samples during storage.

Week	Control	T1	T2
0	0.0244 ± 0.0035^{BCa}	0.0278 ± 0.0063^{ABa}	0.0269 ± 0.0040^{Aa}
1	0.0238 ± 0.0046^{Ca}	0.0262 ± 0.0045^{Ba}	0.0273 ± 0.0097^{Aa}
2	0.0282 ± 0.0039^{ABCa}	0.0289 ± 0.0025^{ABa}	0.0320 ± 0.0058^{Aa}
3	0.0247 ± 0.0038^{BCa}	0.0251 ± 0.0048^{Ba}	0.0290 ± 0.0071^{Aa}
4	0.0301 ± 0.0047^{ABa}	0.0305 ± 0.0063^{ABa}	0.0295 ± 0.0059^{Aa}
5	0.0312 ± 0.0062^{Aa}	0.0263 ± 0.0034^{ABb}	0.0311 ± 0.0030^{Aa}
6	$0.0273 \pm 0.0048A^{BCb}$	0.0321 ± 0.0042^{Aa}	0.0256 ± 0.0048^{Ab}

Means followed by the same capital letter do not differ statistically among each other in the same column (comparison of a single sample during different analysis days) and means followed by the same small letter do not statistically differ among each other in the same row (comparison between samples on the same analysis day) ($p > 0.05$; Tukey test). n = 12 eggs/group.

that a yolk has, the bigger b* value will be, and the same will occur with a* component for redness of the yolk.

As expected, no further differences in color have been found when using the CIEL*a*b* system. a* and b* values have remained constant after plasma treatments and during storage, showing a similar trend among control and treated samples after this time. They all correspond to a similar orange grade. Regarding luminosity (L*), it also remained unchanged in general, and although significant differences were found in some cases, this can be due to the finest scale that this system uses for measuring. By calculating the color difference (ΔE), very similar values can be seen among samples. When comparing color data from week 0 and week 6, results were; 2.6 for the control sample, 1.9 for T1, and 2.1 for T2. According to Yuk et al., (Yuk et al., 2014), when the color difference value is between 1.5 and 3, the change in color can be estimated to be noticeable, being slightly noticeable when it is between 0.5 and 1.5, and well visible when it is in the range of 3–6. The three samples of this study relay in the noticeable range, being the control sample the one with the highest value, and close to the well visible values. T1 and T2 showed very similar values, being closer to the lower limit of this range.

These results align with those provided in literature. In studies where egg quality is measured among time in untreated eggs, no changes in yolk color happened. After 30 days at 2 °C, color measured with the YolkFan Color scale varied from 7.00 to 7.34 in Lee et al. (2016) study, and Jin et al. (2011) saw a variation from 7.27 to 7.85 after 10 days at 5 °C.

Regarding plasma treated eggs, previous studies also found no effect of the treatments over yolk color. Wan et al. (2017) used a similar egg analyzer apparatus, which also provided yolk color using the DSM scale, and they reported a non-significant difference after treatment of eggs at 85 kV using MA65 during 15 min, where their untreated eggs had a YolkFanTM color of 3.0 ± 2.8, and the treated ones 9.5 ± 7.8. This case even shows a higher variability in yolk color among eggs. In the case of Lin et al. (2021), they used a panelist assessment for color and other sensory evaluation of treated eggs, where a value of 7 means that they 'like moderately', and 8 means that they 'like very much', among other values. Right after a non-thermal plasma treatment in a conveyor belt at 400 W using argon, panelists gave a score of 7.42 ± 0.98 to treated eggs, while they gave 7.00 ± 1.29 to washed untreated eggs. Not significant difference was detected. After 15 days of storage at room temperature (25 ± 1 °C), still not statistical difference was determined and scores were 7.17 ± 0.75 for untreated eggs and 8.00 ± 1.33 for plasma treated eggs. As additional information, no changes were also appreciated by the panelists in egg aroma, texture or taste after plasma treatments and after storage time, and overall acceptance remained constant during the storage time.

As it can be seen, HVACP treatment does not affect to another key parameter of eggs quality such as its yolk color, indistinctly of the length of the treatment applied and the post treatment conditions.

Table 5
Shell strength values for control and treated samples during storage.

Week	Control	T1	T2
0	41.03 ± 6.66^{Aa}	40.89 ± 5.32^{Aa}	40.77 ± 4.16^{Aa}
1	41.72 ± 6.42^{Aa}	39.99 ± 7.23^{Aa}	42.71 ± 2.96^{Aa}
2	39.34 ± 5.80^{Aa}	40.48 ± 7.62^{Aa}	43.64 ± 6.33^{Aa}
3	38.83 ± 4.76^{Aa}	42.07 ± 3.91^{Aa}	41.11 ± 8.52^{Aa}
4	39.74 ± 5.84^{Aa}	41.28 ± 7.09^{Aa}	39.86 ± 6.92^{Aa}
5	41.06 ± 5.69^{Aa}	39.03 ± 7.23^{Aa}	39.69 ± 6.81^{Aa}
6	41.90 ± 6.58^{Aa}	42.37 ± 4.90^{Aa}	41.95 ± 5.50^{Aa}

Means followed by the same capital letter do not differ statistically among each other in the same column (comparison of a single sample during different analysis days) and means followed by the same small letter do not statistically differ among each other in the same row (comparison between samples on the same analysis day) ($p > 0.05$; Tukey test). n = 12 eggs/group.

3.1.4. *Vitelline membrane strength (VMS)*

Vitelline membrane consists in a fibrous network that retains yolk integrity and prevents it from breaking, as well as from bacteria transfer between the albumen and yolk (Gast et al., 2004). This essential item steadily disappears during the aging of the egg, causing the decrease of VMS during storage time (Biladeau & Keener, 2009).

Although the reduction of VMS is a known and natural process, the eggs tested in this study did not show a reduction of their VMS during a storage of 6 weeks. No significant difference was found between the VMS of control eggs on Week 0 and Week 6, being 0.0244 ± 0.0035 N and 0.0273 ± 0.0048 N, respectively (Table 4). However, it should be also noticed that for example in Week 5 of storage, this value was significantly higher, what does not correspond to the natural aging of eggs. A similar trend could also be observed in T1 and T2 samples, with increased and decreased values during the storage. The explanation may be in the very high variation observed among samples of the same groups, as previously detected in other parameters, where in some cases is higher than a 10% of the average. Furthermore, although the Instron probe was always placed in a similar position over the egg yolk, the shape and size variation that is usually observed among eggs yolks could have a great impact in the measurements variability. Overall, VMS of the eggs was not affected after any of the treatments, and they also did not show a significant difference after 6 weeks of storage.

Kirunda and Mckee (2000) found a correlation between a decrease of VMS and a decrease of the Haugh Unit, and the increase of albumen and yolk pH. They also pointed out that the disappearing of the vitelline membrane is favoured with time and temperature. They performed SEM analysis on the surface of yolks and could appreciate a significant loss of the structural integrity of eggs that had been kept during 2 weeks at room temperature. They also measured VMS with a texture analyzer and saw a decrease from 577.1 g to 263.1 g after this time. Probably low temperatures have an essential role in the longer maintenance of VM integrity, corresponding with the results obtained in the present study.

Wan et al. (2017) measured VMS after a HVACP treatment, and after 15 min at 85 kV, they did not find a significant difference, showing that even long treatments retain the integrity of the vitelline membrane.

3.1.5. *Shell strength*

Shell strength is an essential parameter of eggs to keep their integrity and avoid them to break. The interest of measuring this parameter after treatments is to confirm that its strength is not weakened by the action of HVACP or the generated gas species, such as ozone.

The egg shell strength remained unchanged after plasma treatments as well as during the 6 weeks of storage for all egg groups (Table 5). The same observation was made by Chen (2014), who obtained similar results after treating eggs during 15 min with MA65 at 85 kV and after storage during 6 weeks, finding no variation in the shell strength.

Moritz et al. (2021) did not measure the shell strength, but they checked the integrity of the shell cuticle by a staining technique, and they observed an interesting modification of the egg surface. The treated

Table 6
Reactive gas species concentration in the gas (ozone) after treatment and post treatment, and in the eggs (nitrates, nitrites and peroxides) right after treatments and after 6-week storage.

SAMPLE	NO_3^- (PPM)		NO_2^- (PPM)		H_2O_2 (PPM)		O_3 (PPM)	
	Week 0	Week 6	Week 0	Week 6	Week 0	Week 6	After treatment	After post-treatment
C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.
T1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	611 ± 28	0.0 ± 0.1
T2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1788 ± 198	n.a.

n.d. means non-detected, n.a. means not applicable, n = 3 eggs/group for nitrates, nitrites and peroxides and n = 17 for ozone measurements.

eggs showed unstained areas where the electrodes had applied plasma, possibly caused by an interaction of the cuticle and the reactive species of plasma, or due to the plasma field. As the current study was performed in an indirect way, it is probable that this effect did not happen, but a SEM and cuticle integrity study should be performed to confirm this theory.

3.2. Gas chemistry characterization of the optimized HVACP treatment

3.2.1. Ozone concentration in the gas

Ozone was quantified for the treated samples right after their treatment or post treatment times. As expected, it can be seen the higher generation of ozone after the 8.5 min plasma treatment (T2) when compared to 0.5 min (T1), being 1788 ± 198 ppm and 611 ± 288 ppm, respectively (Table 6). Although the ozone generated after T1 is almost a third of the generated in the longer treatment, it can be seen that the effect of maintaining the eggs for a longer time in contact with ozone (up to 24 h) can achieve the same bactericidal effect as a higher ozone concentration for a shortest time. After the 24 h of post treatment, no residual ozone was left inside the package.

3.2.2. Nitrate, nitrite and peroxides concentration in the eggs

To ensure that no residues of nitrates, nitrites or peroxides formed in the plasma gas would dissolve in the albumen or yolk by penetration through the eggshell pores, those were weekly measured in untreated and treated eggs. None of these species were found in this study after the two treatments nor during or after the storage period, proving that egg integrity was maintained after treatments (Table 6).

4. Conclusions

HVACP has been previously proved and optimized to be an effective decontamination tool for eggs surface with *Salmonella* Enteritidis. For a future commercialization of the process, it is essential to understand its effect over the egg quality, as well as how it changes during their shelf life. Neither a 0.5 min treatment with 24 h post treatment nor an 8.5 min treatment with no post treatment time at 100 kV and air with 60% RH significantly affected the albumen and yolk pH, Haugh unit, yolk color, vitelline membrane strength or shell strength of the eggs. Furthermore, these quality parameters of the eggs remained either unchanged, or changed in a similar way to the untreated eggs during 6 weeks of storage at 4 °C, showing variations due to the natural aging process of the eggs and not due to the HVACP treatments.

Overall, it can be concluded that HVACP is an effective decontamination technology for eggs surface while maintaining the egg quality over their shelf life under the tested conditions, and would be a promising technology for egg safety assurance.

Author statement

Alba E Illera: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original Draft. **Vanessa R Souza:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Review & Editing. **Linyi Tang:** Methodology, Validation, Investigation, Writing – Review & Editing. **Nooshin Nikmaram:** Methodology, Validation, Investigation, Writing – Review & Editing. **Kevin M Keener:** Conceptualization, Methodology, Validation, Supervision, Writing – Review & Editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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