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Extending Fresh Chicken Eggs Shelf Life using Unconventional Methods

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ABSTRACT

This study investigated the effectiveness of coating fresh chicken eggs carboxymethyl cellulose (CMC) following ozone or UV treatments on their functional properties, internal quality, eggshell breaking strength, and microbial counts during storage at 20 - 25°C for 60 days. The coatings significantly affected Haugh unit, yolk index, pH, relative whipping capacity (RWC), albumen viscosity, and total viable count (TVC) in chicken eggs. The control eggs (not treated with ozone, UV, or CMC coating) showed increasing in weight loss, pH, and yolk index, while foam stability decreased after 60 days of storage at 20 - 25° C. Microbiological results for uncoated egg samples revealed a TVC of 3.51 $\log_{10}$ CFU/g at the beginning of storage, reaching 8.54 $\log_{10}$ CFU/g by the end. In contrast, the coated eggs (both ozone + CMC and UV + CMC) reached a TVC of 6.23 $\log_{10}$ CFU/g. The results demonstrate the significant impact of ozone or UV treatments combined with CMC coating on chicken eggs stored at 20 - 25°C for 60 days.

1. Introduction

Chicken eggs are a widely consumed and healthy food source globally. They are an excellent source of protein, including ovalbumin, ovomucin, and ovoglobulin. Eggs also boast a variety of essential nutrients choline, vitamin B12, vitamin A, vitamin D, and selenium fatty acids and minerals. research suggests that consuming eggs can contribute to a better quality diet, weight management, and feelings of fullness. Additionally, antioxidants present in egg yolks may play a role in preventing age-related macular degeneration (Ruxton et al., 2010). Egypt's poultry industry is a major contributor to the global egg market. Commercial farms raise around 1.4 billion chickens, while rural areas add another 320 million. This substantial production translates to roughly 14 billion table eggs produced in Egypt according report published by GAIF (2023). Eggs, a valuable source of protein, are susceptible to spoilage. being laid, they undergo a series of physical, chemical, and microbiological changes that deteriorate their quality. Storage accelerates these changes, resulting in an increase in pH, a thinner albumen, thinner albumen, and moisture loss through the eggshell's pores. This loss of moisture and CO_2 contributes to a higher pH and a decline in both albumen and yolk quality (Nongtaodum et al., 2013). Additionally, the air cell depth increases with storage duration, further impacts overall egg quality (Batkowska & Brodacki, 2014). Considering the vast quantities of eggs produced globally, even a slight improvement in shelf life and overall quality translates to significant financial gains for the egg industry. This reality fuels the exploration of innovative food preservation technologies to address these challenges. Recently, researchers have turned to non-thermal techniques like ozonation, high pressure processing, ultrasound, pulsed electric field, and cold plasma for egg preservation (Safwa et al., 2024)

Ozone (O_3) treatments are emerging as a popular technique for extending the shelf life of perishable foods, including eggs. This small gas boasts the ability to penetrate cell membranes, enhancing its effectiveness against microorganisms. A significant advantage of ozone is its rapid decomposition into oxygen gas, leaving no harmful residues on food products. This characteristic makes ozone a safe and attractive option for food preservation applications.

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(Asokapandian et al., 2018). Adding to its appeal for food preservation. Ozone is a powerful antimicrobial agent that is extremely reactive and produces no by-products. Both the US Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) have authorized the use of gaseous and aqueous ozone as a direct antimicrobial agent in Furthermore, complying with good manufacturing practices allows the use of ozone on meat and poultry products in the US (21 CFR 173.368; FDA 2003). This regulatory approval has significantly contributed to the growing research interest in utilizing ozone to extend the shelf life of various food products. Research over the past decade has explored the potential of ozone treatment for extending the shelf life of fresh eggs. Studies have shown that ozone gas can effectively penetrate the eggshell pores, offering a promising approach to combating internal spoilage (Luis et al., 2005). However, a potential drawback exists: ozone's oxidizing properties raise concerns about damaging the eggshell's protective cuticular proteins. Fuhrmann et al. (2010) observed that even low ozone concentrations could negatively impact the structure and composition of these proteins, potentially weakening the eggshell's barrier function. In 2012 study, examined the effects of ozone gas (at a concentration of 38.8 ppm) on various quality parameters of fresh eggs, Treatment times ranged from 10 to 30 minutes. They evaluated factors such as Haugh Unit (a measure of freshness), yolk color, albumen and yolk pH, foaming ability, and lipid oxidation, with treatment times ranging from 10 to 30 minutes. Their findings revealed that ozone-treated eggs behaved similarly to control eggs during storage, suggesting no significant long-term impact on these quality parameters.

Ultraviolet (UV) irradiation is a promising food preservation technique with the potential to reduce pathogens and minimize nutritional losses compared to heat treatment methods (Mattioli et al., 2020). UV radiation exists in a spectrum categorized into four main ranges: vacuum-UV (100–200 nm), UV-A (315–400nm), UV-B (280–315 nm), and UV-C (200–280nm) (Mattioli et al., 2020). Notably, UV-C radiation (200-280nm) is the primary type used for food preservation because it effectively disrupts the DNA or RNA of microorganisms, rendering them unable to reproduce. Because of its strong antimicrobial properties, the UV-C range is helpful in guaranteeing the microbiological safety of food products. Microbes' genetic material (DNA or RNA) absorbs UV photons strongly in the UV-C range, the maximum UV absorption occurs at a wavelength of about 260–265nm (Green et al., 2020). UV-C has emerged as a promising substitute for thermal pasteurization in the global food industry, provided it receives approval from relevant regulatory bodies and ensures product safety. The Food and Drug Administration (FDA) regulations (21 CFR 179.39) authorize the use of UV-C for sterilizing water used in food production, reducing human pathogens and other microorganisms in juices, and controlling surface microorganisms in food and food products (FDA ECFR, 2023). The egg-production industry uses biomaterial coatings that can be based on proteins, lipids, or polysaccharides. Many researchers use different materials among the biomaterials with characteristics that lend themselves to egg coating, (Kodsangma et al., 2020; Chi et al., 2020 and Leksawasdi et al., 2021), carboxymethyl cellulose (CMC) (Tantala et al., 2019; Suriyatem et al., 2020 and Klunklin et al., 2021), carboxymethyl chitosan (Chi et al., 2020), carboxymethyl bacterial cellulose (Leksawasdi et al., 2021), sericin (Rachtanapun et al., 2021), keratin (Kaewsalud et al., 2021), fibroin (Yakul et al., 2021), pectin, (Chaiwarit et al., 2020). The creation of coating materials, has been one of the best strategies for egg preservation. Nevertheless, rather than stifling microbial activity, the majority of coating applications to date have concentrated on avoiding respiration and dehydration. Furthermore, attention is now focused on coating materials made of natural materials (Waimaleongora-Ek et al., 2009; Wardy et al., 2010; Wardy et al., 2011; Jo et al., 2011; and Musa et al., 2011). This study investigated the effectiveness of ozone and UV treatments followed by carboxymethyl cellulose (CMC) coating on the quality and shelf life of chicken eggs. It employed two primary goals:

1- Monitoring internal egg quality during storage. This involved tracking changes in quality indicators like Haugh Unit (HU), Yolk Index (YI), albumen and yolk pH, and functional properties like albumen and whole egg Relative Whipping Capacity (RWC).
2- Measuring eggshell breaking strength throughout a 60-day storage period at 20- 25°C.

2. Materials and Methods

Materials

Chicken eggs, chemicals & materials

Six hundred white-shelled chicken eggs, laid by Hy-Line breed chickens, were obtained from Elwaha Fresh Foods Company in the Third Settlement of New Cairo, Egypt. After being cleaned using mechanical brushes and inspected (fresh, unfertile, crack-free, 45g-65g weight range), the eggs were transported to the lab, stored in clean cardboard boxes at 20 - 25°C, and divided into five groups of 120 each: untreated (control), UV-treated, UV/CMC-coated, ozone-treated, and ozone/CMC-coated. Glycerol and carboxymethyl cellulose (CMC) were obtained from Adwik Company, Egypt. Violet red bile agar was purchased from Al-Gomhoria company. for Chemicals & Medical Appliances in Egypt. Xylose Lysine Deoxycholate Agar (Difco - USA) and sterile stomacher bags were acquired from Desert Cart, Egypt. Peptone water was sourced from Becton, Dickinson Company in France.

Methods

Application of UV-C

The UV-C irradiation procedure involved treating the eggs in batches of ten. Each batch was placed inside a metal flat tray within a closed UV device (BLX-254, France). The eggs were then exposed to UV lamps (5×8W, 254nm tube) for three minutes within a UV exposure area of 20×50 cm². Throughout the storage period, these eggs were kept under the same temperature conditions (20 - 25°C) as the other treatment groups. This method applied by Elwaha Fresh Foods Company.

Ozone Treatment Procedure

An ozone generator (model KH-PA10, 10g/hr) with a multiply corona discharge unit and circulating fan was used to create the ozone atmosphere. The eggs were exposed to a controlled ozone concentration of 6 ppm for a duration of 20 minutes. An ozone detector (model APP-1403, detection range 0-50 ppm) was used to monitor the ozone concentration within the test room throughout the process. The ozone generator was placed inside the room and switched on. The ozone concentration readings were recorded at specific time intervals as displayed on the ozone detector.

Preparation of CMC Coating Solution

To prepare the coating solution, 10 grams of CMC were weighed and added to a conical flask. Then, one liter of distilled water was mixed in to create a 1% CMC solution. Finally, 1 ml of glycerol was added and thoroughly mixed to form the coating solution for coating the eggs.
Throughout the process, surgical gloves were worn to prevent contamination, including during the preparation of the coating solution and while dipping the eggs. For coated treatments, the eggs were individually dipped into the solution for 1 minute. Afterward, they were left to dry at room temperature (20 - 25°C) for two hours to allow a coating film to form on their surfaces. Once dry, the coated eggs were placed in clean cardboard boxes and stored at a temperature between 20 - 25°C.

Weight loss (%)

Egg weight loss was determined by expressing the weight loss as a percentage of the initial weight. According to Yüceer et al. (2015) ten eggs from each treatment were weighed in the lab using an electronic balance with an accuracy of ±0.001 g. This weighing was performed every 15 days for a total period of 60 days. Egg weight loss % was determined using the following equation (1):

$$\text{Egg weight loss (\%)} = \frac{W_i - W_f}{W_i} \times 100 \qquad (1)$$

Where W_i the initial weight (g), W_f final weight

Determination of Haugh Unit

After carefully breaking the eggs onto flat surfaces, the height of the thick albumen and the yolk were measured. A tripod micrometer was used to measure the albumen height, while a digital caliper

determined the yolk width. To ensure accuracy, each parameter was measured three times at different locations, and the final value was obtained by averaging these three measurements. This method aligns with the approach described by Haugh (1937), the Haugh unit is

$$100 \log (H - 1.7W^{0.37} + 7.57) \quad (2)$$

Where H is the albumen height (mm) and W is the egg weight (g).

Egg Albumen and Yolk Measurements

Albumen Viscosity Measurements

The viscosity of egg albumen (mPa s) for all treatments was measured at room temperature using a Brookfield Digital Rheometer (model AMETek by Brookfield Engineering Laboratories INC.). A SC4-18 spindle rotating at 30 rpm was chosen for the measurements.

pH Determination

Following separation, the albumen and yolk were placed in separate 50 ml beakers. To obtain a representative sample, the thin and thick albumen were then thoroughly mixed. The pH of both the albumen and yolk was measured using a pre-calibrated digital pH meter (Starter 3000, OHAUS, USA) at a controlled temperature of 20-25°C. Three replicate measurements were performed for each treatment at each 15 day interval during 60 days.

Foaming properties

The relative whipping capacity (RWC) and foam stability of egg white and whole egg samples were measured at 20°C following a method by Li-Chan et al. (1995) with slight modifications. Briefly, 75 mL of egg white was whipped at room temperature in a Hobart mixer (N50CE, Hobart Foster Scandinavia A/S, Aalborg, Denmark) for a total of 180 seconds, starting at speed 2 for 90 seconds and then increasing to speed 3 for the final 90 seconds. Following the established method by Lechevalier et al. (2007), foam stability was evaluated by measuring the remaining foam volume (mL) after one hour of rest in a graduated cylinder. This approach is well-suited for assessing short-term stability. Additionally, the volume of liquid released at the bottom of the foam was measured in the same container after one hour. The experiment was repeated three times, and the average values were calculated for both foam volume and released liquid as follows:

$$\text{FoamingVolume}\ (\%) = \frac{(\text{volume of prepared foam} - \text{volume of liquid drainage})}{\text{original volume of liquid}} \times 100 \quad (3)$$

Yolk index determination

The yolk index, an indicator of egg freshness, was calculated by dividing the yolk height by its width, following the method described by Stadelman (1995). This measurement was performed every 15 days on three replicate eggs from each of the five treatments.

Eggshell Breaking Strength Measurement

Eggshell breaking strength, also known as puncture strength, was determined using a texture analyzer (Brookfield Texture Analyzer CT3, USA). Each egg was securely mounted on a platform and the eggshell was punctured at both the top (small end) and bottom (large end) using a 3 mm diameter die probe. The puncture was performed at a constant speed of 5mm/second in compression mode with a 10000 g load cell. The force required to puncture the shell was recorded as eggshell breaking strength (kgf). Twenty eggs per treatment were measured.

Microbiological analysis

Aseptically, 10 grams of chicken egg sample (combined yolk and albumen) were transferred to a sterile Stomacher bag. 90 millilitres of sterilized peptone water were added to the bag, and the mixture was homogenized for 2 minutes using a Stomacher 3500 blender. Serial dilutions (0.1 mL) of the homogenized egg sample were spread on the surface of Plate Count Agar (PCA, Oxoid, CM325, UK) to determine the total viable bacterial count (TVC). All agar plates were incubated at 37°C for 48 hours for TVC enumeration. For the detection of coliform bacteria, Violet Red Bile Agar (VRBA, Biolife code No. 442185) was used. The agar plates were inoculated with 0.1 mL aliquots of the serial dilutions and incubated at 37°C for 48 hours (Morsy et al., 2015). The presence of *Salmonella typhimurium* was specifically investigated using Xylose Ly-

The presence of *Salmonella typhimurium* was specifically investigated using Xylose Lysine Deoxycholate Agar (XLD Agar). Similar to the other analyses, 0.1 mL aliquots of the serial dilutions were spread on the XLD Agar plates, followed by incubation at 37°C for 48 hours El-Prince et al. (2019).

3. Results and Discussion

Egg Weight loss% during storage

One crucial indicator of egg quality during storage is weight loss %. Chicken eggshells are naturally porous, containing approximately 7,000 to 17,000 tiny pores. This porosity allows moisture to evaporate from the egg's interior, leading to weight loss over time (Kulshershtha et al., 2018).

Figure 1 demonstrates the weight loss% differences between four treatment groups: UV light, UV light with carboxymethyl cellulose (CMC) coating, ozone gas, and ozone gas with CMC coating, compared to the control group with no treatment. As expected, the control group exhibited the highest weight loss, reaching 12.2% by the end of the storage period. All treated groups displayed reduced weight loss compared to the control. Notably, the ozone + CMC treatment demonstrated the most effective reduction, with weight loss reaching only 7.44% after 60 days of storage at room temperature. This suggests that the combined application of ozone gas and CMC coating offers a superior strategy for minimizing weight loss in stored chicken eggs. The effectiveness of coatings in reducing egg weight loss aligns with previous research by Batkowska (2014), Homsaard (2020), and Ezazi (2021). These studies also demonstrated the potential of coating materials for preserving egg quality during storage.

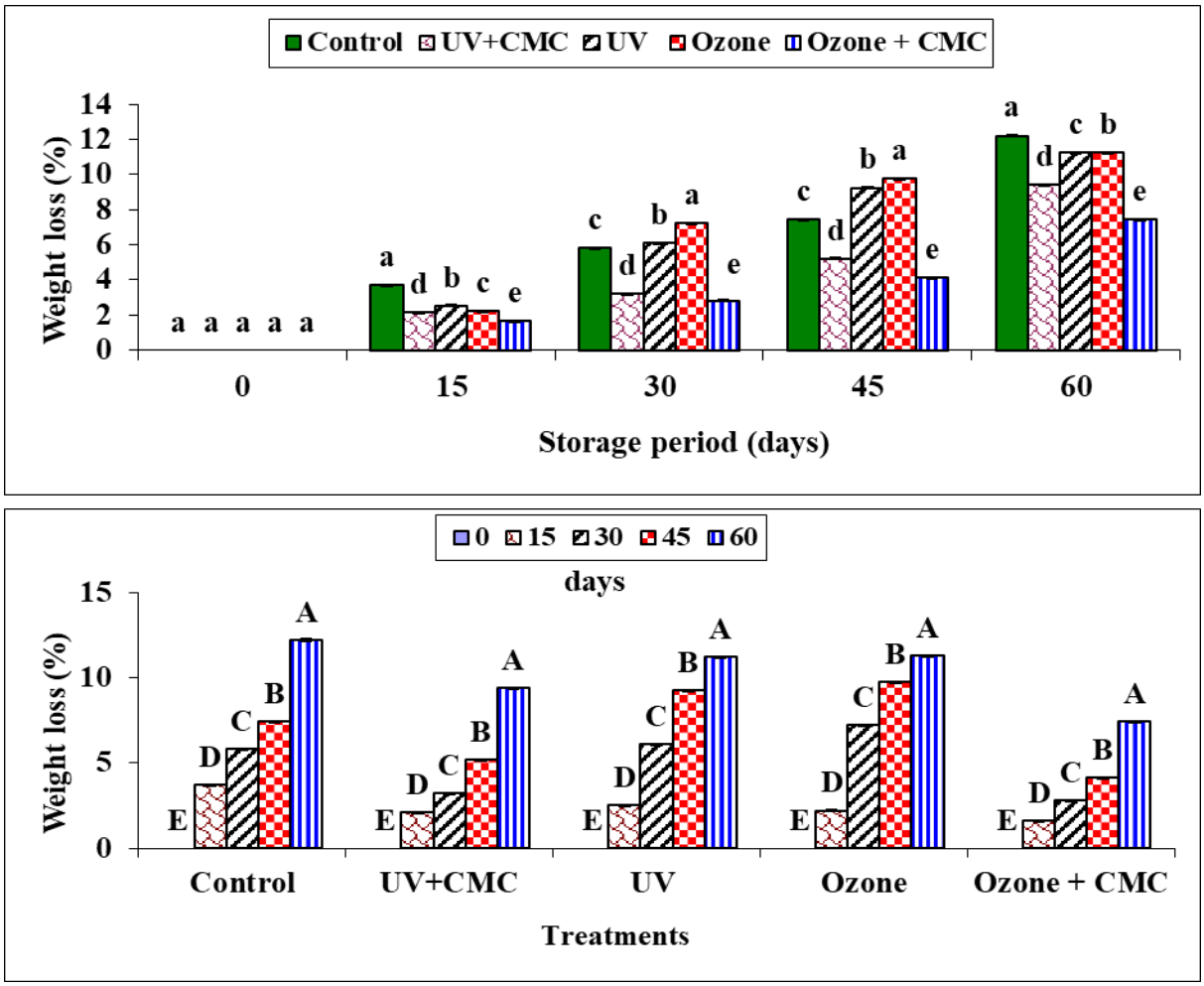

Figure 1. The effect of different treatments of chicken eggs on weight loss % of chicken eggs during storage at 20 - 25 °C for 60 days.

a, b and c: No two means differ significantly (P>0.05), and the superscript letter in the same **column** is the same for all of the means.
A, B and C: No two means differ significantly (P>0.05), and the superscript letter in the same **row** is the same for all of the means.

Yolk index (YI)

The yolk index (YI) is a critical indicator of egg freshness. A decrease in YI signifies a weakening of the yolk sac membranes and liquefaction of the yolk, primarily caused by water transfer from the albumen (Silversides & Scott, 2001). As shown in Table 1, eggs treated with UV, UV + CMC, Ozone, and Ozone + CMC maintained higher YI values compared to untreated control eggs throughout 60 days storage period at 20 - 25°C. Treated eggs reached YI values of 0.26, 0.24, 0.25, and 0.28, respectively, while the control group dropped to 0.21. These findings, particularly the superior performance of the Ozone + CMC treatment, suggest that this combined approach effectively preserves yolk quality during storage. The ozone treatment followed by the 1% CMC coating solution likely reduces water and CO_2 loss from the albumen through the eggshell, preventing albumen liquefaction and water absorption by the yolk. This aligns with the observations of Yuceer and Caner (2015).

Table 1. Yolk index of chicken eggs treated by different methods during storage at 20 -25°C for 60 days

Treatments	Yolk Index				
	Storage period (days)				
	0	15	30	45	60
Control	0.48^{aA}±0.00	0.44^{cB} ±0.00	0.38^{dC}±0.00	0.31^{cD}±0.01	0.21^{dE}±0.01
UV	0.47^{aA}±0.00	0.45^{bcB} ±0.00	0.43^{bC}±0.00	0.37^{aD}±0.01	0.26^{bE}±0.00
UV+CMC	0.48^{aA}±0.00	0.46^{abA} ±0.00	0.43^{bB}±0.00	0.35±0.01^{bC}	0.24^{cD}±0.00
Ozone	0.46^{aA}±0.00	0.45^{abA} ±0.01	0.41^{cB}±0.01	0.35^{bC}±0.01	0.25^{cD}±0.00
Ozone+CMC	0.47^{aA}±0.00	0.46^{aA} ±0.00	0.45^{aB}±0.00	0.38^{aC}±0.00	0.28^{aD}±0.00

a, b and c: All two means inside the same **column** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.
A, B and C: All two means inside the same **row** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.

Albumen Viscosity Measurements

One of the most noticeable changes in egg albumen during storage is the physical breakdown of the thick white. This gelatinous structure weakens and becomes runny, transforming into the thin white (Wells & Belyavin, 1987). Ovomucin, a key protein in egg albumen, significantly contributes to the gel-like consistency of the thick white, ultimately affecting its viscosity. Viscosity is a crucial quality factor impacting the albumen's ability to whip, emulsify, and gel (Belitz et al., 2004). As shown in (Table 2), albumen viscosity significantly decreased throughout storage, similar to findings by Yuceer and Caner (2014). The control group (untreated eggs) displayed a dramatic viscosity drop, falling from 74.95 mPa to 9.27 mPa by the end of storage, significantly lower than other treatments. The Ozone+CMC and UV+CMC treatments yielded the best results for maintaining albumen viscosity. These treatments significantly preserved albumen quality, exhibiting the highest viscosity throughout storage. An increase in albumen pH may explain the altered viscosity. As pH rises during storage, it approaches the isoelectric point of lysozyme, destabilizing the ovomucin-lysozyme complex (Li-Chan & Nakai, 1989; Stadelman & Cotterill, 1995).

CO_2 loss through the eggshell's microscopic pores contributes to pH rise and deterioration of the ovomucin-lysozyme complex. CMC coatings create a barrier during storage, hindering CO_2 diffusion and minimizing pH changes. This helps preserve albumen quality, which aligns with the findings of Sahansoy et al. (2024).

pH measurement

Freshly laid chicken eggs typically have an albumen pH ranging from 7.6 to 8.5 and contain significant amounts of carbon dioxide (1.44–2.05 mg CO_2 g^{-1}). During storage, however, the albumen pH gradually increases due to CO_2 loss through the eggshell pores. This loss is associated with the breakdown of carbonic acid and a corresponding rise in albumen pH, which can reach as high as 9.5 over time. This rise in pH signifies a decline in albumen quality and structural changes (Yuceer

and Caner, 2015). Figure 2 demonstrates the impact of different treatments on albumen pH during storage. Interestingly, the results suggest that while the pH values for all treatments tend to converge towards a more alkaline state at the end of the storage period, eggs treated with CMC exhibited a slower rise in pH compared to untreated eggs. This observation aligns with the findings of Priyanka Singh Rao (2020), who proposed that CMC coating might act as a barrier, hindering CO_2 escape through the eggshell pores. This delayed rise in pH within CMC -treated eggs supports the notion that coatings can potentially help maintain albumen quality during storage, which is consistent with the work by Kulshershtha et al. (2018).

Table 2. Albumin viscosity as affected by different treatments throughout 60 days storage

Treatments	Albumin viscosity, mPa.s				
	Period (days)				
	0	15	30	45	60
Control	$74.95^{aA}\pm6.85$	$40.20^{cB}\pm0.15$	$25.93^{cC}\pm1.91$	$14.07^{bD}\pm1.00$	$9.27^{bE}\pm0.43$
UV	$74.95^{aA}\pm6.85$	$64.93^{bB}\pm2.43$	$57.27^{bC}\pm1.53$	$50.63^{aD}\pm0.84$	$35.47^{aE}\pm2.92$
UV+CMC	$74.95^{aA}\pm6.85$	$68.40^{abB}\pm1.31$	$62.20^{aC}\pm1.00$	$52.80^{aD}\pm1.57$	$38.23^{aE}\pm1.60$
Ozone	$74.9^{aA}\pm6.85$	$64.83^{bB}\pm2.23$	$57.37^{bC}\pm1.73$	$51.00^{aD}\pm1.40$	$35.53^{aE}\pm2.53$
Ozone+CMC	$74.95^{aA}\pm6.85$	$70.27^{aB}\pm0.19$	$65.07^{aC}\pm2.42$	$52.37^{aD}\pm0.63$	$38.77^{aE}\pm1.43$

- a, b and c: All two means inside the same **column** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.
- A, B and C: All two means inside the same **row** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.

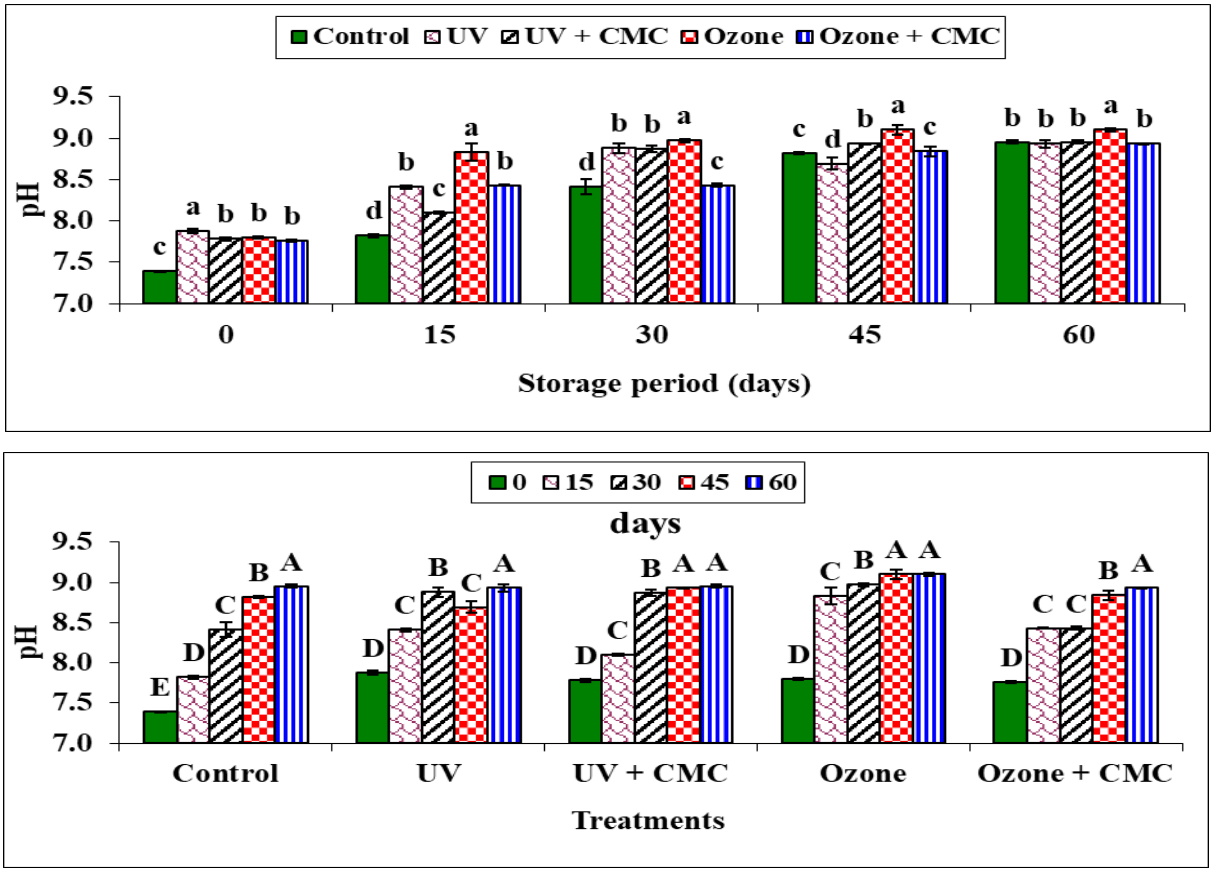

Figure 2. Effect of chicken eggs treatments on changes of pH during storage at 20 - 25°C during 60 days

a, b and c: No two means differ significantly (P>0.05), and the superscript letter in the same **column** is the same for all of the means.
A, B and C: No two means differ significantly (P>0.05), and the superscript letter in the same **row** is the same for all of the means.

Haugh Unit

As previously mentioned, the Haugh Unit (HU) is a critical indicator of egg quality (Stadelman, 1995). Higher HU values signify fresher eggs. There are two factors that affect the level of HU: the weight of eggs and the height of Albumin. Stadelman (1995). Table 3 compares the HU values of eggs treated with UV, UV + CMC, Ozone, and Ozone + CMC to those of untreated control eggs during storage. The results support the effectiveness of certain treatments in maintaining higher HU values. Eggs treated with Ozone + CMC and UV + CMC exhibited the slowest decline in HU throughout the storage period. After 30 days at 20°C, these treatments maintained HU values of 71.63 and 67.30, respectively, which still fall within the normal range for fresh eggs (85-75 HU). While HU values did decrease by day 60, they remained relatively high at 60.0 and 51.3 for Ozone + CMC and UV + CMC treatments, respectively. This suggests that these combined treatments effectively slow down the loss of albumen quality, thus preserving egg freshness for a longer duration. These findings align with observations by Yuceer et al. (2015).

According to Martinez et al. (2021) there is an osmatic migrate on of fluids from albumin to yolk through the yolk surrounding layer (Claza), which lead to flattening of this layer, decrease in albumin height and HU values. This phenomenon is associated with a reduction in protein quality, especially those of ovomucin through hydrolysis by enzymes. As a result, release of CO_2 increasing raising pH value and albumin becomes thinner and its viscosity decrease. However, HU values lower than 70 should be avoided during storage to maintain eggs suitable for human nutrition, which could be achieved to a great extent up to 45 days storage at 20 - 25 °C, by combined treatment of Ozone and CMC coating applied in the present work, (Table 3).

Table 3. Haugh Unit of chicken eggs treated by different methods during storage at 20 - 25°C for 60 days

Treatments	Haugh Unit				
	Storage period (days)				
	0	15	30	45	60
Control	85.30^{dA}±0.12	60.40^{eB}±0.12	50.35^{eC}±0.10	39.23^{eD}±0.09	35.40^{eE}±0.12
UV	85.77^{cA}±0.09	69.33^{dB}±0.09	54.27^{dC}±0.09	49.30^{dD}±0.10	45.33^{dE}±0.09
UV+CMC	86.33^{bA}±0.09	73.47^{bB}±0.09	65.27^{cC}±0.07	55.53^{cD}±0.09	51.3^{cE}±0.06
Ozone	86.30^{bA}±0.10	71.23^{cB}±0.09	67.30^{bC}±0.06	60.57^{bD}±0.12	56.40^{bE}±0.12
Ozone+CMC	87.37^{aA}±0.09	79.30^{aB}±0.06	71.63^{aC}±0.09	65.33^{aD}±0.09	60.00^{aE}±0.15

a, b and c: All two means inside the same **column** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.
A, B and C: All two means inside the same **row** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.

Relative Whipping Capacity (RWC) (Foaming Properties):

Foams are inherently unstable due to their thermodynamic nature. Factors like drainage, disproportionation, and coalescence can influence their stability (Alleoni & Antunes, 2004). The air and water interfaces within the foam play a crucial role in its formation and breakdown. Several factors, including air volume, liquid viscosity, interfacial tension, bubble size and distribution, and shape, all contribute to the overall rheology (flow properties) of foams.

Egg White Foaming Capacity

The ability of egg white to form and maintain a foam can be assessed by measuring both foam volume and its stability over time, as evidenced by the amount of liquid released (Alleoni & Antunes, 2004). During whipping, egg white proteins undergo a process called denaturation, where they unfold and aggregate due to the increased air-liquid interface area. Notably, the protein ovomucin plays a vital role in stabilizing the foam by forming an

insoluble film around the air bubbles, separating them from the liquid phase.

Impact of Storage and Coating on RWC

The study revealed a significant interaction between storage time and the RWC of coated samples (Ozone + CMC and UV + CMC). As storage time increased, the RWC (foaming capacity) exhibited a linear decrease (Table 4). Compared to the control and uncoated samples (UV and Ozone), the coated samples displayed a slower decline in RWC. After 30 days, the RWC of Ozone + CMC and UV + CMC treatments reached 970.33 ml and 843.00 ml, respectively, while the control and uncoated samples ranged from 429 ml to 800 ml. This trend continued at 60 days, with the coated samples showing a lesser decrease in RWC compared to the uncoated ones.

Protein Charge and Foam Stability

The pH of the egg white solution influences the type and amount of protein charges present, which in turn affects foam stability (Alleoni & Antunes, 2004). Egg globulins further contribute to foam stability by increasing the viscosity of the liquid phase and reducing surface tension (Lechevalier et al., 2007).

Impact of Storage on Protein Interactions

During storage, the pH of egg white increases, and some of the protein N-ovalbumin transforms into S-ovalbumin. Unfortunately, S-ovalbumin is less hydrophobic (water-repelling) than N-ovalbumin. This reduced hydrophobicity weakens the formation of a cohesive film at the air-water interface, leading to poorer foam stability. This phenomenon aligns with observations by Cho et al. (2002) and Lomakin et al. (2006).

Table 4. Albumin foaming capacity of chicken eggs during storage at 20 -25°C for 60 days

Treatment	Foaming Albumin capacity				
	Storage period (days)				
	0	15	30	45	60
Control	913.67^{dA}±0.88	803.33^{eB}±0.88	783.33^{eC}±0.33	583.00^{dC}±1.53	492.00^{eD}±1.15
UV	934.00^{cA}±0.58	911.33^{dB}±0.88	833.67^{dC}±1.20	776.67^{cD}±1.20	760.67^{dE}±1.20
Ozone	941.00^{cA}±0.58	924.67^{cB}±0.88	843.00^{cC}±0.58	782.33^{cD}±1.45	771.67^{cE}±0.88
UV + CMC	961.00^{bA}±0.58	942.67^{bB}±1.45	911.67^{bC}±0.88	884.67^{bD}±0.88	800.00^{bE}±32.5
Ozone+CMC	991.67^{aA}±1.20	983.00^{aB}±1.15	970.33^{aC}±0.88	892.33^{aD}±1.45	844.67^{aE}±0.88

a, b and c: All two means inside the same **column** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.

A, B and C: All two means inside the same **row** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.

Eggshell breaking strength

The quality of eggshells is paramount in commercial egg production The egg's shell provides a degree of protection against external impact, Enables controlled gas exchange through its pores for respiration and moisture regulation. Acts as a barrier against microbial contamination. Unfortunately, eggshell quality tends to decline as hens ages (Cordts et al., 2002; Caner & Cansız, 2008). This decline can lead to economic losses due to cracked or broken eggs during handling and storage. Stronger eggshells are essential for minimizing such losses. Studies have shown that the top portion of the eggshell generally exhibits higher puncture strength compared to the bottom as shown in Table 5 a and b. This finding is reflected in the data, where the puncture strength of control eggs at the top decreased from 3.94 to 3.31 kg_f and for the bottom side deceased from 4.13 to 3.48 kg_f after 60 days of storage. The research also investigated the effectiveness of coatings in improving eggshell strength. As shown in Table 5b, coated eggs displayed higher puncture strength at the bottom compared to the control group.

Interestingly, after 60 days of storage at room temperature, no significant difference was observed between the Ozone + CMC and UV + CMC treatments, with both reaching a puncture strength around 3.7 kg_f. These results suggest that both Ozone + CMC and UV + CMC coatings effectively increased eggshell strength compared to the control and other treated groups. These findings align with previous research by Caner and Cansız (2008), who demonstrated that chitosan coatings, particularly those combined with lactic acid, can enhance eggshell strength compared to coatings using acetic or propionic acids. Similarly, Xie et al. (2002) reported that coatings made from soy protein, whey isolate, or wheat protein combined with carboxymethyl cellulose also improved eggshell strength compared to uncoated eggs. According to Camargo et al., (2021), eggshell membrane is formed by two layer and outer thick layer, which provides resistance to the eggshell. Their thickness and elasticity changes in response to moisture loss, which makes the shell brittle and more susceptible to cracked and breaking.

Table 5a. Top side texture strength (kg_f) for preserved chicken eggs during storage at 20 -25°C for 60 days

Treatment	Eggshell breaking strength, kg_f				
	Storage period (days)				
	0	15	30	45	60
Control	3.88^{eA}±0.04	3.83^{cB}±0.01	3.63^{cC}±0.01	3.43^{cD}±0.01	3.31^{cE}±0.01
UV	4.22^{aA}±0.01	4.11^{aB}±0.01	3.97^{aC}±0.01	3.82^{aD}±0.01	3.73^{aE}±0.01
UV+CMC	3.94^{cA}±0.02	3.77^{dB}±0.01	3.64^{cC}±0.01	3.55^{bD}±0.01	3.20^{dE}±0.01
Ozone	3.91^{dA}±0.02	3.67^{eB}±0.01	3.51^{dC}±0.01	3.42^{cD}±0.01	3.41^{bE}±0.01
Ozone+CMC	4.15^{bA}±0.01	4.02^{bB}±0.00	3.91^{bC}±0.01	3.82^{aD}±0.00	3.71^{aE}±0.01

a, b and c: All two means inside the same **column** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.
A, B and C: All two means inside the same **row** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.

Table 5b. Bottom side texture strength (kg_f) for preserved chicken eggs during storage at 20-25°C for 60 days

Treatments	Eggshell breaking strength, kg_f				
	Storage period (days)				
	0	15	30	45	60
Control	4.13^{cA}±0.03	3.85^{cB}±0.01	3.74^{cC}±0.01	3.61^{dD}±0.01	3.48^{dE}±0.01
UV	4.27^{bA}±0.01	4.15^{bB}±0.01	4.07^{bC}±0.02	3.91^{bD}±0.01	3.82^{bE}±0.01
UV+CMC	3.94^{dA}±0.03	3.88^{cB}±0.01	3.77^{cC}±0.01	3.66^{cD}±0.01	3.54^{cE}±0.01
Ozone	3.97^{dA}±0.09	3.73^{dB}±0.01	3.61^{dC}±0.01	3.54^{eD}±0.01	3.51^{cE}±0.01
Ozone+CMC	4.43^{aA}±0.01	4.36^{aB}±0.01	4.13^{aC}±0.01	4.01^{aD}±0.01	3.91^{aE}±0.01

a, b and c: All two means inside the same **column** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.
A, B and C: All two means inside the same **row** have the same superscript letter, indicating that there is no significant difference (P>0.05) between them.

Microbiological quality of coated eggs

Figure 3 demonstrates the impact of storage time and various treatments on bacterial counts in chicken eggs stored at 20 - 25°C during 60 days. The study evaluated total viable counts (TVCs), coliform bacteria, and Salmonella spp. Interestingly, all tested eggs were free of Salmonella and coliform bacteria throughout the storage period. However, TVCs increased over time for all groups. Notably, Ozone + CMC and UV + CMC coated eggs displayed the slowest rise in TVC, reaching 6.23 log_{10} CFU/g after 60 days.

This suggests that both ozone and UV treatments, followed by the CMC coating, effectively reduced the initial microbial load and load and offered extended preservation by potentially limiting bacterial penetration through eggshell pores. These findings align with observations by Morsy et al. (2015).

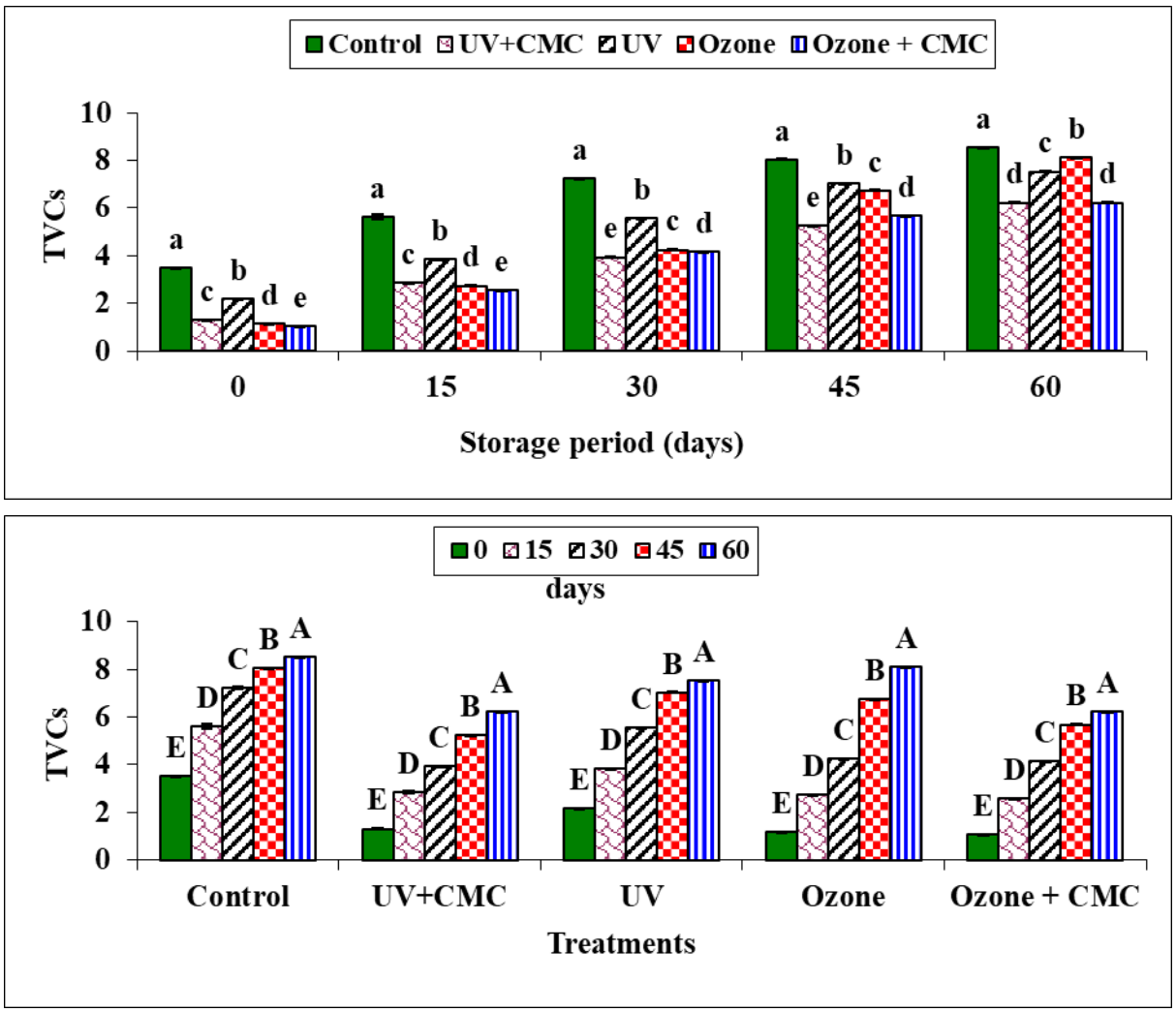

Figure 3. Total viable count (TVCs) expressed as log_{10} of preserved chicken eggs during storage at 20 -25°C for 60 days

a, b and c: No two means differ significantly (P>0.05), and the superscript letter in the same **column** is the same for all of the means.
A, B and C: No two means differ significantly (P>0.05), and the superscript letter in the same **row** is the same for all of the means.

4. Conclusion

This study demonstrates that coatings, particularly those combining CMC with ozone or UV treatments, offer a promising approach to preserving the quality of fresh eggs during storage. The coatings effectively maintained various functional properties (Haugh Unit, yolk index, pH, viscosity, microbial load, and relative whipping capacity) that typically decline with storage duration. These benefits likely stem from the ability of the coatings to reduce gas exchange through eggshell pores and enhance foam stability. The results suggest that both ozone + CMC and UV + CMC treatments are effective, with minimal difference between them, and significantly outperform uncoated eggs. This research highlights the potential of coatings as a viable alternative to current methods for preserving the internal quality of eggs during storage.

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