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Preparation of eggshell powder and using it in coating and extending shelf life of table eggs during refrigerator storage

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ABSTRACT

This study aimed to formulate carboxymethyl cellulose (CMC)–eggshell powder (ESP) composite coatings (T_2, T_3, and T_4) and evaluate their efficacy in extending table egg shelf life and preserving quality parameters compared to an uncoated control (T_1). Egg weight parameters, interior egg quality, component proportions, and bacterial counts of shell eggs stored under refrigeration for up to 8 weeks. Coating treatments significantly increased total egg weight and shell thickness at ($P<0.05$), with T_4 and T_3 recording the highest initial values. Although weight loss and weight loss percentage were non-significant during week 1, significant weight loss emerged by week 2 and scaled proportionally through weeks 4 and 8. The uncoated control (T_1) sustained the highest cumulative weight loss, whereas T_3 and T_4 demonstrated superior long-term weight retention, significantly outperforming T_2 by week 8. Interior quality parameters (yolk height, yolk diameter, yolk index, and albumen height/Haugh units) showed no initial significant differences during week 1; however, as storage extended to 2, 4, and 8 weeks, T_3 and T_4 consistently preserved higher albumen and yolk quality compared to T_1. Coated eggs maintained significantly higher relative shell weights and percentages throughout cold storage, slowing the characteristic reduction in albumen weight and percentage observed over time. Microbiologically, coating treatments exerted an immediate and sustained antibacterial effect ($P < 0.05$), significantly lowering Total Bacterial Count (TBC) and Total Coliform Count (TCC) from zero-time through 8 weeks, with T_3 and T_4 demonstrating the highest antimicrobial efficacy. Overall, applying CMC–eggshell powder coatings—particularly formulations T_3 and T_4—serves as an effective multi-functional barrier to minimize weight loss, preserve interior freshness, and inhibit microbial contamination in refrigerated table eggs during extended storage.

Keywords: Table eggs, Coating, Eggshell powder, CMC, Egg shelf life, Refrigerator storage.

Introduction

Table eggs are produced for human consumption by domestic poultry, they serve as a vital source of high-quality protein, providing all the essential amino acids the human body requires, with a biological value and utilization rate approaching 100% (Stadelman et al 2017). In addition, table eggs particularly the yolks are a rich source of essential fatty acids, fat-soluble vitamins, water, and vital minerals, though they lack vitamin C. Despite their low calorie and energy content, eggs offer an affordable source of high-quality animal protein containing all essential amino acids. Their versatility, ease of preparation, pleasant taste, and functional properties make them a globally popular ingredient across countless culinary applications for all age groups (Ismail et al., 2025).

Although freshly laid eggs carry few microorganisms, surface contamination increases rapidly upon

exposure to poultry housing environments, transportation, and handling. To combat this, the egg relies on three natural defense layers: the outermost cuticle (a protective waxy coating), the calcareous shell, and the dual inner and outer shell membranes. Working together, these barriers play a crucial role in preventing or delaying microbial penetration (Al-Rubaiey et al., 2020; Salman et al., 2023).

While no storage method can permanently prevent egg spoilage, proper techniques significantly slow quality loss and extend shelf life to ensure consumers receive high-quality eggs. In standard distribution, eggs are packed on 30-egg pulp trays, stacked 12 layers high in cardboard master cartons, and shipped from production facilities to refrigerated wholesale units before reaching retail. To maximize microbial resistance and withstand storage conditions, only clean, defect-free eggs should be selected for long-term storage and advanced preservation processing (Shenga et al., 2010; Oliviera et al., 2022).

Egg aging and functional quality loss begin immediately after laying, with the efficacy of surface coatings depending heavily on storage duration and temperature (Caner and Yüceer, 2015; Yuceer and Caner, 2020). Within the egg industry, these protective coating biomaterials are typically categorized into three main types: lipids, proteins, or polysaccharides. A wide range of biomaterials has been explored for surface coatings, including lipids such as conventional oils, waxes, and medicinal plant oils (Goresline, 1951; Stadelman, 2017; Cindric et al., 2007; Oliveira et al., 2022). Polysaccharide-derived options feature heavily in the literature, encompassing starch, carboxymethyl cellulose (CMC), carboxymethyl chitosan, carboxymethyl bacterial cellulose, pectin, and chitosan (Chaiwarit et al., 2018; Chaiwong et al., 2020; Wongkaew et al., 2020; Salman, 2023). Additionally, protein-based materials such as whey protein, zein, sericin, keratin, fibroin, and protein isolates have demonstrated strong barrier properties (Caner et al., 2015; Pires et al., 2019; Soares et al., 2021). Other promising candidates include natural extracts like propolis (Copur et al., 2008; Akpinar et al., 2015), material blends (Sun et al., 2021; Oliveira et al., 2022), and advanced nanocomposites (Chekh et al., 2021; Abed et al., 2025; Abed et al., 2026).

Table eggshells are increasingly recognized as valuable by-products in both human and animal nutrition, serving as a rich, natural source of essential minerals. Composed primarily of calcium carbonate (94%), eggshells also contain vital trace elements and minerals such as phosphorus, magnesium, sodium, and potassium (Stadelman et al., 2017; Bartter et al., 2018; Shahnila et al., 2022).

In human nutrition, processed eggshell powder is widely utilized as an organic calcium supplement to help prevent calcium deficiencies, support bone mineral density, and improve overall skeletal health. Similarly, in poultry nutrition, finely ground eggshells are routinely recycled into feed formulations as an economical and highly bioavailable mineral supplement. This practice not only enhances dietary calcium intake for laying hens strengthening subsequent eggshells and promoting optimal bone development but also champions sustainability by converting agricultural waste into a high-value nutritional resource (Shahnila et al., 2022; Kausar and Naureen, 2021; Masrianih et al., 2025).

The objective of this study was to determine the effect of eggshell powder-carboxy methyl cellulose-based as natural coating table eggs to extending shelf life during refrigerator storage for 8 weeks.

Materials and Methods

Ethical approval: The animals of the study were conducted using the guidelines for the care and handling of animal accordance to the Palmtree Environmental and Agricultural Organization Committee (certificate no. 1F1801034, January 25th 2018), this research is a proposal in Poultry Products Technology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Period of the study: This experiment was conducted at the College of Veterinary Medicine, University of Baghdad, from February 15th to 12th April 2026, to determine the effect of eggshell powder-carboxy methyl cellulose-based coating table eggs to extending shelf life during refrigerator storage for 8 weeks.

Egg collection: A total of 240 fresh table eggs (Brown shell eggs) were randomly collected from 20000 Luhman chicken layer flock at Al-Amir project commercial farm, Al-Musaib city, Iraq. The layers were fed a production ration as explain in Table (1).

Table (1): Components and ingredients quantity of ration.

Components	(%)
Corn	15.0
Soya Meal	25.0
Wheat	10.0
Wheat bran	10.0
Rice bit	20.0
Chickpea	10.0
Premix *	2.5
Vegetable oil	1.25

Limestone	6.25
Total	100 Kg
Calculated analysis	
Total protein %	18.75
Metabolic energy Kcal/Kg feed	2650
Lysine (%)	0.77
Methionine (%)	0.30
Methionine + cysteine (%)	0.59
Ca (%)	3.50
P Available (%)	0.35

*Premix supplied diet the following: vitamin A: 12,000 IU; vitamin D3: 2500 IU; vitamin E: 30 IU; vitamin K3: 2 mg; thia- mine: 2.25 mg; riboflavin: 7.5 mg; pyridoxine: 3.5 mg; cobalamine: 0.02 mg; niacin: 45 mg; D-pantothenic acid: 12.5 mg; biotin: 0.125 mg; folic acid: 1.5 mg, zinc: 50; copper: 12; iodine: 0.3; cobalt: 0.2; iron: 100; selenium: 0.1.

Preparing of egg shell powder:

Cleaning and Drying: Approximately 250 g of chicken eggshells were collected and thoroughly washed with tap water to remove dirt and membranes.

Mechanical Grinding: The eggshells were dried in oven at 100°C for 24 hrs. and ground into a fine powder using a fine electric grinder to reduce their size to the smallest possible particle size. The ground eggshells are then dissolved by immersing the dried eggshells in 0.5% food-grade acetic acid. The mixture is left at room temperature for 24–48 hours, during which time liquid calcium acetate and carbon dioxide gas are produced. The resulting solution is then dried at 100°C to obtain eggshell powder.

Carboxy methyl celluloses: Food grade Carboxy Methyl Cellulose (CMC) was obtained from local markets and prepared by dissolving 1 gm in 1000 ml of sterile normal saline (pharmacy grade) and stir until dissolved as based coating solution. It functions as a stabilizing and adhesive material for eggshell powder.

Treatment groups: After egg collection 240 fresh table eggs were immediately distributed into four groups of treatments; T1: 60 eggs were untreated eggs as control group. T2: 60 eggs were coated with 0.1% carboxy methyl cellulose-based solution. T3: 60 eggs were coated with 1% eggshell powder in 0.1% carboxy methyl cellulose-based solution. T4: 60 eggs were coated with 2% eggshell powder in 0.1% carboxy methyl cellulose-based solution. All eggs were arranged in plastic eggs tray (30 eggs per tray) and refrigerator storage (3-7°C) immediately after treatment.

Storage periods: All eggs were refrigerator storage for 0, 1, 2, 4 and 8 weeks. At each period 6 eggs from each treatment were randomly used for individually egg weight and interior quality parameter. At mean time 6 eggs were used for bacterial counts included total bacterial count and total coliform count were studied.

Parameters of the study

Egg weight: 6 Egg weights were recorded for each egg separately and for each treatment, and after each storage period, a digital Sartorius balance scale (Göttingen, Germany) was used for this purpose, measuring to two orders of grams (Stadelman et al., 2017).

Weight Loss Percentages (%): The weight loss percentages relative to the initial weight at Zero Time are calculated according to Purnawarman et al., 2025, using the formula:

$$\text{Weight Loss (\%)} = \frac{\text{Initial Weight} - \text{Weight at Time } t}{\text{Initial Weight}} \times 100$$

Internal egg quality characteristics: All studied egg quality (internal) traits were measured for all 6 eggs collected individually and according to the method indicated by Kop-Bozbay (2024) and Stadelman et al., 2017, which included white high, yolk high and yolk diameter. The white and yolk height was measured by using vertical digital micrometer (China) for the white of each egg, from the midpoint between the edge of the outer white and the yolk membrane, the high of the yolk measured in the midpoint of the yolk. The diameter of the yolk was measured by using the digital Vernier scale (China).

Egg components percentage: After breaking the 6 eggs and separating the yolk from the white, by carefully lifting the yolk with a tablespoon so as not to contaminate it with the white, and placing the yolk on filter paper, where it was rotated on the paper to get rid of the white, if any, and the shell was dried by leaving it until the next day at room temperature, then the weights were recorded each components (yolk, shell with the membranes and white), then the percentage of the three components was extracted according to Ismail et al., (2024) by applying the following equations:

$$\text{Component (\%)} = \left(\frac{\text{Component Weight (g)}}{\text{Egg Weight (g)}}\right) \times 100$$

Haugh Units: After performing egg weight and the albumen height measurement, the Haugh units (UH) were determined by following equation proposed by Haugh (1937), Haugh unit was determined using the formula below:

$$HU = 100 \log_{10}(h - 1.7w^{0.37} + 7.6)$$

where:

UH **=** Haugh unit**.**

H: Height of the thick albumen in millimeters.

W: Weight of the egg in grams.

Log10: The formula uses a base-10 logarithm to normalize albumen height relative to egg weight, ensuring comparability across eggs of different sizes.
7.57: is a factor.

Bacterial counts: All bacterial count tests were conducted in the laboratory of the Milk Hygiene at the College of Veterinary Medicine, University of Baghdad, and were done on 6 eggs (three replicates) for each treatment and after each storage period. Two eggs of each replicate were immerged with 100 ml of sterile peptone water in polyethylene bags (Accumix company) at sterile conditions and rinsed with accurate for 2 min. then 1ml of the solution was transferred to screw caped bottles containing 9 ml of sterile peptone water to make decimal dilutions of 10 ml each. The culture media used in bacteriological examinations were sterilized at 121 °C for 15 minutes and under a pressure of 15 pounds / square inch. As for the different glassware, they were sterilized using electric oven at 180°C for three hours (Anburaj, 2020; Chauhan and Jindal, 2020).

Total bacterial count: Total bacterial counts were determined using the pour-plate method, as described by Chauhan and Jindal (2020). Briefly, 1 mL of each decimal dilution was pipetted into two empty, sterile Petri dishes (duplicates). Approximately 15 mL of sterile Nutrient Agar medium (Accumix company, Zimbabwe). The culture medium and bacterial dilution were thoroughly mixed by gently rotating the plates clockwise and counter-clockwise. Once the agar solidified, the plates were incubated inverted at 37°C for 24 hours. Colonies were counted on plates containing 30 to 300 colonies. The estimated number of bacteria per egg (CFU/mL) was calculated by multiplying the average colony count by the reciprocal of the corresponding dilution factor.

Coliform bacterial count: Coliform bacteria were enumerated using the pour-plate method, as specified by Chauhan and Jindal (2020). In this procedure, 1 mL of each serial decimal dilution was pipetted into duplicate empty, sterile Petri dishes. Approximately 15 mL of sterile MacConkey Agar medium (Accumix company, Zimbabwe), was immediately added to each dish. The bacterial dilution and culture medium were mixed thoroughly by gently swirling and rotating the plates. Once the agar solidified, the plates were inverted and incubated at 37°C for 24 hours. Colonies were counted on plates containing 30 to 300 colonies. The estimated number of coliform bacteria per egg (CFU/mL) was calculated by multiplying the average colony count by the reciprocal of the corresponding dilution factor.

Statistical analyses: The Statistical Analysis System (SAS, 2018) program was used to detect the effect of difference treatments in parameters study (Duncan, 1955) multiple range (ANOVA: Completely randomized design-CRD) was used to significant compare between means at probability on 0.05 and 0.01.

Results and Discussion

Egg weight: Table (2) show that all coating treatments using carboxymethyl cellulose T2 and eggshells powder-CMC based significantly ($P<0.05$) increased egg weight due to an increase in outer layer thickness. The egg coating treatment (T4) recorded the highest values, with no significant difference from T3. Treatments T2 and T1 recorded the lowest values for egg weight. The egg coating treatments continued to outperform the control treatment (T1) in terms of egg weight, with increased storage time in the refrigerator to one week, two weeks, four weeks, and eight weeks. T4 and T3 continued to outperform T2, which in turn outperformed the control treatment (T1).

Where does the table itself show that the egg weight loss and egg weight loss percentage were not significant during the first week. Significant ($P<0.05$) differences appeared when the storage period extended to two weeks, and increased proportionally with the storage period up to 4 and 8 weeks. T1 group recorded the highest percentages of egg weight loss and percentage. While the egg coating treatments T2, T3, and T4 recorded the lowest percentage of egg loss and egg weight loss percentages as the storage time increased. No significant differences were observed between T2, T3, and T4 until the fourth week. After that, the T4 and T3 outperformed others in reducing egg loss and weight loss percentages.

Interior egg quality: Table (3) show no significant differences between treatments in the studied characteristics of interior egg quality at zero time and after one week of refrigerator storage. As the refrigerator storage increased for 2 and 4 weeks, T2 and T3 recorded the best interior egg quality (yolk high, yolk diameter, yolk index and white high) then T4 and T1 recorded the lowest interior egg quality ($P<0.05$). As the egg storage period progressed to two, four, and eight weeks, significant ($P<0.05$) differences began to emerge.

Table (2): Effect of eggshell powder-carboxy methyl cellulose-based coating treatments on egg weight and egg loss during refrigerator storage.

Treatments	Egg weight (g)	Egg loss (g)	Egg loss (%)
Zero time			
T1	65.43 ±0.78 ^{c}	—	—
T2	65.53 ±0.85 ^{b}	—	—
T3	65.76 ±0.87 ^{ab}	—	—
T4	65.83 ±0.85 ^{a*}	—	—
1 week			
T1	65.28 ±0.74 ^{c}	0.18 ±0.03 ^{a}	0.28 ±0.04 ^{a}
T2	65.40 ±0.83 ^{b}	0.13 ±0.02 ^{a}	0.20 ±0.02 ^{a}
T3	65.66 ±0.86 ^{ab}	0.10± 0.03 ^{a}	0.15 ±0.03 ^{a}
T4	65.77 ±0.83 ^{a*}	0.10 ±0.02 $^{aN.S.}$	0.12 ±0.03 $^{aN.S.}$
2 weeks			
T1	63.56 ±0.77 ^{b}	1.87 ±0.51 ^{a*}	2.86 ±0.57 ^{a*}
T2	65.08 ±0.82 ^{b}	0.50 ±0.50 ^{b}	0.76 ±0.53 ^{b}
T3	65.29 ±0.84 ^{ab}	0.54 ±0.52 ^{b}	0.82 ±0.56 ^{b}
T4	65.36 ±0.86 ^{a*}	0.60 ±0.51 ^{b}	0.91 ±0.52 ^{b}
4 weeks			
T1	61.21 ±0.75 ^{c}	4.22 ±0.54 ^{a*}	6.45 ±1.03 ^{a*}
T2	63.76 ±0.83 ^{b}	1.82 ±0.49 ^{b}	2.78 ±1.01 ^{b}
T3	63.87 ±0.84 ^{ab}	1.96 ±0.52 ^{b}	2.98 ±1.04 ^{bc}
T4	64.77 ±0.84 ^{a*}	1.19 ±0.51 ^{b}	1.80 ±1.02 ^{c}
8 weeks			
T1	57.13 ±0.76 ^{c}	8.30 ±0.78 ^{a}	12.69 ±1.19 ^{a*}
T2	61.32 ±0.82 ^{b}	4.26 ±0.76 ^{a}	6.50 ±1.21 ^{b}
T3	62.35 ±0.83 ^{ab}	3.48 ±0.79 ^{a}	5.29 ±1.20 ^{c}
T4	63.89 ±0.85 ^{a*}	2.07 ±0.79 ^{a*}	3.14 ±1.20 ^{d}

All data as mean ± SE, T1: untreated eggs as control group. T2: coated with 0.1% carboxy methyl cellulose-based solution. T3: coated with 1% eggshell powder in 0.1% carboxy methyl cellulose-based solution. T4: coated with 2% eggshell powder in 0.1% carboxy methyl cellulose-based solution., $^{a, b, c}$ superscripts in a column significantly differ, N.S. not significant, * ($p<0.05$).

The highest white content was recorded for the egg coating treatments, as was the yolk index and the white content and its unit. Meanwhile, the yolk diameter was significantly higher in one case compared to the egg coating treatments. Treatments T3 and T4 were the best egg coating treatments in terms of internal egg quality characteristics. They achieved the best yolk height, yolk index, and white height and unity. The differences between them were not significant. Treatment 1 recorded the lowest values ($P<0.05$).

Egg component percentages: Significant ($P<0.05$) differences appeared in the egg components (yolk, shell and white) weight and percentages due to egg coating treatments. These treatments contributed to an increase in shell weight and percentage, thus affecting the weight and proportions of white and yolk immediately after the zero-time treatment. As the storage period progressed, the proportions and components of the egg white remained consistent. With increasing storage time, the weights and percentages of eggshells in the egg-coating treatments remained significantly higher than in the control treatment (T1), recording the highest values after 2, 4, and 8 weeks of refrigerated storage. Also, significant ($P<0.05$) differences appeared in the percentages and weights of white and eggshells, with a significant ($P<0.05$) decrease in white weight and percentage observed as storage time increased (Table 4).

Table (3): Effect of eggshell powder-carboxy methyl cellulose-based coating treatments on interior egg quality during refrigerator storage.

Treatments	Yolk high (mm)	Yolk diameter (mm)	Yolk index	White high (mm)	Haugh unit
Zero time					
T1	17.32 ±0.31^{a}	39.36 ±0.77^{a}	0.44 ±0.01^{a}	10.11 ±0.43^{a}	99.95 ±0.22^{a}
T2	17.32 ±0.30^{a}	39.36 ±0.75^{a}	0.44 ±0.02^{a}	10.12 ±0.45^{a}	99.95 ±0.25^{a}
T3	17.31 ±0.32^{a}	39.34 ±0.76^{a}	0.44 ±0.01^{a}	10.10 ±0.42^{a}	99.95 ±0.22^{a}
T4	17.33 ±0.30$^{aN.S.}$	39.39 ±0.81$^{aN.S.}$	0.44 ±0.01$^{aN.S.}$	10.12 ±0.43$^{aN.S.}$	99.95 ±0.24$^{aN.S.}$
1 week					
T1	17.29 ±0.32^{a}	40.21 ±0.80^{a}	0.43 ±0.01^{a}	10.03 ±0.26^{a}	99.91 ±0.26^{a}
T2	17.30 ±0.31^{a}	39.32 ±0.78^{a}	0.44 ±0.01^{a}	10.08 ±0.28^{a}	99.90 ±0.23^{a}
T3	17.30 ±0.32^{a}	39.31 ±0.78^{a}	0.44 ±0.02^{a}	10.09 ±0.26^{a}	99.92 ±0.24^{a}
T4	17.30 ±0.32$^{aN.S.}$	38.31 ±0.79$^{aN.S.}$	0.44 ±0.01$^{aN.S.}$	10.06 ±0.27$^{aN.S.}$	99.92 ±0.25$^{aN.S.}$
2 weeks					
T1	17.23 ±0.32^{b}	42.02 ±0.73^{a}*	0.41 ±0.02^{b}	9.83 ±0.61^{b}	97.50 ±0.24^{b}
T2	17.28 ±0.33^{a}	40.19 ±0.75^{b}	0.43 ±0.02^{a}	9.93 ±0.63^{a}	99.25 ±0.22^{a}
T3	17.29 ±0.34^{a}	40.21 ±0.74^{b}	0.43 ±0.01^{a}	9.94 ±0.60^{a}	99.35 ±0.23^{a}
T4	17.28 ±0.31^{a}*	40.19 ±0.75^{b}	0.43 ±0.02^{a}*	9.88 ±0.63^{b}*	99.30 ±0.22^{a}*
4 weeks					
T1	15.84 ±0.37^{c}	42.81 ±0.74^{a}*	0.37 ±0.01^{c}	7.64 ±0.64^{c}	87.10 ±0.21^{b}
T2	16.39 ±0.34^{b}	40.98 ±0.75^{b}	0.40 ±0.02^{b}	8.17 ±0.65^{a}	89.15 ±0.23^{a}
T3	16.68 ±0.35^{a}	39.71 ±0.73^{c}	0.42 ±0.02^{a}	8.25 ±0.62^{a}	89.70 ±0.26^{a}
T4	16.76 ±0.36^{a}*	39.90 ±0.71^{c}	0.42 ±0.02^{a}*	7.81 ±0.67^{b}	89.75 ±0.22^{a}*
8 weeks					
T1	11.67 ±0.24^{c}	44.88 ±0.61^{a}*	0.26 ±0.02^{c}	4.61 ±0.66^{b}	66.50 ±0.21^{c}
T2	14.84 ±0.22^{b}	42.40 ±0.60^{b}	0.35 ±0.02^{b}	5.76 ±0.65^{a}	74.55 ±0.27^{b}
T3	15.73 ±0.23^{a}	41.39 ±0.64^{bc}	0.38 ±0.03^{a}	5.81 ±0.64^{a}*	78.40 ±0.26^{a}
T4	15.89 ±0.22^{a}*	40.74 ±0.62^{c}	0.39 ±0.02^{a}*	5.72 ±0.62^{a}	78.55 ±0.22^{a}*

All data as mean ± SE, T1: untreated eggs as control group. T2: coated with 0.1% carboxy methyl cellulose-based solution. T3: coated with 1% eggshell powder in 0.1% carboxy methyl cellulose-based solution. T4: coated with 2% eggshell powder in 0.1% carboxy methyl cellulose-based solution., $^{a, b, c}$ superscripts in a column significantly differ, N.S. not significant, * ($p<0.05$).

Bacterial count: Table (5) illustrates the effect of egg coating treatments on total bacterial count and total coliform count. It is evident that the egg coating treatments T2, T3, and T4 significantly ($P<0.05$) contributed to a decrease the total bacterial count and total coliform count immediately after the zero-time coating process. This decrease persisted with increasing coating time to one, two, four and eight weeks. The lowest total bacterial counts and total coliform count were recorded in treatment T4 and T3, then T2, while T1 recorded the highest total bacterial counts and total coliform count, starting from zero-time, up to eight weeks of refrigerator storage.

The initial significant ($P<0.05$) increase in egg weight in the treated groups (T_2, T_3, and T_4) compared to the control (T_1) is directly attributed to the physical deposition of the coating material onto the outer shell surface. Carboxymethyl cellulose (CMC) is a hydrocolloid known for its strong film-forming, hydrophilic, and binding properties. It is widely used in various applications, including food, paper, and pharmaceutical industries, due to its tunable hydrophilicity and mechanical strength. CMC can be combined with materials like fine eggshell powder (calcium carbonate, $CaCO_3$) to form a dense, semi-permeable matrix on the eggshell, enhancing its properties for specific applications (Rahman et al., 2021). Treatment Effect (T_4 and T_3 vs. T_2 and T_1), Higher concentrations or specific combinations of ESP-CMC (as seen in T_4 and T_3) create a thicker barrier layer, contributing to a measurable mass addition to the egg exterior. As storage extended to 1, 2, 4, and 8 weeks, all coated treatments (T_2, T_3, and T_4) consistently maintained higher egg weights compared to the untreated control (T_1).

Table (4): Effect of eggshell powder-carboxy methyl cellulose-based coating treatments on egg components weight and percentage during refrigerator storage.

Treatments	Yolk weight (gm)	Yolk (%)	Shell weight (gm)	Shell (%)	White weight (gm)	White (%)
Zero time						
T1	18.29 ±0.82^{a}	27.97 ±1.45^{a}*	6.01 ±0.24^{b}	9.19 ±0.40^{b}	41.10 1.08^{c}	62.84 ±2.37^{a}
T2	18.28 ±0.80^{a}	27.90 ±1.51^{ab}	6.09 ±0.26^{a}	9.29 ±0.43^{a}	41.16±1.07^{b}	62.81 ±2.35^{a}
T3	18.30 ±0.82^{a}	27.83 ±1.44^{b}	6.12 ±0.23^{a}	9.30 ±0.41^{a}	41.34 1.11^{a}	62.86 ±2.36^{a}
T4	18.31 ±0.81$^{aN.S.}$	27.79 ±1.42^{b}	6.12 ±0.25^{a}*	9.29 ±0.41^{a}*	41.42±1.05^{a}*	62.90 ±2.33$^{a\ N.S.}$
I week						
T1	18.31 ±0.79^{a}	28.04 ±1.25^{a}*	6.03 ±0.22^{b}	9.24 ±0.42^{b}	40.94±1.10^{c}	62.71 ±2.35^{b}
T2	18.29 ±0.81^{a}	27.97 ±1.22^{a}	6.10 ±0.24^{a}	9.33 ±0.40^{a}	41.04±1.09^{b}	62.75 ±2.33^{b}
T3	18.30 ±0.83^{a}	27.87 ±1.26^{b}	6.11 ±0.23^{a}	9.31 ±0.42^{a}	41.25±1.11^{ab}	62.82 ±2.36^{a}
T4	18.31 ±0.82$^{a\ N.S.}$	27.84 ±1.23^{b}	6.12 ±0.24^{a}*	9.31 ±0.41^{a}*	41.34±1.10^{a}*	62.86 ±2.35^{a}*
2 weeks						
T1	18.39 ±0.82^{a}	28.93 ±1.27^{a}*	6.02 ±0.18^{b}	9.47 ±0.42^{a}*	39.15±1.25^{c}	61.60 ±2.36^{c}
T2	18.35 ±0.81^{a}	28.20 ±1.26^{b}	6.10 ±0.16^{a}	9.37 ±0.41^{b}	40.63±1.23^{b}	62.43 ±2.37^{b}
T3	18.32 ±0.81^{a}	28.06 ±1.27^{b}	6.10 ±0.18^{a}	9.34 ±0.41^{b}	40.87±1.26^{ab}	62.60 ±2.33^{a}
T4	18.32 ±0.82$^{aN.S.}$	28.03 ±1.28 ^{b}	6.11 ±0.17^{a}*	9.35 ±0.40^{b}	40.93±1.24^{a}*	62.62 ±2.35^{a}*
4 weeks						
T1	19.21 ±0.79^{a}*	31.38 ±1.26^{a}*	6.01 ±0.19^{b}	9.82 ±0.43^{a}*	35.99±1.13^{d}	58.80 ±2.29^{c}
T2	18.84 ±0.82^{b}	29.55 ±1.22^{b}	6.10 ±0.17^{a}	9.57 ±0.41^{b}	38.82±1.17^{c}	60.88 ±2.32^{b}
T3	18.55 ±0.79^{c}	29.04 ±1.25^{c}	6.10 ±0.19^{a}	9.55 ±0.44^{b}	39.22±1.15^{b}	61.41 ±2.34^{ab}
T4	18.56 ±0.81^{c}	28.66 ±1.23^{d}	6.10 ±0.18^{a}*	9.42 ±0.43^{c}	40.11±1.14^{a}*	61.93 ±2.29^{a}*
8 weeks						
T1	20.87 ±0.87^{a}*	36.53 ±1.26 ^{a}*	6.01 ±0.20^{b}	10.52 ±0.45^{a}*	30.25±1.17^{d}	52.95 ±2.34^{d}
T2	19.44 ±0.84^{a}	31.70 ±1.28 ^{b}	6.09 ±0.18^{a}	9.93 ±0.44^{b}	35.79±1.16^{c}	58.37 ±2.41^{c}
T3	18.61 ±0.86^{a}	29.85 ±1.27 ^{c}	6.09 ±0.19^{a}	9.77 ±0.45^{bc}	37.15±1.18^{b}	59.58 ±2.33^{b}
T4	18.84 ±0.85^{b}	29.49 ±1.26 ^{c}	6.10 ±0.20^{a}*	9.55 ±0.45^{c}	38.95±1.17^{a}*	60.96 ±2.34^{a}*

All data as mean ± SE, T1: untreated eggs as control group. T2: coated with 0.1% carboxy methyl cellulose-based solution. T3: coated with 1% eggshell powder in 0.1% carboxy methyl cellulose-based solution. T4: coated with 2% eggshell powder in 0.1% carboxy methyl cellulose-based solution., $^{a, b, c}$ superscripts in a column significantly differ, N.S. not significant, * (P<0.05).

Table (5): Effect of eggshell powder-carboxy methyl cellulose-based coating treatments on egg bacterial count during refrigerator storage (cfu/egg).

			Total count		
Treatments	**Zero time**	**1 week**	**2 weeks**	**4 weeks**	**8 weeks**
T1	52 X 10^{2} ±15.05^{a}*	139 X 10^{2} ±17.39^{a*}	106 X 10^{3} ±28.11^{a}*	139 X 10^{4} ±35.14^{a}*	176 X 10^{5} ±40.06^{a}*
T2	25 X 10^{1} ±18.36^{b}	97 X 10^{1} ±25.76^{b}	81 X 10^{2} ±30.09^{b}	58 X 10^{3} ±35.17^{b}	93 X 10^{4} ±41.23^{b}
T3	< 100 ±0.00^{c}	< 100 ±0.00^{c}	59 X 10^{1} ±16.89^{c}	81 X 10^{2} ±32.44^{c}	78 X 10^{3} ±33.17^{c}
T4	< 100 ±0.00^{c}	< 100 ±0.00^{c}	31 X 10^{1} ±21.51^{d}	96 X 10^{1} ±30.10^{d}	58 X 10^{2} ±27.26^{d}
			Total coliform		
Treatments	**Zero time**	**1 week**	**2 weeks**	**4 weeks**	**8 weeks**
T1	47 X 10^{2} ±16.56^{a}*	115 X 10^{2} ±17.47^{a}*	89 X 10^{3} ±12.53^{a}*	140 X 10^{3} ±17.32^{a}*	133 X 10^{4} ±13.62^{a}*
T2	19 X 10^{1} ±18.20^{b}	46 X 10^{1} ±18.22^{b}	33 X 10^{2} ±19.26^{b}	93 X 10^{2} ±19.32^{b}	37 X 10^{3} 19.35^{b}
T3	< 100 ±0.00^{c}	< 100 ±0.00^{c}	26 X 10^{1} ±19.33^{c}	31 X 10^{2} ±20.13^{c}	95 X 10^{2} ±20.44^{c}
T4	< 100 ±0.00^{c}	< 100 ±0.00^{c}	11 X 10^{1} ±20.12^{d}	85 X 10^{1} ±20.20^{d}	46 X 10^{2} ±20.27^{d}

All data as mean ± SE, T1: untreated eggs as control group. T2: coated with 0.1% carboxy methyl cellulose-based solution. T3: coated with 1% eggshell powder in 0.1% carboxy methyl cellulose-based solution. T4: coated with 2% eggshell powder in 0.1% carboxy methyl cellulose-based solution., $^{a, b, c}$ superscripts in a column significantly differ, N.S. not significant, * (p<0.05).

An eggshell naturally possesses approximately $7{,}000$ to $17{,}000$ microscopic pores (Stadelman et al., 2017). During storage, weight loss occurs primarily due to the evaporation of water vapor and the escape of carbon dioxide (CO_2) produced by albumen protein degradation (Obianwuna et al., 2022). The role of ESP-CMC matrix composite acts as a pore-blocking agent. The micro-particles of eggshell powder physically seal the acoustic pores, while the CMC film binds the particles together into a continuous barrier (Caner, 2005; Wardy et al., 2011; Abed et al., 2026). This drastically restricts gas exchange and water transpiration, preserving internal moisture and reducing total weight loss over extended storage (up to 8 weeks). Low storage temperatures (≈ 4°C) reduce the kinetic energy of water molecules, slow enzymatic processes, and lower the vapor pressure gradient between the egg's interior and the external environment (Oliveira et al., 2022; Esmat et al., 2026). Consequently, moisture loss remains minimal in the first 7 days, regardless of coating presence. From Week 2 onward, weight loss increased proportionally with time, with the control group (T_1) suffering the highest rates of loss, while T_3 and T_4 maintained the lowest.

At baseline (day 0) and through Week 1, no significant differences were observed among any of the treatments (T_1–T_4) for interior quality traits. Freshly laid eggs contain intact ovalbumin network structures and firm vitelline membranes. During the initial days of refrigerated storage, the low ambient temperature (4°C) depresses enzymatic action, slows carbonic acid dissociation, and keeps carbon dioxide (CO_2) loss to a minimum regardless of coating application (Yamak et al., 2021; Kim et al., 2024; Maggonage et al., 2024). Consequently, albumen firming and vitelline membrane elasticity remain essentially preserved in all groups during Week 1. As storage extended beyond Week 2 and through Weeks 4 and 8, the uncoated control (T_1) experienced a rapid drop in interior quality (lowest albumen height, yolk height, yolk index, and Haugh units). Egg albumen contains carbonic acid (H_2CO_3) in equilibrium with dissolved CO_2. Uncoated eggshell pores allow CO_2 gas to escape into the atmosphere continuously. This loss increases albumen pH from approximately 7.6 to 9.5+. The alkaline environment destabilizes the lysozyme–ovomucin protein complex responsible for the thick albumen gelatinous structure, causing it to liquefy into thin albumen (reduced albumen height) (Biladeau et al., 2009; Stadelman et al., 2017). As albumen breaks down, water moves osmotically from the albumen across the vitelline membrane into the yolk due to concentration gradients. This water influx causes the yolk to swell, increasing yolk diameter while weakening vitelline membrane tension, leading to a flatter shape (decreased yolk height and lower calculated yolk index. From Week 2 through Week 8, treatments T_3 and T_4 consistently achieved the highest values for albumen height, yolk height, yolk index, and Haugh units. The composite coating of carboxymethyl cellulose (CMC) and eggshell powder (ESP) forms a micro-composite film over the shell surface. The calcium carbonate micro-particles in ESP physically plug the pores, while the hydrocolloid CMC matrix forms a sealed, continuous film. By sealing the microscopic shell openings, T_3 and T_4 trap CO_2 within the interior, preventing the alkaline pH shift (Yamak et al., 2021). This maintains the gel structure of ovomucin (preserving albumen height) and halts osmotic water migration into the yolk, preserving vitelline membrane strength (maintaining high yolk height and low yolk diameter). Performance Hierarchy T1>T4>T2>T1, while T_2 (pure CMC or lower ESP concentration) performed significantly better than the control (T_1), T_3 and T_4 delivered greater structural barrier integrity over longer durations (4 to 8 weeks) due to the synergistic reinforcement of ESP within the CMC matrix.

The significant ($P<0.05$) reduction in total bacterial and coliform counts immediately after applying treatments T_2, T_3, and T_4 compared to the control (T_1) highlights the surface-sanitizing and entrapment capabilities of the coating solution. The liquid application and subsequent drying of the CMC composite solution physically wash away or encapsulate free-living bacteria and coliforms adhering to the outer shell surface. Also, eggshell powder consists primarily of bio-calcium carbonate ($CaCO_3$) alongside trace bio-minerals and shell matrix proteins (Caner and Cansiz, 2007; Salman et al., 2025). When dissolved or dispersed in hydrocolloid matrices, localized mild alkalinity creates adverse conditions for transient Gram-negative pathogenic species like *Coliforms* (e.g., *Escherichia coli*), resulting in an immediate reduction in viable cell density (Wardy et al., 2011). As storage time extended to 1, 2, 4, and 8 weeks, the control group (T_1) exhibited the highest continuous increase in TBC and TCC, whereas coated treatments systematically suppressed microbial proliferation. Natural eggshells contain thousands of pores (10–50 μm in diameter) through which bacteria and moisture readily pass (Stadelman et al., 2017). Uncoated eggs (T_1) allow opportunistic surface microflora to penetrate into the shell membranes over time. Carboxymethyl cellulose

(CMC) forms a dense polymer network. When reinforced with fine eggshell powder micro-particles, it creates a structural composite film that physically seals the shell pores. This continuous physical shield blocks microbial entry and deprives surface microbes of external moisture and atmospheric exchange necessary for rapid colony growth (Caner and Cansiz, 2007; Pires et al., 2020). The treatments recorded a clear hierarchy in suppressing microbial counts: $T_4 \geq T_3 > T_2 > T_1$. Refrigerated conditions ($4^{\circ}C$) extend the microbial lag phase and slow bacterial replication rates (Kim et al., 2024). When combined with the physical barrier provided by T_3 and T_4, bacterial proliferation is doubly suppressed. Treatment T_2 (CMC or lower ESP ratio) provides effective protection, but T_3 and T_4 benefit from a higher particulate density of ESP embedded in the CMC matrix. This creates a more impermeable tortuous path across the coating film, preventing cracking or degradation during 8 weeks of cold storage (Liang et al., 2024). Coating shell control moisture activity (a_w), while uncoated eggs lose internal moisture, creating surface condensation under humidity fluctuations, which promotes coliform proliferation (Abed et al., 2026). By stabilizing internal moisture retention, T_3 and T_4 maintain a drier shell exterior, depriving coliforms and mesophilic bacteria of the liquid film required for colonization.

Conclusions

Hospitals are essential places for dissemination and spread of resistant bacteria due to excessive or incorrect usage of antibiotics in clinical setting. Discharged of untreated hospital effluent into river water affect the sustainability and health of the environment. Strict strategies like increased hygienic measures, stewardship program, drug combination therapy or (nanoparticle and phage therapy) should be applied in hospitals to combating this phenomenon. Hospital wastewater should be monitored and treated properly before discharge into river water. Continuous surveillance of antibiotic resistance in healthcare system is needed to optimize antibiotic use and limit spread of resistant bacterial isolates into aquatic ecosystem.

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