Application of Eggshell-Derived Calcium Carbonate in Chitosan-Based Coating to Extend Fresh Egg Shelf Life

Susi Lesmayati*, *Asep* Wawan Permana, *Christina* Winarti, *Waryat, Mulyana* Hadipernata, *Widaningrum, Imro'ah* Ikarini, and *Alvi* Yani

National Research and Innovation Agency, Indonesia

Abstract. Eggshell waste, comprising approximately 9–15% of total egg mass, is a calcium carbonate ($CaCO_3$)-rich byproduct frequently disposed of. This study investigates its utilization as a functional additive in chitosan-based edible coatings to enhance egg preservation. Calcium carbonate was extracted from eggshells using oxalic acid and incorporated into 1% (w/v) chitosan solutions at 0.1% and 0.2% (w/v) concentrations. Four treatments were tested: uncoated control, chitosan only, and chitosan combined with $CaCO_3$ extract at 1% and 2% concentrations. Eggs were coated by immersion for two minutes and stored at room temperature for six weeks. Quality parameters such as weight loss, albumen index, yolk index, and Haugh Unit (HU) were measured weekly. The results showed that chitosan and chitosan $CaCO_3$ coatings effectively reduced egg quality degradation compared to uncoated eggs. Uncoated eggs remained acceptable for two weeks, while coated eggs maintained quality for up to five weeks, though HU values declined to category B by week six. The addition of $CaCO_3$ did not significantly improve protection beyond chitosan alone. This study demonstrates the potential of eggshell waste valorization in edible coatings and provides scientific insight for further optimization to enhance food preservation and waste reduction.

1 Introduction

Eggshells are one of the most common forms of food waste and are readily available. Apart from households, eggshells can also be generated from the waste disposal of laying hen farms and other food processing industries. The percentage of eggshell weight of the total egg weight ranges from 9 – 15% [1]. This eggshell waste can be a rich source of inorganic biomaterials, such as calcium carbonate ($CaCO_3$), which comprises approximately 90–96% in the form of crystalline Calcite, as well as small amounts of magnesium carbonate and other organic matter [2]. This composition gives eggshells the potential to be used for various applications. In recent years, the utilization of eggshells has been increasingly directed towards non-food applications, such as fillers in the paper industry, nutraceuticals, pharmacy,

* Corresponding author : susilesmayati@yahoo.com

and cosmetics [3], and some also use it primarily as an alternative source of calcium and filler material in biocomposites or active coatings [4].

Innovations in the use of eggshells as functional materials for food are still very limited and require further exploration. One potential approach is to extract $CaCO_3$ using organic acids [2], which are then combined into natural-based coating materials, such as chitosan. Chitosan is a natural biopolymer derived from chitin and is widely recognized for its potential as a food preservation coating material [5]. The unique properties of chitosan include its ability to form films, antimicrobial activity [6], and biodegradability [7]. However, its weak mechanical properties, as well as its permeability to gases and water vapour, limit its use [7]. Therefore, a composite $CaCO_3$ extract can likely be used to address this drawback, making it an attractive option for extending the shelf life of various fresh food products.

Indonesia is among the top 15 countries in egg consumption per capita per year. According to data from the National Food Agency, the average Indonesian person consumes 6.69 kg of purebred chicken eggs per capita annually. The obstacle faced today is that the distribution and storage of eggs are still carried out without refrigeration, despite eggs being highly susceptible to damage and having a short shelf life of approximately 10-14 days when left untreated at room temperature. The development of coating technology for eggs primarily focuses on the manufacture of protective coatings that prevent moisture loss, gas exchange, and microbial contamination. Various ingredients, including chitosan, have been explored for their effectiveness. Some of the approaches that have been studied include adding different types of essential oils to enhance their antimicrobial properties, blending biopolymers [8,9], and repeated coating [10]. However, the effect of the presence of calcium carbonate remains largely unexplored. The use of eggshell waste as an active ingredient in edible coatings is one strategy that aligns with the principles of the circular economy, while also providing a solution for managing household waste.

Based on the description above, this study aims to investigate the effect of adding calcium carbonate from eggshell extraction using oxalic acid to the chitosan coating solution on egg quality during storage.

2 Research method

This study was designed to evaluate the effect of incorporating calcium carbonate ($CaCO_3$) extracted from eggshells using oxalic acid, an acid solvent, into chitosan coating solutions, thereby extending the shelf life and maintaining the internal quality of chicken eggs during storage. The research was conducted at the Laboratory of Agroindustry and Biomedicine, National Research and Innovation Agency (BRIN), from April to July 2024.

2.1 Experimental design

The subject of this study consisted of commercial chicken eggs sourced from a local poultry supplier, characterized by uniform size, weight, and cleanliness. The study employed a Completely Randomized Design (CRD) with one factor: the type of coating treatment. The treatments consisted of (i) uncoated control, (ii) coated with 1% chitosan, (iii) coated with 1% chitosan + 0,1% $CaCO_3$ extract, and (iv) coated with 1% chitosan + 0,2% $CaCO_3$ extract. Each treatment group consisted of five replications. Random sampling effectively minimized selection bias across treatments.

2.2 Materials and methods

2.2.1 Preparation of calcium carbonate extract

Eggshells were thoroughly washed with distilled water to remove adhering organic matter, oven-dried at 105 ºC for 2 hours, and ground into a fine powder using a mechanical grinder. Following the method described by Oliveira et al. [2] with slight modifications, the powdered eggshell was mixed with 20% (w/v) oxalic acid and stirred at 700 rpm on a hot plate stirrer for 2–4 hours until visible foaming ceased, indicating complete dissolution of calcium carbonate. The suspension was then filtered to separate impurities, and the precipitate obtained was washed five times with distilled water until the filtrate became clear. The washed solid was subsequently dried at 80 °C under continuous stirring to yield a purified calcium compound powder. The dried sample was further calcined in an electric muffle furnace at 500-600°C for 5 hours to ensure the decomposition of residual organic matter and to obtain high-purity $CaCO_3$ (Fig. 1). The structural and morphological characteristics of the extracted $CaCO_3$ were confirmed using X-ray diffraction (XRD) and scanning electron microscopy equipped with energy-dispersive spectroscopy (SEM-EDS).

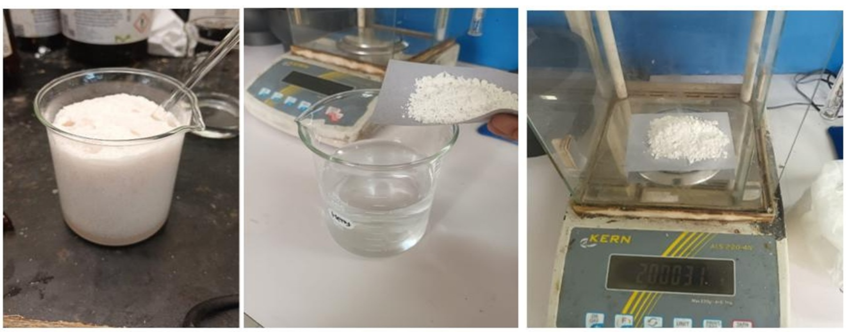

Fig. 1. Extraction with acid, repeated washing until neutral, and the appearance of the $CaCO_3$ eggshell extract

2.2.2 Preparation and application of coating solutions

A chitosan coating solution was prepared by dissolving chitosan at a concentration of 1% (w/v) in 1% acetic acid medium. The $CaCO_3$ extract was incorporated into a chitosan solution at concentrations of 0.1% and 0.2% (w/v). The eggs were immersed in the coating solution for a standardized period and air-dried at room temperature.

2.2.3 Storage and observation

All eggs were stored at ambient room temperature (25–27°C) for six weeks. Measurement of egg quality was taken weekly on the following parameters: Weight loss (%) by comparing initial and weekly weight using a digital scale; Albumen index (AI) and Yolk Index (YI) calculated using egg height and diameter data measured with a calliper, according to SNI 2008 standards [11]; and Haugh unit (HU), computed based on egg weight and albumen height using the standards formula from the Indonesian National Standard [11].

2.3 Data analysis

The collected data were analyzed using SPSS statistical software. One-way Analysis of Variance (ANOVA) was used to determine the effect of the treatments on each parameter. When significant differences were observed, the means were further analyzed using Duncan's

Multiple Range Test (DMRT) at a significance level of $\alpha = 0.05$. All data were expressed as mean ± standard deviation (SD).

3 Results and discussion

3.1 Characterization of calcium carbonate extract from eggshells

3.1.1 XRD analysis

To determine the crystalline structure of the extracted calcium carbonate, XRD (X-ray Diffraction) analysis was performed on the eggshell powder before and after treatment with 20% oxalic acid, as well as after calcination. The results of the diffraction pattern before extraction are shown in Fig. 2, after extraction in Fig. 3, and after calcination in Fig. 4.

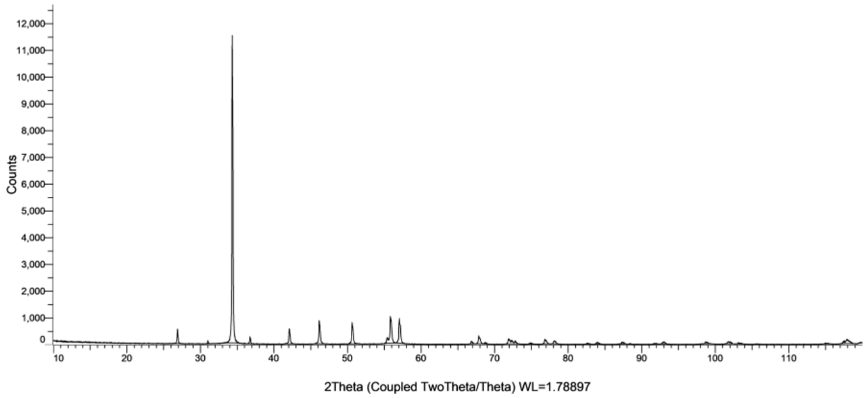

Fig. 2. Cristalline structure of eggshell powder before treatment

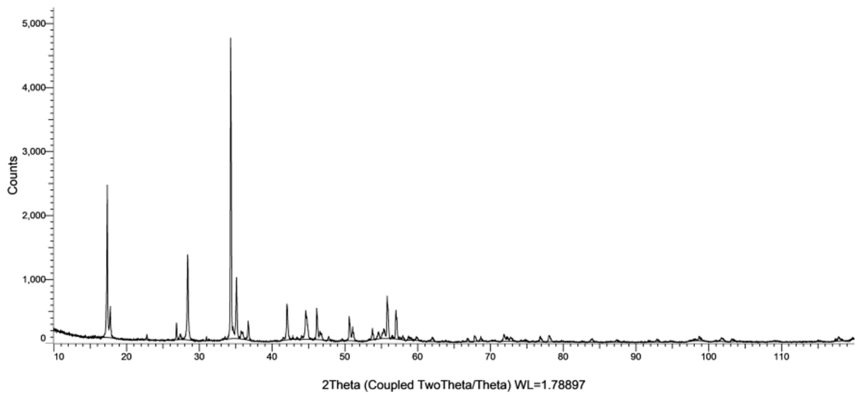

Fig. 3. Cristalline structure of egg shell after extracted with oxalic acid 20%

The XRD pattern on the eggshell powder before treatment (Fig. 2) showed peaks of high intensity around $2\theta = 29 - 31^{\circ}$, 39°, 47°, and 48°, which are characteristic reflections of calcium carbonate ($CaCO_3$) in the form of Calcite, the most common form of inorganic minerals in eggshells. After the extraction treatment, the XRD pattern shows a decrease in the peak intensity of $CaCO_3$ and the appearance of a new peak in the low-angle region ($12 - 24^{\circ}$), which can be attributed to the calcium oxalate monohydrate phase (Fig. 3). This change indicates that the Ca^{2+} of $CaCO_3$ reacts with oxalate to form new crystals. After the calcination process, the typical peaks of oxalate disappear (Fig. 4). At a calcination temperature of 500

– 600 °C, calcium oxalate tends to decompose into calcium carbonate. Thus, the main phase of post-calcination is most likely to be purer $CaCO_3$, with the possibility of the formation of a limited amount of CaO in the event of partial decomposition [12].

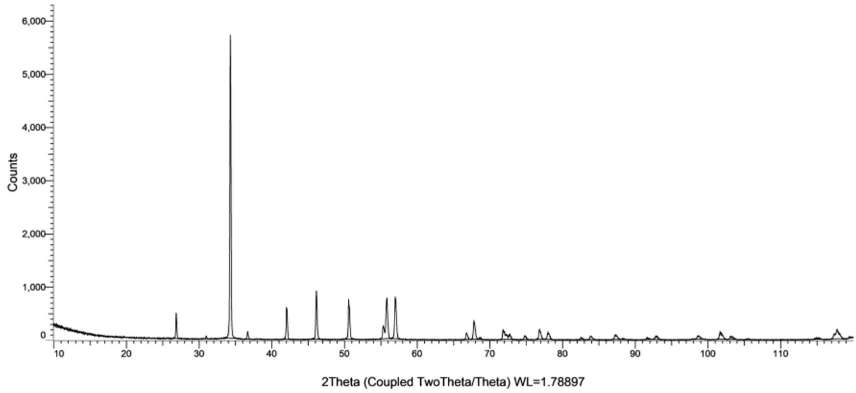

Fig. 4. Cristalline structure of calcinated eggshell extracted with oxalic acid 20%

3.1.2 SEM - EDS analysis

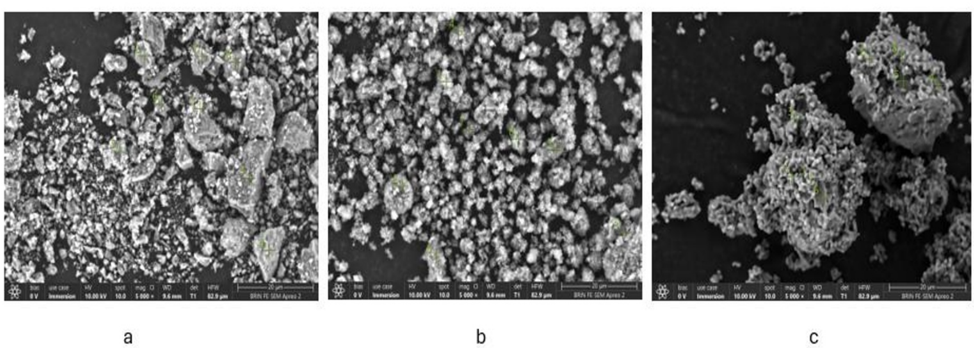

Fig. 5. SEM micrographs of eggshell powder (a), eggshell extract before calcination (b), and after calcination (c)

The SEM imagery supports the XRD results by showing significant morphological changes. In the eggshell powder (Fig. 5a), the particles appear large, irregular, and rough, in accordance with the biomineral properties of $CaCO_3$ that still contain an organic matrix. After extraction, the morphology changes to smaller, uniform granular particles, indicating the loss of organic components and the formation of new calcium oxalate crystals (Fig. 5b). In Figure 5c, it can be seen that the particles are agglomerated and sintered after calcination so that they form solid clumps with lower porosity. It is a characteristic of inorganic materials resulting from thermal decomposition.

The composition of the constituent elements is presented in Table 1. The EDS data analysis shows a trend consistent with XRD results. In the eggshell powder, the elemental content is dominated by Ca (33,2%) and O (50.3%) with C at 15.2%, which corresponds to the structure of $CaCO_3$. After extraction, the O content increased (54.6%) while Ca decreased (29.2%), with C relatively stable. This ratio supports the formation of $CaC_2O_4{\cdot}H2O$ (calcium oxalate). After calcination, the carbon content decreased significantly to 5.7–7.8%, indicating the release of carbon as CO2, while Ca increased to 55.1%, with the dominance of the $CaCO_3$ or CaO phase, whereas N and S were no longer detected. These changes confirm the decomposition of calcium oxalate into a Ca-rich phase such as $CaCO_3$ or CaO. The minor elements (Al, Mg, Si) remain detected in trace amounts and do not affect the primary phase.

Table 1. Elemental composition (EDS) of eggshell extract before and after calcination

Element	**Eggshell powder (wt%)**	**Before calcination (wt%)**	**After calcination (wt%)**
C	15.2	15.4	5.7 – 7.8
O	50.3	54.6	40.4 – 61.3
Ca	33.2	29.2	31.4 – 55.1
Minor elements: Al, Mg, Si	Trace	present	trace
N, S	Not detected	present	Not detected

Visually, the extracted powder is whiter; this is likely due to the use of oxalic acid, which not only converts $CaCO_3$ but can also help dissolve membrane proteins, pigments, and organic residues, as supported by several studies that show similar effects on both weak to strong acids such as HCl or acetic acid [13]. After calcination, a grey powder is obtained, which may be caused by several conditions, such as carbon residue that has not been completely burned, the formation of an additional phase from impurities, or the rehydration of partially hydrated CaO back into $Ca(OH)_2$ during cooling. As reported by Olayinka et al [12], eggshell calcined at 600 °C may show a peak of CaO in XRD, along with the presence of $Ca(OH)_2$ and Ca_2SiO_4 and a change in color to dark grey. The results of this XRD and SEM-EDS analysis provide preliminary evidence that calcium carbonate extracted using acids opens up opportunities for utilizing eggshell waste as a raw material in the synthesis of calcium-based materials, adsorbents, and biomedical applications, as well as food applications, such as additives in food coating systems [2].

3.2 Egg quality

Egg quality is a key indicator for assessing the freshness and feasibility of egg consumption, especially during the distribution and storage process. Changes that may occur as the shelf life increases include physical and chemical changes due to water evaporation, gas transfer, and degradation of internal components. The quality of eggs can be assessed through several important parameters, including weight loss, albumen index, yolk index, and Haugh Unit, each of which provides an overview of the egg's physical integrity and biological freshness.

The application of edible coatings such as chitosan, either pure or combined with $CaCO_3$ additives from eggshells, aims to slow down the rate of degradation. Tables 1–4 show the quality of the coated eggs according to the treatment during the 6-week storage period.

3.2.1 Weight loss

Egg weight loss during storage is a crucial indicator in assessing mass loss due to the evaporation of water and gases through shell pores. Eggs that lose significant amounts of water generally experience a decrease in physical quality and nutritional value [14]. The greater the weight loss, the higher the potential for a reduction in the internal quality of the eggs.

Based on Table 2, it can be seen that eggs without coating treatment (control) experienced a progressive increase in weight loss from week 1 to week 6, reaching 8.58 ± 0.066% at the end of the period. The chitosan coating treatment and the combination of chitosan with CaCO3 resulted in a lower weight loss, ranging from 7.67% to 8.32% at week 6.

Table 2. The effect of coating and storage period on Weight Loss (%)

Coating treatment	Observation Time (week -)					
	1	2	3	4	5	6
Uncoated (control)	$0.92 \pm 6.8 \times 10^{-2}$ a	$2.41 \pm 1.5 \times 10^{-2}$ a	$3.72 \pm 2.7 \times 10^{-2}$ a	$5.0 \pm 3.8 \times 10^{-2}$ a	$7.02 \pm 2.7 \times 10^{-2}$ a	$8.58 \pm 6.6 \times 10^{-2}$ a
Chitosan 1%	$0.71 \pm 4.0 \times 10^{-1}$ a	1.83 ± 1.03 a	3.32 ± 1.33 a	4.77 ± 1.73 a	6.37 ± 2.3 a	7.67 ± 2.40 a
Chitosan 1% + $CaCO_3$ 0.1 %	$0.65 \pm 1.3 \times 10^{-1}$ a	$1.83 \pm 3.4 \times 10^{-1}$ a	$3.26 \pm 5.5 \times 10^{-1}$ a	$4.65 \pm 7.8 \times 10^{-1}$ a	$6.13 \pm 9.4 \times 10^{-1}$ a	7.96 ± 1.2 a
Chitosan 1% + $CaCO_3$ 0.2 %	$0.64 \pm 1.4 \times 10^{-1}$ a	$1.84 \pm 2.3 \times 10^{-1}$ a	$3.13 \pm 2.5 \times 10^{-1}$ a	$4.59 \pm 5.7 \times 10^{-1}$ a	6.87 ± 1.42 a	8.32 ± 1.19 a

Note: The same letter in the same column behind the value showed no significant difference ($p > 0.05$)

However, based on the results of statistical analyses, the value of weight loss between all treatments in each week showed the same letter (a), which means that there was no significant difference ($p > 0.05$) between treatments at each observation time. Thus, although there is a numerical tendency for coating treatments to reduce the rate of weight loss, the difference is not statistically significant enough. Other factors, such as the homogeneity of the coating layer, the film thickness, and environmental conditions (temperature and humidity), are likely to affect these results.

3.2.2 Albumen index and Yolk index

The albumen index reflects the viscosity and height of the egg whites, while the yolk index describes the integrity and shape of the yolk. A high yolk index value indicates that the yolk is still round and the vitelline membrane is strong. Based on Indonesian national standards [11], the egg white index ranged from 0.134 to 0.175, and the yolk index was 0.458 to 0.521 for eggs with a Quality I level. The albumen index ranged from 0.050 to 0.091, and the yolk index ranged from 0.330 to 0.393 for the lowest quality (III). The grades are included in Quality II.

Table 3 shows the value of the albumen index during storage, indicating a downward trend in the albumen index value for both the control and the egg with each treatment. All egg samples, whether coated or not, have an albumen index that is below the quality threshold I. The age of the eggs may influence this at the time of sampling or other conditions before treatment. However, compared to the controls, the coating treatment was able to keep the albumen index in the same quality category for longer, while the controls decreased towards a value close to the lower limit of consumption quality at the end of week 6.

Table 3. The effect of coating and storage period on Albumen Index

Coating treatment	**Observation Time (week-)**					
	1	**2**	**3**	**4**	**5**	**6**
Uncoated (control)	$0.057 \pm 9.3 \times 10^{-3}$ a	$0.045 \pm 6.4 \times 10^{-3}$ a	$0.041 \pm 3.5 \times 10^{-3}$ a	$0.032 \pm 2.6 \times 10^{-3}$ a	$0.024 \pm 4.4 \times 10^{-3}$ a	$0.019 \pm 2.9 \times 10^{-3}$ a
Chitosan 1%	$0.062 \pm 5.9 \times 10^{-3}$ a	$0.048 \pm 3.9 \times 10^{-3}$ a	$0.047 \pm 4.8 \times 10^{-3}$ ab	$0.049 \pm 2.7 \times 10^{-3}$ b	0.034 ± 2.9 x 10^{-3} b	0.028 ± 3.9 x 10^{-3} b
Chitosan 1% + $CaCO_3$ 0.1 %	$0.077 \pm 1.2 \times 10^{-2}$ b	$0.062 \pm 1.9 \times 10^{-3}$ b	$0.056 \pm 7.1 \times 10^{-3}$ c	$0.043 \pm 5.2 \times 10^{-3}$ b	0.034 ± 7.1 x 10^{-3} b	0.024 ± 7.1 x 10^{-3} ab
Chitosan 1% + $CaCO_3$ 0.2 %	0.067 ± 7.5 x 10^{-3} ab	0.057 ± 3.9 x 10^{-3} b	0.05 ± 7.7 x 10^{-3} b	0.047 ± 6.1 x 10^{-3} b	0.033 ± 7.0 x 10^{-3} b	0.027 ± 2.6 x 10^{-3} b

Note: The same letter in the same column behind the value showed no significant difference ($p > 0.05$)

Statistically, a noticeable difference was observed between the control and the coating treatment within the first two weeks. The chitosan and $CaCO_3$ treatment at 0.1% showed a significantly different albumen index value (b) compared to the control (a), indicating that the coating provides real protection against albumen degradation. However, by week 6, some treatments again showed the same letter (ab), indicating that the coating's effectiveness decreased over time.

Table 4. The effect of coating and storage period on the Yolk index

Coating treatment	**Observation Time (week-)**					
	1	**2**	**3**	**4**	**5**	**6**
Uncoated (control)	$0.337 \pm 1.8 \times 10^{-2}$ a	$0.278 \pm 5.6 \times 10^{-3}$ a	0.198 ± 3.0 x 10^{-2} a	0.185 ± 1.8 x 10^{-2} a	0.131 ± 1.6 x 10^{-2} a	0.107 ± 6.7 x 10^{-3} a
Chitosan 1%	0.545 ± 1.2 x 10^{-2} b	0.378 ± 1.9 x 10^{-2} c	0.256 ± 3.1 x 10^{-2} ab	0.236 ± 3.1 x 10^{-2} b	0.206 ± 5.8 x 10^{-3} c	0.197 ± 3.4 x 10^{-2} b
Chitosan 1% + $CaCO_3$ 0.1 %	0.571 ± 9.4 x10^{-3} d	0.393 ± 3.9 x 10^{-2} c	0.311 ± 7.7 x 10^{-2} b	0.198 ± 1.1 x 10^{-2} a	$0.153 \pm 2.0 \times 10^{-2}$ b	0.178 ± 6.4 x 10^{-2} b
Chitosan 1% + $CaCO_3$ 0.2 %	0.504 ± 7.3 x 10^{-3} c	0.321 ± 3.8 x 10^{-2} b	0.284 ± 7.9 x 10^{-2} b	0.256 ± 3.9 x 10^{-2} b	0.162 ± 1.6 x 10^{-2} b	0.182 ± 5.0 x 10^{-2} b

Note: The same letter in the same column behind the value showed no significant difference ($p > 0.05$)

Table 4 shows that all eggs have exhibited a downward trend in the yolk index value, with control eggs experiencing a more significant decrease compared to coated eggs, particularly in weeks 1 through 4. This indicates that the coating can maintain the quality of the yolk.

3.2.3 Haugh Unit (HU)

HU value is a measure that describes the viscosity of egg whites; the smaller the HU value, the thinner the egg whites are, indicating lower egg white quality. HU standards are classified into C quality (< 31), B quality (31 – 60), A quality (60-72), and AA quality (>72) [15].

Table 5. The effect of coating and storage period on the Haugh Unit

Coating treatment	Observation Time (week -)					
	1	2	3	4	5	6
Uncoated (control)	66.819 ± 4.23 a	59.699 ± 4.24 a	46.693 ± 7.95 a	46.893 ± 4.15 a	34.697 ± 9.09 a	30.697 ± 6.88 a
Chitosan 1%	79.748 ± 0.53 b	77.865 ± 5.33 b	75.996 ± 3.79 b	70.976 ± 2.22 b	64.541 ± 4.46 b	58.321 ± 33.74 c
Chitosan 1% + $CaCO_3$ 0.1 %	78.312 ± 4.59 b	78.569 ± 5.84 b	67.858 ± 3.54 b	67.718 ± 4.53 b	56.848 ± 6.06 b	47.634 ± 8.56 b
Chitosan 1% + $CaCO_3$ 0.2 %	76.366 ± 4.78 b	75.834 ± 1.19 b	71.387 ± 4.56 b	70.918 ± 1.30 b	59.184 ± 3.61 b	44.954 ± 7.42 b

Note: The same letter in the same column behind the value showed no significant difference ($p > 0.05$)

Table 4 illustrates the trend of decreasing HU values between control eggs and eggs subjected to multiple coating treatments. It can be seen that the control eggs showed the most drastic decrease in HU from 66.81 in the first week to 30.69 at the end of the observation. Meanwhile, the coating expansion can maintain a higher HU value, especially during the storage period from week 1 to week 5. Statistically, it can be seen that coating both chitosan and chitosan incorporating with $CaCO_3$ is able to significantly maintain the freshness of eggs for longer than without coating. Although in week 6, the HU value in the egg coating remained below 60, indicating that the quality has entered quality B, the value is still higher than that of the control. The decrease in HU is related to the breakdown of the ovomucin fibers (which provide the viscous texture of albumen), which leads to the loss of CO_2 gas and an increase in pH.

Overall, the observations indicate that chitosan coating treatment, with or without the addition of $CaCO_3$, was effective in prolonging the decline of internal egg quality. According to the albumen index, yolk index, and Haugh Unit indicators, the uncoated egg (control) could only maintain acceptable consumption quality for around 2 weeks of storage. In contrast, eggs treated with a coating were able to retain their quality for up to 5 weeks at room temperature, although by the sixth week, the quality had decreased to category B. These findings confirm that the application of edible coating can effectively extend the shelf life of eggs as indicated by internal quality parameters. Although the incorporation of $CaCO_3$ did not provide greater effectiveness compared to chitosan alone.

4 Conclusion and recommendation

Eggshells can be utilized as a source of calcium carbonate through an extraction process using oxalic acid, and the extracts can be applied as additives in chitosan-based coating solutions to maintain egg quality during storage at room temperature. Observation results on the quality of eggs coated with a chitosan coating, with or without the incorporation of calcium carbonate extract, showed that it was effective in prolonging the reduction in egg quality compared to bare eggs. In terms of shelf life, the coating treatment extended the storage duration of eggs at room temperature from approximately 2 weeks (uncoated) to around 5 weeks (coated) with acceptable consumption quality (HU category B). Although the incorporation of eggshell-derived calcium carbonate did not enhance coating effectiveness beyond that of chitosan itself, the novelty of this study lies in the valorization of eggshell waste as a functional ingredient in edible coatings and in providing scientific evidence that such formulation requires further optimization to maximize its efficacy.

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