Research Article

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Antimicrobial lysozyme–chitosan coatings affect functional properties and shelf life of chicken eggs during storage

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Abstract

BACKGROUND: The interior quality, shell impact strength and functional characteristics of eggs coated with chitosan and lysozyme–chitosan combinations were evaluated for enhancing egg freshness during storage. A 10% (w/w) lysozyme solution was incorporated into 1% (w/w) chitosan film-forming solution at ratios of 0, 10, 20 and 60% (w/w).

RESULTS: Storage time and coating had significant effects on Haugh unit, yolk index, weight loss, albumen pH, dry matter, relative whipping capacity (RWC) and albumen viscosity. Uncoated eggs had higher albumen pH and weight loss and lower albumen viscosity. All coated eggshells showed greater puncture strength than uncoated eggshells, resulting in extended shelf life. The 20 and 60% lysozyme–chitosan coatings were more effective in maintaining the internal quality of eggs (e.g. pH, dry matter and RWC). Attributes such as pH, dry matter and RWC were better after the 20% lysozyme–chitosan treatment than after the other treatments.

CONCLUSION: The 10, 20 and 60% lysozyme–chitosan coatings, considered active packaging, showed promising attributes. They could be a viable alternative to existing techniques for maintaining the internal quality of fresh eggs during long-term storage. Chitosan coatings also improved shell strength. This study also confirms that measurements of albumen quality (pH, dry matter, viscosity and RWC) are excellent indicators of egg freshness.

Keywords: lysozyme; chitosan; coating; shell eggs; egg quality; functional properties

INTRODUCTION

As an excellent protein source, fresh shell eggs are among the most nutritious foods consumed on a daily basis. However, shell eggs are highly susceptible to internal quality deterioration and bacterial growth during storage. Immediately after shell eggs are laid, the aging process begins, altering their chemical, physical, microbiological and functional properties.[1,2] Although the shell can be considered a natural barrier, shell eggs have a short shelf life and are extremely fragile, which can result in serious economic losses to the egg industry.[3,4] Even a small improvement in the overall quality of fresh eggs will result in significant savings to the industry. Egg preservation relies mainly on time and temperature management and the use of edible coatings on the shell.

Interior quality deterioration of fresh shell eggs can be significantly delayed during storage by maintaining the storage temperature near the freezing point.[5] Numerous food-grade coating materials (mineral oils, waxes, whey protein, soy protein, gluten, chitosan, cellulose-based materials and corn zein) have also proven to be efficient in reducing mass transfer by sealing pores. Such coatings prevent the penetration of micro-organisms into shell eggs. As a result, they extend their storage time and reduce economic losses.[3,4,6–9] In addition, Xie *et al.*[10] showed that protein isolate- or carboxymethylcellulose-coated eggshells had greater puncture strength than uncoated eggshells. Other coating materials such as chitosan[11–14] have been used for preserving the internal quality of eggs.

Antimicrobial-enhanced coatings, which are considered active packaging, have been receiving increased interest since they exhibit great potential for ensuring food safety. Hen egg white 'lysozyme' is one of the few natural antimicrobial lytic enzymes currently approved by regulatory agencies for use in foods. Lysozyme is a peptidoglycan *N*-acetyl-muramoylhydrolase (EC 3.2.1.17) bacteriolytic enzyme commonly found in nature. The antimicrobial spectrum of lysozyme may be enhanced when it is used with other substances such as chitosan. Chitosan is known for its natural antimicrobial properties and is a carrier of other functional ingredients. Lysozyme has been incorporated into coating materials such as soy protein and corn zein[15] and Na-alginate and κ-carrageenan[16] and the antimicrobial activities of those lysozyme-containing films have been reported. A few studies have also been conducted related to chitosan combined with other active antimicrobial substances with potential food applications.[17–19]

Chitosan, a polysaccharide, is one of the most abundant and renewable polymers obtained from shellfish, shrimp, crab and

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lobster waste products. Since chitosan possesses unique physical and chemical properties, antimicrobial activity, biocompatibility and biodegradability, it has great potential for use in a wide range of applications.[20] Thus chitosan-based films or coatings have proven to be very effective in food preservation. It has attracted notable interest since it provides excellent oxygen barrier properties as well as some level of antimicrobial activity.[20,21] Chitosan coatings can modify or maintain internal atmospheres so as to reduce respiration rates of fruits.[22] The use of lysozyme–chitosan coatings appears promising to slow mass transfer rates in order to improve the quality and extend the storage life of shell eggs. However, none of the previous studies provided detailed information on interior quality criteria after applying such coatings.

Therefore the objectives of the present study were (1) to extend the internal quality (weight loss, pH, Haugh unit and yolk index) (shelf life) of fresh shell eggs without extra barrier packaging, (2) to maintain their rheological property (viscosity) and functional characteristics (e.g. relative whipping capacity) and (3) to increase their shell strength during storage under ambient conditions.

MATERIALS AND METHODS

Clean, white shell (Lohmann White laying hen breed), unfertile, large size, freshly laid (1-day-old) eggs supplied by AB Foods Inc. (Bandirma, Turkey) were used in the present study. Shell egg treatments consisted of control (uncoated), coating with chitosan and coating with chitosan containing lysozyme (10, 20 and 60% w/w).

Preparation of chitosan coating solutions and coating of shell eggs

Chitosan coating solutions were prepared according to Caner and Cansiz[2] using food-grade chitosan (odourless and tasteless powder extracted from recycled crab and shrimp shells with 89.9% deacylation; Vanson, Redmond, WA, USA). Chitosan film-forming solution was prepared by dissolving 1% (w/w) chitosan in 1% (w/w) glacial acetic acid (Riedel-de Haen AG, Seelze-Hannover, Germany), adding 25% (w/w) glycerol as plasticizer and heating the mixture on a hot plate at 40 °C for 1 h with magnetic stirring. Lysozyme stock solution (Sigma Chemical Co., St Louis, MO, USA) was prepared by dissolving 10% (w/w) lysozyme in distilled water. The lysozyme solution was then mixed into the chitosan solution at concentrations of 0, 10, 20 and 60% (dry weight lysozyme/dry weight chitosan)[20] using a homogenizer.

After washing with water, clean eggs were immersed individually by hand in the coating solutions for 1 min (first layer of coating), then immersed again for 1 min (second layer of coating) and finally dried at ambient temperature for 24 h. Uncoated eggs served as control. The eggs were subsequently placed in open moulded plastic egg trays and stored under controlled ambient laboratory conditions (~25 °C with 70–75% relative humidity) for 5 or 6 weeks.[3]

Samples were divided into five groups: a control group, a chitosan-coated group and three (10, 20 and 60% w/w) lysozyme–chitosan-coated groups. Ten eggs per treatment were taken at each storage interval for the evaluation of internal quality parameters.

Weight loss

Weight loss (%) of eggs during storage was calculated by subtracting the final weight from the initial weight, dividing by the initial weight and multiplying by 100. Ten eggs per treatment were weighed to within ±0.001 g using a laboratory electronic balance.

Eggshell breaking strength

Eggshell breaking strength (puncture strength) was determined using a texture analyzer (TA.XT2, Texture Technologies Corp., Scarsdale, NY, USA). Each egg was mounted on a platform and the eggshell was punctured at the top (small end) and bottom (large end) using a 3 mm die probe at 5 mm s^{-1} constant speed with a 30 kg load cell in compression mode.[2,23] The force required to puncture the shell was recorded as eggshell breaking strength (kgf). Twenty eggs per treatment were measured.

Haugh unit and yolk index

Haugh unit was calculated by the formula[3]

$$\text{Haugh unit (HU)} = 100 \times \log\left(h - 1.7G^{0.37} + 7.6\right)$$

where h is the height of the thick albumen (mm) and G is the mass of the whole egg (g). The parameter h was estimated by averaging three measurements carried out at different points of the thick albumen at a distance of 10 mm from the yolk using digital callipers (CD-15CP, Mitutoyo Ltd, Andover, Hampshire, UK). Eggs were graded as follows: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

Yolk index was calculated as yolk height divided by yolk width. Yolk height and width were measured with digital callipers (CD-15CP, Mitutoyo Ltd). A fresh egg of good quality has a yolk index of around 0.45.

Albumen viscosity

Eggs were broken, chalazae were separated and the albumen was collected in a vessel for measuring viscosity (per egg sample). Albumen viscosity (mPa s) measurements were carried out at 20 ± 0.5 °C using a Brookfield viscometer (Model DV II+Pro D 220, TC-502 temperature controller unit and Rheocalc software; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). The spindle (UL adapter, 30 rpm) was selected based on the torque measurement between 10 and 100% as suggested by the manufacturer. The first result was recorded after a 20 s rotation of the spindle and the second result was recorded after a further 10 s of rotation. Silicone oil standard solution (Brookfield Engineering Laboratories, Inc.) was used to calibrate the viscometer. Ten eggs per treatment were used for viscosity measurements.

pH measurement

After albumen height (mm) had been measured, the albumen was separated from the yolk. Volumes (mL) of firm and thin albumen were homogenized for 20 s in a blender (Model 32 BL 80, Waring, Torrington, CT, USA) and then measured for pH (pH 210 meter, Hanna Instruments, Woonsocket, RI, USA).[3] The pH electrode was standardized using buffer solutions of pH 4, 7 and 9 before testing began.

Total solids (dry matter) of white and yolk

Total solids of egg samples were determined as described by Cahn and Epstein[24] and Triebold.[25] Dry matter (% w/w) contents of egg white and yolk were measured separately using an Abbe refractometer with a Peltier system (DR-A1, Atago Co. Ltd, Tokyo, Japan) at 20 ± 1 °C.[26] Four replicates were performed for each sample.

Foaming properties

Relative whipping capacity (RWC) and foam stability of egg white and whole egg samples at 20 °C were measured by the method of Li-Chan *et al.*[27] with slight modification. Foam was obtained at room temperature by whipping 75 mL of egg white in a Hobart mixer (N50CE, Hobart Foster Scandinavia A/S, Aalborg, Denmark) at speed 2 for 90 s and then speed 3 for 90 s. Foam density was calculated from the mass of a given volume of foam. Foam stability by detecting the foam height is a well-proven method for foams with short-term stability. Foam stability was measured with a graduated cylinder after the foam had been allowed to rest for 1 h, as described by Lechevalier *et al.*[28] Volume (mL) was used as a measure of this property. Foam stability was measured in the same vessel as the volume of released fluid at the bottom 1 h after whipping. The experiment was repeated three times and mean values were calculated.

$$\text{Volume (\%)} = \big[(\text{volume of prepared foam} - \text{volume of liquid drainage}) / \text{original volume of liquid}\big] \times 100$$

Data analysis

This study aimed to evaluate the combined effect of chitosan-based coatings produced with lysozyme at 0, 10, 20 and 60% (w/w) and storage time on the quality characteristics of shell eggs. Analysis of variance was carried out on all measured parameters among the control (uncoated) and coated eggs to determine any significant differences during or after storage. The study was repeated twice and 50 eggs were used for each replicate ($n_{\text{total}} = 100$ and $n_{\text{total for each treatment}} = 20$). Statistical procedures were performed using LSM-PROG GLM of the SAS program (SAS Institute, Cary, NC, USA). Statistical significance was defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Weight loss

Egg weight decreased significantly during storage owing to the movement of water through the 7000–17 000 pores in the shell.[23] Weight losses of uncoated (control) and coated eggs increased as storage progressed (Table 1). The weight loss of uncoated eggs increased at a much faster rate than that of eggs coated with lysozyme–chitosan. Lysozyme–chitosan-coated eggs showed a similar and very small weight loss reduction until the end of the experiment, losing only 5.06–3.08% of their initial weight after 5 weeks of storage. In contrast, uncoated eggs showed an increasing level of weight loss, reaching a value of 7.18% at the end of the storage period (Table 1). Chitosan coating thus renders excellent sealing properties (good moisture/oxygen barrier) for shell eggs destined for extended storage. Eggs coated with lysozyme showed a significant reduction ($P < 0.05$) in weight loss compared with control eggs and eggs coated with chitosan alone. Differences in weight loss between chitosan coating with or without lysozyme were not significant except after 5 weeks. According to the FAO,[29] a 2–3% loss of egg weight during marketing is acceptable. In this study, chitosan coating kept the weight loss within or close to the acceptable range after 4 weeks of storage (Table 1). Only eggs coated with 20% lysozyme–chitosan were able to keep the weight loss within the desirable range beyond 5 weeks (Table 1). Based on Table 1, the ability of the coating materials to minimize weight loss was 20% lysozyme > 60% lysozyme > 10% lysozyme > chitosan. Various studies have shown the enhancement effects of coating and refrigeration[7] in minimizing the weight loss of eggs during storage. The present results are in agreement with those of Caner,[3] Samli *et al.*[30] and Waimaleongora-Ek *et al.*,[9] who reported significant ($P < 0.05$) egg weight reductions.

Eggshell breaking strength

Eggshell breakage is directly related to the quality of the shell. Eggshell must be strong enough to minimize breakage during handling and storage. Improved shell strength will result in significant reductions in the number of eggs lost due to breakage or cracks during handling and storage.[4,10,31] Puncture strength is the maximum stress at break. The top of the eggshell has higher puncture strength than the bottom.[10] Uncoated (control) eggshells exhibited significantly lower puncture strength than chitosan-coated eggshells at both the top (Table 2) and bottom (Table 3). The puncture strength of eggshells coated with chitosan and lysozyme was significantly greater than that of uncoated (control) eggshells (Tables 2 and 3). However, no significant differences were found among the different chitosan–lysozyme coatings. During storage, no significant differences between coated and uncoated eggshells were found in terms of puncture strength except after 1 week of storage (Tables 2 and 3). No significant differences in puncture strength were observed at the top and bottom of eggshells coated with chitosan with or without lysozyme. All coated eggs significantly exhibited the highest puncture strength (Tables 2 and 3). This clearly indicates the potential application of chitosan coating with or without

Table 1. Effect of different coatings on egg weight loss during 5 weeks of storage

	Egg weight loss (%)					
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	0.00 ± 0.00Aa	2.40 ± 0.41Ba	3.86 ± 0.62Ca	4.98 ± 0.39Da	6.30 ± 0.64Ea	7.18 ± 0.64Fa
1% CHI	0.00 ± 0.00Aa	1.72 ± 0.21Bab	2.55 ± 0.41Cb	3.19 ± 0.39Cb	3.93 ± 0.50Db	5.06 ± 0.69Eb
10% LYS	0.00 ± 0.00Aa	1.61 ± 0.28ABb	2.10 ± 0.23BCb	2.54 ± 0.34Cbc	3.38 ± 0.47Dbc	3.97 ± 0.53Dc
20% LYS	0.00 ± 0.00Aa	1.41 ± 0.26Bb	1.97 ± 0.18BCb	2.35 ± 0.25CDc	2.71 ± 0.32DEc	3.08 ± 0.38Ed
60% LYS	0.00 ± 0.00Aa	1.55 ± 0.25ABb	1.90 ± 0.29BCb	2.39 ± 0.37CDc	2.90 ± 0.38DEc	3.44 ± 0.23Ecd

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

Table 2. Effect of different coatings on eggshell breaking strength at top during 4 weeks of storage

	Eggshell breaking strength (kgf)					
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	Overall
Control	4.58 ± 0.36	4.62 ± 0.73	4.60 ± 0.42	4.37 ± 0.52	4.28 ± 0.60	4.49 ± 0.55a
1% CHI	4.58 ± 0.36	5.14 ± 0.57	4.94 ± 1.10	4.83 ± 0.37	4.73 ± 0.47	4.85 ± 0.64b
10% LYS	4.58 ± 0.36	5.19 ± 0.32	5.19 ± 0.70	4.99 ± 0.66	4.83 ± 0.56	4.96 ± 0.57b
20% LYS	4.58 ± 0.36	5.35 ± 0.40	5.07 ± 0.44	5.01 ± 0.76	4.91 ± 0.50	4.97 ± 0.53b
60% LYS	4.58 ± 0.36	5.10 ± 0.34	5.08 ± 0.47	5.07 ± 0.58	4.74 ± 0.32	4.89 ± 0.44b
Overall	4.58 ± 0.34A	5.05 ± 0.56B	4.99 ± 0.69BC	4.84 ± 0.61BC	4.70 ± 0.52AC	

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

Table 3. Effect of different coatings on eggshell breaking strength at bottom during 4 weeks of storage

	Eggshell breaking strength (kgf)					
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	Overall
Control	4.11 ± 0.34	4.19 ± 0.63	4.25 ± 0.70	4.12 ± 0.29	4.05 ± 0.52	4.12 ± 0.48a
1% CHI	4.11 ± 0.34	4.85 ± 0.65	4.77 ± 0.45	4.62 ± 0.50	4.56 ± 0.34	4.57 ± 0.53b
10% LYS	4.11 ± 0.34	4.73 ± 0.42	4.82 ± 0.61	4.72 ± 0.57	4.61 ± 0.44	4.60 ± 0.53b
20% LYS	4.11 ± 0.34	4.68 ± 0.47	4.69 ± 0.31	4.63 ± 0.37	4.57 ± 0.36	4.53 ± 0.42b
60% LYS	4.11 ± 0.34	4.76 ± 0.41	4.62 ± 0.46	4.46 ± 0.48	4.60 ± 0.54	4.50 ± 0.48b
Overall	4.11 ± 0.32A	4.67 ± 0.53B	4.65 ± 0.52B	4.51 ± 0.46B	4.43 ± 0.49B	

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

lysozyme in minimizing eggshell breakage. Chitosan coatings enhance eggshell strength, allowing significant reductions in the number of eggs lost owing to poor shell quality.

Haugh unit and egg grade

The Haugh unit, depending on egg weight and albumen height, refers to albumen quality and hence to egg quality: the higher the HU value, the better the albumen quality of the egg.[32] Most fresh eggs leaving the farm should be between 85 and 75 HU,[33] while an older yolk would have a lower HU value.[3,23] The interaction between coating and storage time was significant, as was the interaction among groups (Table 4). HU decreased with increasing storage time in both uncoated and lysozyme–chitosan-coated eggs. All chitosan-coated eggs had significantly higher ($P < 0.05$) HU than uncoated eggs during 6 weeks of storage. Lysozyme–chitosan-coated eggs had significantly higher HU than control and chitosan-coated eggs (Table 4). HU of control eggs decreased significantly during storage. No significant differences were observed in HU of eggs coated with 20 and 60% lysozyme after 2 weeks of storage, but not eggs coated with 10% lysozyme. The 20 and 60% lysozyme coatings were more effective than the 10% lysozyme and chitosan coatings in preserving albumen quality during 6 weeks of storage. Eggs coated with 20 and 60% lysozyme remained at 81.36 and 82.07 HU respectively after 6 weeks of storage, compared with 55.30 HU for control eggs (Table 4). HU of uncoated eggs decreased more rapidly than that of coated eggs. This indicated that lysozyme–chitosan coating helped maintain egg freshness during storage, in agreement with Caner,[3] Bhale *et al.*[34] and Wong *et al.*[4]

While lysozyme–chitosan-coated eggs remained at grade AA throughout the storage period, eggs coated with chitosan fell to grade A in week 6. Uncoated eggs dropped from grade AA to grade A after 3 weeks of storage and then to grade B after 6 weeks (Table 4). Wong *et al.*[4] reported that uncoated eggs changed from grade A to grade B after 1 week, while eggs coated with different materials (soy, corn) remained at grade B after 28 days of storage. The present results indicate that the shelf life was extended by at least 3 weeks by chitosan and lysozyme–chitosan coatings. These findings demonstrate the synergism of lysozyme with chitosan in preserving albumen quality during long-term storage.

Yolk index

The yolk index (YI), measured as the ratio of yolk height to yolk width, can be used to assess individual egg freshness.[3,6] A decrease in YI indicates progressive weakening of the vitelline membranes and liquefaction of the yolk caused mainly by diffusion of water from the albumen.[34–36] A fresh egg of good quality has a YI of around 0.45, while an older egg will have a lower YI. The higher the YI, the better is the quality of the yolk. Storage time has a significant effect on YI. After 3 weeks of storage, the YI of uncoated eggs decreased from 0.48 to 0.38 (Table 5).

The YI of uncoated, chitosan-coated and lysozyme–chitosan-coated fresh eggs decreased during storage (Table 5). Eggs coated with lysozyme–chitosan had significantly higher YI than eggs

Table 4. Effect of different coatings on Haugh unit (HU) and egg grade during 6 weeks of storage

Coating[a]	Haugh unit (egg grade[b])						
	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	85.04 ± 0.14Aa (AA)	83.41 ± 0.73Ba (AA)	79.54 ± 0.85Ca (AA)	73.10 ± 0.70Da (AA)	69.42 ± 0.60Ea (A)	65.86 ± 1.23Fa (A)	55.30 ± 0.99Ga (B)
1% CHI	85.04 ± 0.14Aa (AA)	84.31 ± 0.36Aab (AA)	82.66 ± 0.72Bb (AA)	81.19 ± 0.70Cb (AA)	77.93 ± 0.33Db (AA)	73.78 ± 0.44Eb (AA)	68.90 ± 0.83Fb (A)
10% LYS	85.04 ± 0.14Aa (AA)	84.71 ± 0.20Ab (AA)	84.01 ± 0.26ABc (AA)	83.29 ± 0.31Cc (AA)	82.28 ± 0.36Cc (AA)	80.83 ± 0.65Dc (AA)	78.53 ± 0.50Ec (AA)
20% LYS	85.04 ± 0.14Aa (AA)	84.74 ± 0.18ABb (AA)	84.16 ± 0.25ABCb (AA)	83.88 ± 0.31BCc (AA)	83.05 ± 0.47CDc (AA)	82.41 ± 0.16DEd (AA)	81.36 ± 0.40Ed (AA)
60% LYS	85.04 ± 0.14Aa (AA)	84.84 ± 0.19ABb (AA)	84.66 ± 0.23ABc (AA)	83.82 ± 0.17BCc (AA)	83.22 ± 0.17CDc (AA)	82.51 ± 0.38DEd (AA)	82.07 ± 0.26Ed (AA)

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.
[b]Egg grades: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

coated with chitosan alone (Table 5). While the YI values of eggs coated with 60, 20 and 10% lysozyme–chitosan were 0.38, 0.37 and 0.36 respectively after 6 weeks of storage, the YI of control eggs was only 0.28. These YI values of coated eggs at 6 weeks were similar to the YI of control eggs at 3 weeks. The YI values of lysozyme–chitosan-coated eggs after 1 week were significantly higher than the YI of uncoated eggs (Table 5). Eggs coated with 60 and 20% lysozyme had significantly higher YI values after 3 weeks. The reductions in YI after 6 weeks were 41.67% (uncoated), 33.33% (chitosan), 25% (10% lysozyme), 22.91% (20% lysozyme) and 20.83% (60% lysozyme). These results indicated that lysozyme coating has an enhancement effect in maintaining yolk quality during storage. Lysozyme and chitosan together minimized yolk quality loss, as they effectively reduced the rate of water and CO_2 loss from the albumen through the eggshell, thereby inhibiting albumen liquefaction and water uptake by the yolk. No significant differences in YI were found among the three lysozyme–chitosan coating treatments during storage. Chitosan treatment led to the second highest YI values. This indicated that lysozyme–chitosan coating preserved the yolk quality of fresh eggs during storage, in agreement with Caner[3] and Obanu and Mpieri.[6] Obanu and Mpieri[6] reported insignificant differences in the YI of eggs coated with groundnut, cottonseed and coconut oils after 36 days of storage under ambient conditions. HU, weight loss and YI are highly correlated.[37]

Albumen viscosity

In the albumen, one of the most obvious changes is the physical deterioration of the thick white during storage; the gelatinous structure of the thick white gradually degrades, changing into thin white.[38] One of the most important proteins in egg albumen is ovomucin, as it plays a major role in the gel-like structure of egg white and thus a role in the viscosity of the thick albumen of native egg white. Rheological properties of liquids are commonly described by the shear stress *versus* shear rate curve. The slope of this curve is defined as the viscosity.[39] The viscosity of the albumen is an important quality variable, because it is related to functional characteristics of the albumen such as its whipping, emulsifying and gelling properties, among others.[40] However, very few studies have been concerned with modifications of the rheological behaviour of the albumen during storage. The decrease in viscosity during storage was significant ($P = 0.01$), confirming earlier results obtained by Kemps *et al.*[40]

Egg albumen is a pseudoplastic fluid and its viscosity depends on the shear force. The albumen viscosity values measured during storage are given in Table 6. The viscosity measurements show that the thin albumen behaves almost like a Newtonian fluid.[39,41]

Comparison of our viscosity values with those reported in the literature is difficult, because different shear rates and different sample preparation methods were adopted.[40] A striking difference was observed between the variances for HU and viscosity. The mean viscosity values were 30.4 and 18.1 mPa s for fresh eggs and eggs stored for 24 days respectively.[40] The increase in albumen pH can be a reason for the change in viscosity of the albumen. The destabilization of the complex is due to an increase in pH, which approaches the lysozyme isoelectric point during storage.[41,42] It has been suggested that coating with lysozyme–chitosan minimizes changes in carbohydrate and protein moieties involved in the formation of ovomucin complex, resulting in a loss of gel-like structure.[27,43] During fresh egg storage, a decrease in viscosity was observed. This is a consequence of the thinning of the albumen

Table 5. Effect of different coatings on yolk index during 6 weeks of storage

	Yolk index						
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	0.48 ± 0.00Aa	0.45 ± 0.01Ba	0.42 ± 0.00Ca	0.38 ± 0.01Da	0.33 ± 0.01Ea	0.31 ± 0.01Fa	0.28 ± 0.01Ga
1% CHI	0.48 ± 0.00Aa	0.47 ± 0.01Ab	0.45 ± 0.01Bb	0.42 ± 0.02Cb	0.38 ± 0.02Db	0.35 ± 0.01Eb	0.32 ± 0.01Fb
10% LYS	0.48 ± 0.00Aa	0.47 ± 0.00Ab	0.45 ± 0.00Bb	0.42 ± 0.00Cb	0.40 ± 0.01Dc	0.38 ± 0.01Dc	0.36 ± 0.01Ec
20% LYS	0.48 ± 0.00Aa	0.48 ± 0.00Ab	0.45 ± 0.00Bb	0.43 ± 0.00Cb	0.40 ± 0.01Dc	0.39 ± 0.01Dc	0.37 ± 0.01Ecd
60% LYS	0.48 ± 0.00Aa	0.48 ± 0.00Ab	0.45 ± 0.00Bb	0.43 ± 0.00Cb	0.41 ± 0.00Dc	0.39 ± 0.01Dc	0.38 ± 0.02Ed

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

Table 6. Effect of different coatings on albumen viscosity (20 s) during 6 weeks of storage

	Albumen viscosity (mPa s)						
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	58.57 ± 2.34Aa	38.93 ± 2.72Ba	24.67 ± 2.03Ca	18.07 ± 1.16Da	10.63 ± 0.76Ea	6.23 ± 1.03EFa	4.67 ± 0.55Fa
1% CHI	58.57 ± 2.34Aa	53.60 ± 0.53Bb	46.30 ± 0.80Cb	29.10 ± 0.89Db	19.80 ± 1.08Eb	15.23 ± 1.07Eb	10.30 ± 0.78Fb
10% LYS	58.57 ± 2.34Aa	53.37 ± 1.06Bb	49.37 ± 0.67Bb	35.37 ± 0.95Cc	24.53 ± 2.31Db	18.57 ± 0.85Ebc	13.97 ± 0.84Ebc
20% LYS	58.57 ± 2.34Aa	53.83 ± 0.12Ab	47.93 ± 0.90Bb	32.20 ± 0.92Cbc	27.47 ± 1.11Cc	20.27 ± 0.81Dc	15.03 ± 1.17Ebc
60% LYS	58.57 ± 2.34Aa	53.80 ± 1.11Bb	49.37 ± 0.81Bb	34.97 ± 2.57Cc	27.63 ± 1.60Dc	21.23 ± 1.06Ec	17.03 ± 1.18Ec

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

during egg aging caused by a gradual uptake of water from the albumen in stored eggs.[44] The viscosity of the albumen depends on the integrity of the ovomucin–lysozyme complex. Hence the β-fraction of ovomucin separates (breakage of ovomucin–lysozyme complex) and is released into solution, causing the thinning and the decrease in thick albumen.[41] Lysozyme–chitosan-coated eggs had significantly higher albumen viscosity values than uncoated eggs throughout the storage period (Table 6). The average albumen viscosity of uncoated eggs (4.67 mPa s) was significantly lower than that of eggs coated with chitosan (10.30 mPa s), 10% lysozyme–chitosan (13.97 mPa s), 20% lysozyme–chitosan (15.03 mPa s) and 60% lysozyme–chitosan (17.03 mPa s) after 6 weeks of storage. Significant differences were observed in the albumen viscosity of coated and uncoated eggs during storage, with 60% lysozyme–chitosan-coated eggs showing the highest values throughout the storage period (Table 6). According to these results, the 20 and 60% lysozyme–chitosan coatings were significantly most able to preserve albumen quality.

pH measurement

The effects of storage on egg quality can also be measured by the increase in albumen pH. The pH value of albumen from a freshly laid egg is around 7.5–8.5. Within a short time, the albumen pH increases to 9 owing to the release of CO_2. A highly significant interaction on group, storage time and group × storage time was observed for albumen pH ($P < 0.05$). The albumen pH values

Table 7. Effect of different coatings on albumen pH during 6 weeks of storage

	Albumen pH						
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	8.95 ± 0.04Aa	9.11 ± 0.03Ba	9.20 ± 0.02Ca	9.26 ± 0.03CDa	9.30 ± 0.03Da	9.40 ± 0.02Ea	9.69 ± 0.03Fa
1% CHI	8.95 ± 0.04Aa	9.00 ± 0.05Ab	9.11 ± 0.06Bb	9.15 ± 0.03BCb	9.19 ± 0.03CDb	9.25 ± 0.02Db	9.37 ± 0.02Eb
10% LYS	8.95 ± 0.04Aa	8.97 ± 0.05Ab	9.00 ± 0.05Ac	9.01 ± 0.08Ac	9.12 ± 0.02Bb	9.17 ± 0.02BCc	9.23 ± 0.02Cc
20% LYS	8.95 ± 0.04Aa	8.97 ± 0.05Ab	8.99 ± 0.04Ac	9.00 ± 0.05Ac	9.14 ± 0.03Bb	9.18 ± 0.03Bbc	9.26 ± 0.03Cc
60% LYS	8.95 ± 0.04Aa	8.98 ± 0.05ABb	9.04 ± 0.04Bbc	9.05 ± 0.03Bc	9.14 ± 0.03Cb	9.20 ± 0.02Cbc	9.28 ± 0.02Dc

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

Table 8. Effect of different coatings on total solids (dry matter) of albumen during 5 weeks of storage

	Total solids (% w/w)					
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	11.50 ± 0.01Aa	12.70 ± 0.17Ba	15.01 ± 0.23Ca	17.01 ± 0.12Da	17.94 ± 0.26Ea	19.46 ± 0.12Fa
1% CHI	11.50 ± 0.01Aa	11.71 ± 0.12Ab	12.20 ± 0.05Bb	13.96 ± 0.04Cb	15.53 ± 0.04Db	16.51 ± 0.26Eb
10% LYS	11.50 ± 0.01Aa	11.74 ± 0.06Ab	12.26 ± 0.03Bb	13.50 ± 0.31Cc	14.32 ± 0.15Dc	15.87 ± 0.39Ec
20% LYS	11.50 ± 0.01Aa	11.64 ± 0.06ABb	11.86 ± 0.05Bc	12.26 ± 0.08Cd	13.55 ± 0.02Dd	14.73 ± 0.03Ed
60% LYS	11.50 ± 0.01Aa	11.54 ± 0.04Ab	11.76 ± 0.06ABc	11.99 ± 0.06Bd	13.25 ± 0.02Ce	14.50 ± 0.04Dd

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

Table 9. Effect of different coatings on total solids (dry matter) of yolk during 5 weeks of storage

	Total solids (% w/w)					
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Control	45.26 ± 0.04Aa	44.81 ± 0.03Aa	43.39 ± 0.02Ba	42.59 ± 0.03Ca	41.94 ± 0.03Da	41.10 ± 0.02Ea
1% CHI	45.26 ± 0.04Aa	45.08 ± 0.03Ba	44.57 ± 0.04Cb	44.11 ± 0.02Db	43.57 ± 0.03Eb	42.98 ± 0.04Fb
10% LYS	45.26 ± 0.04Aa	45.13 ± 0.02Ba	44.84 ± 0.02Cb	44.46 ± 0.02Dc	43.82 ± 0.02Ec	43.70 ± 0.02Fc
20% LYS	45.26 ± 0.04Aa	45.21 ± 0.02Ba	44.99 ± 0.04Bc	44.64 ± 0.03Cd	44.04 ± 0.05Dd	43.79 ± 0.02Ed
60% LYS	45.26 ± 0.04Aa	45.21 ± 0.02Ba	45.07 ± 0.05Cc	44.69 ± 0.02De	44.16 ± 0.04Ed,e	43.91 ± 0.02Fe

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

were higher for uncoated eggs than for chitosan-coated eggs during storage (Table 7). The albumen pH is dependent on the equilibrium between dissolved CO_2, bicarbonate ions, carbonate ions and proteins.[27] The rise in albumen pH is caused by a loss of CO_2 from the breakdown of carbonic acid in egg white, resulting in changes in the bicarbonate buffer system.[7,45] The diffusion of CO_2 through the pores of the shell, which starts soon after the egg is laid, causes a sharp rise in pH, especially in the albumen. The enhancement effects of chitosan coatings with or without lysozyme in maintaining albumen freshness are due to the stability and sealing characteristics of the pores. The albumen pH for uncoated eggs ranged from 8.95 initially to 9.69 at the end of storage (Table 7). For coated eggs, albumen pH values reached 9.37 (chitosan), 9.23 (10% lysozyme), 9.26 (20% lysozyme) and 9.28 (60% lysozyme) at the end of storage. The chitosan coating works as a barrier to CO_2 diffusion during storage, helping to maintain albumen quality. The present results confirm that the chitosan coatings with lysozyme reduced CO_2 permeation through the eggshell pores, thus having an important effect in controlling the albumen pH of coated eggs. Some coating materials such as chitosan and mineral oils are effective barriers against the loss of CO_2 and other gases through the eggshell, thus helping to keep the albumen pH down.[7,23,35] All coated eggs had significantly lower albumen pH compared with uncoated eggs owing to the impairment of albumen CO_2 loss through the shell. There were no significant differences among lysozyme-coated eggs. The albumen freshness of lysozyme-coated eggs after 6 weeks was comparable to that of control eggs after 3 weeks and chitosan-coated eggs after 5 weeks.

Total solids (dry matter) of albumen and yolk

The total solid (dry matter) albumen (albumen refraction) (DMA) index, which measures the liquid concentration of albumen (index of refraction), has also been used as an indicator of egg freshness. There is no single reason for albumen thinning or liquefaction. This phenomenon could occur through protease enzymes, depolymerisation by hydroxyl ions at increasing pH or reduction by thiol-type reducing agents and the interaction of ovomucin–lysozyme complex. The albumen pH changes after the egg is laid, leading to destabilization of the ovomucin–lysozyme interaction.[46] Water contained in the albumen permeates the yolk, and some nutrients contained in the yolk can permeate the albumen. These osmotic paths and changes in albumen and yolk concentrations can be measured by refractometry. Within a short time, the DMA increases owing to mixing of the yolk into the albumen.

A highly significant interaction on group, storage time and group × storage time was observed for DMA. Albumen DMA values for uncoated eggs were higher than those for all coated eggs (Table 8). The increase in albumen dry matter during storage has been attributed to liquefaction of the yolk and subsequent mixing into the albumen. Thick albumen is a gel and thin albumen is a fluid. During storage, the gelatinous structure of thick albumen changes its physical and chemical characteristics and gradually breaks down into a clear liquid, losing its consistency. The DMA of uncoated egg albumen ranged from 11.50 initially to 19.46 at the end of storage (Table 8). For coated egg albumen, DMA values reached 16.51 (chitosan), 15.87 (10% lysozyme), 14.73 (20% lysozyme) and 14.50 (60% lysozyme) at the end of storage. Eggs coated with 20 and 60% lysozyme–chitosan displayed significantly lower albumen

Table 10. Effect of different coatings on albumen relative whipping capacity (albumen foaming capacity) during 6 weeks of storage

	Relative whipping capacity (mL)						
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	1025.00 ± 41.83Aa	900.00 ± 31.62Ba	766.67 ± 40.82Ca	666.67 ± 60.55Da	533.33 ± 40.82Ea	400.00 ± 31.62Fa	300.00 ± 44.72Ga
1% CHI	1025.00 ± 41.83Aa	985.71 ± 37.80ABab	914.29 ± 37.80BCb	850.00 ± 70.71Cb	692.86 ± 60.75Db	550.00 ± 40.82Eb	407.14 ± 34.50Fb
10% LYS	1025.00 ± 41.83Aa	1035.71 ± 37.80Ab	978.57 ± 26.73ABb	935.71 ± 37.80Bbc	878.57 ± 26.73Cc	792.86 ± 44.99Cc	700.00 ± 28.87Dc
20% LYS	1025.00 ± 41.83Aa	1014.29 ± 24.40Ab	964.29 ± 37.80Ab	942.86 ± 44.99Ac	842.86 ± 44.99Bc	771.43 ± 39.34Bc	614.29 ± 24.40Cc
60% LYS	1025.00 ± 41.83Aa	1014.29 ± 37.80ABb	971.43 ± 39.34ABb	928.57 ± 26.73BCbc	878.57 ± 56.69Cc	778.57 ± 39.34Dc	692.86 ± 44.99Dc

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

Table 11. Effect of different coatings on whole egg relative whipping capacity (foaming capacity) during 6 weeks of storage

	Relative whipping capacity (mL)						
Coating[a]	0 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
Control	725.00 ± 27.39Aa	683.33 ± 40.82ABa	641.67 ± 49.16BCa	591.67 ± 37.64Ca	416.67 ± 51.64Da	375.00 ± 27.39Da	266.67 ± 25.82Ea
1% CHI	725.00 ± 27.39Aa	714.29 ± 24.40Aa	657.14 ± 44.99ABa	628.57 ± 26.73Bab	442.86 ± 34.50Cab	400.00 ± 28.87CDb	342.86 ± 44.99Dab
10% LYS	725.00 ± 27.39Aa	721.43 ± 26.73Aa	671.43 ± 26.73Aa	671.43 ± 26.73Ab	500.00 ± 50.00Bb	435.71 ± 37.80BCb	400.00 ± 40.82Cb
20% LYS	725.00 ± 27.39Aa	714.29 ± 24.40Aa	664.29 ± 24.40Aa	664.29 ± 24.40Aab	485.71 ± 47.56Bab	442.86 ± 34.50BCb	385.71 ± 37.80Cb
60% LYS	725.00 ± 27.39Aa	721.43 ± 26.73Aa	685.71 ± 55.63Aa	678.57 ± 56.69Ab	464.29 ± 37.80Bab	421.43 ± 26.73BCb	371.43 ± 26.73Cb

Data are expressed as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$). Means in the same row with different capital letters are significantly different ($P \leq 0.05$).
[a]1% CHI, chitosan coating; 10% LYS, chitosan coating with 10% lysozyme; 20% LYS, chitosan coating with 20% lysozyme; 60% LYS, chitosan coating with 60% lysozyme.

DMA values than eggs coated with 10% lysozyme–chitosan and chitosan. However, Jones[47] reported that albumen total solids were fairly consistent throughout storage (low, 12.2%; high, 12.59%).

Yolk DMA decreased significantly during storage. A highly significant interaction on group, storage time and group × storage time was observed for DMA. Water transfer from egg white lowers the solid content of the yolk by 2–4% during storage.[48] The DMA of uncoated egg yolk ranged from 45.26 initially to 41.10 at the end of storage (Table 9). Significant differences in yolk DMA were observed between uncoated and all coated samples ($P < 0.05$). Eggs coated with 60% lysozyme–chitosan had significantly higher yolk DMA values than all others, while eggs coated with 20% lysozyme–chitosan had the next highest values (Table 9). Yolk total solids decreased during storage from a maximum of 48.25% at 0 weeks to 43.17% at 10 weeks according to Jones.[47] The total solids detected in the present study were in accordance with those of Stadelman and Cotterill,[49] who reported the following levels of egg solids: albumen, ~12%; yolk, 44–48% (from commercial egg separators); whole egg, 23–25%.

Relative whipping capacity (foaming properties)

Foams are thermodynamically unstable, and their relative stability is affected by factors such as drainage, disproportionation and/or coalescence. It is the properties of the two air/water interfaces of thin films that make or break foams. Several factors influence the rheology of foams, including air phase volume, liquid phase viscosity, interfacial tension and viscosity and bubble size, size distribution and shape.[50–53]

The whippability of egg white can be assayed by measurement of foam volume and foam stability (amount of liquid released from the foam in a given time). Owing to a large surface area increase in the liquid/air interphase, proteins denature and aggregate during whipping. In particular, ovomucin forms a film of insoluble material between the liquid lamella and air bubble, thereby stabilizing the foam. A highly significant interaction between coating and storage time was observed for relative whipping (albumen foaming) capacity ($P < 0.01$). There was a linear decrease in RWC (foaming capacity) over time. In all storage periods, the volume of drained liquid from albumen foam (RWC) was greater for uncoated eggs (Table 10). As time passed, the films became progressively thinner and ruptured. Throughout the storage period, fluid is lost by lamellar water drainage, resulting in foam collapse.[54] There was a significant chitosan coating, lysozyme–chitosan coating and storage time interaction effect for the foaming capacity of albumen and also whole eggs. There were significant differences in the RWC of albumen for chitosan (407), uncoated (300), 10% lysozyme (700), 20% lysozyme (614) and 60% lysozyme (692) treatments (Table 10). There were also significant differences in the RWC of whole eggs for chitosan (342), uncoated (266), 10% lysozyme (400), 20% lysozyme (385) and 60% lysozyme (371) treatments (Table 11).

The reductions in RWC after 4 weeks were 42.62% (uncoated), 39.03% (chitosan), 31.03% (10% lysozyme), 33.10% (20% lysozyme) and 36% (60% lysozyme). After 6 weeks of storage, the reductions were 63.3% (uncoated), 52.8% (chitosan) and 31–36% (lysozyme). These results demonstrated that lysozyme–chitosan coatings maintained foaming properties (whipping capacity, RWC) during storage. The pH in the aqueous phase determines the magnitude and nature of protein charges and therefore affects the foam stability.[55]

Egg globulin also contributes to this effect by increasing the fluid viscosity and decreasing the surface tension.[27] It is known that the pH increases with storage and, as a consequence, part

of the egg white N-ovalbumin is transformed into S-ovalbumin (less hydrophobic then N-ovalbumin). This interferes with the formation of a cohesive film at the air/water interface, causing a decrease in foam stability, and thus the correlation between the content of S-ovalbumin and the volume of drained liquid is positive.[56,57]

CONCLUSIONS

Egg freshness is related to egg quality. The present results indicate that egg weight loss, albumen pH, HU and RWC are parameters that are greatly influenced by chitosan coating and storage time. This study showed that lysozyme–chitosan coating solutions could be effectively produced and had beneficial effects in extending the shelf life of eggs during storage by delaying the loss of interior quality. Chitosan coatings with or without lysozyme significantly increased shell strength and would therefore reduce eggshell breakages during handling and storage. The 20% lysozyme–chitosan coating maintained internal quality (especially HU and pH) for at least 3 weeks longer than observed for uncoated control eggs during storage. The present study clearly indicates that chitosan coatings containing 10, 20 and 60% lysozyme show promise as a viable alternative to existing techniques for maintaining functional properties (pH, viscosity, total solids and RWC as interior quality) of eggs that are adversely affected by length of storage. The study also confirms that measurements of albumen quality (pH, total solids, viscosity and RWC) are excellent indicators of egg freshness.

Further studies are desirable to apply different coatings with different antioxidant and antimicrobial effects on various perishable food products.

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