

Article

Effects of Chitosan Coating Structure and Changes during Storage on Their Egg Preservation Performance

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Abstract: To explore the influences of chitosan coating structure and structure changes during storage on egg preservation, eggs coated by chitosan solution for single time (CS1), two times (CS2), and three times (CS3) were prepared separately and stored with untreated eggs (CK1), eggs washed by water (CK2) and eggs treated by acetic acid solution (CK3) at 25 °C, 80% RH. The weight loss, Haugh unit, yolk index, albumen pH, eggshell morphologies and infrared (FTIR—Fourier Transform Infrared) spectra of all the samples were monitored. CS2 and CS3 presented the lowest weight loss, highest Haugh unit and yolk index, stabilized pH, and the highest thickness of chitosan coating layers (>2 μm) among all the groups, which extended egg shelf life for 20 days longer compared to CK1 and CK2. CS1 with very thin chitosan coating showed similar egg qualities with CK3, which are second only to CS2 and CS3. Furthermore, destructions were found on chitosan coatings during storage as revealed by the eggshell morphologies and FTIR spectra, which caused the quality deterioration of eggs. The results demonstrated that eggs with the thickest coating showed the best qualities during storage, while destructions on coating layers led to the quality drop of eggs.

Keywords: chitosan; coating; structural changes; egg preservation; shelf life

1. Introduction

Eggs are produced and consumed daily all over the world on a large scale, as excellent and inexpensive sources of high quality protein, certain minerals, and vitamins [1]. Nowadays, they have also been considered as ideal vehicles of bioactive components to enhance human nutrition [2]. Although the eggshells have been regarded as natural protective barriers of eggs, quantities of tiny pores presenting on the eggshells for gas exchange lead to the moisture and CO₂ loss of eggs, as well as the penetration of microbial, which would then cause the quality deterioration of eggs [3,4]. Moreover, eggshells are too fragile to retain their integrity during transport. Even a tiny crack in an eggshell would largely increase the risk of microbial contamination, particularly when eggs are stored at room temperature.

Attracted by the huge economic benefit, many strategies have been studied for egg preservation, which can be generally classified into two types based on the mechanism. The first one is to deactivate the microorganisms on the eggshells, including techniques such as ultrasonic treatment [5], ozone treatment [6], application of AgNPs-doped paper egg trays [7], and vacuum packaging [8]. The other one is to seal the pores on eggshells by coating, which serves as good barrier layers towards water vapor, gases, and microorganisms. The most widely studied coating materials are oil [9,10], proteins [11], biopolymers [3], etc. Biopolymers composed of biomass or derived polymers are intensively studied as edible coatings [12,13]. Among them, chitosan has attracted much attention of researchers due

to its extraordinary performance, such as the excellent film forming property, low gas permeability, satisfied biocompatibility [14], and strong antimicrobial activities [15]. Therefore, coating of chitosan on eggshells can protect egg from both physicochemical changes and microbial contamination, leading to promising effects on quality preservation of eggs. Owing to the low cost, wide availability of chitosan and the operational feasibility of coating process, chitosan coating is considered an efficient and practical way to preserve eggs at room temperature. The influencing factors on the efficiency of chitosan coating such as the molecular weight [16], plasticizer types [17], sources of chitosan [18], and crosslinking agents [19] have been widely investigated. Combination coatings of chitosan with natural antimicrobial agents [20], pullulan [3], oil [21], and montmorillonite [22] have also been applied to eggs and showed improved performance.

However, although the coating materials have been intensively studied, the structure of the chitosan coating layer and their effects on egg preservation has not been carefully investigated so far. Suresh et al. [23] have coated eggs by chitosan a single time and three times, respectively. The coating layer on the eggshell with three-time coating was 20 μm thicker than that with single time coating, but no significant differences were found in weight loss, Haugh unit and albumin index of eggs between these two groups at 22 ± 1 °C. The preservation effects of chitosan coatings should be mainly dependent on their barrier properties and antimicrobial activities, which are highly related to the thickness and structures of coating layers on the eggshells.

Therefore, in this study, eggs were coated with chitosan one, two, and three times, separately, to observe their differences in coating structure and structural stability during storage in order to study the relationship between the coating structure and preservation effects. The quality of coated eggs were compared with those of the untreated, water washed and acetic acid treated eggs.

2. Materials and Methods

2.1. Materials and Reagents

The freshly laid eggs were provided by a local farm of Chongqing, China. Chitosan with molecular weight of 180 kDa and deacetylation degree of 90% was supplied by Weifang Haizhiyuan Biological Products Co., Ltd. (Weifang, China). Other chemicals were of analytical grade and were used as received.

2.2. Treatment of Eggs

Chitosan coating solution with a concentration of 0.5% (w/w) was prepared by dissolving certain amount of chitosan granules in 1 vol % acetic acid and stirring for 24 h. The freshly laid eggs were divided into 6 groups with 50 eggs in each. There were three coated groups, which were coated by chitosan solution for one, two and three times, separately, and were designated as CS1, CS2, and CS3, correspondingly. Each coating process was carried out as immersing eggs in the prepared coating solution for 2 min and drying under ambient conditions overnight with their small-ends down. The other three groups were set as control, including the untreated eggs (CK1), water washed eggs (CK2), and eggs immersed in 1 vol % acetic acid for 2 min (CK3). After the above treatments, all the eggs were placed in egg trays with their small-ends down, and were stored at 25 °C and 80% relative humidity (RH). Five marked eggs of each group were taken out to weigh every 5 days, and were returned back right after the measurement. Another five eggs of each group were picked up randomly to measure the Haugh unit, yolk index and pH of albumen every 5 days.

2.3. Characterizations of Eggs

2.3.1. Weight Loss

Weight loss (%) of a whole egg was calculated as the weight difference in percentage of an egg during storage compared to its whole weight at day 0 [24]. The weight of five marked eggs in each groups was measured using a balance every 5 days and given as average value \pm standard deviation.

2.3.2. Haugh Unit

Haugh unit is a value related to egg weight and thick of albumen, which was calculated using Equation (1) [17]:

$$HU = 100 \log(H - 1.7 \times W^{0.37} + 7.6) \quad (1)$$

where H is the albumen height (mm) and W is the weight of whole egg (g).

According to the United States Standards for Quality of Individual Shell Eggs (USDA 2000 [25]), eggs are classified in to AA, A and B grade, which require Haugh unit value to be above 72, 71 to 60, and below 60, correspondingly.

2.3.3. Yolk Index

Yolk index was calculated as the ratio of yolk height to yolk width [9]. The height and width of egg yolk were measured using a micrometer.

2.3.4. pH of Albumen

pH values of the albumen separated from the yolk was measured by a digital pH meter.

2.3.5. Scanning Electron Microscopy (SEM) of Eggshells

Morphologies of the surfaces and cross sections of eggshells were observed using a scanning electron microscope (JEM-2100, JEOL Ltd., Tokyo, Japan) after platinum sputtering of the samples.

2.3.6. Fourier Transform Infrared (FTIR) Spectra of Eggshells

FTIR spectra of the outer surface of eggshells were recorded using a FTIR spectrophotometer (Thermo-Nicolet 6700, Thermo Fisher Scientific, Waltham, MA, USA) with attenuated total reflectance (ATR) accessories. For each sample, 32 scans at 4 cm^{-1} resolution were used to collect the ATR spectra of the outer surface of eggshells.

2.3.7. Statistical Analysis

All the experiments were repeated at least three times to calculate the average values and standard deviations unless otherwise stated. All the data were statistically compared among groups by one-way analysis of variance (ANOVA). The significances of the mean values were determined by Duncan's multiple range testing with $p < 0.05$.

3. Results

3.1. Weight Loss

Generally, the weight loss of eggs gradually increased during storage, which was attributed to the escaping of CO_2 and water vapor in albumen through numerous pores on the eggshells [24]. The loss of CO_2 and water was responsible to many physical and chemical changes in albumen and yolk resulting in deterioration of eggs. Therefore, weight loss rate of eggs is an important index of egg quality. As shown in Table 1, the weight losses of CK1 and CK2 increased rapidly over time, which were significantly higher than others at day 36. CK3 and CS1 showed lower average values of weight loss, but were not significantly different from those of CK1 and CK2 ($p < 0.05$) until day 36.

However, the weight losses of CS2 and CS3 were significantly lower than those of CK1 and CK2 since day 16. Up to day 36, the weight loss of CS2 was 29% less than that of CK2. The above results revealed that acetic acid treatment and one-time coating of chitosan showed limited effects to reduce the loss of CO₂ and water vapor of eggs, while coating two or three times largely slowed down the weight loss rate during storage. It is known that the main component of eggshell is CaCO₃ [26], which would react with acetic acid solution leading to some structural changes on the eggshell surface. Further coating with chitosan was more effective to seal the pores on the eggshells and to form dense and barrier outer layers, leading to considerably inhibited weight loss. Therefore, observations on the morphologies of eggshells would be helpful to further understand the weight loss change of each group.

Table 1. Changes in weight loss of different groups during storage.

Storage Time (day)	Weight Loss (%)					
	CK1	CK2	CK3	CS1	CS2	CS3
6	0.90 ± 0.07 ^a	1.01 ± 0.37 ^a	0.88 ± 0.17 ^a	0.78 ± 0.11 ^a	0.92 ± 0.17 ^a	0.80 ± 0.19 ^a
11	1.90 ± 0.15 ^a	2.12 ± 0.81 ^a	1.72 ± 0.47 ^a	1.66 ± 0.24 ^a	1.67 ± 0.45 ^a	1.68 ± 0.42 ^a
16	2.65 ± 0.23 ^a	2.76 ± 0.02 ^a	2.46 ± 0.45 ^{a,b}	2.31 ± 0.32 ^{a,b}	1.91 ± 0.27 ^b	2.01 ± 0.32 ^b
21	3.22 ± 0.24 ^{a,b}	3.27 ± 0.39 ^a	3.18 ± 0.58 ^{a,b}	2.95 ± 0.41 ^{a,b}	2.46 ± 0.35 ^b	2.61 ± 0.42 ^{a,b}
26	3.93 ± 0.33 ^{a,b}	4.21 ± 0.02 ^a	3.87 ± 0.69 ^{a,b,c}	3.58 ± 0.48 ^{a,b,c}	2.99 ± 0.42 ^c	3.19 ± 0.50 ^{b,c}
31	4.72 ± 0.36 ^a	4.74 ± 0.56 ^a	4.19 ± 0.07 ^{a,b}	3.96 ± 0.25 ^{a,b}	3.61 ± 0.52 ^b	3.84 ± 0.60 ^b
36	5.92 ± 0.48 ^{a,b}	6.18 ± 0.04 ^a	5.09 ± 0.09 ^{b,c}	5.02 ± 0.47 ^{b,c}	4.38 ± 0.63 ^c	4.64 ± 0.73 ^c

Note: Means in the same row with different superscripted letters (a–d) are significantly different ($p < 0.05$).

3.2. Haugh Unit

Haugh unit is an important index of egg quality [27], which is determined by the age-related changes of egg white proteins [28]. The reduced Haugh unit value during storage is a result of albumen thinning, which was caused by the increased concentration of clusterin and ovoinhibitor, as well as the disordering of ovalbumin structure [29]. These protein changes have been mainly attributed to the proteolysis of dense protein or to the increase of albumin pH [30], which are influenced by the losses of water and CO₂ during storage. As given in Table 2, the Haugh unit values of CK1 and CK2 decreased rapidly, which were significantly lower than those of other treated groups after stored for only 6 days. Three chitosan coated groups had similar Haugh unit values during the first 11 days. However, CS1 showed a sharp drop from day 11 to 16, making the value comparable to that of CK3. Moreover, the Haugh unit values of CS2 and CS3 were always significantly higher than those of CS1 and CK3 from day 16 to day 31. According to the egg grading standard given in 2.4, both CK1 and CK2 fell to grade B at day 16, followed by CK3 and CS1 both at day 21, while CS2 and CS3 were in grade B since day 36 and 31, respectively. Therefore, coating eggs with chitosan two times showed the best performance to significantly slow down the structural changes in albumen proteins, thus extending the shelf life by up to 20 days longer at 25 °C, 80% RH according to Haugh unit values.

Table 2. Changes in Haugh unit of different groups during storage.

Storage Time (day)	Haugh Unit					
	CK1	CK2	CK3	CS1	CS2	CS3
0	83.83 ± 0.81	83.83 ± 0.81	83.83 ± 0.81	83.83 ± 0.81	83.83 ± 0.81	83.83 ± 0.81
6	65.43 ± 2.85 ^c	62.34 ± 0.55 ^c	73.69 ± 4.56 ^b	74.90 ± 1.56 ^{a,b}	78.92 ± 3.98 ^{a,b}	80.44 ± 4.70 ^a
11	62.92 ± 1.67 ^c	61.72 ± 1.27 ^c	68.68 ± 1.89 ^b	73.37 ± 4.43 ^{a,b}	73.37 ± 4.43 ^{a,b}	73.54 ± 3.50 ^a
16	58.10 ± 2.14 ^c	57.89 ± 1.40 ^c	60.02 ± 3.46 ^{b,c}	62.04 ± 3.42 ^{b,c}	72.22 ± 4.58 ^a	73.09 ± 1.75 ^a
21	56.54 ± 3.93 ^b	54.39 ± 2.31 ^b	54.35 ± 2.69 ^b	54.03 ± 0.96 ^b	66.27 ± 1.41 ^a	68.34 ± 5.01 ^a
26	54.54 ± 5.25 ^c	57.04 ± 2.33 ^b	53.33 ± 1.79 ^c	55.16 ± 1.16 ^{b,c}	63.28 ± 3.81 ^a	65.79 ± 3.76 ^a
31	53.91 ± 2.89 ^b	52.81 ± 2.98 ^b	53.29 ± 3.89 ^b	54.55 ± 7.41 ^b	60.56 ± 5.26 ^a	56.95 ± 1.65 ^{a,b}
36	51.39 ± 2.74 ^a	50.53 ± 3.20 ^a	52.37 ± 1.08 ^a	53.96 ± 0.91 ^a	57.95 ± 8.11 ^a	56.53 ± 2.89 ^a

Means in the same row with different superscripted letters are significantly different ($p < 0.05$).

3.3. Yolk Index

Yolk index is another important measure of egg freshness besides Haugh unit, which is based on the yolk quality. As shown in Figure 1, CK1 and CK2 showed the similar and maximum decreasing rate of yolk index with storage time, followed by CK3 and CS1, while CS2 and CS3 presented the highest values during the whole storage. Moreover, it was worth noting that the yolk index of all the groups almost decreased linearly in two time periods, which were day 0 to 16, and day 20 to 36, respectively. To clearly demonstrate the changing trend, each curve in Figure 1 was linear fitted separately in these two periods. The slopes and correlation coefficients obtained from the linear fittings were listed in Table 3, which revealed some important results. First, the slope values of each curve were much higher in the first time period (day 0 to 16) than those in the second period (day 21 to 36), indicating that the yolk quality declined more seriously in the first time period compared to the second one. Second, the order of all the slope values in the first time period from small to large was CS2, CS3, CS1, CK3, CK1, and CK2. Particularly, CS2 and CS3 showed quite lower values compared to those of other groups. Third, the slope values of CS1, CS2, and CS3 were still lower than those of CK1, CK2, and CK3 in the second time period, but the differences among them became much smaller compared to those in the first period. Therefore, the above results showed that coating eggs with chitosan two or three times was very effective to slow down the quality drop of egg yolks, especially in the most important period of egg quality control, which is the first 16 days of storage.

The decreased values of yolk index with storage time was a result of liquefaction and flattening of yolk, which was attributed to the constant permeation of water from albumen to yolk through vitelline membrane driven by the osmotic pressure [11]. The osmotic pressure between albumen and yolk was associated to the albumen viscosity, which was reduced by the breakage of ovomucin-lyzyme complex. Therefore, when chitosan coating reduced the loss of CO₂ and water vapor and slowed down the structural changes in albumen, the increase in osmotic pressure between albumen and yolk would be slowed down resulting in improved yolk quality.

Furthermore, it was interesting to notice that the yolk index of CS1 showed a sharp decrease from day 11 to 16 as pointed by an arrow in Figure 1, which was in accordance with the rapid drop as mentioned above in Haugh unit value of CS1. As the changes in both yolk index and Haugh unit were associated to the barrier properties of eggshells, the sharp reductions of these values of CS1 might indicate some structural changes occurred on the chitosan coatings at this time period.

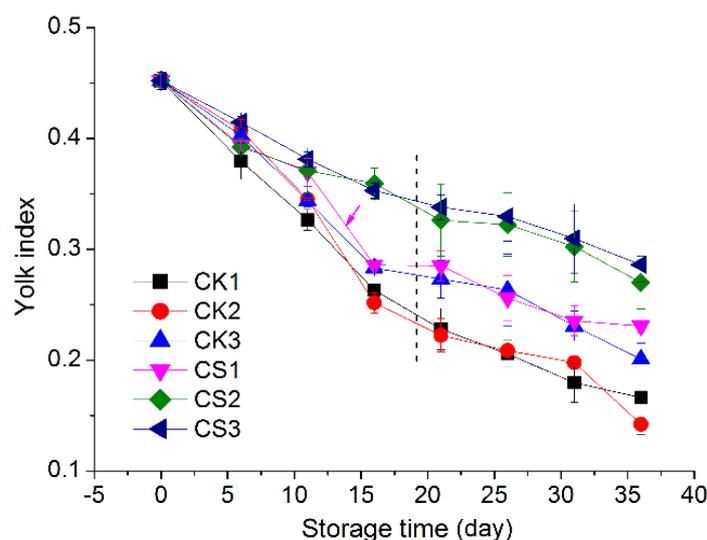


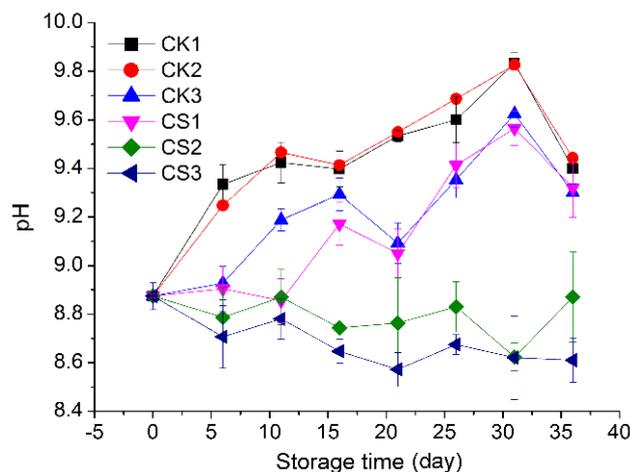
Figure 1. Changes in yolk index of eggs in different groups during storage.

Table 3. Slopes and correlation coefficients (R^2) of the yolk index curves of different groups at two time periods.

Samples	0–16 Days		21–36 Days	
	Slope	R^2	Slope	R^2
CK1	−0.0117	0.9990	−0.0042	0.9852
CK2	−0.0124	0.9585	−0.0050	0.8514
CK3	−0.0106	0.9916	−0.0050	0.9591
CS1	−0.0098	0.9536	−0.0037	0.9162
CS2	−0.0057	0.9064	−0.0038	0.8962
CS3	−0.0062	0.9991	−0.0035	0.9625

3.4. pH of Albumen

Since the albumen of fresh-laid eggs are saturated with CO_2 , the evacuation of CO_2 through eggshells led to an increase in the albumen pH with increasing storage time. As given in Figure 2, the albumen pH of CK1 and CK2 increased to as high as 9.8 after storage for 31 days. The followed decrease at day 36 might be attributed to the breakdown of proteins in the albumen to fat and peptone. The albumen pH values of CK3 and CS1 presented similar changes, but were always lower than those of CK1 and CK2 at the same time. The pH value of CS1 also showed a sharp increase from day 11 to day 16. However, the albumen pH value of CS2 and CS3 was stabilized between 8.5 and 8.9 during the whole storage. Similar results have also been observed in eggs coated by proteins [11] and edible oils [31]. The stable albumen pH of CS2 and CS3 should be a result of the effective reductions of CO_2 loss in albumen, which further confirmed the considerably improved barrier properties of eggshells by chitosan coating two or three times.

**Figure 2.** Changes in pH of albumen of eggs in different groups during storage.

3.5. Morphology of Eggshells

It has been generally agreed that coating eggs with polymers is able to seal the pores on eggshells and form barrier layers, thus providing effective protections to eggs to extend their shelf life. Therefore, it is necessary to examine and compare the eggshell morphologies of eggs in different groups, which is closely related to their barrier properties. The micrographs of the eggshell surfaces of all the groups are given in Figure 3. A large number of micro-cracks were observed on the surfaces of CK1 and CK2, which were probably produced during handling due to the fragile nature of eggshells. These micro-cracks would not only accelerate the permeation rate of CO_2 and water vapor from eggs to surroundings, but also provide pathways for the entry of bacteria. As a result, the protection function of eggshells would be largely compromised. However, after eggs were soaked in 1% acetic acid for 2 min, the micro-cracks on the eggshell surface of CK3 disappeared. It might be because the main

component of eggshell, CaCO_3 , reacts with acetic acid resulting in the filling up of micro-cracks on the eggshell surface. Therefore, the barrier properties of the eggshell of CK3 were superior to those of CK1 and CK2, which explained the improved quality of CK3. Moreover, the eggshell surfaces of CS1, CS2, and CS3 presented similar dense morphologies with that of CK3 when viewed at a low magnification ($200\times$).

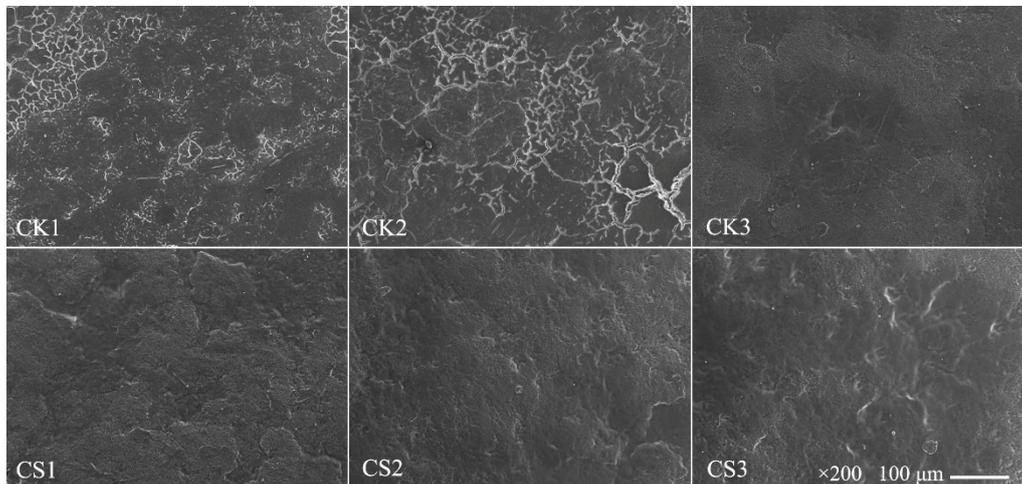


Figure 3. Scanning electron microscopy (SEM) micrographs of eggshell surfaces at day 0.

Therefore, the micrographs of the eggshell cross-sections were examined and given in Figure 4, chitosan coating layers with different thickness were found on the outside of the eggshells of CS1, CS2, and CS3. The chitosan coating layer on the eggshell of CS1 showed a thickness of approximately $0.7\ \mu\text{m}$, which was too thin to homogeneously cover the whole eggshell and to act as a dense and effective barrier layer. This might be the reason that CS1 showed comparable performance with that of CK3 during storage. The thicknesses of the coating layers of CS2 and CS3 were close to each other, and were larger than $2\ \mu\text{m}$. Although a dense eggshell surface was obtained by merely acetic acid treatment of eggs, eggshells of CS2 or CS3 coated by chitosan two or three times, which formed a dense polymer layer with a thickness of several micrometers on the outside of eggshells, provided more effective barriers compared to that of CK3.

Another thing that needed to be figure out is whether there are structural changes on the coating layers with increasing storage time, when they are constantly influenced by the environmental conditions and the gas transfer between eggs and surroundings. If the dense structure of coating layers was destructed during storage, the barrier properties of eggshells would certainly be compromised thus affecting the physical and chemical changes of eggs. Therefore, micrographs of eggshell surfaces were taken at different times during storage with a magnification of $2000\times$ as given in Figure 5. It was clearly observed that the cracks on the eggshells of CK1 were gradually widened with increasing storage time, which indicated the weakened protection functions of the eggshell with time. For CK3, a stable structure with little change on the surface morphologies was observed on the eggshells treated by acetic acid. For CS1 and CS2, their eggshell surfaces were somewhat blurred at day 0 because of the soft texture of chitosan coating. As the storage time increased, some parts of the surfaces became clear with some micro-pits on it, which were similar to the morphology of CK3. Therefore, it might reveal that the dense structure of chitosan coating was gradually destructed during storage. Furthermore, this phenomenon was firstly observed for CS1 at day 11, while for CS2 at day 16 as observed in Figure 5. Since the coating layer on the eggshell of CS1 was thinner than that of CS2, it was reasonable that the coating on CS1 was more vulnerable to damages.

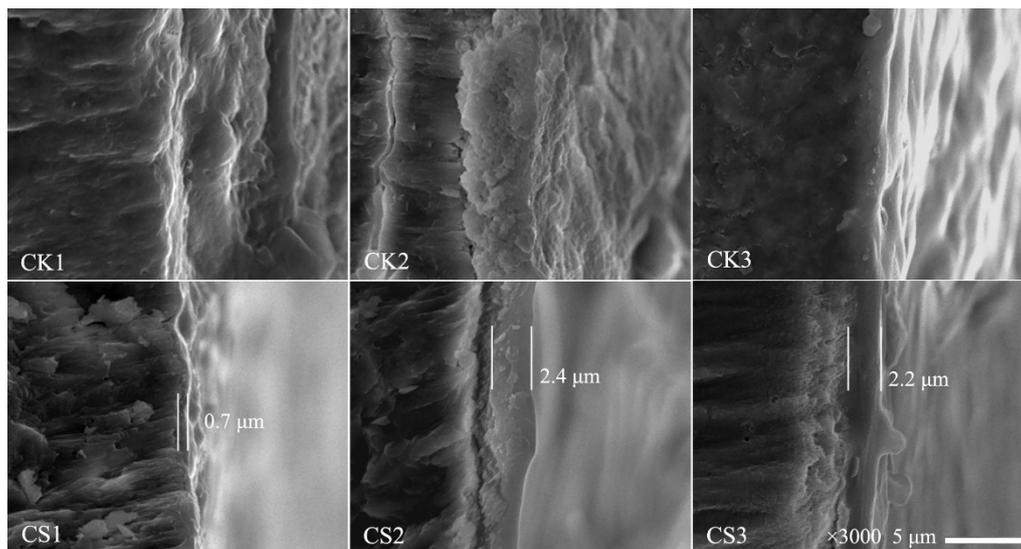


Figure 4. SEM micrographs of eggshell cross-sections at day 0.

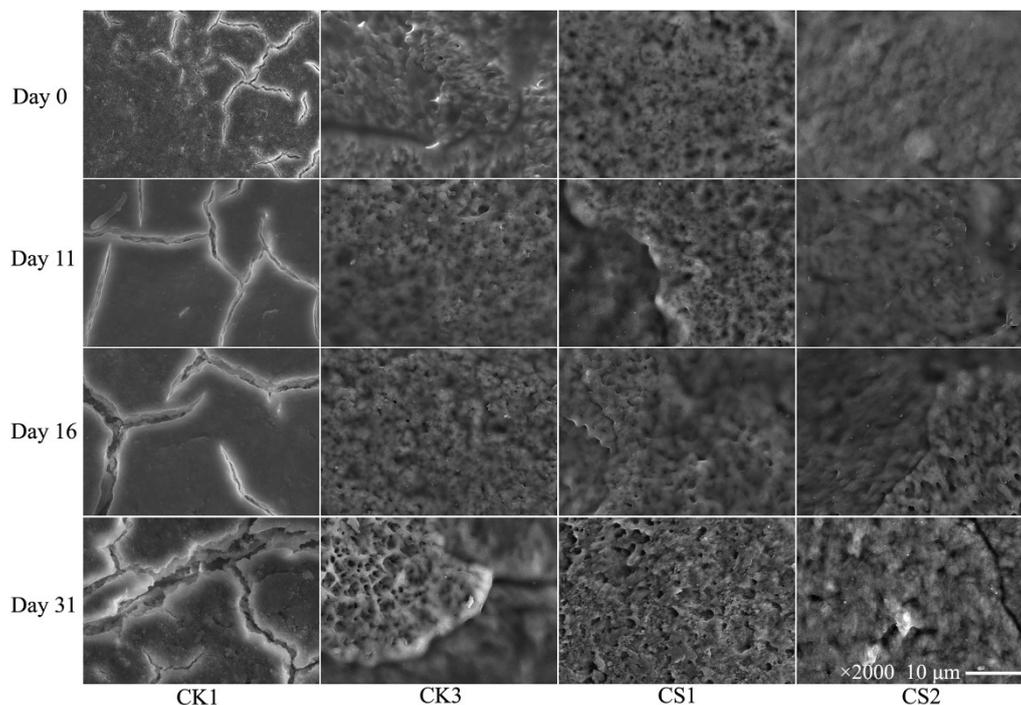


Figure 5. SEM micrographs of eggshell surfaces at day 0, 11, 16, and 31.

3.6. FTIR Spectra of Eggshells

To further confirm the structural changes on the coatings, the compositional changes of the eggshells were analyzed by the FTIR spectra, which was taken on the outer surface of eggshells at day 0 and day 36, respectively. As given in Figure 6, the spectra of CK1, CK2, and CK3 showed peaks at 872 cm^{-1} and 1397 cm^{-1} , which were due to the out-plane deformation and stretching vibration of carbonate groups, respectively [32], while peaks at 1026 cm^{-1} and 1647 cm^{-1} might be attributed to the C–O–C vibration of polysaccharides [33] and amide I band of proteins on the eggshell cuticles, respectively [34]. However, the intensity of peaks at 1026 cm^{-1} and 1647 cm^{-1} were relatively lower than that of peaks at 872 cm^{-1} and 1397 cm^{-1} in the spectra of CK3 compared to that of CK1 and CK2, which revealed the loss of polysaccharides and proteins on the eggshell cuticles after soaking in acetic

acid solution. For CS2 and CS3, a new absorption at 1064 cm^{-1} appeared, which might be assigned to the asymmetric stretching of C–O–C of chitosan [35].

After storage for 36 days, the spectra of CK1, CK2, and CK3 showed little changes compared to those of them at day 0, which indicated that the compositions of their eggshell cuticles were stable. However, for CS1, CS2, and CS3, the intensity of peaks at 1064 cm^{-1} significantly decreased in day 36 compared to those in day 0, which further confirmed the destructions of chitosan coating layer in the coated groups during storage.

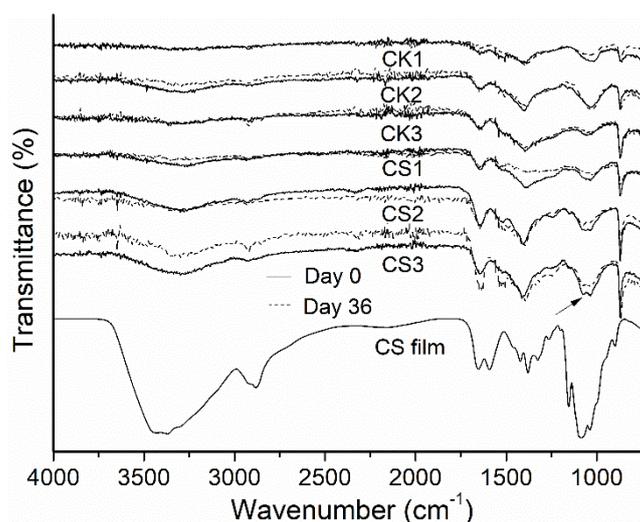


Figure 6. Fourier Transform Infrared (FTIR) spectra of CS film and eggshell surfaces at day 0 (solid line) and day 36 (dashed line).

4. Discussion

When changes in egg qualities are correlated to variations in coating morphologies, the relationship between them can be observed. CS2 and CS3 showed the highest thickness of coating layers and they were close to each other. At the same time, they both exhibited the best qualities during storage. CS1 and CK3 had thinner coatings presented poorer qualities but better than those of CK1 and CK2. It indicated that thicker coating layers led to better performance of coated eggs, and similar thickness of coating resulted in similar quality of eggs during storage. Therefore, it confirms that the thickness of chitosan coating layer, which determines their barrier properties, is responsible to the preservation performance of coatings on eggs.

Secondly, destructions on chitosan coating for different samples occurred at different time of storage. For CS1, destructions were observed on day 11, which was the same time that weight loss, Haugh unit, yolk index, and pH of CS1 showed sharp changes. Similarly, from day 16 to 21, more significantly changes on weight loss, Haugh unit, yolk index of CS2 were also observed compared to other time periods, which could correspond to the destructions on coatings of CS2 that appeared on day 16. Therefore, it indicated that egg quality was quite sensitive to the structural changes of coating layers during storage, which was dominant to the barrier properties of eggshells. However, thicker coatings are more resistant to the damages, thus exhibiting better preservation effects on eggs during the whole storage. The above findings inspired us to further extend the shelf life of eggs, efforts should be made not only to improve the initial barrier properties of the coatings, but also to enhance their structural stability during storage.

5. Conclusions

The results revealed that eggs coated by chitosan two or three times with a coating thickness of more than $2\text{ }\mu\text{m}$ showed a shelf life up to 36 days at $25\text{ }^{\circ}\text{C}$, 80% RH, which was 15 days longer than those of CS1 and CK3, and 20 days longer than those of CK1 and CK2. The thicker the coating layer,

the better quality of the coated eggs. Therefore, the preservation effects of chitosan coating on eggs should be predominantly attributed to the barrier properties of coatings. However, destructions in the coating structure with increasing storage time were revealed by the micrographs and FTIR spectra of the outer surfaces of eggshells. Although the mechanism needed to be further explored, this study inspired us to effectively improve the storage qualities of eggs, coatings with stable structure and durable barrier properties should be developed.

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Conflicts of Interest: The authors declare no conflict of interest.

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