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Effect of solvent types in chitosan-based nanocomposite coating on internal quality and eggshell morphology

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Abstract

In recent years, the use of bio-friendly nanocomposite coating as egg packaging has vigorously developed considering environmental consequences arising from using synthetic polymers. This study aimed at investigating the effect of solvent types and montmorillonite incorporated in a chitosan-based coating on internal quality and eggshell morphology. Chitosan-based coatings were prepared with different types of solvent (1% acetic acid; CAc, and 1% lactic acid; CLa) and without or with 2% nanoclay (NC). The coatings were applied by immersion of eggs, followed by air-drying, and stored at 25°C for 5 wk. Internal quality parameters including weight loss (WL), Haugh unit (HU), and yolk index (YI) were weekly determined during storage. A Scanning Electron Microscope (SEM) and a Fourier Transform Infrared (FTIR) spectrophotometer were also used to describe the outer surface morphology of eggshells. On day 35, HU and YI values (66.20 and 0.34 respectively) in acetic acid-soluble nano-montmorillonite-coated (CAcNC) eggs were significantly ($p<0.05$) superior to other treatments. At the same time, the WL was progressively increased in all the groups, but the least WL value (5.35%) was observed in CAcNC-coated eggs. The images by SEM and spectra of FTIR showed that nanocomposite coatings could seal the surface of eggshells to prevent interior chemical changes. In general, the bio-packaging of eggs with acetic acid-soluble chitosan coatings, especially along with nano-montmorillonite, can play a significant role in maintaining the integrity properties of the eggshell, improving the internal quality, and consequently extending the egg's shelf life.

Keywords: Chitosan, Eggshell morphology, Montmorillonite, Shelf life, Solvent

1. Introduction

Eggs are a valuable source of protein in the human diet due to their high content and ideal proportions of essential amino acids. The nutritional value of the egg is based on its quality characteristics. However, fresh eggs are highly perishable with the loss of carbon dioxide and moisture through pores through the eggshell surface. Therefore, using surface coating is very effective in prolonging the shelf life, while maintaining the interior properties [1]. On the other hand, the flawless eggshell normally protects the egg contents against physical impact and microorganism contamination [2]. Thus, eggshell quality and integrity are of vital importance all over storage. Eggshells materials are permeable; hence they permit water vapor and carbon dioxide to permeate through the shell. It causes changes in interior quality as well as weight loss, so the pores on eggshell need to be sealed to reduce evaporation and permeation [3]. For this purpose, biodegradable edible films and coatings have been considered and widely used [1,4-7].

Bio-polymers such as chitosan are widely studied as edible coatings in the food industry [8]. Chitosan is among the most abundant bio-polymers in the world, obtained mainly from de-acetylation of crustacean wastes chitin. Due to admirable performance, shown as low gas and vapor permeability, good bio-adaptability, cheapness, broad availability, lack of toxicity, the possibility of film forming and coating preparation, using chitosan coating is considered a feasible approach to extend of egg shelf life during storage [5,9].

Chitosan-based coatings prevent moisture loss and oxygen penetration as well as possess antimicrobial and antioxidant properties [10]. Researchers have separately studied factors affected the efficiency of chitosan coating such as the molecular weight [11], types of chitosan [12], plasticizer types and concentrations [13,14], solvent types [14] and coating methods [5]. Among these factors, solubility plays an essential role in various applications of chitosan. In general, it is insoluble in most organic solvents, while it is easily soluble in dilute acidic solutions [15]. Depending on deacetylation degree (DD), solution concentration and additional components, various physicochemical and biological properties of the resulting compound are obtained [16]. The properties of films and coatings can be affected by the viscosity of the chitosan solution derived from different types of solvents. In this regard, Kim et al. [14] stated that the use of acetic acid as a solvent is more useful than lactic acid in terms of increasing egg shelf life. At the same time, the mechanical properties of chitosan films produced with acetic acid are superior in comparison with other solvents including citric, lactic, and malic acids [17]. In contrast, according to Derelioglu and Turgay [18], the use of different solvents including acetic acid, lactic acid, and propionic acid in chitosan-coated eggs did not cause a significant difference in the internal quality indexes in terms of weight loss and Haugh unit.

In the last years, some researchers have adopted the use of composed coatings of chitosan with nano-montmorillonite [19], in order to improve the mechanical strength properties of chitosan composites and showed improved performance. Therefore, polymer nanocomposites are designed in such a way that both of the components synergistically improve or raise the particular properties. [15]. Due to the clay colloid properties caused by the size of the nanoclay particles, they are used in a wide range of industries. In addition, nanoclays barrier properties have also received more attention due to the existence of a platelet-like structure. Polymer-clay biocomposites are composed of biopolymer matrices such as chitosan and clay filler such as montmorillonite. These combinations have the ability to improve the barrier properties of films and coating [20]. Electrostatic interaction between chitosan biopolymer and nanomontionite causes their complete entanglement. In the resulting composition, the biopolymer plays the role of a supportive network scaffold for the emergence of clay properties [15]. Soares et al [21] reported that the sodium montmorillonite-whey protein isolate composite coated eggs retained grade A for up to 5 weeks. The objective of this work is therefore to determine the effects of solvent types as well as nano-montmorillonite being in chitosan-based composite edible coating on the internal quality and eggshell morphology in view of their possible use in egg storage period at room temperature.

2. Materials and methods

2.1 Materials

Chitosan (Mw: 440 kDa; DD: approximately 90%) and nano-montmorillonite (MMT, K10), without organic modification were purchased from Sigma-Aldrich Chemicals (Germany). All other chemicals provided were laboratory-grade (Merck, Germany). Unwashed, clean, white-shell eggs (55.8 to 60.3 g with an average weight of 58.7 g) were purchased from a local poultry farm and immediately used for this experiment.

2.2 Preparation of chitosan coating solutions and treatment of eggs

The chitosan solutions were prepared dissolving the chitosan (2% w/v) with 2% (v/v) glycerol in a 1% (v/v) acetic (CAc) and/or lactic acid (CLa) solution using a magnetic stirrer during 2 h at room temperature (25^{o}C). The pH adjusted to 5.0 and/or 5.5 with 1 N NaOH [12]. As the method reported by Xu et al [22], Nanoclay (NC) suspension in 2% w/w chitosan were prepared and vigorously stirred for 24 h. Thereafter, the appropriate volume of chitosan solution was gradually added into the already prepared NC suspensions, while the blends were stirred continuously for 4 h. Eggs were immersed separately in recently prepared chitosan solutions for 1 minute and dried under the fan for 15 min. All samples (30 eggs/treatment) were included on egg shelves and stocked at 25^{o}C in an incubator. At 1-wk intervals 5 eggs per treatment were randomly selected to determine the internal quality parameters. At the same time, eggs were also collected for morphological evaluations.

2.3 Determination of weight loss (WL)

All selected eggs were separately weighed at specific intervals with a balance (HL 300, and Japan). The weight loss was calculated as the ratio of subtracting the final weight from the initial weight of sample with coating to the initial weight of samples [23].

2.4 Determination of haugh unit (HU) and yolk index (YI)

The parameters of HU and YI determination including altitude of albumen and yolk and the width of yolk were gauged with a micrometer (AMES S-6428, Waltham, Mass, USA) and a caliper (Digital INSIZE, Germany), respectively. Yolk index [24] and Haugh unite [25] were calculated by the following Equations:

$$\mathrm{YI} = \frac{\text{yolk height (mm)}}{\text{yolk width (mm)}} \tag{1}$$

$$\mathrm{HU} = 100 \log(\mathrm{H} - 1.7\mathrm{w}^{0.37} + 7.6) \tag{2}$$

where, H is the height of the albumen (mm); w is the weight of the egg (g) Eggs can be classified according to Haugh unit value standards (USDA). Accordingly, eggs in Class AA, A, and B have values higher than 72, 60 to 71, and less than 60, respectively.

2.5 Scanning electron microscope (SEM)

A scanning electron microscope (AIS-2100, Seron Technology, Korea) was used to examine the morphology of the eggshell surface during storage [5].

2.6 Fourier transform infrared (FTIR) spectra of eggshells

Using a FTIR spectrophotometer (Tensor 27, Brucken Ltd, Germany), the FTIR spectra of the outer surface of the treated eggshell were recorded. For each eggshell sample, scans were used to obtain attenuated total reflectance (ATR) spectra. The spectra of the eggshells were recorded after breaking off a small segment of it and placing on the ATR plate. The measurement consisted of recording FTIR spectra of the outer part of selected eggshells [5].

2.7 Statistical analysis

One-way analysis of variance (ANOVA) was used to determine differences in the means of data points. The significance of differences found ($p<0.05$) was evaluated using Duncan multiple ranges tests. Three replicates for each sample were used for the evaluation.

3. Results and discussion

3.1 Weight loss (WL)

In all study groups, WL progressed slowly, ranging from 1.49% to 5.35% after 5 wk of storage. While in CAc and CLa groups, as shown in Table 1, the WL sharply increased throughout storage. At a similar time, average values of weight loss of acetic acid-soluble nano-montmorillonite-coated (CAcNC) and lactic acid nano-montmorillonite-coated (CLaNC) eggs were lower than others till the 5th wk. However, on day 35, the WL of CAcNC was approximately 14% less than that of CLaNC (5.35 vs 6.12%). Therefore, regardless of the solvent type, the use of nano-montmorillonite significantly reduced weight loss in composite coated eggs. It was stated that some of the mechanical properties of composite films depended significantly on chitosan content and clay particle levels [23,26]. Furthermore, previous studies have shown that oxygen permeability in chitosan layers with lactic acid is equal to or less than acetic acid [26]. According to the results, morphological observations of the eggshell of the studied eggs will be useful to better understand the changes in weight loss during the storage. SEM images show that NC particles appear to be almost uniformly scattered throughout the chitosan matrix. Therefore, the effective protective properties of composite-coated eggshells were higher and better than CLa and CAc, which indicated the relative improvement of composite coatings using nano-montmorillonite particles.

Considering the results shown in Table 1, it easily found that egg weight loss was affected by storage time and gradually progressed from 5.35% to 7.68% during 35-day storage. These trend changes were ascribed to the exchange of CO_2 and water vapor between albumin and the surrounding environment through pores and micro-cracks on the eggshells [27]. The escape of gases causes many adverse internal physical and chemical changes, which ultimately lead to a deterioration in the quality of the eggs. Therefore, determining the WL values is one of the effective indicators in assessing the interior quality of eggs [5]. There were no significant weight losses observed among CLa and CAc-coated eggs, as also observed by Kim et al [12], throughout the 5 wk. of storage. As can be concluded in SEM images, CAcNC-coated eggs had a surface with higher density and less porosity, which lead to less weight loss rate during storage.

Table 1 Effects of experimental treatments on weight loss (%) during a 5-wk storage.

Storage Time (day)	Weight Loss (%)			
	CLa	CAc	CLaNC	CAcNC
7	1.61±0.10^{a}	1.58±0.17^{a}	1.52±0.18^{a}	1.49±0.07^{a}
14	3.88±0.37^{a}	3.69±0.23^{a}	3.30±0.25^{ab}	3.00±0.32^{b}
21	4.33±0.14^{a}	4.18±0.21^{a}	3.95±0.17^{ab}	3.67±0.45^{b}
28	5.83±0.24^{a}	5.22±0.21^{a}	4.95±0.18^{ab}	4.57±0.35^{b}
35	7.68±0.73^{a}	7.07±0.07^{a}	6.12±0.37^{b}	5.35±0.15^{c}

$^{a, ab, b, c}$ Means (n=3) with different letters within a row indicate significant differences (p<0.05). C: chitosan, Ac: acetic acid, La: lactic acid, NC: nanoclay.

3.2 Haugh unit (HU)

Haugh unit is among the important parameters of egg quality which are attributed to the structural changes of egg white protein over time [28]. In freshly laid eggs, the HU values are between 75 and 85 (grade AA), while due to the decomposition of organic compounds, and subsequently decrease in albumen height, the HU values gradually decrease during storage [11]. Several studies have shown that HU decreased as storage duration increases and that the usage of coatings delays this decline. Whey protein isolate, sodium montmorillonite nanoparticles, and sodium metabisulfite were used by Soares et al. [21]. Furthermore, during storage, as a result of the carbon dioxide escape and the migration of water from the albumin to the egg yolk, the pH of the internal content of the egg slightly increases, which leads to proteolysis of the dense protein and affects the albumen quality [29].

As shown in Table 2, the undesirable changes in HU values of eggs containing CLa and CAc coating was similar to changes in their WL throughout storage. However, their HU value was significantly (p<0.05) decreased from AA grade to A grade, then to B grade at the end of the 1st and 2ed wk. of storage respectively. According to the egg grading standard, both nanocomposite coatings extend the egg shelf life at least 2 weeks longer at 25°C. They maintained the initial AA grade for 3 wk. Xu et al [23] reported similar results by using soy protein isolated (SPI)-montmorillonite coated eggs in comparison with SPI coated eggs.

On the other hand, the solvent type in chitosan coating solutions with or without NC had no significant effect on the HU value. This result was concordant with the observations of Derelioglu and Turgay [18] study on chitosan-coated eggs that were prepared with acetic, lactic, and propionic acid.

Table 2 Effects of experimental treatments on Haugh unit during a 5-wk storage.

Storage Time (day)	Haugh Unit (grade)			
	CLa	CAc	CLaNC	CAcNC
0	84.75±1.01^{a} (AA)	84.75±1.01^{a} (AA)	84.75±1.01^{a} (AA)	84.75±1.01^{a} (AA)
7	66.30±1.80^{b} (A)	68.54±1.21^{b} (A)	79.92±2.00^{a} (AA)	81.33±0.80^{a} (AA)
14	57.73±3.85^{b} (B)	59.85±2.12^{b} (B)	72.37±4.43^{a} (AA)	74.53±2.89^{a} (AA)
21	53.39±2.47^{c} (B)	57.84±2.31^{b} (B)	68.70±3.12^{a} (A)	69.94±0.91^{a} (A)
28	50.22±2.25^{c} (B)	54.15±3.13^{b} (B)	66.55±3.41^{a} (A)	67.78±1.21^{a} (A)
35	49.96±4.17^{b} (B)	52.09±1.81^{b} (B)	65.69±4.14^{a} (A)	66.20±3.12^{a} (A)

$^{a, b, c}$ Means (n=3) with different letters within a row indicate significant differences (p<0.05). C: chitosan, Ac: acetic acid, La: lactic acid, NC: nanoclay.

3.3 Yolk index

Figure 1 shows that the YI decreased over time as also reported for the weight loss and the Haugh unit (Tables 1 and 2). The yolk index values were not significantly different between CLa and CAc and/or between composite-coated eggs till 5 wk. Moreover, while during the storage period, the lowest values of YI were observed in CLa and CAc treated eggs, the highest values of YI were presented in CAcNC and CLaNC-coated eggs throughout the storage period (p<0.05). In addition, the YI values curves especially in CAc and CLa experienced a sharp drop from day 14 to 21.

As stated, apart from the HU, the YI is also one of the indexes of the freshness of egg evaluation. As a result of the progressive diffusion of water from the albumen to the yolk, its membrane is gradually weakened [30]. The downward changes in YI and HU values are mainly related to the barrier abilities of the eggshell. Therefore, the lowering of these indexes in CLa and CAc coated eggs can be attributed to changes in the microstructure of their outer coating are attributed which is exhibited in Figure 2.

As shown in Figure 1, the decline in YI in composite coated is significantly less than those of other treatments. These are attributed to a depletion of carbon dioxide and water vapor escape, as well as presented the synergy effects of composite coating in preserving the quality of yolk during storage [23]. In this study, there were no significant differences between chitosan dissolved in 1% acetic acid or 1% lactic acid-coated eggs during 5 wk.

of storage as also reported by Kim et al. [14]. Among composite coatings, eggs with acetic acid-dissolved coatings had a higher YI than lactic acid-dissolved coatings during 5 weeks of storage, in addition, similar results were viewed for the HU (Table 2). On the base of internal quality, Table 2 data and Figure 1curves demonstrate that acetic acid is more useful in extending the shelf life of eggs than lactic acid as a solvent for coating. From another perspective, Suresh et al. [27] showed that the YI of nanocomposite-coated eggs was superior to that of chitosan-coated eggs. As a result, it is certain that the composite coatings enhanced the preservation of the yolk's integrity during storage.

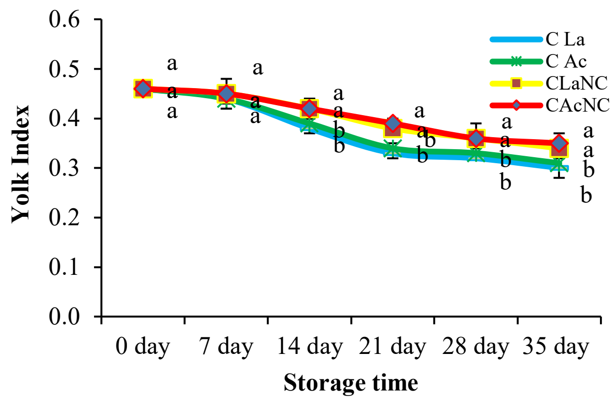

Figure 1 Effects of experimental treatments on yolk index during a 5-wk storage (n=3).

3.4 Morphology of eggshells

As a general agreement, the use of biopolymers in the coating of eggs can prevent the exchange of gases and microorganisms between the interior and the surrounding spaces of the egg by sealing very small pores on the eggshell surface, thus extending eggs shelf life. Due to the fact that the barrier properties of eggshells strongly depend on the morphology of the eggshell surface, the study and comparison of eggshell micrographs are inevitable in different groups. The microscopic images of the eggshell's outer surfaces are exhibited in Figure 2. Some of the early micro-cracks probably disappear on the eggs after immersion of the eggs in coating solutions. According to Xu et al. [5], acetic acid and/or lactic acid may seal primary micro-cracks by reacting with eggshell calcium carbonate.

It has been proven that if the outer surface structure of the eggshell is destroyed for any reason, the ability to block the eggshell is surely weakened, resulting in changes in the internal quality indicators. Therefore, it seems necessary to study the structural changes in the surface of the coating under the influence of environmental factors [5]. SEM images in Figure 2 exhibit a more uniform structure of CAc-coated eggshells than CLa-coated. On day 35, as pointed by arrows, some micro-pits, wrinkles, and somewhat discontinuity were clearly expanded on the eggshells treated with CLa. After adding NC to chitosan-based coatings composition, new and different morphology was observed on the surface of the eggshells. SEM images of composite coatings demonstrated a lower porosity surface morphology at CAcNC as compared to CLaNC. One of the most likely reasons for these results may be the uniform distribution of NC in the CAc matrix. In this case, it seems that the clay nanoparticles are well placed in the chitosan polymer.

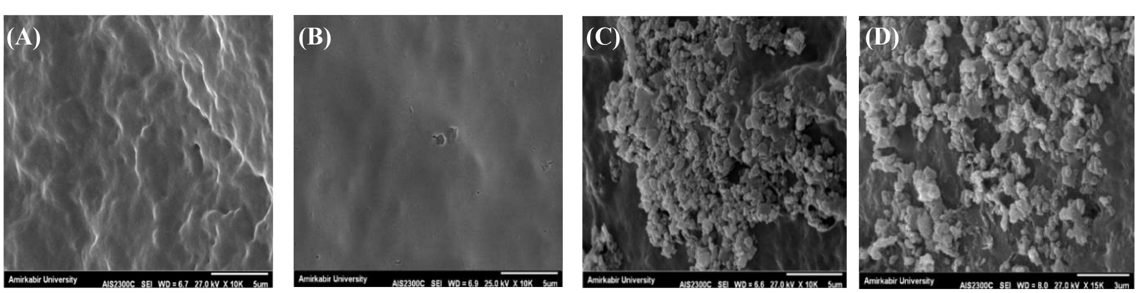

Figure 2 Scanning electron microscopy (SEM) images of eggshell outer surface at days 0: (A) CLa, (B) CAc, (C) CLaNC, (D) CAcNC).

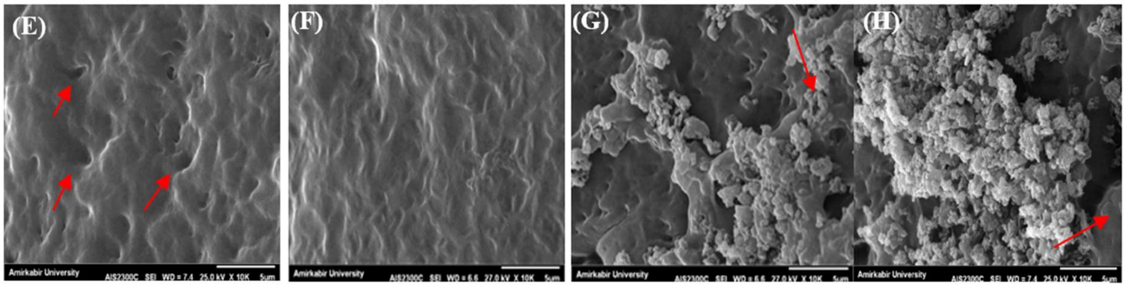

Figure 2 (continued) Scanning electron microscopy (SEM) images of eggshell outer surface at days 35: (E) CLa, (F) CAc, (G) CLaNC, (H) CAcNC) (Scale bar=5μm). Arrows show some micro-pits, wrinkles, and discontinuity in images.

3.5 FTIR spectra of eggshells

Figure 3 shows the FTIR spectra associated with the outer surface of the eggshells on day 35. In general, the FTIR spectra in Figure 3 partially confirmed the microscopic structure of the eggshell surface coatings which is shown in Figure 2. As pointed out by arrows in Figure 3, the spectra of CLa and CAc presented peaks between 3000 cm^{-1} and 3500 cm^{-1}, which were due to the stretching bonds of free hydroxyl and symmetric and asymmetric bonds of -NH in amine groups, respectively, while peaks at 1023 cm^{-1} and 1632 cm^{-1} might be attributed to the C-O-C vibration of polysaccharides and amide I band of proteins on the eggshell cuticles, respectively [31].

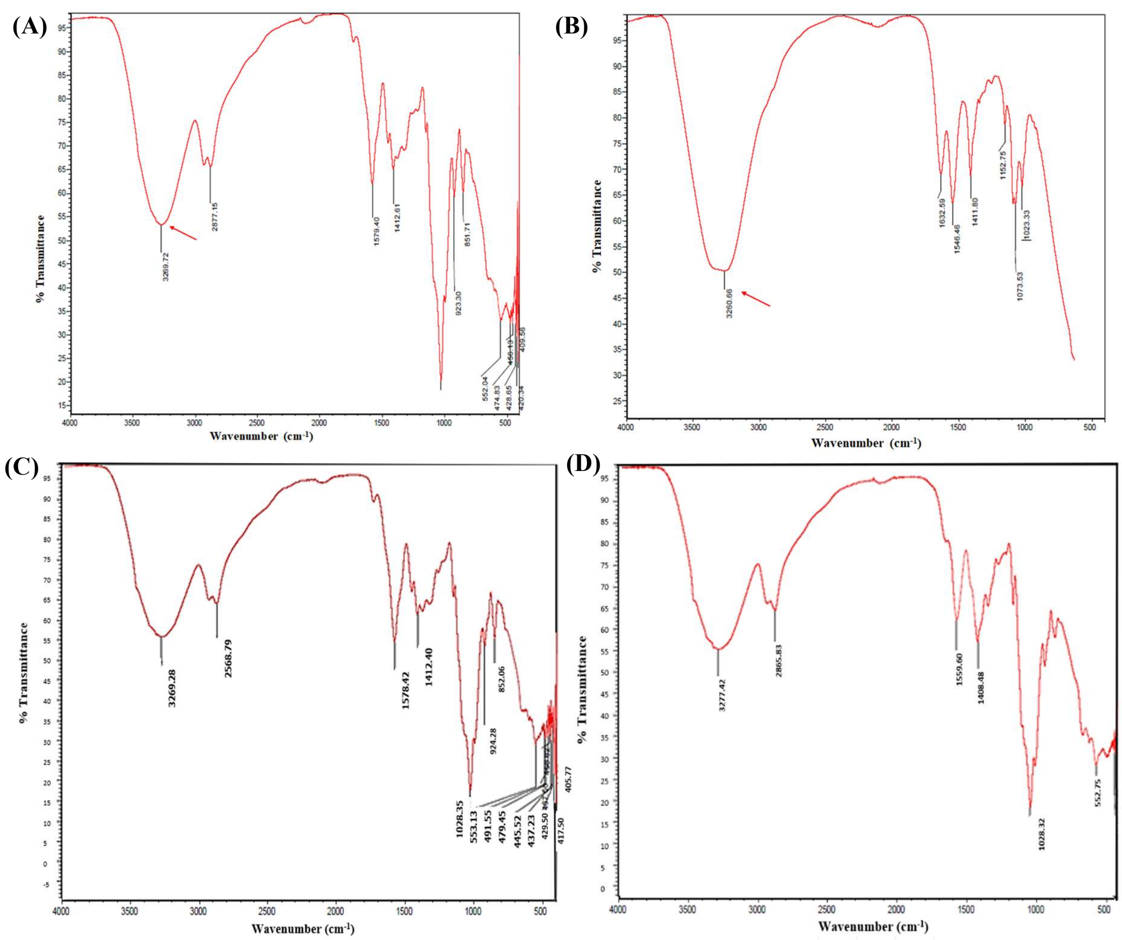

Figure 3 Fourier Transform Infrared (FTIR) spectra of CLa (A), CAc (B), CLaNC (C) and CAcNC (D) coated eggshell surfaces at day 35. Arrows might be attributed to the stretching bonds of free hydroxyl and symmetric and asymmetric bonds of -NH (A, B) and C-O-C vibrations (C, D).

In addition to the above, two strong bonds in the range of 1560 cm^{-1} and 1410 cm^{-1} in nanocomposite coatings can be due to hydrogen bonds between active -OH groups in the nanoclay structure and -NH and -OH groups in chitosan. FTIR spectra of eggshells coating clearly confirmed the results that were observed in SEM images. Similar results were observed in other studies with SPI-montmorillonite [23,32].

4. Conclusion

The internal changes of eggs can be mainly attributed to the barrier properties of the coatings. However, attenuation of the coating's outer surface structure was indicated by microscopic images and eggshell spectroscopy during storage. The inclusion of nano montmorillonite into the chitosan-based coatings improved their efficiency in extending the egg shelf life by at least 2 wk. In this regard, acetic acid was a more efficient solvent than lactic acid. Therefore, a practicable egg coating technology with high inhibitory properties of biopolymers was successfully developed. This coating material can be applied to egg bio-packaging and storage.

5. Acknowledgments

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