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The effect of bio-filler-reinforced chitosan coating with types of solvent on internal changes and outer eggshell morphology

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Abstract

Due to the environmental hazards raised by using synthetic polymers, attention has turned to biodegradable polymers such as chitosan. The unique efficiency of chitosan coatings is severely affected by using the types of solvent as well as the fillers. To evaluate the effect of solvent types and application of cellulose nanocrystals in chitosan-based coatings on internal quality and eggshell morphology, an experiment was conducted with 4 treatments including chitosan coatings dissolved in acetic and/or lactic acids, with and without cellulose nanocrystal filler during 35 days at 25°C. Results demonstrated that the reinforcement of biopolymer coatings matrix with cellulose nanocrystal had significantly improved weight loss, Haugh unit, and yolk index in coated eggs. Also, chitosan coating dissolved in acetic acid had better efficiency than lactic acid, as a view of the Haugh unit on days 21-28. At the same time, microscopic images of outer surface eggshells had somewhat confirmed the interior changes. In general, chitosan dissolved in acetic acid cellulose nanocrystal-reinforced coating can be used as an egg biodegradable packaging.

Keywords:

Cellulose nanocrystal, Chitosan, Egg, Nanocomposite, Shelf life, Solvent

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Introduction

In recent years, the potential environmental hazards arising from the use of petroleum-based polymers packaging have led to a shift in attention to the application of environmentally friendly biodegradable compounds. In this regard, biopolymers such as chitosan have attracted attention, because of their superior performance, including the excellent film and coating forming ability, wide availability, biodegradability, biocompatibility, and non-toxicity. Thus, the chitosan-based coating can be a promising and efficient approach to preserving the quality of perishable foods, including eggs during storage (Kopacic et al., 2018; Xu et al., 2018). Chitosan is a de-acetylated form of chitin. The impact of effective parameters on chitosan efficiency, such as solvent types (Kim et al., 2006), molecular weight (Bhale et al., 2003), types and concentrations of plasticizer (Kim et al., 2008; Kim et al., 2006) and coating methods (Xu et al., 2018) have been separately investigated. Among these factors, chitosan solubility depends mainly on the pH of the solution, so chitosan is easily soluble in weakly acidic solutions at pH 6.0, while it is insoluble in most organic solvents (Kumar et al., 2019). Kim et al. (2006) emphasized that the use of acetic acid as a chitosan solvent was more efficient than lactic acid in view of the shelf-life extension of eggs. It is strong evidence that the synergistic interaction between the nanofillers including nanocellulose (NC) and the biopolymer matrices increases the mechanical properties of the coating and reduces its permeability to moisture and gases (Jafarzadeh et al., 2021). The inclusion of NC in polymer materials is reported significantly to affect the barrier abilities of the film and coating (Pirozzi et al., 2021). Furthermore, the excellent mechanical properties render cellulose nanocrystals (CNCs) as potential reinforcements in packaging films (Scaffaro et al. 2017). Because CNCs have a highly crystalline nature, the oxygen barrier properties of packaging materials can be improved when they are used as filler in composite films by increasing both crystallinity and the pathway for the diffusion of gas molecules (Rhim et al. 2013).

An egg is a nutritious, perishable, and relatively inexpensive food. Eggshell has a porous, permeable, and brittle structure. The presence of many pores on the eggshell surface causes the penetration of microorganisms and gasses exchanges between the interior and surrounding environment, thereby leading to internal chemical changes and weight loss during storage. Therefore, sealing these very tiny holes with the coating is inevitable to preserve the internal quality, protecting microbial contamination, and extending the shelf life of eggs ultimately (Samli et al., 2005). The claimed effects of biodegradable biopolymer coatings have been investigated on the barrier properties of coated eggs in extensive studies (Oliveira et al., 2020; Xu et al., 2018; Ndife et al., 2020).

This study aimed to the applicative aspects of the merging of NC in composite chitosan-based coating to prevent the egg interior quality decline and eggshell morphological evaluation during 5-wk storage at 25^{o}C.

Materials and methods

Materials

Both chitosan (Powder with mesh size of 80, MW: 60 kDa, DD: 83%), and cellulose nanocrystal (CNC) were obtained from Nano Novin Polymer Co. (Mazandaran, Iran). All other chemicals were analytical 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 (Mazandaran, Iran) and immediately used for this experiment.

Preparation of chitosan-based coating solutions and treatments

The chitosan solutions (2% w/v) were prepared by dissolving the proper amount of chitosan into a 1% (v/v) acetic (CA) and/or lactic acid (CL) under a constant magnetic stirrer. Then, glycerol (2% v/v) was added as a plasticizer to the solutions and stirring continued for 15 min. The pH was adjusted to 6 with 1 N NaOH (Kim et al., 2007). Afterward, the dispersed cellulose nanocrystal in distilled water was slowly mixed with the solutions to produce composites containing 5 wt% nanocellulose (Salmanian et al., 2019). Eggs were immersed individually in treated solutions (and in distilled water for the control group) for 1 min. After air drying, all eggs (30 eggs/treatment) were placed in cardboard egg racks and stored in an incubator at 25◦C for 5 wk. During storage, at 1-wk intervals, 5 eggs per each treatment, including the control group, were taken for determination of internal quality parameters. At the same time, eggshells were also collected for morphological assessments.

Determination of internal quality parameters

All eggs were weighted with a balance (HL 300, AND, Japan) at specific time intervals, the weight loss was (WL) calculated by the ratio of the subtracting between final and initial weights to the initial weight of eggs (Xu et al., 2017). The height of albumen and yolk and the width of yolk were measured with a tripod micrometer (AMES S-6428, Waltham, Mass, USA) and a digital caliper (Digital INSIZE, Germany), respectively. Yolk index (YI) was calculated as the ratio of yolk height (mm) to yolk width (mm) (Ryu et al., 2011). The Haugh unit (HU) was calculated by the following equation (Haugh, 1937):

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

Where; H is the height of the albumen (mm); w is the weight of the egg (g)

Eggshell morphological assessments

Eggshell morphology was observed using a scanning electron microscope (SEM; AIS-2100, Seron Technology, Korea). Fourier transforms infrared (FTIR) spectra of the outer surface of eggshells were recorded using an FTIR spectrophotometer (Tensor 27, Brucken Ltd, Germany) with attenuated total reflectance (ATR) accessories. The X-ray diffraction (XRD) patterns of the eggshells were obtained with a diffractometer (Philips Co., Holland) using nickel monochromatic Cu Kα radiation ($\lambda = 1.542$ A^{o}) at 40 kV, 30 mA, and a scan speed of 1^{o}/min between 5^{o} and 55^{o}.

Statistical analysis:

A one-way analysis of variance (ANOVA) was used to determine differences in the means of data points. Statistically significant differences ($p<0.05$) were evaluated using Duncan multiple range tests. Three replicates for each sample were used for the evaluations.

Results

Internal quality parameters

The results of weight loss, Haugh unit, and yolk index are presented in Table 1 as three of the most important parameters of egg internal quality assay. Over storage time, undesirable changes were gradually seen in all of the considered indexes, so that WL ranged from 1.46% to 7.68 % during 5-wk storage. Therefore, 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. At the same time, HU and YI values were andante decreased, whilst pointed falls were observed in HU and YI values of CL coated eggs after only 7 and 14 days respectively. From the point of view of solvent types, the eggs coated with acetic acid-soluble chitosan had higher HU values than lactic acid during 21-28 days of storage. However, the merge of CNC into the chitosan-based coating matrices significantly improved the HU and YI values on day 35 ($p<0.05$).

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Table 1: Effect of different coatings on weight loss (WL) (%), Haugh unit (HU) and yolk index (YI) during storage (day)

Parameters	Treatments	Storage time (days)					
		0	7	14	21	28	35
WL	CA	-	1.58±0.17[a]	3.69±0.23[a]	4.18±0.21[a]	5.22±0.21[a]	7.07±0.07[a]
	CL	-	1.61±0.10[a]	3.88±0.37[a]	4.33±0.14[a]	5.83±0.24[a]	7.68±0.73[a]
	CA-NC	-	1.46±0.10[a]	2.72±0.27[b]	3.13±0.24[b]	4.07±0.35[b]	5.10±0.50[b]
	CL-NC	-	1.51±0.18[a]	3.00±0.25[ab]	3.65±0.17[b]	4.50±0.18[b]	5.67±0.33[b]
HU	CA	84.75±1.01[a]	68.54±1.21[b]	59.85±2.12[b]	57.84±2.31[b]	54.15±3.13[b]	52.09±1.81[b]
	CL	84.75±1.01[a]	66.30±1.80[b]	57.73±3.85[b]	53.39±2.47[c]	50.22±2.25[c]	49.96±4.17[b]
	CA-NC	84.75±1.01[a]	82.10±0.80[a]	75.67±2.95[a]	71.25±1.17[a]	68.13±1.33[a]	67.33±3.12[a]
	CL-NC	84.75±1.01[a]	80.27±2.00[a]	73.45±4.45[a]	69.15±3.17[a]	67.40±2.35[a]	66.80±4.14[a]
YI	CA	0.48±0.01[a]	0.44±0.01[a]	039±0.00[b]	0.34±0.00[b]	0.33±0.01[b]	0.31±0.02[b]
	CL	0.48±0.01[a]	0.44±0.01[a]	0.38±0.01[b]	0.33±0.02[b]	0.32±0.02[b]	0.30±0.02[b]
	CA-NC	0.48±0.01[a]	0.45±0.01[a]	0.42±0.00[a]	0.39±0.01[a]	0.36±0.01[a]	0.35±0.01[a]
	CL-NC	0.48±0.01[a]	0.45±0.00[a]	0.42±0.01[a]	0.38±0.02[a]	0.36±0.00[a]	0.34±0.01[a]

[a-b]Means±SD with different superscripts in the same column are differences ($p<0.05$). C: chitosan, A: acetic acid, L: lactic acid, NC: nanocellulose

Outer eggshell morphological assessments

Because of the fact that the barrier abilities of eggshells intensively associated with the morphology of the eggshell surface, so the assessment and comparison of eggshell micrographs is inevitable in different groups. The microscopic illustrations of the eggshells outer surfaces are exposed in Fig 1. According to Xu et al. (2018), acetic acid may seal primary micro-cracks by reacting with eggshell calcium carbonate (Xu et al., 2018). Also SEM images in Fig. 1 showed that CNC particles appear to be were almost uniformly scattered throughout the chitosan matrix. Therefore, the effective protective abilities of composite-coated eggshells can be attributed to their more integrity than CL and CA coatings.

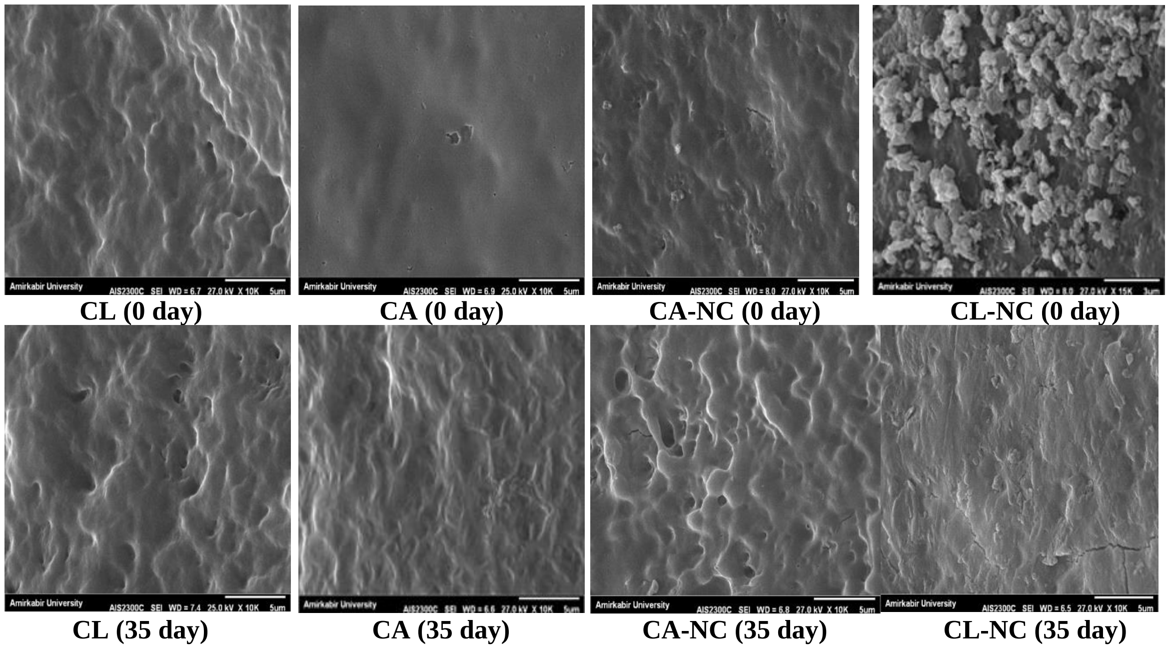


Fig. 1. Scanning electron microscopy images of eggshell outer surface at days 0 and 35 (Scale bar=5µm).

In general, the FTIR spectra in Fig. 2 partially confirmed the SEM images of the eggshell surface coatings shown in figure 1. As pointed by arrows, the spectra of CA and CL exhibited peaks between 3000 cm^{-1} and 3500 cm^{-1}, which were due to the stretching bonds of free -OH and symmetric and asymmetric bonds of -NH in amine groups, respectively, while peaks among 850 cm^{-1} and 1650 cm^{-1} might be attributed to the C-O-C vibration of polysaccharides and amide I band of proteins on the eggshell cuticles, respectively (Dominguez-Gasca et al., 2017).

In nanocellulose composites spectra, the peaks between 600 cm^{-1} and 1800 cm^{-1} result from tensile of O=C and hydrogen bonds coupled to the C-O-O group. The peak in the range 1540 cm^{-1} is also the result of bending and vibration of the H-N group and the tension and vibration of the N-C groups. The peak at 1068 cm^{-1} is characteristic of the tensile O-C bond in cellulose, while the peak in the 1150 cm^{-1} range is associated with the vibrational skeleton involved with the tensile O-C involved in the β1→4 glycosidic bonds of beta-glucopyranose units in cellulose.

X-ray diffraction analysis was carried out on chitosan-based coatings to assess amorphous and crystalline states as exposed in Fig. 3. Although the complete crystal structural properties or the distinct crystalline order were not present in any coatings, the materials were seen as a combination of amorphous and crystalline states. In diagrams, the wide peaks are attributed to amorphous regions, while the crystalline areas form sharp peaks. The ratio of these peaks can also be used to determine crystallinity.

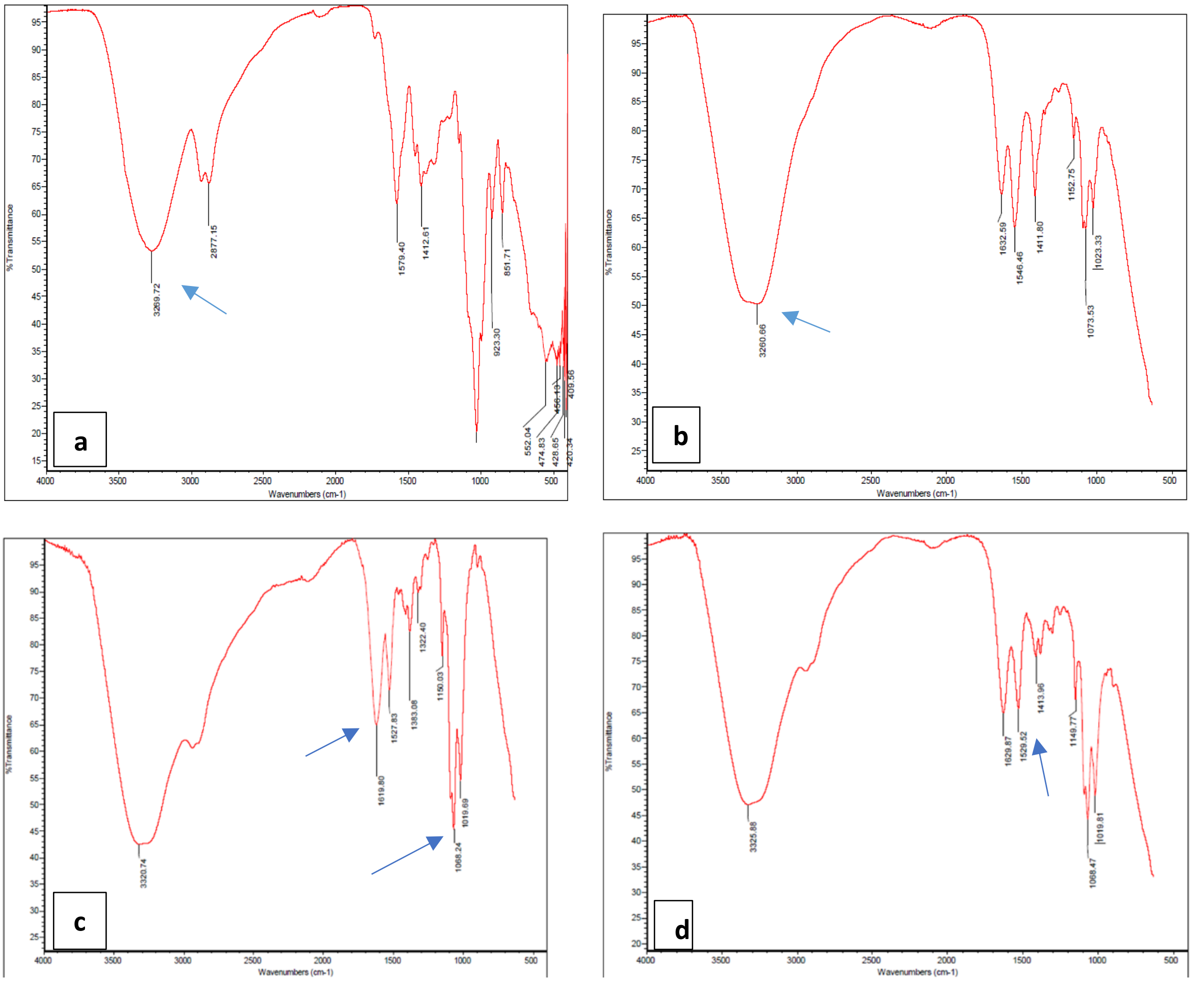
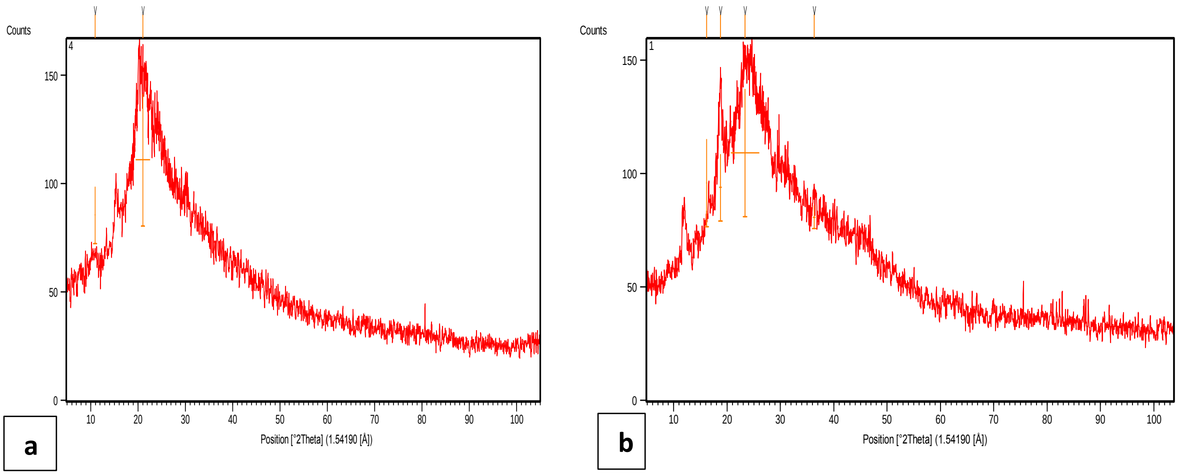


Fig. 2. Fourier Transform Infrared (FTIR) spectra of CL (a), CA (b), CL-NC (c) and CA-NC (d) coated eggshell surfaces at day 35



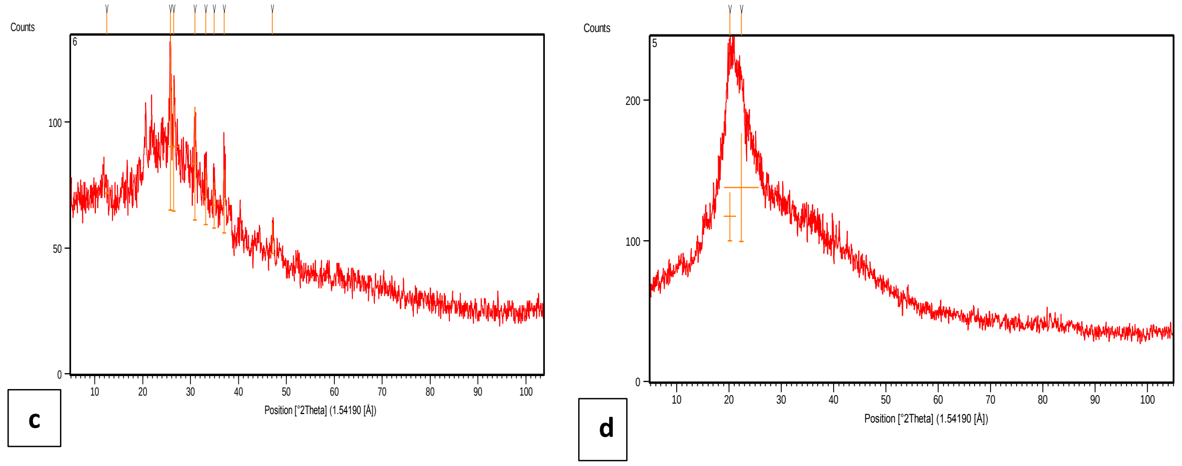


Fig. 3 X-ray diffraction diagrams of CL (a), CA (b), CL-NC (c) and CA-NC (d) coated eggshell surfaces at day 35

Discussion

The trend decline changes of WL are correlated with the exchange of the mass between albumin and the surrounding environment through tiny holes and some fractures on the eggshells (Suresh et al., 2015). The run-away of gases causes many undesirable changes, which ultimately lead to a deterioration in the interior quality of the eggs. Thus, determining the WL values is indirectly one of the effective indicators in assessing the interior quality of eggs (Xu et al., 2018). However, the solvent types used in the production of chitosan-based coatings had no significant effect on egg WL, as also observed by Kim et al. (2007), whilst the use of nanocellulose significantly slowed down weight loss in composite coated eggs throughout the 5 wk. of storage (Jafarzedeh et al., 2021). As can be seen from SEM images, CA-coated eggs had a surface both with higher entanglement and less porosity, leading to less weight loss rate during storage. On the other hand, the incorporation of CNC into coating solutions, due to their high-crystallinity structure, as somewhat confirmed in XRD diagrams, may increase the mechanical abilities, as well as the barrier property of the coatings (Pirozzi et al., 2021). Salmanian et al. (2019) reported that adding nanocellulose to kefiran-chitosan matrix improved some of its mechanical properties, but the authors emphasized that the dispersed phase of nanocellulose could not indicate its own crystalline peaks in the kefiran-chitosan matrix as based on XRD diagrams.

Haugh unit and yolk index are two main parameters for evaluating egg quality based on changes in egg proteins (Sheng et al., 2018). In newly laid eggs, HU values are between 75 and 85, while due to changes in the organic compounds of proteins and the consequent decrease in albumin height, the HU values gradually decline during storage (Bhale et al., 2003). In addition, as a result of the release of water vapor and carbon dioxide, the pH of the interior content of the egg increases and leads to the proteolysis of dense proteins (Omana et al., 2011). According to the United States Standards for Quality of Individual Shell Eggs (USDA 2000), eggs are generally classified into AA, A, and B grades, which require Haugh unit value to be above 72, 71 to 60, and below 60, correspondingly. Haugh unit values in CA and CL coated eggs showed a sharp drop after 14 days and fell to grade B, while the composite coated eggs fixed in the same grade until the end of the storage after falling from grade AA to A on day 14. Considering Haugh unit values, the CNC-reinforced coating elongates the shelf life up to at least 3 weeks longer at 25 ◦C.

As pointed out, the downward changes in YI and HU values are mainly related to the barrier abilities of the eggshell. Therefore, these indexes were lower in CA and CL coated- eggs than in composite coatings, which can be attributed to changes in their coating microstructure as exhibited in the SEM images.

The analysis of experimental data clearly showed that CNC-reinforced coatings had lower YI values than other treatments. These are likely attributed to the synergistic effects of the composited coating components to preserve yolk quality by creating a more impermeable eggshell during storage (Xu et al., 2017). In view of the YI, there were no significant differences between chitosan dissolved in different types of solvents (acetic and lactic acids) during 5 wk of storage as also reported by Kim et al (2006).

It has almost been proven that if the physical structure of the eggshell's outer surface is distorted for any reason, the barrier properties of the eggshell will be equally weakened, resulting in changes in the internal quality indexes. Therefore, the assessment of outer structural changes of the eggshell coating seems inevitable under the influence of environmental factors (Xu et al., 2018). In this respect, SEM images exhibit a more uniform structure of CA-coated eggshells than CL-coated. After incorporating the CNC into biopolymer-based coatings, new and different morphology was seen on the surface of the eggshells, as illustrated in Figure 1. One of the most likely reasons for these results may be the more uniform distribution of CNC in the CA-matrix. In this case, it seems that the cellulose nanocrystals are well filled into the chitosan tangled polymer as seen in SEM images. On the other hand, the FTIR spectra of eggshell coatings greatly confirmed the results of the SEM images. Also, the bond presented ranging from 1590-1690 cm-1 is associated with the adsorption of water molecules in the non-crystalline structure of cellulose and its increase in intensity in nanocomposite films indicates the lack of complete crystallization of nanocrystals. Furthermore, the incorporation of nanocellulose into the chitosan matrices slightly increased the peak intensity in the range 3260-3325 cm. This change indicates a decrease in the amount of water absorbed by chitosan in the presence of nanocellulose particles. This event can be due to the competition of nanocellulose particles to bind to water molecules. Also, the increase in peak intensity may be related to the hydrogen bonds formed between chitosan and nanocellulose (Xu et al., 2017; Lee and Kim, 2010).

Conclusion

As the results of this scientific work demonstrated, in view of the Haugh unit values, the acetic acid-soluble CNC-reinforced chitosan coating extended the shelf life of eggs up to 35 days at 25°C, which was at least 2 weeks longer than CA and CL eggs. The protective ability of nanocomposite coatings on the internal quality of eggs can be mainly attributed to the barrier properties of the coatings. However, attenuation of the outer physical structure of the eggshell coatings was exhibited by microscopic images and was slightly confirmed through eggshell spectroscopy during storage.

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Conflict of interest statement

None to be declared.

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