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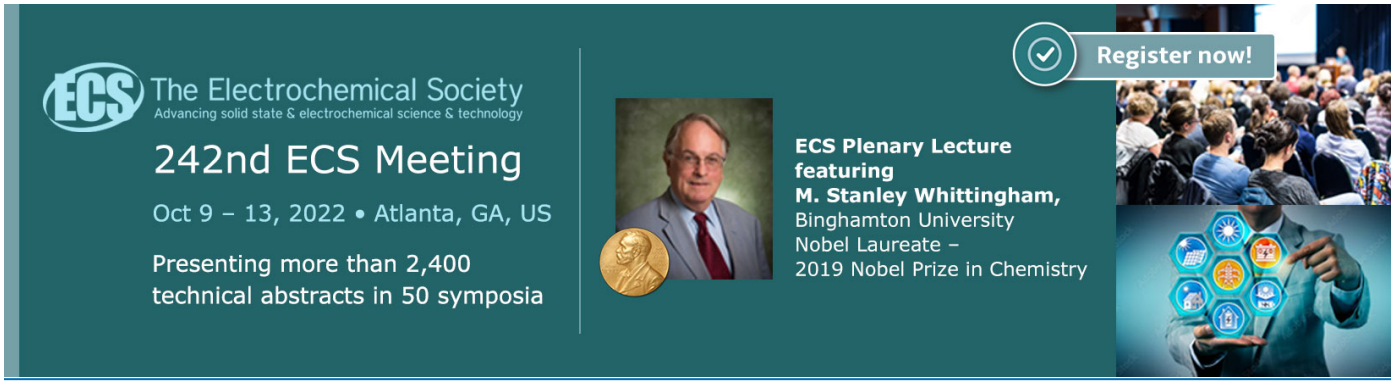

Utilization of cellulose nanocrystals (CNC) as a filler for chitosan-based films for chili peppers packaging

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Abstract. This study investigates the effects of the percentage of cellulose nanocrystals (CNC) on chitosan film for wrapping chili peppers during storage at room temperature for 12 days. The chitosan-CNC film's characterization was analyzed by SEM, FTIR, and physical properties were measured based on tensile strength, percent elongation, water solubility, and degradation rate. This research used a randomized design with four treatments and two replications. The treatments in this study were E0 = control, E1 = CNC 1 %, E3 = CNC 3 %, E5 = CNC 5 % and E10 = CNC 10 %. The quality of chili peppers was analyzed based on the concentration of vitamin C, titratable acidity, total soluble solids, and weight loss rate. The E3 treatment achieved the best results on day 12, i.e., weight loss of chili peppers with the chitosan-CNC film was 0.51 %, vitamin C was 295 mg/100 g, titratable acidity was 0.25 %, and the total soluble solid was 24%.

Keywords: chitosan film, cellulose nanocrystals, chili peppers

1. Introduction

Chili pepper is a perishable agricultural product, and its quality rapidly decreases after post-harvest. Damaged chili peppers occurred because it loses the natural waxy layer, thus the transpiration rate of water vapor increases, resulting in softening of the fruit tissue, the fruit withers, rot, wrinkles and soft [1]. In addition to undergoing the transpiration process, chili pepper also undergoes a process of respiration that is the process of oxidizing the breakdown of complex compounds and organic compounds such as starches, organic acids, and others into simple molecules of CO_2, H_2O, and energy.

One way to slow down the respiration rate is by coating, wrapping, and packaging with green materials that are safe to eat, non-toxic, and biodegradable. Edible films and coating have been developed from protein, lipid, polysaccharide [2], and a combination with other components [3]. Kwang et al. [4] demonstrated the potential use of chitosan-acorn starch-eugenol edible films to

improve the film's mechanical and barrier properties and improved the flexibility, barrier, hydrophobicity, antimicrobial and antioxidant properties.

Liming et al. [5] also developed edible films made from chitosan, zein, and α-tocopherol. They reported that high α-tocopherol contents in the aqueous chitosan-zein film increased zeta potential from +26.023 to +37.187 mV, which revealed that α-tocopherol could improve the stability of solutions. The PDI decreased from 0.354 to 0.222, indicating that the film-forming solutions became uniform. The addition of α-tocopherol resulted in low water vapor permeability and excellent opacity. The film's lowest water vapor permeability was 0.944 kg m•m−2•s−1•Pa−1, and its maximum opacity was 12.601. It was observed that the highest value of DPPH radical scavenging was 97.66 % when the α-tocopherol concentration was 1 g/100 mL.

Walid et al. [6] used mixture design to optimize a chitosan-gelatin-pectin formulation film to enhance elongation at break, tensile strength texture values, and antibacterial property. According to their results, the best formulation was obtained using a mixture of 10.0 % chitosan, 24.3 % gelatin, 0.5 % pectin, and 65.2 % glycerol. This composition provided the highest elongation at break, tensile strength, and texture values: 51.60 ± 6.04 %, 8.53 ± 2.36 MPa, and 13.67 ± 1.43.

The development of edible films has received increased interest in recent years due to the increased consumer demand for high quality, long shelf life, and ready to eat foods. Researchers are now investigating these biopolymers. The addition of various nanomaterials to polymeric matrices has been another solution for improving the desired packaging properties.

Cellulose nanocrystals (CNC) can be obtained using acid hydrolysis to remove the amorphous structures from the cellulose, which are highly crystalline nanoparticles [7]. CNC's potential applications have attracted a great deal of attention as reinforced nanofillers due to their excellent mechanical properties, with high stiffness and elastic modulus.

Qi et al. [8] studied the effects of cellulose nanocrystals on the mechanical properties and barrier properties of corn nano starch (CNS). It was observed that when compared with the pure CNS film, the 8.0 wt % modified-CNC loaded nanostarch-based nanocomposite film achieved a 230.0 % increase in tensile strength, and the moisture absorption ability was decreased by 25.6 %, water vapor permeability was decreased by 87.4 %, and the water contact angle value was increased by 18.1 %. This experimental study had revealed that the CNS/modified-CNCs nanocomposite film showed better antimicrobial activities against *E. coli* and *S. aureus*.

Zinab et al. [9] reported that incorporating 1, 3, 5, and 8 wt % CNC into k-carrageenan biopolymer matrix, via solvent casting method, resulted in nanocomposite films with vastly improved Young's modulus tensile strength and toughness parameters. An increase of 101 % and 74 % were reached for Young's modulus and tensile strength for k-carrageenan-based nanocomposite film containing 8 wt % CNC.

Carboxylated cellulose nanocrystal whiskers (C-CNCW) have been incorporated into cassia-gum (CG) based edible packaging film and used for oil-bags. Lele et al. [10] reported that CG reinforced with C-CNCW films increased as a mechanical barrier, thermal stability, and heat sealability of the films. They also reported that the seal strength was significantly improved from 1295.40 to 2218.78 N/m after 4 % of C-CNCW was added.

Noorbakhsh et al. [11] prepared starch and gelatin films by incorporating chitosan and nano cellulose to improve mechanical, anti-fungal, and waterproof properties. The results showed that increasing nanocellulose composition to 10 % led to an increase in the tensile strength at the break to 8,121 MN/m^2 and decreased the elongation at break. Also, increasing chitosan composition from 5 % to 30 % can enhance pomegranate seeds preservation for up to 15 days. Increasing the glycerol content to 2.16 mL in 1 g dry film results in the enhancement of food quality starting to decrease from that point on, attributed to the enhancement of relative humidity preventing the food from losing moisture. This may, in turn, destroy film structure and porosity enhancement leading to fast moisture loss and quality reduction.

This study aimed to investigate the change in chitosan films' properties resulting from CNC's addition to the post-harvest quality and physicochemical characteristics of chili peppers. The structure

of the chitosan-CNC film was observed using SEM and FTIR. The weight loss, total soluble solids (TSS), and titratable acid content of the chili peppers subjected to different treatments during storage at 20 °C for 12 days were determined in this research.

2. Materials and Methods

2.1. Materials

Acetic acid, glycerol, NaOH were purchased from Merck. Chitosan and cellulose nanocrystals (CNC) were obtained from Chemistry Laboratory, Jakarta State University. Chili peppers were obtained from a local market in Rawamangun (Jakarta, Indonesia) and stored at 40 °C until before the experiment.

2.2. Preparation of films

Chitosan (CH) films were prepared by the casting method. Briefly, 300 mL acetic acid solution 1 % was used to dissolve 3 g CH, and glycerol at 0.20 % (w/w) was added and stirred with a magnetic stirrer until homogeneous. CNC at 0, 3.5, and 10 % (based on CH level, w/w) was added. Next, the mixture was stirred using a magnetic stirrer for 60 min at room temperature to allow homogeneous dispersion of CNC. The air bubbles were avoided with a degassing step, then the film-forming solutions were cast onto a glass plate (10 cm x 30 mm) and dried at the ambient conditions for 48 h. The dry film sample was soaked in a sodium hydroxide solution for 60 min to neutralize acetic acid. The resulting sample was dried in air to obtain biocomposite films. Film samples of 0, 3, 5 and 10 % CNC were coded as E0 = 0 % CNC, E1 = 3 % CNC, E2 = 5 % CNC, and E4 = 10 % CNC, respectively. Characterizations of each film were analyzed using SEM and FTIR.

2.3. Physical property analysis and microstructure observation

The physical properties of chitosan and chitosan-CNC packaging materials were tensile strength (TS), percentage elongation (% E), and degradation rate. TS and % E were examined using the Instron universal testing machine model 4201. TS was calculated by dividing the maximum load by the cross-sectional area of the film. % E was calculated as the percentage of change by dividing film elongation at the moment of rupture by initial gauge length of 50 mm. TS and % E measurements for each film were replicated three times, and each replication being the mean of seven tested sampling units taken from the same film.

Biodegradation tests of chitosan films and chitosan-CNC were conducted in soil, referring to Bras et al. (2010). The soil was taken from the surface layer in the garden. Then the soil was put in a plastic tray to a thickness of around 4 cm. The films were cut into small pieces (about 2 × 5 cm^2) and dried completely before tests. The film samples were accurately weighed and recorded. Then, the film samples were buried about 2 cm beneath the soil. The average temperature, pH, and moisture of the soil were about 25 °C, 6.8, and 20 %, respectively. Water was sprayed once on the soil surface to maintain the moisture. The degraded samples and fragments were taken out one after another every week for four weeks and rinsed with distilled water gently, followed by drying for two days. Finally, the dried samples were accurately weighed, and the weight loss of the film degraded in the soil at 7-day intervals was calculated. The degree of film biodegradation was determined as the weight loss (WL) using equation (1):

$$\% \text{ weight loss} = \frac{Wo-W1}{Wo} x100\% \quad \text{.....(1)}$$

Note:

Wo = sample weight before burial

W_1= sample weight after burial

The time used for the biodegradation test was 30 days, then the degradation rate was calculated using the equation (2):

$$\text{Degradation rate} = \frac{Wo - W1}{30\ days}\ g\ x\ 1000\ mg/g \quad \text{.....(2)}$$

2.4. Packaging of chili peppers

Chili peppers were selected for this study by their shape, size, and color, and the physically damaged or diseased ones were removed. About 10 g of selected chili peppers were packed with chitosan, chitosan-CNC film (15 bags), which were then sealed and stored at temperatures ranged from 250 °C-270 °C for 12 days. Three bags of each packaging were used to analyze each parameter every three days (0, 3, 6, 9, and 12) during the storage.

2.5. Weight loss rate

The weight loss rate was measured according to the following formula (3):

$$\%\ \text{Weight loss rate} = \frac{\text{(original weight- weight after storage)}}{\text{original weight}} \times 100\ \% \quad \text{.....(3)}$$

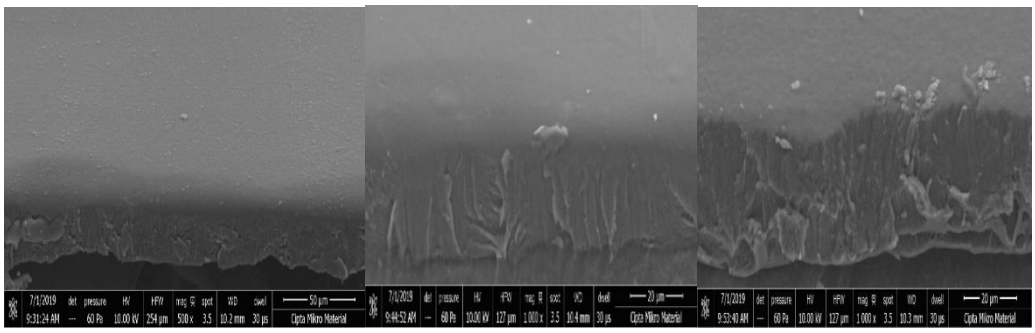
2.6. Biomass measurement of total soluble solids and titratable acid

Chili peppers (50 g) from six fruits were homogenized and then was centrifuged at 4000 rpm for 20 min. The supernatant was collected to measure total solids using a digital refractometer. For titratable acid determination, chili peppers were squeezed with a hand press, and the obtained juice was used. Total titratable acid was determined by diluting 1 mL of chili pepper juice in 9 mL distilled water and then titrated to pH 8.1 using NaOH solution.

3. Results and Discussions

3.1. Analysis of SEM

The SEM image (Fig. 1) showed a rough surface structure in the blend films. The chitosan film was smooth, and the more CNC added, the rougher the resulting film surface. It is observed that there was no agglomeration, which indicated that CNC was homogeneously dispersed in the chitosan film [12], and CNC formed strong interactions with chitosan [13].



(a) (b) (c)

Fig 1. Cross-sectional SEM images of (a) chitosan, (b) chitosan-CNC 5 %, (c) chitosan-CNC 10 %.

3.2. Analysis of FTIR

The characterization results of chitosan, chitosan-CNC films by FTIR are shown in Fig 2. In chitosan films, there is a peak in the wavenumber 3000-3700 cm^{-1}, which indicates the vibrational

stretching of the OH-O intermolecular, which overlaps with N-H stretching vibration [14] at wavenumber 3300-3280 cm^{-1} [15]. The peak at wavenumber 1538 cm^{-1} shows C-O strain resulting from hydrogen bonding band observed at 1538 cm^{-1} (N-H) in near chitosan spectrum, which is shifted to 1543 cm^{-1} in chitosan-CNC spectra. This confirms that strong interactions occurred between the NH_3 group of chitosan and $SO3^{-2}$ group of CNC at wavenumber 1204 cm^{-1}. These observations indicated that strong electrostatic interactions and hydrogen bonding occurred between the functional groups of CNC and the free functional groups of chitosan film. These results are in agreement with the results reported by Perumal et al. [16].

Besides, there was also a shift of chitosan film tape with CNC's addition and experienced a shift in the wavenumber 3200-3600 cm^{-1} compared to chitosan film. This may be due to electrostatic interactions between anionic sulfate from CNC and amine group from chitosan, or hydrogen bonding between OH groups of CNC and ammonium groups from chitosan. Such interactions are essential in improving the mechanical properties of composite films [17].

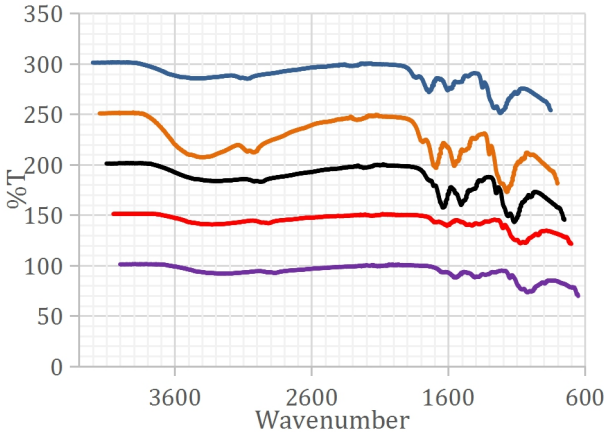


Fig 2. Spectra IR of chitosan and chitosan-CNC films.

3.3. Tensile strength and percentage elongation

Tensile strength (TS) of chitosan films containing different levels of CNC are shown in Fig 3. Except for the 5-10 % CNC film, TS was increased after CNC addition. This increase may be attributed to cross-linking between chitosan and CNC of chitosan film [18]. An increase in TS or puncture strength of chitosan film was due to the development of cross-linking has been reported for nano-silica treated chitosan film [19]. In our results, the TS of chitosan film increased from 35±1.04 to 50±1.42 MPa with 3 % CNC, which resulted in the strongest film in this study. At higher levels of CNC addition (10 %), TS was decreased. This may indicate that the increased CNC ratio to chitosan resulted in steric hindrances preventing the hydroxyl group on chitosan film from reacting with amino groups on chitosan chains [20].

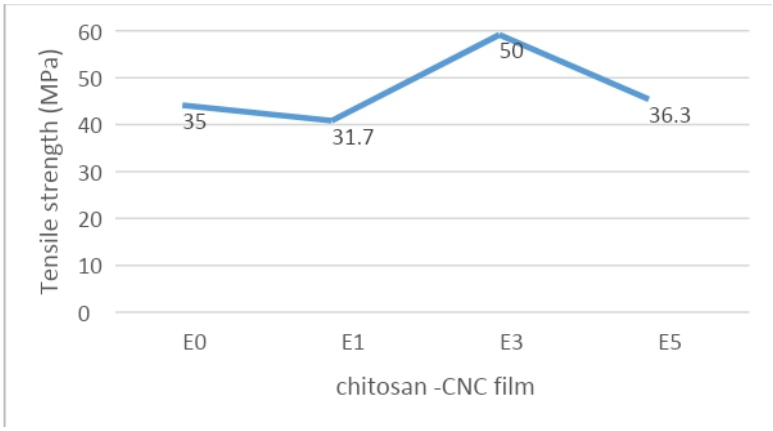


Fig 3. Tensile strength of chitosan-CNC films.

Percentage elongation (% E) at the break as a measure of the extensibility for chitosan-CNC films was decreased from 26.72±1.80 to 18±2.01 % as the amount of CNC increased from 0 to 10% (Fig 4). In general, increased TS of chitosan films was due to cross-linking, often accompanied by a reduced film, resulting in less elastic films. Different CNC levels in chitosan films showed a difference in mean values of % E of chitosan-CNC films. These results support cross-linking between CNC and protein as the primary mechanism for strengthening composite films' physical properties.

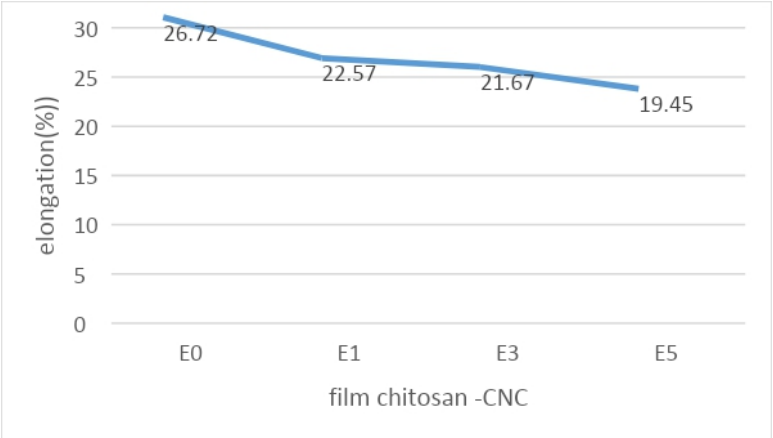


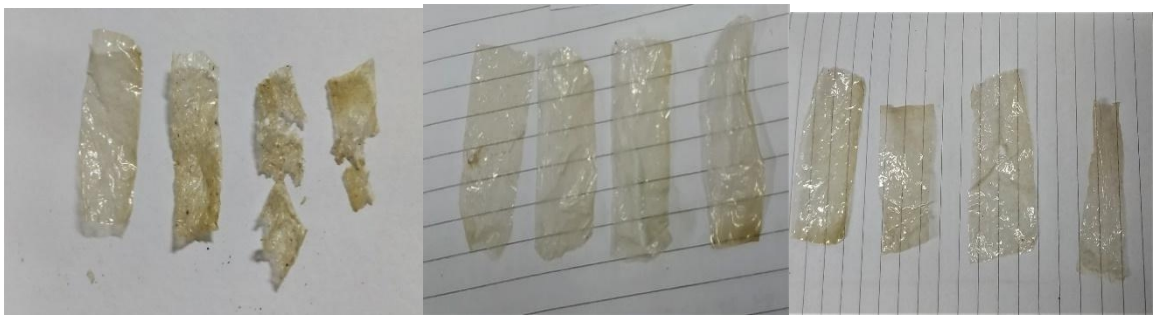
Fig 4. Changes of % elongation at break of chitosan-CNC films.

3.4. Biodegradation test

The results showed that the chitosan-CNC film of 3 % (E3) weight loss was 50.48±1.44 %. The addition of CNC 5 %-10 % to the chitosan film caused a decrease in weight loss (Fig 5). This may be due to many interactions between chitosan and CNC, thus breaking the chain by microorganisms takes longer than films with the addition of less CNC.

The film's burial showed a reduction in the plastic's mass and the change in the film from being smooth to stiff and brittle, including cracking, hole formation, and discoloration. Microorganisms developed gradually and covered the surface of the sample so that biodegradation occurred in plastic films. The presence of CNC on plastic films increased microbial attack and biodegradation rates by stimulating biofouling and adhesion of microorganisms to the surface, which

led to cracking and crevice formation. Chitosan-CNC film burial for 150 days has been shown to reduce plastic mass above 70 % [16].



(a) (b) (c)

Fig 5. Films degradation after 30 days: (a) CNC 3 %, (b) CNC 5 %, and (c) CNC 10 %.

3.5 Weight loss of chili peppers wrapped with chitosan-CNC film

Observation of weight loss of chili peppers that wrapped with chitosan and chitosan-CNC films are shown in Fig 6. Observations were made every 3 days for 12 days. The results showed that the greater chitosan-CNC addition in films resulted in smaller changes in weight loss. Chili peppers wrapped with chitosan film were less effective in maintaining weight loss because of respiration and transpiration, which cannot be appropriately blocked. Meanwhile, the chili peppers wrapped with chitosan-CNC films can inhibit the increase in weight loss. According to Jiang et al. [21], the fruit given with wax coating can inhibit the transpiration process resulting in damage to fruit tissue, and this can be slowed down. The chitosan film results with CNC 3 % showed a significant effect at $p < 0.05$ on the weight loss of chili peppers.

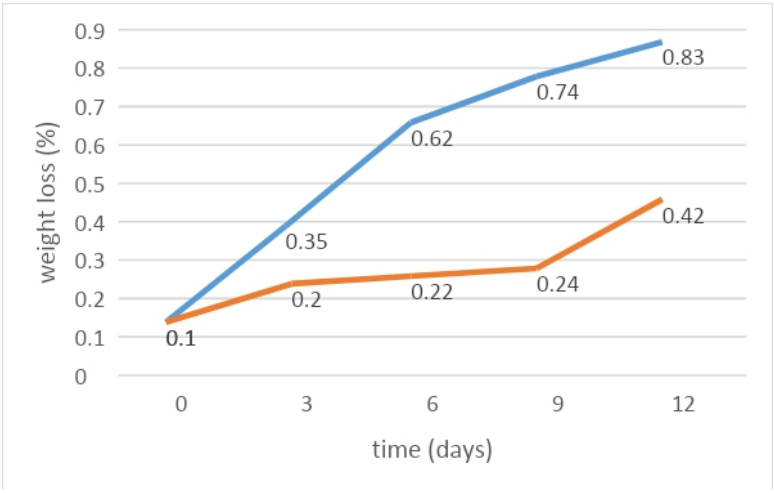


Fig 6. Weight loss of chitosan-CNC films.

3.6 Total soluble solids of chili peppers

Total soluble solids (TSS) are among the most important parameters that affect fruit quality and consumer acceptability. Changes of TSS of chili peppers during room temperature storage are presented in Fig 7. Fig 7 showed that TTS was increased with increasing time storage due to the initial metabolic process for converting carbohydrates into polysaccharides and other soluble compounds. TTS was declined rapidly during the late storage period because respiration required the presence of nutrients. A high percentage of CNC in chitosan slowed down the decline of TTS. Chitosan-CNC films wrapped on chili peppers work as an oxygen barrier, slowing down respiration rate and metabolic activity. Therefore, it delayed the decaying process, which was the same as the TSS value. A similar finding was reported by [22] that when using 0.3 % of chitosan nanoparticles, the cucumbers lose 4 % less weight than the control group after 15 days of storage. This may be because chitosan has a highly selective permeability, reducing water loss in the fruit [23].

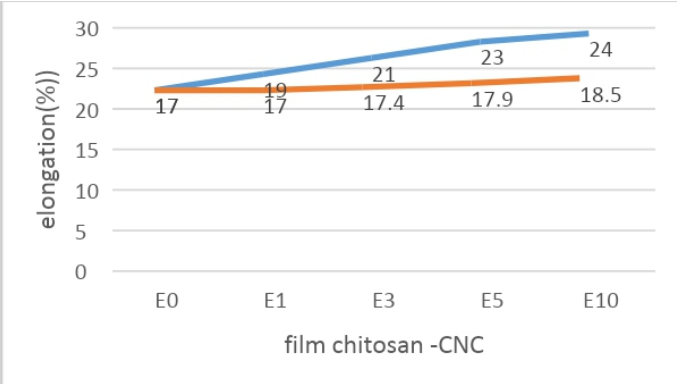


Fig 7. Total soluble solids of chili peppers wrapped with chitosan and chitosan-CNC films.

3.7 Titratable acidity

Titratable acidity (TA) is an important factor in measuring the quality of fruit and vegetable. TA content in chili peppers was significantly decreased ($p < 0.05$) during the entire storage period, with lower values in chitosan treated chili peppers compared to chitosan-CNC films treated chili peppers (Fig 8). Under normal temperature storage conditions, the TA decline rate of chili peppers wrapped with chitosan-CNC films was higher than that of chili peppers wrapped with chitosan films. Likewise, the chitosan-CNC films made the TA of chili peppers declined in the late mature stage. Due to the high CNC content in chitosan film, chili peppers' respiration was slowed down to extend the shelf life and quality of the chili peppers.

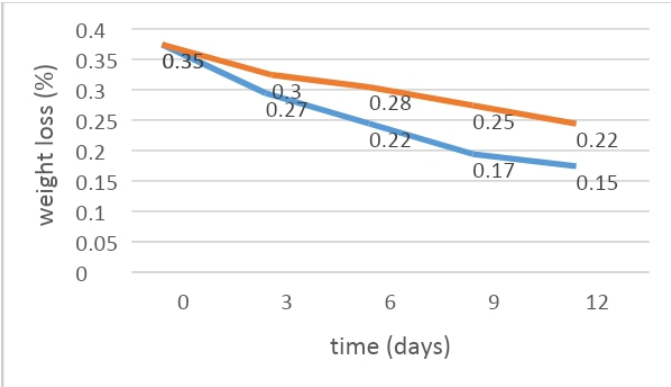


Fig 8. Titratable acidity of chili peppers wrapped with chitosan and chitosan-CNC films.

To visually observe chitosan-CNC films' effect on chili peppers spoilage and preservation, the chitosan film with 3 % CNC was compared to chitosan films. It is shown that the chitosan-CNC films could effectively extend the shelf life of the chili peppers and inhibit the spoilage of the chili peppers (Fig 9).

Fig 9. The effect of chitosan-CNC films on chili peppers spoilage and preservation.

4. Conclusion

Packaging materials based on chitosan containing different cellulose nanocrystals (CNC) were developed for increasing the shelf life of chili peppers. Cellulose nanocrystals additions improved the tensile strength, percentage elongation, and reduced biodegradation of the packaged product quality. The CNC enhanced the interactions (e.g., hydrogen bonds and electrostatic interactions) between the chitosan chains to perform a smooth morphology. Also, chitosan-CNC films had specific physical properties, so the chili peppers' preservation was better than chitosan films considering the films' mechanical properties and the freshness of chili peppers.

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References

1. Michael M. Blankei, and Peter A. Holth. 1997. Bioenergetics, Maintenance Respiration and Transpiration of Pepper Fruits. *J Plant Physiol* **50** : 247-250
2. Mohamed SAA, El-Sakhawy M, El-Sakhawy MA-Monem.2020. Polysaccharides, Protein and Lipid -Based Natural Edible Films in Food Packaging: A Review.*Carbohydrate Polymers* **238** : 116-178
3. Wu.Y., Weller.C.L., Hamouz.F., Cuppet.S.L., Schnepf. M. 2002. Development and application of multicomponent edible coatings and films. A review.*Advances in Food and Nutrition Research* **44** : 347-394
4. Kewang Zheng, Sa Xiao, Wei Li, Wei Wang, Hujian Chen, Fan Yang, Caiqin Qin. 2019. Chitosan-acorn starch-eugenol edible film: Physico-chemical, barrier, antimicrobial, antioxidant and structural properties. International Journal of Biological Macromolecules 135: 344-352

5. Liming Zhang., Zhanli Liu., Yang Sun., XiaominWang.,Ling Li, 2020 Effect of α-tocopherol antioxidant on rheological and physicochemical properties of chitosan/zein edible films. *LWT* **118** : 107999
6. Walid Yeddes,. Kais Djebali, Wissem Aidi Wannes, Karima Horchani-Naifer, Majdi Hammami, IslemYounes, Moufida Saidani Tounsi. 2020. Gelatin-chitosanpectin films incorporated with rosemary essential oil: Optimized formulation using mixture design and response surface methodology, *International Journal of Biological Macromolecules* **154** : 92-103
7. Ma Q, D. Hu, L. Wang.2016. Preparation and physical properties of tara gum film reinforced with cellulose nanocrystals. *Int. J. Biol. Macromol*. **86** : 606-612
8. Qi-Jie Chen, Li-Ling Zhou, Jia-Qi Zou , Xin Gao.2019. The preparation and characterization of nanocomposite film reinforced by modified cellulose nanocrystals. *International Journal of Biological Macromolecules*. **132** : 1155-1162
9. Zineb Kassab, Faissal Aziz, Hassan Hannache, Hicham Ben Youcef, Mounir El Achaby. 2019. Improved mechanical properties of k-carrageenan-based nanocomposite films reinforced with cellulose nanocrystals. International Journal of Biological Macromolecules **123** : 1248-1256
10. Lele Cao., Tingtignge., Fnsong Meng., Shiyu Xu., Jian Li., Lijuan Wang. 2020. An edible oil packaging film with improved barrier properties and heat sealability from cassia gum incorporating carboxylated cellulose nanocrystal whisker. *Food Hydrocolloids* **98**:105251.
11. S.M.Noorbakhsh.S.M, Soltani M.M.,Zerafat S., Sabbaghi. 2018. A comparative study of gelatin and starch-based nanocomposite films modified by nano-cellulose and chitosan for food packaging applications*. Carbohydrate Polymers* 189 : 48-55
12. Hänninena.A ,S.Rajalaa , T.Salpavaaraa , M.Kellomäkia , S.Tuukkanena.2016. Piezoelectric sensitivity of a layered film of chitosan and cellulose nanocrystals. Procedia Engineering **168** :1176 -1179
13. Jaya Sundaram, Jitendra Pant, Marcus James Goudie, Sudhagar Mani, and Hitesh Handa.2016. Antimicrobial and Physicochemical Characterization of Biodegradable, Nitric Oxide-Releasing Nanocellulose-Chitosan Packaging Membranes. J. *Agric. Food Chem* **64** : 5260-5266
14. Debandi, María V, Celina Bernal, and Nora J Francois. 2016. Development of Biodegradable Films Based on Chitosan/Glycerol Blends Suitable for Biomedical Applications. *Journal Tissue Science & Engineering* **7** : 1-9
15. Khan, Avik et al. 2012. Mechanical and Barrier Properties of Nanocrystalline Cellulose Reinforced Chitosan Based Nanocomposite Films. *Carbohydrate Polymers* **90** : 1601-1608
16. Perumal, Anand Babu, Periyar Selvam Sellamuthu, Reshma B Nambiar, and Emmanuel Rotimi Sadiku. 2018. Development of Polyvinyl Alcohol/Chitosan Bio-Nanocomposite Films Reinforced with Cellulose Nanocrystals Isolated from Rice Straw. *Applied Surface Science* **449** : 591-602
17. Bao, Yuping et al. 2018. Fabrication of Cellulose Nanowhiskers Reinforced Chitosan-Xylan Nanocomposite Films with Antibacterial and Antioxidant Activities. *Carbohydrate Polymers* **184**: 66-73
18. Muhammad Mujtaba, Asier M.Salaberria, Mar´ıa A.Andres, Murat Kaya, Ayse Gunyakti, Jalel Labidi. 2017. Utilization of flax (*Linum usitatissimum*) cellulose nanocrystals as reinforcing material for chitosan films. *International Journal of Biological Macromolecules* **104** : 944-952
19. Mostafa Y.Ismail, Minna Patanen, Juho Antti Sirviö, MiikkaVisanko , Takuji Ohigashi, Henrikki Liimatainen. 2019. Hybrid films of cellulose nanofibrils, chitosan and nanosilica—Structural, thermal, optical, and mechanical properties. *Carbohydrate Polymers* 218 : 87-94
20. Abdul Khalil, H. P. S., Saurabh, C. K., Adnan, A. S., Nurul Fazita, M. R., Syakir, M. I., Davoudpour, Y., Dungani, R. 2016. A review on chitosan-cellulose blends and nanocellulose reinforced chitosan biocomposites: Properties and their applications. *Carbohydrate Polymers*, **150** : 216-226
21. Jiang.X, H. Lin, J. Shi, S. Neethirajan, Y. Lin, Y. Chen, H. Wang, Y. Lin. 2018. Effects of a novel chitosan formulation treatment on quality attributes and storage behavior of harvested litchi fruit. *Food Chem*. **252** : 134-141

22. Velickova. E, E. Winkelhausen, S. Kuzmanova, V.D. Alves, M. Moldão-Martins. 2013. Impact of chitosan-beeswax edible coatings on the quality of fresh Strawberries (*Fragaria ananassa* cv *camarosa*) under commercial storage conditions. *LWT-Food Sci Technol*. **52** : 80-92
23. He.Y., S. Bose, W. Wang, X. Jia, H. Lu, H. Yin. 2018 Pre-Harvest treatment of chitosan oligosaccharides improved strawberry fruit quality, *Int. J. Mol. Sci*. **19** : 2194