SYNTHESIS AND CHARACTERIZATION OF NANO CHITOSAN-AVOCADO SEED STARCH AS EDIBLE FILMS

Suhartini[1], Imas Solihat[2*], Foliatini[2], Sri Redjeki Setyawati[3], Nurdiani[4], Lilis Sulistiawaty[1], Muhammad Fadhil Khoirurrizal[2]
[1]Department of Chemical Analysis, Politeknik AKA Bogor, Jl. Pangeran Asogiri No. 283, Bogor 16154, Indonesia
[2] Department of Food Nanotechnology, Politeknik AKA Bogor, Jl. Pangeran Asogiri No. 283, Bogor 16154, Indonesia
[3]Department of Food Industry Quality Assurance, Politeknik AKA Bogor, Jl. Pangeran Asogiri No. 283, Bogor 16154, Indonesia
[4]Department of Industrial Waste Treatment, Politeknik AKA Bogor, Jl. Pangeran Asogiri No. 283, Bogor 16154, Indonesia
[*]Email: imaskhairani@gmail.com

Received 12 February 2023
Accepted 01 May 2023

Abstract

The use of plastic as food packaging tends to cause problems because it is difficult to decompose; therefore, it can pollute the environment. The development of biodegradable plastics is an alternative to this problem. Chitosan, a bioplastic, can be used as a packaging material but has poor barrier properties. A biodegradable film was made from a mixture of plasticizer, nano chitosan, and avocado seed starch. Nanochitosan synthesis was carried out using the UAE method for 2 h at an amplitude of 50% to produce 0.7 µm sized particles. Films were formed using the casting method, and characterization was performed, which included functional group, thickness, color, and antioxidant tests. The FTIR spectrum showed that the interaction between nanochitosan and avocado seed starch occurred physically, marked by a shift in the wavenumber of the amide carbonyl group from 1646.60 cm^{-1} 1549.99 cm^{-1}. The film thickness was 0.10–0.15 cm with a darker color as the volume of nanochitosan used increased. Antioxidant analysis revealed that the LC_{50} value was between 150-250 ppm. The barrier properties of the resulting film against water vapor can inhibit strawberry fruit decay for 3–4 days at room temperature.

***Keywords**: avocado seed starch, biodegradable, nano chitosan.*

Introduction

Population growth is one of the factors that increases the need for food packaging, which is useful for distributing and maintaining the quality of food products (Marsh and Bugusu, 2007). Plastic packaging is often the choice because it has good barrier properties, is low in price, and is easy to manufacture and apply, but it can cause waste problems such as blockage of waterways and flooding (Kamsiati *et al.*, 2017). Therefore, efforts are needed to minimize the risk of using plastic food packaging, among others, by finding new sources of biodegradable materials derived from polymers that can decompose in the environment. Biodegradable packaging materials can be obtained from various natural materials, such as starch, cellulose, chitin, chitosan, and gelatin, as well as from synthetic materials such as polylactic acid, polyglycolide, and polycaprolactone (Shaikh *et al.*, 2021).

One of the agricultural waste products (food losses and waste) produced by Indonesians is avocado seeds and avocado seed starch, which has never been developed before. Avocado seeds

 This is open access article under the CC-BY-NC-SA license

have a high starch content, consisting of amylose (43.3%) and amylopectin (37.7%) (Lubis, 2008), avocado seeds have the potential to be used as food packaging materials, such as edible films. Starch is a hydrocolloid that can be used to prepare edible films because it is easy to obtain and inexpensive. According to Garcia (García *et al.*, 2011), starch-based edible films produce films that are strong enough, odorless, colorless, and tasteless with a high degree of clarity. The weakness of edible starch is that it is easy to crack and the barrier to water vapor is low because starch is hydrophilic.

Bharathi (Bharathi *et al.*, 2021) modified a film made from avocado seed starch, tween-20, and sorbitol to produce a transparent and homogeneous film, but reduced its resistance to water vapor. Coniwanti (Coniwanti *et al.*, 2016) studied avocado seed starch combined with agar as edible films. The combination of avocado seed starch and chitosan can produce films with high tensile strength and elongation values, but low solubility properties (Susilowati and Lestari, 2019). The deficiency in edible films produced from avocado seed starch can be corrected by adding glycerol. To improve the properties of edible films, nanosized fillers can be added to biopolymers to form nanocomposite polymers (Yoksan and Chirachanchai, 2010). Bionanocomposites are nanoscale (≤100 nm) materials with a polymer matrix and fillers that can improve functional properties, morphology, and film stability (Slavutsky and Bertuzzi, 2014). Nanochitosan is a natural and safe nanoscale filler material.

Edible films with chitosan have good antioxidant and antimicrobial activities (Zareie *et al.*, 2020). Zhao (Zhao *et al.*, 2022) prepared a composite edible film of nanochitosan and cinnamon extract, which can inhibit microbial growth for six days on fresh fish packaging. Nanochitosan can also be combined with other materials to obtain packaging films with superior mechanical and functional properties. Romainor (Romainor *et al.*, 2014) studied the synthesis of nanochitosan/cellulose-based films and demonstrated their antimicrobial properties.

Although several researchers have studied food packaging based on chitosan and avocado seed starch, the use of chitosan in nanoform composites with avocado seed nanostarch as edible films has never been studied. The potential of nanochitosan and nanopati as ingredients for making edible films is very high because of their characteristics as superior nanomaterials with high antimicrobial capabilities and interactions between components that are predicted to be more effective because they have a larger surface area. In this study, nanochitosan and starch-based biocomposites from avocado seeds were studied, starting from their synthesis and characterization through their application as edible films in fruit products.

Research Methods

Materials

Avocado seeds and strawberry fruits were obtained from Bogor-West Java traditional market: chitosan, sodium metabisulfite, bovine gelatin from HAYS, acetic acid (Merck, Germany), glycerol (Merck, Germany), anhydrous calcium chloride (Merck, Germany), and distilled water.

Instrumentation

Dry Herbs Grinding Milling Type C2100Y, Test Sieve Shaker Type Haver ELM 200 Premium, Alpha Bruker Fourier Transform Infrared (FTIR) Spectrophotometer, Spectrophotometer UV-Vis Type Specord 200 Plus, Particle Size Analyzer (PSA) Type Saturn Digisizer II, Planetory Rotary Ball Mill, Minolta CR 310 Chromameter, Sonicator, LabTech brand oven, Type ATX224 analytical balance, autoclave, hot plate, incubator, vortex mixer, handheld micrometer, magnetic stirrer, water bath, silicone container, knife, scissors, and glassware.

Synthesis

1) Synthesis of avocado seed starch

   Avocado seeds were separated from the fruit, cut into small pieces, washed, and soaked in salt for 30 min. The samples were washed and soaked with 200 ppm sodium metabisulfite for 24 h, washed, drained, crushed to a 20 mesh size, and filtered. The filtrate was then collected and incubated for 24 h. The starch obtained was dried in an oven at 40 °C for 48 h.

2) Nanochitosan synthesis

   Chitosan (1 g) was mixed with 100 mL of 1acetic acid, stirred at 1400 rpm for 1 h at 80 ºC, and sonicated for 2 h with an amplitude of 50%.

3) Making plasticizers

   A total of 10 g of gelatin was dissolved in 400 mL of distilled water and heated to boiling temperature. After boiling, 5 mL glycerol was added, and the mixture was heated for 10 min.

4) Making biodegradable films

   Edible films were prepared using the casting method with solvent evaporation as described by Zheng (Zheng *et al.*, 2019) and Chen (Chen *et al.*, 2019), modified. Avocado seed starch with a concentration of 4%, 1% nanochitosan, and plasticizers were mixed in various ratios and concentrations in a total volume of 28 mL. The mixture was stirred at 1000 rpm and 50°C, poured into a silicone mold measuring 8 × 8 cm, sonicated for 2 × 30 min at room temperature, then dried at 45°C for 24 h. The variations in filmmaking are presented in Table 1.

Table 1. Variations in film composition

Film Code	**Composition (mL)**		
	Plasticizer	**Nanochitosan**	**Starch**
1	5	1	1
2	4	2	1
3	3	3	1
4	2	4	1
5	1	5	1

Characterization

1) Characterization of nanochitosan

   The particle size and size distribution were determined using a laser diffraction-based Particle Size Analyzer (PSA) with an aqueous background.

2) Characterization of biodegradable films

   a. Functional group analysis: Functional group analysis was carried out to determine the interactions that occur between the components in the edible film using an Alpha Bruker Fourier Transform Infrared (FTIR) spectrophotometer at a wavelength of 400–4000 cm^{-1} (Shahbazi and Shavisi, 2018).

   b. Thickness test: The thickness of the biodegradable film was measured using an ASTM Designation: D1005-95 (2001) handheld micrometer with an accuracy of 0.001 mm at five different locations. The thickness was calculated from the average of five thickness measurements.

   c. Color test: Color measurement was carried out using a Minolta CR 310 chromameter. The biodegradable film samples were placed on a white mat. The measurements produce values for L, a, and b. L represents the brightness parameter (chromatic tint, 0: black to 100: white), while a and b are chroma coordinates. Parameter a is the reflected light that produces a mixed red-green chromatic color with a value of +a (positive) from zero to 100 (red) and a value of -a (negative a) from zero to 80 (green). Parameter b is a mixed blue-yellow chromatic color with a value of +b (positive b) from zero to 70 (yellow) and a value of -b (negative b) from zero to 70 (blue).

3) The application of film as edible coating

The strawberry fruit samples were cleaned, and each fruit was covered with a biodegradable film and placed in an open glass cup. Changes in physical properties were observed for 96 h. Controls were prepared using strawberries without biodegradable film coatings.

Results and Discussion

Biodegradable film synthesis

Biodegradable film synthesis was carried out using a casting method with a silicone vessel. Prior to drying, sonication was performed for 2 × 30 min to prevent the formation of an uneven film surface. The unevenness of the film could be caused by air bubbles trapped in the solution during the drying process. The nature of the polymer, which is able to absorb air, and the combination of solvents in the manufacture of films that have different boiling points (the boiling point of distilled water as an avocado seed starch solvent is 100 °C, which is different from the boiling point of acetic acid as a chitosan solvent at 118 °C) cause the solvent to be trapped, while other parts are This phenomenon is called case hardening. The effect of sonication on the resulting films is shown in Figure 1.

(a) (b)

Figure 1. Synthesized biodegradable film, (a) without sonication, (b) with sonication

The plasticizer used was a mixture of gelatin and glycerol because it is easily soluble in water (hydrophilic) and has a low molecular weight, thus reducing the stiffness of the polymer and increasing the flexibility of the film. The mixed plasticizer and nanochitosan can be distributed evenly on the film so that its properties can be evenly distributed throughout (Ismaya *et al.*, 2021). The results of biodegradable film synthesis are shown in Figure 2. Morphology of the edible film no 5 is observed with SEM magnification of 3500× which can be seen in Figure 3.

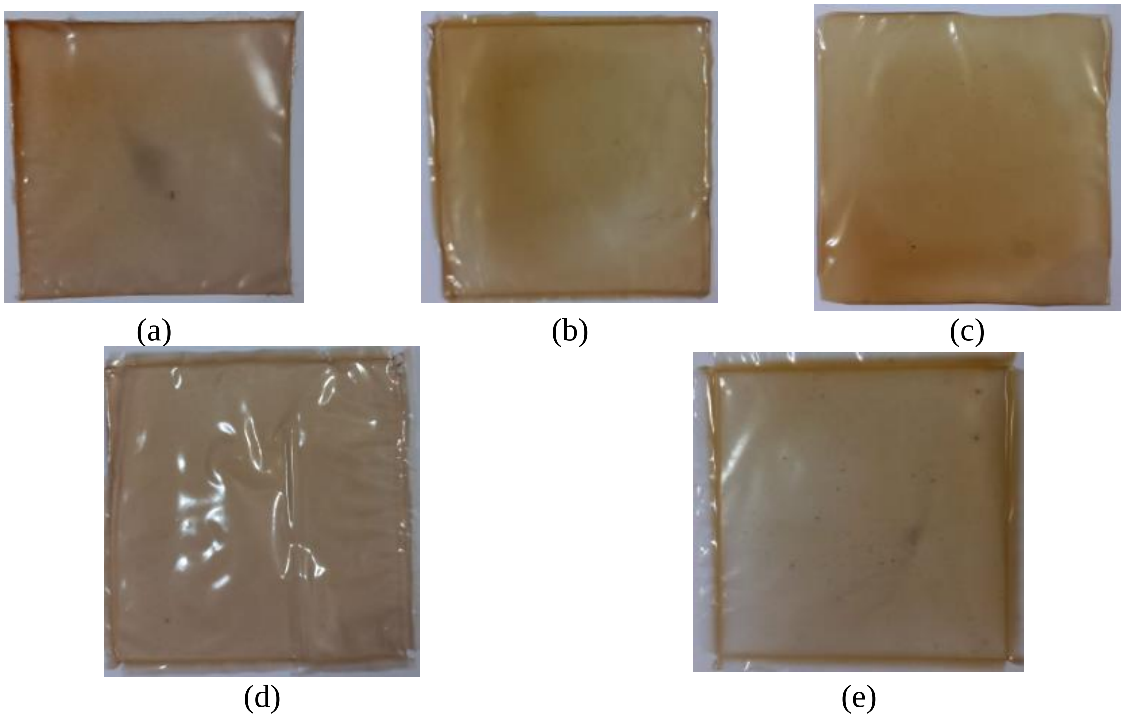

Figure 2. The synthesized film with variation plasticizer and nanochitosan, (a) edible film 1, (b) edible film 2, (c) edible film 3, (d) edible film 4, (e) edible film 5

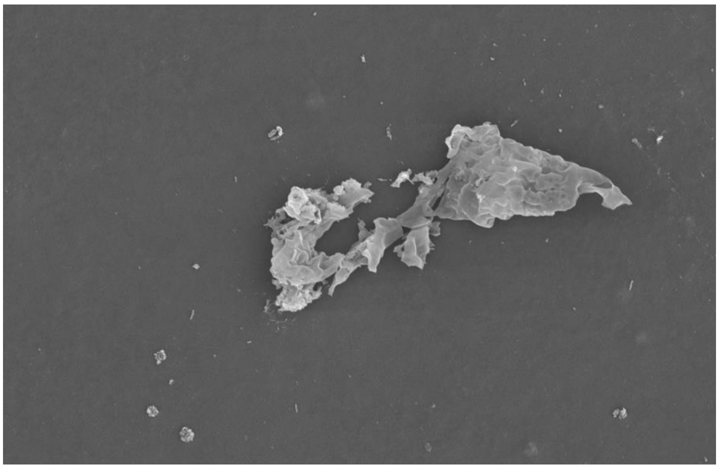
Figure 3. SEM analysis result

From the results of observations at 3500× magnification, it can be seen that the morphology of the edible film with a composition of nanochitosan and starch is well dispersed. The film surface of formula no. 5 is quite smooth and textured, which shows a homogeneous composition.

Characterization of biodegradable films

The mechanism for making films using the UAE method involves the formation of cavitation waves in the liquid media by ultrasonic waves generated by the probe, thereby creating different pressures in the media. The bubbles that are formed will stick to the solid particles, so that the components contained in the solid particles will diffuse into the solvent (Azmin *et al.*, 2016). Nanoparticle size analysis was performed using a particle-size analyzer (PSA). From these observations, it was found that the size of chitosan after ultrasonic treatment was 0.7 µm.

Functional group analysis was performed to determine the type of interactions that occurred in a mixture. When several components of a substance are mixed, chemical and/or physical interactions occur, which can be determined by observing the changes in the absorption peaks in the FTIR spectrum. The FTIR spectrum of the resulting biodegradable film is shown in Figure 4.

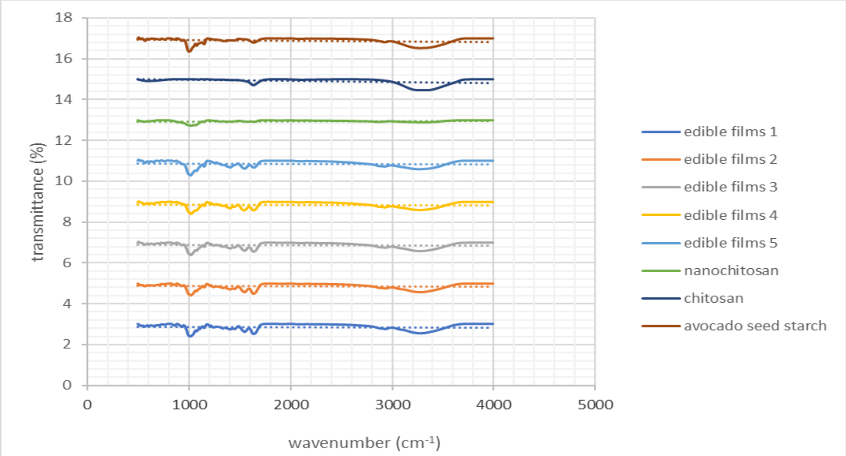

Figure 4. FTIR analysis of edible film

The FTIR spectra of chitosan and nanochitosan on edible films 1–5 were the same. The FTIR peak is at wave number 1646.60 cm^{-1} which is characteristic of the amide carbonyl functional group. The peak was slightly weakened on the chitosan/starch nanocomposite film; however, the peak at 1549.99 cm^{-1} was clearly visible on the nanocomposite film. The -OH and -NH peaks in the 3000–3500 cm^{-1} region of the nanocomposite film also appeared to have lower intensities, indicating the possibility of interactions between these groups. Based on the FTIR results obtained, it was predicted that in the nanocomposite film, the interaction between nanochitosan and avocado seed starch occurs physically.

One of the factors that determines the quality of a film is its thickness because it directly affects its mechanical and physical properties (Fundo *et al.*, 2015). The results of thickness analysis are shown in Figure 5.

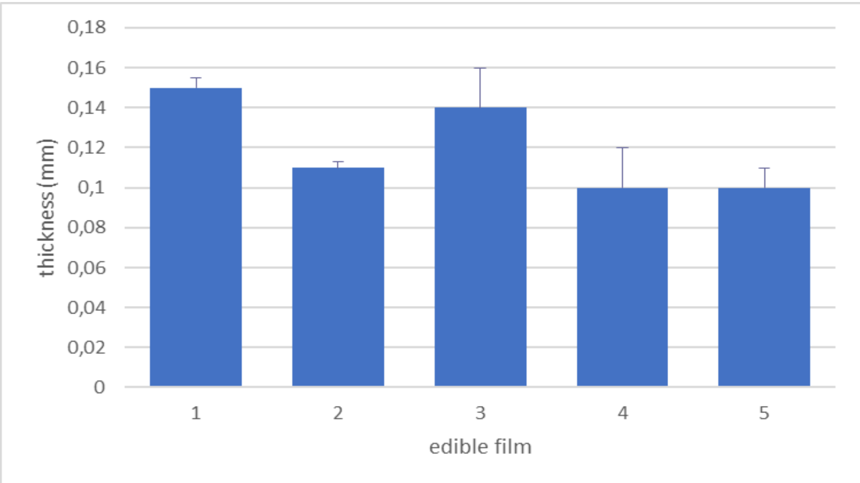

Figure 5. Results of film thickness analysis

Figure 5 shows that, as more plasticizer was added, the total solids in the solution increased, causing the film thickness to increase. According to Jongjareonrak (Jongjareonrak *et al.*, 2006), the dispersed plasticizer molecules in the film matrix contribute to increasing the interstitial distance of the polymer chains in the film matrix, thereby increasing the film thickness. The line marks on the bar graph indicate the magnitude of the variation (error bars) in the edible film thickness data 1-5. On the edible film chart, nos. 3 and 4 have long lines. This shows that a large error bar value indicates that the resulting data variation is large.

The color intensity of the resulting film was measured using a chromameter (Minolta CR-300) with CIE L, a, and b systems. Otles (Otles, 2009) explained that L is the coordinate of brightness, a is the coordinate of a reddish to a greenish color, and b is the coordinate of a yellowish to bluish color. The resulting CIE system was converted to ΔE, which is the value of the total color difference between the films. The ΔE value indicates the clarity of the film; the greater the ΔE value produced, the more turbid or non-transparent the film will be. The ΔE values of the resulting biodegradable films are shown in Figure 6.

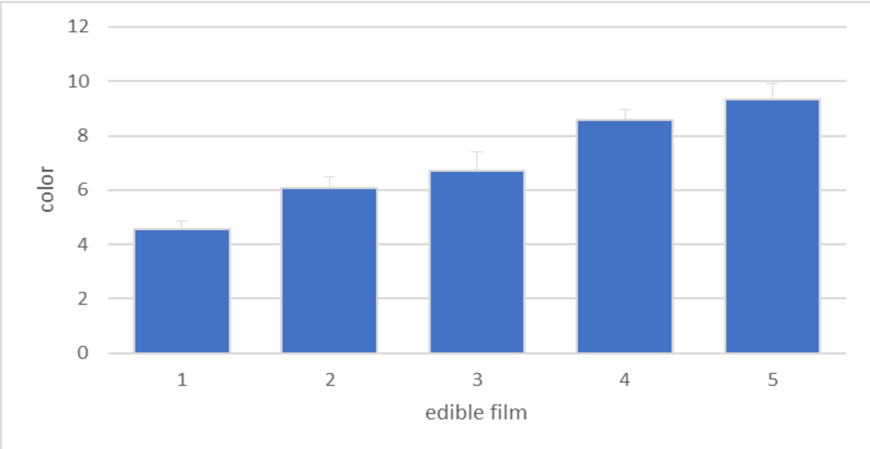

Figure 6. The ΔE value of the film

The results of the analysis of variance showed that the color of the film was significantly different at the 5% level due to the influence of two factors, namely the difference in the amount of added plasticizer and nanochitosan. Figure 6 shows that the addition of nanochitosan affected the color of the resulting film. The more nanochitosan used, the darker the color of the film. This is because the energy generated in the chitosan sonication process can cause chemical degradation, thereby decreasing the brightness value. The difference in film transparency is related to the mobility of the polymer chains and the distance between the molecules in the film matrix with plasticizers, which affects the light permeability of the resulting film (Farhan and Hani, 2017). The ΔE values of edibles 3 and 5 are larger than those of edibles 1, 2, and 4. This is indicated by a bar on the longer graph (larger bar error value).

The application of film as edible coating

Strawberries have a high economic value but are easily degraded. One of the factors that cause damage is the amount of water contained in the environment. The high water content can make it easy for decomposing microorganisms to multiply, thereby shortening storage time. The microorganism that easily develops on strawberries is *Botrytis cinerea*, which is a fungal species (Bai *et* *al.*, 2022). The resulting biodegradable film was used to wrap strawberries for four days at room temperature, with observations made every 24 h for 96 h. The results are shown in Figure 7.

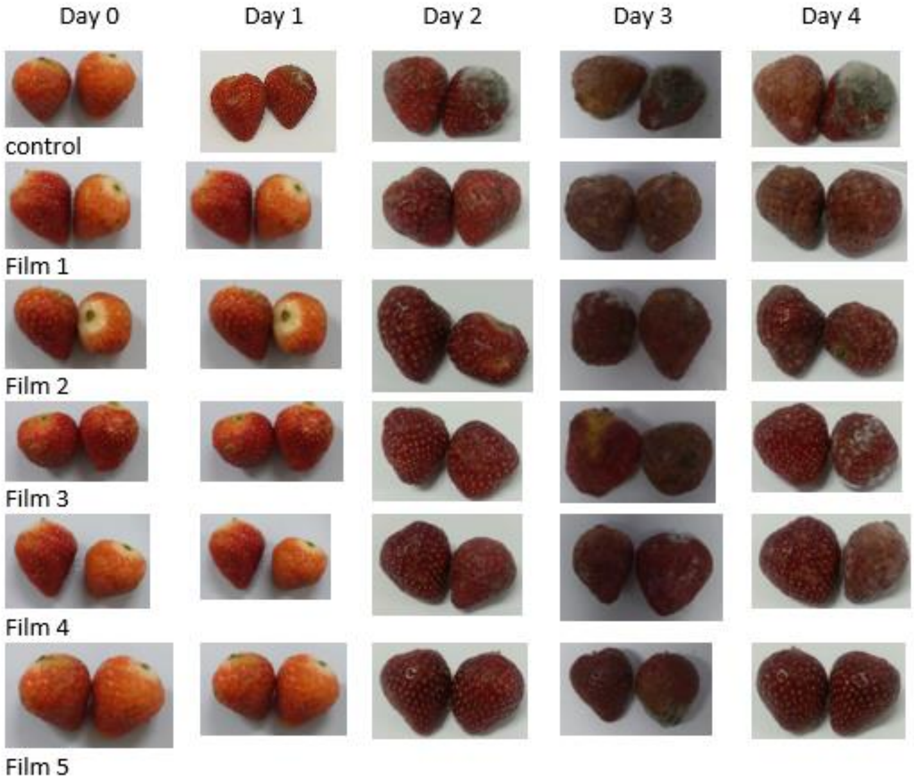

Figure 7. Application of biodegradable films on strawberries

Figure 7 shows that strawberries that were not packaged with the nanocomposite film experienced more serious decay after 48 h of storage. Decay is characterized by the shrinkage of fruit volume, decrease in freshness, and appearance of mold on the surface of the fruit. Strawberries packed with the nanocomposite films retained their freshness for up to 72 h. The rate of decay increased as the amount of plasticizer used increased. The addition of a plasticizer increases the flexibility of the film such that the film pores become larger, resulting in an easier water distribution process (Suyatma *et al.*, 2005). The addition of an increasing amount of nanochitosan acts as a barrier to the rate of H_2O that passes through the film because nanochitosan can fill the shafts formed on the film. In this experiment, the strawberries packed with the 5th variation of the nanocomposite film still showed freshness after 120 h of storage.

Conclusions

Biodegradable films made from nanochitosan and avocado seed starch were successfully synthesized by sonication. The results showed that the nanocomposite film with the plasticizer, nanochitosan, and avocado seed starch 1:5:1 was able to extend the shelf life of strawberries for 120 h of storage.

Acknowledgement

Thank you to Bogor AKA Polytechnic for all the facilities and research funds that have been provided to carry out this research.

References

Azmin, S.N.H.M., Manan, Z.A., Alwi, S.R.W., Chua, L.S., Mustaffa, A.A. and Yunus, N.A., 2016. Herbal processing and extraction technologies. *Separation and Purification Reviews*, 45(4), pp.305–320.

Bai, Y., Liu, H., Lyu, H., Su, L., Xiong, J. and Cheng, Z.M.M., 2022. Development of a single-cell atlas for woodland strawberry (Fragaria vesca) leaves during early Botrytis cinerea infection using single-cell RNA-seq. *Horticulture Research*, 9(August 2021).

Bharathi, S., Ramesh, B., Kumaran, S., Radhakrishnan, M., Saravanan, D., Saravanan, P., Pugazhvendan, S.R. and Nalinasundari, M.S., 2021. No TitDevelopment of nanobiomaterial for wound healing based on silver nanoparticles loaded on chitosan hydrogelle. *3 Biotech*, 11(12), pp.1–14.

Chen, X., Cui, F., Zi, H., Zhou, Y., Liu, H. and Xiao, J., 2019. Development and characterization of a hydroxypropyl starch/zein bilayer edible film. *International Journal of Biological Macromolecules*, 141, pp.1175–1182.

Coniwanti, P., Sari, D.M. and Febriana, R., 2016. Pengaruh Rasio Massa Pati Biji Alpukat dan Agar-Agar terhadap Pembuatan Edible Film. *Jurnal Teknik Kimia*, 22(2), pp.52–59.

Farhan, A. and Hani, N.M., 2017. Characterization of edible packaging films based on semi-refined kappa-carrageenan plasticized with glycerol and sorbitol. *Food Hydrocolloids*, 64, pp.48–58.

Fundo, J.F., Galvis-Sanchez, A.C., Delgadillo, I., Silva, C.L.M. and Quintas, M.A.C., 2015. The effect of polymer/plasticiser ratio in film forming solutions on the properties of chitosan films. *Food Biophysics*, 10, pp.324–333.

García, N.L., Ribba, L., Dufresne, A., Aranguren, M. and Goyanes, S., 2011. Effect of glycerol on the morphology of nanocomposites made from thermoplastic starch and starch nanocrystals. *Carbohydrate Polymers*, 84(1), pp.203–210.

Ismaya, F.C., Fithriyah, N.H. and Hendrawati, T.Y., 2021. Pembuatan Dan Karakterisasi Edible Film Dari Nata De Coco Dan Gliserol. *Jurnal Teknologi*, 13(1), pp.81–88.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M., 2006. Effects of plasticizers on the properties of edible films from skin gelatin of bigeye snapper and brownstripe red snapper. *Eur. Food Res. Technol.*, 222, pp.229–235.

Kamsiati, E., Herawati, H. and Purwani, E.Y., 2017. Potensi Pengembangan Plastik Biodegradable Berbasis Pati Sagu Dan Ubikayu Di Indonesia / The Development Potential of Sago and Cassava Starch-Based Biodegradable Plastic in Indonesia. *Jurnal Penelitian dan Pengembangan Pertanian*, 36(2), p.67.

Lubis, L.M., 2008. Ekstraksi Pati dari Biji Alpukat. *Karya Ilmiah*, pp.1–20.

Marsh, K. and Bugusu, B., 2007. Food packaging - Roles, materials, and environmental issues: Scientific status summary. *Journal of Food Science*, 72(3).

Otles, S., 2009. *Handbook of Food Analysis Instruments*, CRC Press, New York.

Romainor, A.N.B., Chin, S.F., Pang, S.C. and Bilung, L.M., 2014. Preparation and characterization of chitosan nanoparticles-doped cellulose films with antimicrobial property. *Journal of Nanomaterials*, 2014.

Shahbazi, Y. and Shavisi, N., 2018. Characterization of active nanochitosan film containing natural preservative agents. *Nanomedicine*

Research Journal, 3(2), pp.109–116.

Shaikh, S., Yaqoob, M. and Aggarwal, P., 2021. An overview of biodegradable packaging in food industry. *Current Research in Food Science*, 4(February), pp.503–520.

Slavutsky, A.M. and Bertuzzi, M.A., 2014. Water barrier properties of starch films reinforced with cellulose nanocrystals obtained from sugarcane bagasse. *Carbohydrate Polymers*, 110, pp.53–61.

Susilowati, E. and Lestari, A.E., 2019. Preparation and Characterization of Chitosan-Avocado Seed Starch (KIT-PBA) Edible Film. *JKPK (Jurnal Kimia dan Pendidikan Kimia)*, 4(3), p.197.

Suyatma, N.E., Tighzert, L., Copinet, A. and Coma, V., 2005. Effects of hydrophilic plasticizers on mechanical, thermal, and surface properties of chitosan films. *Journal of Agricultural and Food Chemistry*, 53(10), pp.3950–3957.

Yoksan, R. and Chirachanchai, S., 2010. Silver nanoparticle-loaded chitosan-starch based films: Fabrication and evaluation of tensile, barrier and antimicrobial properties. *Materials Science and Engineering C*, 30(6), pp.891–897.

Zareie, Z., Yazdi, F.T. and Mortazavi, S.A., 2020. Development and characterization of antioxidant and antimicrobial edible films based on chitosan and gamma-aminobutyric acid-rich fermented soy protein. *Carbohydrate Polymers*, p.116491.

Zhao, R., Guan, W., Zheng, P., Tian, F., Zhang, Z., Sun, Z. and Cai, L., 2022. Development of edible composite film based on chitosan nanoparticles and their application in packaging of fresh red sea bream fillets. *Food Control*, 132, p.N.PAG.

Zheng, K., Xiao, S., Li, W., Wang, W., Chen, H., Yang, F. and Qin, C., 2019. Chitosan-acorn starch-eugenol edible film: Physico-chemical, barrier, antimicrobial, antioxidant and structural properties. *International Journal of Biological Macromolecules*, 135, pp.344–352.