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Research Article

Effect of Nano Chitosan Concentration and Storage Temperature on the Physical Characteristics of Edible Films of Black Mangrove Starch-Chitosan

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Abstract

Edible films made from chitosan and starch materials have several physical limitations, particularly in terms of tensile strength and elongation. To address these limitations, glycerol and sorbitol are often added as plasticizers during the production process. Chitosan has also been reported to have plasticizing properties and can serve as an alternative through its modification into nano-sized particles, thereby increasing its reactivity. Therefore, this study aimed to characterize the physical properties of edible films made from black mangrove (*Rhizophora mucronata*) fruit starch and chitosan by adding nano chitosan suspension at different storage temperatures to determine the best treatment. A completely randomized factorial design was used, and the data obtained were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level with IBM SPSS statistics 25. The treatments used included the addition of nano chitosan suspension (A) at various concentrations of 0%, 25%, 50%, and 75% with storage (B) for 0 days, 9 days at room temperature, and 9 days at cold storage (± 5^0C) in triplicates. The significant treatments were then further tested using Honestly Significant Difference (sig < 0.05). The results showed that the addition of nano chitosan suspension with storage method affected the physical properties of the edible films. The edible film made from black mangrove fruit starch-chitosan with the addition of nano chitosan (50%:50%:75%) stored at cold temperature for nine days had the best physical properties to be applied as a package for fishery products (fillets) based on De Garmo analysis.

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1. Introduction

Plastics are often used as packaging materials due to their durability and versatility for various applications. However, they are obtained from polymers that do not easily decompose, leading to environmental damage. Indonesia has been reported to be the world's second-largest contributor to plastic waste (Jambeck *et al*., 2015). In 2021, the Ministry of Environment and Forestry reported that the national waste reached 68.5 million tons, and these materials accounted for 17% or 11.6 million tons. This indicates that there is a need to develop environmentally friendly packaging alternatives. One promising solution is the use of biopolymers, such as chitosan and starch in edible film production.

Edible films produced from chitosan have hydrophobic properties due to their ability to form intermolecular interactions and amorphous crystals. The intermolecular hydrogen bond interactions create a framework that allows the film products to swell, while the crystals film product facilitate water resistance (Suriyatem *et al*., 2018). Starch is a polysaccharide that can form edible films with good gas barrier properties, but the products often have high water permeability due to their hydrophilic nature, and this limits their application in food packaging (Nazurah and Hanani, 2017). Through the ability to combine chitosan and starch, alternatives to non-biodegradable plastic packaging can be developed (Huri and Nisa, 2014). Supeni and Irawan (2014) reported that edible films made from chitosan have several limitations, including a high-water vapor transmission rate (WVTR) and the need to add plasticizers, such as glycerol and sorbitol during production.

Hosseini *et al*. (2015) reported that the addition of chitosan in gelatin-based edible film increased its flexibility, indicating that the additive played a role in weakening or reducing the number of hydrogen bonds and served as a plasticizer. This indicates that a replacement material is needed to increase its performance, such as the addition of nano chitosan particles with plasticizing properties. However, Lukiyono *et al*. (2020) stated that it has limited solubility, as it is insoluble in water and organic solvents. This showed that physical modifications are needed through the reduction of the particle size. Several studies have shown that the smaller the particle size, the larger the material's surface area, thereby increasing the material's dissolution rate. Nano chitosan from *L. vannamei* shrimp shells can be utilized as an additive to enhance the physical properties of edible films due to the higher reactivity of its particles. It can also affect the physical properties of the film by functioning as a plasticizer (Dar *et al*., 2020; Kurniawidi *et al*., 2022; Lanka and Mittapally, 2016; Nabila *et al*., 2018; Naiu *et al*., 2020; Patel *et al*., 2016;).

Nano chitosan has been reported to have the ability to inhibit food spoilage and suppress microbial activity as a packaging material compared to chitosan in bulk form. This is because the nano form has a larger surface area, thereby increasing its reactivity (Gardesh *et al*., 2016; González-Saucedo *et al*., 2019; Nguyen and Nguyen, 2020; Pilon *et al*., 2015; Quirós-Sauceda *et al*., 2014; Ramezani *et al*., 2015; Romainor *et al*., 2014). Nano chitosan edible film packaging can serve to extend the shelf life of food including fish fillets while maintaining their quality during storage (Homayonpour *et al*., 2021; Ramezani *et al*., 2015; Rumengan *et al*., 2018). The results of Tambunan and Chamidah (2021) showed that red fish fillets coated with chitosan edible film/coating had a shelf life of up to 9 days. Therefore, combining the functions of the edible film, one of the processing methods in this study is the method of preserving the edible film at room temperature and low temperature for 9 days.

Based on previous findings, there are not provide citations on edible film production from black mangrove fruit starch-chitosan material without the addition of plasticizers. Therefore, this study aims to develop edible films with good physical characteristics by adding nano chitosan suspension and exploring different storage methods. Storage methods at room temperature and cold temperature for 9 days are based on the need for edible films that will be produced from this research to be applied as fish fillet packaging during cold storage.

2. Material and Method

This research was conducted in March-November 2022 at the Material Physics Laboratory, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia. Furthermore, the materials used for edible film production were characterized to ensure their suitability as components. To create starch powder black mangroves (*R. mucronate*), the method developed by Podunge *et al*. (2015) was used to produce fruit starch powder. The production yielded a composition of 35.41% starch, 3.73% amylose, 31.72% amylopectin, 9.17% water, 25.46% crude fiber, as well as a gelatinization temperature, solubility, and viscosity of 82.33°C, 4.86%, and 2300 cP, respectively. Chitosan powder was produced from the shells of L. vannamei shrimp obtained in a pond in Palambane Village, Randangan District, Pohuwato Regency, Gorontalo Province. The powder was manufactured based on the

method developed by Setijawati *et al.* (2021), which gave a degree of deacetylation of 86.03% (65% NaOH alkaline solvent) with 767 cP viscosity. Nano chitosan was produced using the ionic gelation method with a D-500 homogenizer based on the technique proposed by Suptijah *et al.* (2011), leading to particle size, polydispersity index (PdI), zeta potential, and viscosity of 481.88 nm, 0.52, 403 mV, and 1267 cP, respectively.

2.1 Material

The tools used in this study included disposable Petri dishes, droppers, micro-pipettes (Denshine-10-100ul-MicroTransfer, US), test tubes (PyRex, US), oven (IKA-125 Basic Dry, Germany), magnetic stirrer (SZCL-2, Yike Instrument Co., Ltd, Shanghai, China). Furthermore, the materials used were *L. vannamei* shrimp shells, black mangrove (*R. mucronate)* fruit, distilled water, acetic acid (Merck), Tween 80 (Merck), and sodium tripolyphosphate (food grade).

2.2 Method

2.2.1 Experimental design

The study's experimental design consisted of two independent variables, namely the addition of nano chitosan suspension and storage method. A total of four treatments of nano chitosan concentration (A0 = 0%, A1= 25%, A2 = 50%, A3 = 75%) and three levels of storage method (B0 = 0 days, B1 = 9 days at room temperature, B2 = 9 days at cold temperature) were used with three replications. Subsequently, the data were analyzed using Analysis of Variance (ANOVA) with a confidence level of 95%. This study used a completely randomized factorial design using the IBM SPSS Statistics 25 application. Treatments that significantly affected the results were further analyzed using the Honestly Significant Difference (HSD) test (sig < 0.05).

2.2.2 Preparation of chitosan solution

Chitosan solution was produced based on the method proposed by Suptijah *et al.* (2011) by dissolving 3 g of chitosan in 60 mL of acetic acid, then slowly adding 80 mL of distilled water while stirring at medium speed with a magnetic stirrer. Subsequently, distilled water was added to the mixture up to a total volume of 600 mL while stirring continuously until homogenous. The solution obtained was then used for further experimental procedures.

2.2.3 Making a solution of black mangrove (R. mucronate) fruit starch

Black mangrove fruit starch powder was prepared into a solution based on the method developed by Podunge *et al.* (2015). Furthermore, a total of 100 g of the powder was dissolved in 500 mL of distilled water using a magnetic stirrer at 82°C until homogeneous. After the homogenization, the solution was cooled (± 30°C for 45 minutes) and ready for use.

2.2.4 Preparation of nano chitosan solution

The preparation of nano chitosan solution was carried out using the technique proposed by Suptijah *et al.* (2011). The previously prepared solution was subjected to emulsification by adding 50 µL of 0.1% Tween with a sprayer while continuously stirring for one hour. Subsequently, stabilization was carried out using 7 mL of 0.1% sodium tripolyphosphate solution while continuously mixing for one hour. The nano chitosan solution obtained was ready for further analysis.

2.2.5 Making of the edible film

The chitosan and black mangrove fruit starch solution (50%: 50%) were heated at 80°C using a hot plate until it was completely dissolved, and the nano chitosan suspension was added based on the predetermined concentration (0, 25, 50, 75%) with continuous stirring until homogenous. Furthermore, the edible film solution was filtered to separate any insoluble particles, and approximately ± 30 mL was poured into a disposable petri dish (size 90 mm x 15 mm). It was then dried in an oven at ± 45°C until dry and removed from the mold. The edible film was stored based on the storage treatment, namely 0 days, nine days at room temperature, and nine days at cold temperature (± 5°C) to characterize the physical properties.

2.2.6 Test parameters for physical characterization of edible film

2.2.6.1 Thickness

The thickness of the film was measured using a micrometer with an accuracy of 0.0001 mm. Furthermore, measurements were taken at three locations with three replicates each to obtain the average thickness of the sample (Setiani *et al*., 2013).

2.2.6.2 Elongation and tensile strength (Setiani *et al.*, 2013)

Tensile strength and elongation were measured using a tensile strength device. Furthermore, the edible film was cut into rectangular shapes with a length of 100 mm and a width of 5 mm. The top and bottom parts of the film were made in the shape of a section to be plastered with the device. Subsequently, a load was applied to the bottom gradually until it broke. The elongation of

the edible film after breakage was measured and the elongation percentage was calculated. The weight level that caused breakage was also measured to calculate the tensile strength.

2.2.6.3 Young's Modulus (Setiani *et al.*, 2013)

Young's modulus referred to an object's flexibility or ability to return to its original shape after being subjected to tensile or compressive forces. Furthermore, Setiani *et al.* (2013) developed a formula for elasticity by comparing tensile strength and elongation, expressed in Mega Pascals (MPa).

2.2.6.4 The water vapor transmission rate (WVTR) (Mulyadi *et al.*, 2016)

The edible film was cut into a diameter of ±3.5 cm and placed between two containers (drink cups). Container 1 was filled with water and container 2 with a known weight of silica gel (constant). Furthermore, the samples were left for 24 hours, and the water vapor transmission was calculated.

2.2.6.5 Water resistance (Setiani *et al.*, 2013)

Water resistance was tested by obtaining the initial weight of the sample (W0), which was then placed in a container filled with distilled water for 10 seconds. The sample was lifted from the container, and the water on the surface of the plastic was removed with tissue paper, followed by weighing. Subsequently, it was placed back into the container with distilled water for another 10 seconds, lifted, and weighed again. The immersion and the weighing procedure were repeated until a constant final weight was obtained.

2.3 Data Analysis

The data obtained in this study were analyzed using Analysis of Variance (ANOVA) with a completely randomized factorial design pattern, and all the analyses were performed using IBM SPSS Statistics 25. The process was then continued with a post hoc test using HSD when the treatment had a significant effect. Treatments with no significant differences were assigned the same superscript letter, while others with significant differences were given different superscript letters. Analysis of all parameters was continued with the De Garmo method to determine the best edible film products based on the effectiveness index (De Garmo *et al.*, 1984). This method proceeds through several stages. First, variables are ranked according to their priority and the amount of contribution to the outcome. A variable weight value (WV) is assigned to each variable, with relative numbers ranging from 0-1. The weights vary according to the importance of each variable in the outcome. Second, the normalized weight value (NW) is calculated by dividing the variable weight by the total weight of all variables. Third, calculate the effectiveness value (EV) of each variable in each treatment. Fourth, calculate the yield value (YV) of each variable in each treatment. Calculate the total yield value (YV) for all variables in each treatment. The highest total value indicates the best treatment studied. The formula used to find the best treatment is as follows:

$$\text{Normal Weight (NW)} = \text{VW} / \text{VW total} \quad \ldots\text{Eq 1}$$

$$\text{Effectiveness Value (EV)} = \text{VT} - \text{Vtj} / \text{Vtb} - \text{Vtj} \quad \ldots\text{Eq 2}$$

$$\text{Yield Value (YV)} = \text{EV} \times \text{NW} \quad \ldots\text{Eq 3}$$

where :

VW = Variable Weight ;
VT = Value of each treatment;
Vtj = The least value of each treatment :
Vtb = The biggest value of each treatment

3. Result and Discussion

3.1 Thickness

A0B2 (edible film without adding nano chitosan suspension stored for nine days at a cold temperature) produced a higher thickness of 17.64 µm, while the lowest was obtained in treatment A3B1 (edible film with adding 75% nano chitosan suspension stored for nine days at room temperature), namely 2.53 µm (Table 1). Based on the ANOVA analysis, the interaction between the addition of nano chitosan suspension and the storage method had a significant effect ($P<0.05$) on the thickness of the edible film.

The highest thickness obtained in treatment A0B2 because the material used contained chitosan and starch, which were believed to retain the trapped water in the edible film and prevent evaporation. However, A3B1 caused a decrease in this parameter due to the dominance of hydrophobic chitosan (in bulk and nano form) in the edible film material. This led to a reduction in the water-binding capacity of the hydrophilic amylopectin from the *R. mucronata* mangrove starch. These findings are consistent with Ramirez *et al.* (2018) that starch with a higher amylopectin content, specifically with short lateral chains, was more prone to retrogradation. This was because the shorter chains allowed for hydration through hydrogen bonding, leading to the formation of a gel. Ramirez *et al.* (2018) reported that smaller starch granules have a larger surface area, surface pores, and increased water absorption channels. The results showed

that the amount of water volume in edible film affects its thickness. This is in accordance with the statement of Coniwanti *et al.* (2014) that the greater the volume of water, the higher the thickness of edible film with the same surface area.

Table 1: The thickness of the edible film from the interaction between the addition of nano chitosan suspension and the storage method (mean ± SD).

Treatment Variations		Thickness (µm)
Nano chitosan (A)	Storage method (B)	
A0 (0%)	B0 (0 day)	13.00 ± 1.39^{bc}
A1 (25%)		11.67 ± 0.83^{be}
A2 (50%)		8.10 ± 0.76^{f}
A3 (75%)		4.67 ± 0.90^{g}
A0 (0%)	B1 (9 days at room temperature)	13.70 ± 0.42^{b}
A1 (25%)		10.00 ± 1.82^{cdef}
A2 (50%)		4.37 ± 0.74^{g}
A3 (75%)		2.53 ± 0.70^{g}
A0 (0%)	B2 (9 days at cold temperature)	17.64 ± 1.11^{a}
A1 (25%)		11.90 ± 1.14^{bd}
A2 (50%)		10.83 ± 1.75^{bf}
A3 (75%)		4.10 ± 0.40^{g}

Description: A0 (0%), A1 (25%), A2 (50%), A3 (75%), B0 (0 days), B1 (9 days at room temperature), B2 (9 days at cold temperature). Different superscripts in the column indicate a significant difference ($p<0.05$).

The decrease in edible film thickness can also be due to the room temperature used during the 9-day storage at ±27°C. During the storage process, the edible film experienced water vapor evaporation, leading to a decrease in thickness. Suwarda *et al.* (2019) reported that the use of a higher temperature during storage caused the evaporation of the bound water content in the edible film.

The results showed that samples stored at a cold temperature without adding nano chitosan suspension experienced an increase in thickness due to the material used consisting of chitosan and mangrove starch. Furthermore, *R. mucronata* starch contained amylopectin (31.72%), which caused water absorption from the environment during cooling due to condensation. Based on the results, samples stored at cold temperatures with the addition of nano chitosan suspension experienced a decrease in thickness. This was because the material was dominated by chitosan (bulk and nano), leading to low water absorption. These findings are in line with Vikele *et al.* (2017) that chitosan additives can be used to obtain packaging paper with better properties, including higher tensile and tear strength, increased hydrophobicity, as well as lower water absorption and air permeability. The thickness obtained based on the JIS 1975 standards was 0.25 mm (250 µm).

Table 2: Tensile strength of the edible film from the interaction between the addition of nano chitosan suspension and the storage method (mean ± SD).

Treatment Variations		Tensile Strength (N/cm^2)
Nano chitosan (A)	Storage method (B)	
A0 (0%)	B0 (0 day)	43.43 ± 1.25^{f}
A1 (25%)		45.62 ± 2.52^{f}
A2 (50%)		66.34 ± 2.52^{c}
A3 (75%)		79.51 ± 1.58^{b}
A0 (0%)	B1 (9 days at room temperature)	43.50 ± 0.71^{f}
A1 (25%)		57.29 ± 0.08^{d}
A2 (50%)		58.93 ± 1.46^{d}
A3 (75%)		89.95 ± 0.25^{a}
A0 (0%)	B2 (9 days at cold temperature)	22.35 ± 1.22^{h}
A1 (25%)		11.99 ± 1.45^{i}
A2 (50%)		34.43 ± 1.57^{g}
A3 (75%)		51.91 ± 0.08^{e}

Description: A0 (0%), A1 (25%), A2 (50%), A3 (75%), B0 (0 days), B1 (9 days at room temperature), B2 (9 days at cold temperature). Different superscripts in the column indicate a significant difference ($p<0.05$).

3.2 Tensile strength

Treatment A3B1 (75% nano chitosan stored for 9 days at room temperature) produced a highest tensile strength of 89.95 N/cm² (0.89 MPa), while the lowest was obtained in A1B2 (25% nano chitosan suspension stored for 9 days at cold storage), namely 11.99 N/cm² (0.11 MPa) (Table 2). Based on ANOVA analysis, the interaction between the addition of nano chitosan suspension and the storage method had a significant effect ($P<0.05$) on the parameter.

For room temperature storage (0 days and 9 days), the tensile strength obtained increased due to the

addition of nano chitosan suspension. The nano chitosan used as the material in this treatment had the ability to form hydrogen bonds between chains, thereby making the product more compact during room-temperature storage. Setiani *et al.* (2013) stated that the tensile strength was directly proportional to the amount of additive used. Furthermore, the greater the percentage of nano chitosan added, the higher the tensile strength. This was because there were more hydrogen interactions in the edible film, which made the bond between the chains stronger and required a large amount of energy to break. According to Hartatik *et al.* (2014), the increase in tensile strength value is due to the reduction of water content in bioplastics. So, the molecular structure in bioplastics is getting tighter and more homogeneous which causes greater tensile strength.

The results showed that the addition of nano chitosan suspension at room temperature storage increased the tensile strength value of edible film due to the increasing amount of nano chitosan. This is thought to be due to the reduction of water content in the edible film so that the nano chitosan suspension with high reactivity added fills the empty pore space in the edible film matrix. This finding is consistent with Lorevice *et al.*, (2016), that the incorporation of CSNPs (chitosan nano particles) into pectin films leads to increased resistance due to the partial occupation of the voids between the polymer chains by the nanostructures, thus compacting the matrix. In addition, the amine groups of chitosan interact electrostatically with the carboxylate groups present in black mangrove (*R. mucronate*) starch powder forming a more resistant matrix.

Table 3: Edible film elongation from the interaction between the addition of nano-chitosan suspension and the storage method (mean ± SD).

Treatment Variations		Elongation (%)
Nano chitosan (A)	**Storage method (B)**	
A0 (0%)	B0 (0 day)	9.00 ± 0.35^{abc}
A1 (25%)		7.50 ± 0.71^{def}
A2 (50%)		7.13 ± 1.24^{bcdef}
A3 (75%)		10.75 ± 0.71^{a}
A0 (0%)	B1 (9 days at room temperature)	6.81 ± 0.78^{bcdef}
A1 (25%)		4.34 ± 0.83^{f}
A2 (50%)		8.14 ± 0.91^{abcd}
A3 (75%)		6.62 ± 0.52^{bcdef}
A0 (0%)	B2 (9 days at cold temperature)	4.50 ± 1.06^{ef}
A1 (25%)		4.50 ± 1.06^{ef}
A2 (50%)		7.88 ± 0.53^{abcde}
A3 (75%)		9.25 ± 2.47^{ab}

Description: A0 (0%), A1 (25%), A2 (50%), A3 (75%), B0 (0 days), B1 (9 days at room temperature), B2 (9 days at cold temperature). Different superscripts in the column indicate a significant difference ($p<0.05$).

Table 4: The young modulus of edible film from the interaction between the addition of nano-chitosan suspension and the storage method (mean ± SD).

Treatment Variations		Young's Modulus (MPa)
Nano chitosan (A)		
A0 (0%)	B0 (0 day)	4.83 ± 0.05^{cd}
A1 (25%)		6.08 ± 0.05^{cd}
A2 (50%)		9.42 ± 1.28^{b}
A3 (75%)		7.41 ± 0.34^{bc}
A0 (0%)	B1 (9 days at room temperature)	6.43 ± 0.64^{bc}
A1 (25%)		13.45 ± 2.57^{a}
A2 (50%)		7.27 ± 0.63^{bc}
A3 (75%)		13.63 ± 1.04^{a}
A0 (0%)	B2 (9 days at cold temperature)	5.08 ± 0.93^{cd}
A1 (25%)		2.70 ± 0.31^{d}
A2 (50%)		4.38 ± 0.10^{cd}
A3 (75%)		5.82 ± 1.55^{cd}

Description: A0 (0%), A1 (25%), A2 (50%), A3 (75%), B0 (0 days), B1 (9 days at room temperature), B2 (9 days at cold temperature). Different superscripts in the column indicate a significant difference ($p<0.05$).

Storage at cold temperatures causes a decrease in tensile strength due to the hydrophilic nature of black mangrove *R. mucronata* starch used in the film, which had high amylopectin and easily absorbed

water from condensation during cooling. This caused a weakening of the hydrogen bond structure, which tended to decrease the intermolecular interactions. The more the water absorbed, the higher the solubility of *R. mucronata* starch, leading to larger bubble cavities that caused the edible film to easily break. Interaction with molecules can weaken hydrogen bonds in biopolymer chain bonds, causing intermolecular interactions to decrease. The weakness of the hydrogen bonds between biopolymer molecules caused a decrease in the tensile strength of the film (Selpiana *et al*., 2015). The standard tensile strength of the edible film, based on JIS (Japanese industrial standard) (1975), was a minimum of 0.392 MPa.

Table 5: The water resistance of edible films from the interaction between the addition of nano chitosan suspension and the storage method (mean ± SD).

Treatment Variations		Water Resistance (%)
Nano chitosan (A)	Storage method (B)	
A0 (0%)	B0 (0 day)	68.51 ± 0.67^{e}
A1 (25%)		86.58 ± 1.63^{bc}
A2 (50%)		80.84 ± 1.61^{d}
A3 (75%)		83.07 ± 0.43^{cd}
A0 (0%)	B1 (9 days at room temperature)	40.32 ± 1.25^{g}
A1 (25%)		88.32 ± 0.04^{b}
A2 (50%)		72.10 ± 2.07^{e}
A3 (75%)		86.09 ± 0.91^{bc}
A0 (0%)	B2 (9 days at cold temperature)	88.34 ± 0.80^{b}
A1 (25%)		61.72 ± 1.13^{f}
A2 (50%)		82.20 ± 0.64^{d}
A3 (75%)		95.86 ± 0.21^{a}

Description: A0 (0%), A1 (25%), A2 (50%), A3 (75%), B0 (0 days), B1 (9 days at room temperature), B2 (9 days at cold temperature). Different superscripts in the column indicate a significant difference (p<0.05).

3.3 Elongation

The results showed that treatment A3B0 (edible film with adding 75% nano chitosan stored for 0 days) had a higher elongation of 10.75%, while the lowest was obtained in A1B1 (edible film with adding 25% nano chitosan suspension stored for nine days at room temperature), namely 4.34% (Table 3). Furthermore, the ANOVA analysis revealed that the interaction between the addition of nano chitosan suspension and the storage method had a significant effect (P <0.05) on the parameter.

Table 6: WVTR of the edible film from the interaction between the addition of nano chitosan suspension and storage method (mean ± SD).

Treatment Variations		WVTR (g/day m^2)
Nano chitosan (A)	Storage method (B)	
A0 (0%)	B0 (0 day)	13.11 ± 3.31^{fghijk}
A1 (25%)		25.76 ±3.43^{abcd}
A2 (50%)		34.64 ±3.16^{abc}
A3 (75%)		35.29 ± 2.91^{a}
A0 (0%)	B1 (9 days at room temperature)	29.08 ±5.82^{abc}
A1 (25%)		22.43 ± 3.88^{cdefghi}
A2 (50%)		23.90 ± 5.98^{abcdefg}
A3 (75%)		21.18 ± 3.68^{cdefghi}
A0 (0%)	B2 (9 days at cold temperature)	18.61 ± 3.22^{cdefghijk}
A1 (25%)		23.49 ± 3.70^{abcdefgh}
A2 (50%)		25.48 ± 4.01^{abcde}
A3 (75%)		24.04 ± 3.79^{abcdef}

Description: A0 (0%), A1 (25%), A2 (50%), A3 (75%), B0 (0 days), B1 (9 days at room temperature), B2 (9 days at cold temperature). Different superscripts in the column indicate a significant difference (p <0.05).

The elongation test results for room temperature storage showed a decrease in the average value because the 9-day storage period (± 27°C) increased the hydrogen bond distance between chitosan and *R. mucronata* starch, thereby reducing the level of bonding. The decrease in elasticity was caused by a reduction in the distance between intermolecular bonds. Hosseini *et al.* (2015) reported that the addition of chitosan to gelatin film increased flexibility. This indicated that this material played a role in the weakening or reduction of hydrogen bonds and served as a plasticizer.

Meanwhile, the decrease in elongation percentage during cold storage was due to water absorption from condensation during cooling, which increased the solubility of *R. mucronata* starch as well as an increase in the amount of nano chitosan added. This can lead to the formation of larger bubble cavities, causing the edible film to break easily and lose its elasticity. Interactions with other molecules can weaken the hydrogen bonds in the biopolymer chain, causing a decrease in intermolecular interactions and ultimately reducing the elongation percentage (Dewi *et al.* 2021). The results of the research of Hosseini *et al.* (2015) that the addition of chitosan significantly increased the tensile strength, causing the film to be stronger than the film from gelatin but significantly ($p < 0.05$) decreased the elongation. According to Hosseini *et al.* (2015), the addition of chitosan to gelatin films resulted in more flexible films so it could indicate that chitosan takes part in weakening or reducing the number of hydrogens bonds and acts as a plasticizer. Percentages of >50% and <10% were considered good and poor, respectively (Krochta and Mulder-Johnston, 1997).

3.4 Young's Modulus

Treatment A3B1 (edible film with adding 75% nano chitosan stored for 9 days at room temperature) produced a higher Young's modulus of 13.63 MPa, while the lowest value was obtained in A1B2 (edible film with adding 25% nano chitosan suspension on storage for 9 days at cold temperature), namely 2.70 MPa (Table 4). Based on the ANOVA analysis, the interaction between the addition of nano chitosan suspension and the storage method had a significant effect ($P < 0.05$) on this parameter.

The value of Young's modulus of the edible film made from chitosan and black mangrove starch (0%) did not change significantly when stored in a room and cold temperatures for 9 days. Meanwhile, the addition of nano chitosan suspension to chitosan-black mangrove starch during 9-day storage at room temperature showed an increase. Furthermore, Young's modulus test results were reported to be inversely related to the percentage of elongation. The higher the chitosan content, the greater the possibility to decrease the number of hydrogen bonds and increase the elasticity of the edible film, thereby increasing the parameter. Hosseini *et al.* (2015) reported that the addition of chitosan to gelatin film increased its flexibility, indicating that the additive played a role in reducing the number of hydrogen bonds and served as a plasticizer.

The Young's modulus of the edible film made from black mangrove fruit starch chitosan remained constant before and after storage. This was possibly due to the use of an equal amount of chitosan and mangrove material. Meanwhile, the addition of nano chitosan

Table 7. Determination of the IE (Effectiveness Index) value

Treatment Variations		Thickness (µm)	Tensile Strength (N/m^2)	Elongation (%)	Young's Modulus (MPa)	Water Resistance (%)	WVTR (g/day m^2)	NH (IE)
Nano chitosan (A)	Storage method (B)							
Variable Weight		0,80	0,70	0,60	0,90	0,50	1,00	4,50
Normal Weight		0,18	0,16	0,13	0,20	0,11	0,22	1,00
A0 (0%)	B0 (0 day)	0,12	0,03	0,10	0,01	0,06	0,31	0,63
A1 (25%)		0,11	0,16	0,02	0,20	0,09	0,13	0,71
A2 (50%)		0,07	0,05	0,06	0,04	0,08	0,01	0,31
A3 (75%)		0,03	0,06	0,13	0,03	0,09	0,00	0,34
A0 (0%)	B1 (9 days at room temperature)	0,13	0,03	0,05	0,02	0,00	0,09	0,32
A1 (25%)		0,09	0,04	0,00	0,07	0,10	0,18	0,47
A2 (50%)		0,02	0,04	0,08	0,03	0,06	0,16	0,40
A3 (75%)		0,00	0,07	0,05	0,07	0,09	0,20	0,48
A0 (0%)	B2 (9 days at cold temperature)	0,12	0,01	0,00	0,02	0,10	0,23	0,48
A1 (25%)		0,11	0,00	0,00	0,00	0,04	0,16	0,32
A2 (50%)		0,10	0,02	0,07	0,01	0,08	0,14	0,42
A3 (75%)		0,02	0,08	0,10	0,02	0,11	0,16	0,49

suspension to edible film for 9 days of storage at room temperature and cold temperature experienced fluctuation, although it was insignificant because the nanoparticles weakened the hydrogen bonds. During cold-temperature storage, water was absorbed from *R. mucronata* starch through condensation, leading to increased solubility and larger bubble cavities (Dewi *et al*., 2021).

The increase in the elongation percentage during cold storage along with the increase in nano chitosan suspension is thought to be due to the absorption of water from condensation during cold storage which can cause a lot of absorbed water (-OH group) to form hydrogen bonds with nano chitosan. According to Santoso *et al.* (2012), the more -OH groups that are trapped, the percentage of elongation increases. The -OH group in the matrix serves to reduce the interaction between polymers so that the cohesive power of the film matrix decreases which results in a more elastic edible film.

3.5 Water Resistance

The results showed that treatment A3B2 (edible film with adding 75% nano chitosan suspension stored for nine days at cold temperature) produced a highest water resistance of 95.86%, while the lowest value was obtained in A0B1 (an edible film without nano chitosan suspension stored for nine days at room temperature), namely 40.32% (Table 5). Based on ANOVA analysis, the interaction between the addition of nano chitosan suspension and the storage method had a significant effect ($P<0.05$) on the parameter.

The low water resistance of the edible film made from black mangrove fruit starch chitosan was due to the presence of amylopectin-type starch from *R. mucronata* with hydrophilic properties. This caused the edible film stored at room temperature to experience increased solubility (decreasing water resistance). These findings are consistent with Susilowati and Lestari (2019) that a decrease in water resistance was caused by an increase in the number of hydrophilic groups in avocado seed starch. The hydrophilic components were then substituted by the hydrophobic groups in chitosan, leading to an increased percentage of swelling. Furthermore, the presence of NH_2 in the additive used caused high reactivity, and chitosan was soluble in acetic acid, which can bind with water (Santoso *et al*., 2020; Suneeta *et al*., 2016).

Meanwhile, the addition of nano chitosan suspension to the edible film at room temperature did affect water resistance due to the dominance of the additive's hydrophobic properties (both nano and bulk). The results also showed that adding polymer nano chitosan did increase water resistance. Edible films treated with nano chitosan suspension during cold storage caused a more increase water resistance due to the dominance of the additive's hydrophobic properties (both nano and bulk). Huri and Nisa (2014) reported that Chitosan-based edible

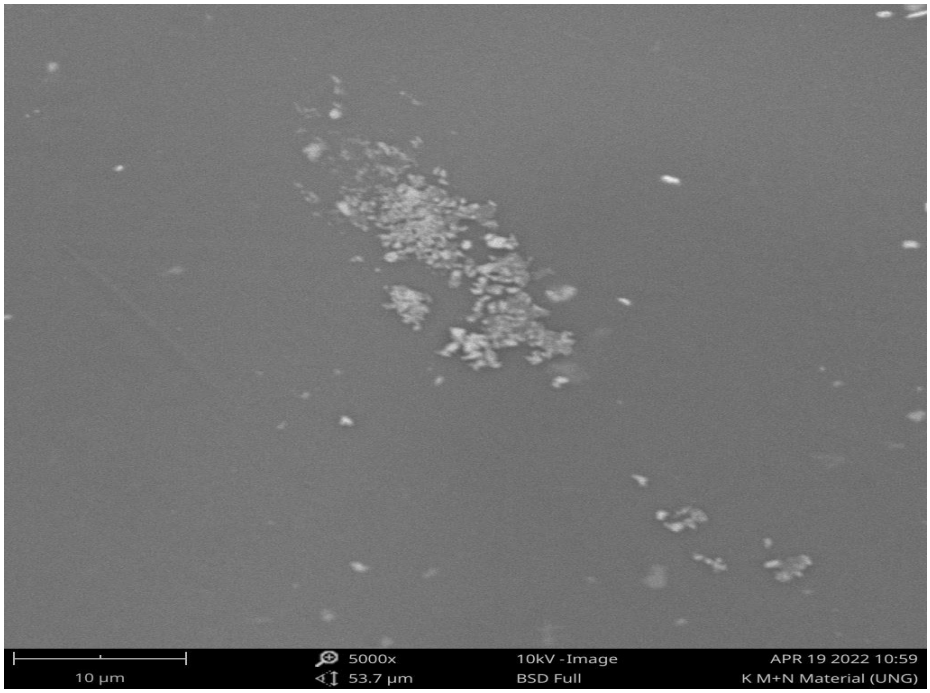

Figure 1. SEM Phenom images of the edible film made from black mangrove fruit starch chitosan with the addition of nano chitosan (50%:50%:75%) stored at cold temperature had the best physical properties based on De Garmo analysis.

films have properties, such as film-forming ability, hydrophobicity, biodegradability, non-toxicity, and increased transparency.

The high-water resistance of edible films made from black mangrove starch chitosan during cold storage can be attributed to the presence of chitosan as a hydrophobic material and the fat content in *R. mucronata* starch that formed a hydrophobic layer on the surface of the granules. This layer hindered the interaction between starch granules and water during cooling (resulting from condensation).

3.6 WVTR

WVTR test showed that treatment A3B30 (75% nano chitosan suspension edible film stored for 0 days) had the highest WVTR value of 35.29 g/day m^2, while the lowest WVTR value was found in A0B0 (an edible film without nano chitosan suspension stored for 0 days) at 13.11 g/day m^2 (Table 6). ANOVA indicated that the interaction between the addition of nano chitosan suspension and the storage method had a significant effect ($P<0.05$) on this parameter.

Storage of the black mangrove starch-chitosan edible film at room temperature caused an increase in WVTR due to the hydrophilic nature of the black mangrove starch material, leading to increased adsorption and decreased water resistance. Meanwhile, the addition of nano chitosan suspension to the edible film caused a decrease in WVTR value due to the dominance of the additive's hydrophobic properties (nano and bulk). This caused an increase in water resistance or a decrease in water adsorption from the environment into the matrix. These findings are consistent with Suwarda *et al.* (2019) that hydrophobic materials can hold water, thereby reducing the rate of water vapor transmission.

Storage of the edible film at cold temperatures caused the WVTR values level of nano chitosan treatments to not change significantly. This indicated that the edible film produced from black mangrove starch-chitosan material with the addition of nano chitosan suspension had good barrier properties when stored at cold temperatures and can last for 9 days. The stability of the WVTR values was possibly due to the hydrophobic nature of chitosan and nano chitosan, which can suppress water adsorption water from the environment (condensation). Furthermore, the additive used in this study increased the density of the edible film and the regularity of the polymer structure produced, making it difficult to break and penetrate water. Chitosan has amino functional groups, as well as primary and secondary hydroxyl groups (Alishahi and Aïder, 2012; Faturrochmah *et al*., 2022). By changing the size of chitosan into nano particles, it is thought to cause an increase in chemical reactivity, because nano chitosan can facilitate the formation of hydrogen bonds between polymer chains and a reduction in intermolecular interactions of edible film constituents. The JIS 1975 standard for WVTR values is a maximum of 10 g.m2/day.

3.7 De Garmo

The determination of the best treatment was based on the highest and lowest values for each observation indicator, with the higher average value indicating the best and vice versa. The results of the effectiveness test on 12 treatment variations showed that the highest IE was 0.71. Based on the value obtained, the best treatment was A1B0 with an IE of 0.71, followed by A3B1 and A3B2 with 0.48 and 0.49, respectively (Table 7). Meanwhile, edible film based on black mangrove fruit starch-chitosan with the addition of nano chitosan (50%:50%:75%) stored at a cold temperature for nine days has the best physical properties to be applied as a package for fishery products (fillets). Morphologically (SEM observation), the best edible film product appears to be well distributed between chitosan (nano and bulk) and black mangrove starch, thus affecting the physical properties of the edible film. (Figure 1).

4. Conclusion

The addition of nano chitosan suspension and storage method affected the physical characteristics of the edible film. The edible film made from black mangrove fruit starch-chitosan with the addition of nano chitosan (50%:50%:75%) stored at cold temperature for 9 days had the best physical properties to be applied as a package for fishery products (fillets) based on De Garmo analysis., The product had a thickness, tensile strength, elongation, Young's modulus, water resistance, and WVTR of 4.10 µm, 51.91 N/cm^2, 9.25%, 5.82 MPa, 95.86%, and 24.04 g/day m^2, respectively.

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Authors' Contributions

All authors have contributed to the manuscript as follows, Lukman; collected and processed the data and wrote the manuscript. Mr. Happy, Mrs. Dwi, and Mrs. Titik; provided the main conceptual ideas and critically revised the manuscript. Furthermore, all authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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