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Optimization of Operating Condition for the Production of Edible Film from Cuttlefish's Bone Gelatin as Instant Noodle Seasoning Packaging

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Abstract

Edible film is a thin layer commonly used to coat food, which is biodegradable and can be consumed. The functions of edible film such as an inhibitor of mass transfer, oxygen, aroma and other dissolved substances. Cuttlefish bones contain protein which can be processed into gelatin and applied as edible film. This study aims to determine the quality and optimal operating conditions of edible films. The research method that used in this research is descriptive quantitative method. The experimental design in this study used a factorial design with independent variables including: gelatin concentration (6 and 8%), glycerol concentration (10% and 20%) and pectin concentration (0.5 and 10%). In this study, the ranged of water vapor transmission rate (wvtr) from eight sample are 9.716 - 11.725 g/m^2.day, the best result of water vapor transmission rate analysis shown at operating conditions of 8% gelatin concentration, 20% glycerol concentration and 1% chitosan concentration which was 9.716 g/m^2.day. In this research the higher gelatin concentration causes an increase in the value of water vapor transmission rate, while the higher the concentration of glycerol causes an increase in the value of wvtr, thickness, elongation but decreases the value of tensile strength an solubility, the higher chitosan concentration causes an decreases in the value of wvtr, tensile strength, elongation, solubility but increase the value of thickness The best results from the thickness, solubility, tensile strength, elongation tests were 0.22 mm; respectively; 1 minute 17 seconds; 6.30 MPa; 166.5%. The morphological analysis using SEM analysis shows that the surface of the molecular structure of the edible film looks smooth, uneven and slightly porous.

INTRODUCTION

The increase in the volume of waste in Indonesia is increasing with the large number and activities of the population. In 2016 the composition of non-organic waste in Indonesia reached 30-40% of which 14% was plastic waste (Purwaningrum, 2016). This makes Indonesia the second highest producer of plastic waste in the world because of the large amount of plastic waste in Indonesian waters which reaches 187.2 million tons (Purwaningrum, 2016).

Excessive use of plastic waste can disrupt the balance of nature, so this problem potentially pollutes the environment. Synthetic plastics are difficult to degrade, take a long time to decompose in the soil and if burned will produce carbon emissions that pollute the environment (Kamsiati et al., 2017). The use of instant noodle plastic packaging is classified as one of the main contributors to environmental waste pollution. Not only the packaging, the packaging of spices, soy sauce, sauce and vegetable oil from instant noodles uses conventional plastic packaging. One of the solution that can be done to overcome these problems is to develop biodegradable packaging materials.

One of the plastics that can be consumed and biodegradable it produced from agro-polymer, namely gelatin which can be used as a film. Edible film itself is a solution to reduce the use of synthetic plastics that are less environmentally friendly.

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Production of edible films from gelatin as a substitute for plastic can be an alternative to help reduce the problem of plastic waste in Indonesia.

Edible film is a thin layer commonly used to coat food and can be consumed. The function of edible film is as an inhibitor of mass transfer, oxygen, aroma and other dissolved substances (Wulandari, 2016). The production of edible films can use several types of raw materials such as hydrochloride (proteins and polysaccharides), lipids, and composites (a combination of several different types of materials) (Fera & Nurkholik, 2018). Gelatin is a protein that can be used as a material for making edible films as a substitute for plastic polymers because it can be degraded and does not disturb the balance of nature. Gelatin is a protein that results from partial hydrolysis of collagen in bones, skin, and animal connective tissue. Gelatin has several characteristics which is reversible, expands in cold water, and can form films (Fatimah, 2012). Gelatin can be made from animal skin and bones. One of the potential raw materials for the manufacture of gelatin is fish bone.

Cuttlefish is a cephalopod that lives on the seabed and contains many essential amino acids that are essential for the body. Cuttlefish is rich in calcium and protein but low in energy. Generally, people only use cuttlefish meat without a head and inner bones where the waste produced from cuttlefish ranges from 65-85% of the weight of cuttlefish and has not been utilized (Fatwa, 2018). The use of inorganic acids can adversely affect health if too much to be consumed, in addition to inorganic acids, there are also organic acids that are safer for health which are likely to be used in the demineralization process, including citric acid, so that the manufacture of gelatin will use citric acid as an acid solvent.

In general, the manufacture of edible films requires the addition of a hydrophilic plasticizer to get the flexibility of the edible film. The addition of plasticizers can increase water vapor permeability and elongation, but their effect is limited to the tensile strength of edible films (Wulandari, 2016). The plasticizers commonly used in hydrochloric films are glycerol and sorbitol. Glycerol is more advantageous because it is easily mixed in film solution, is hydrophilic, and has a low molecular weight so that it can increase flexibility and reduce the stiffness of edible films, while sorbitol is difficult to mix, easy to crystallize at room temperature, and if used in excess it causes digestive problems (Lismawati, 2010).

The addition of chitosan in the manufacture of edible films to make the edible film has a high resistance to water because it is hydrophobic, can increase the transparency in the edible film. In addition, chitosan also functions as an anti-microbial and forms a thin layer in the edible film, thereby increasing the mechanical properties and barrier properties of the edible film (Ristianingsih & Natalia, 2019).

The goal of this research is to study the optimization of the operating conditions using factorial design for making edible film from cuttlefish bone gelatin with the addition of glycerol and chitosan as instant noodle seasoning packaging as a substitute for plastic and efforts to minimize the use of plastic waste. The independent variables selected in this research is concentration of gelatin, concentration of chitosan, and concentration of gliserol.

MATERIALS AND METHODS

Materials

The equipment used in this research for manufacture of gelatin from cuttlefish's bone consisted of glass beaker 500 and 2000 ml, measuring cup, pipette, , pH meter, blender, , funnel, stirrer, watch glass, filter papper, knife, stainless steel pan, water batch, thermometer, digital scales, and oven. The equipment used for manufacture of edible film such as electric stove, measuring cup, glass beaker, piptte, thermometer, digital scales, watch glass, evaporaton dish, micrometer scrup, and oven. The main ingredients needed is cuttlefish bone that obtained from Kaisar Shop Medan. The chemicals used in solid or liquid form include citric acid ($C_6H_8O_7$), acetic acid (CH_3COOH) 99%, aquadest, glycerol that obtained from Indrasari chemical shop and chitosan that obtained from Sentra Kimia Labsains .

Methods

In general, this research procedure is divided into 3 main stages, namely: preparation of materials and equipment, making gelatin from cuttlefish bone, and making edible film from cuttlefish's bone. The fixed variables selected in this research for manufacture fish bone gelatin were fish bone boiling time of 30 minutes, fish bone boiling time of 70°C, citric acid consentration of 4%, ratio of gelatin and aquadest of 1:3 (w/v), demineralization time of 48 hours and the extraction time of 4 hours. While, the fixed variabels for the production of edible film from cuttlefish's bone gelatin was boiling temprature of 45°C, boiling time of 30 minutes, oven temprature of 50°C, oven time of 24 hours and concentration of acetic acid is 1%. The independent variables selected in this study was concentration of gelatin, concentration of chitosan, and concentration of gliserol. This research was conducted on three main factors. The first factor varied the concentration gelatin into 2 variants, namely 6% and 8% (w/w), the second factor is the variation of the concentration of glicerol, namely 10% and 20% (w/v). The third factor is concentration of chitosan, namely 0.5% and 1% (w/v). The research procedures and stages are explained in the following subsecttion.

Production of gelatin from cuttlefish's bone

500 grams of cuttlefish bones were weighed and then washed with fresh water until they were separated from the fish meat. The bones were then boiled for 30 minutes at a temperature of 70 - 80°C. The bones that have been drained and dried was cutt into small pieces with a size around ± 1-2 cm. Made a solution of 4% Citric Acid, soaked the fish bones with 4% Citric Acid with ratio of sample weight and solvent 1:3 (w/v) for 48 hours. The solution was replaced for every 24 hours. After 48 hours, the bone was washed with water to a pH of 5-6. The ossein was extracted with aquadest (1:3 w/v) at a temperature of 70 – 80°C for 4 hours. Extraction results were then filtered with filter paper to separate the precipitate. The solution was then poured into the mold and then dried in the oven for 1-2 days with a temperature of 50°C.

Production of edible film

Fish gelatin with variabek 6% and 8% w/v aquadest was added in 60 ml of aquadest. The chitosan (0.5% an 1% w/v aquadest was dissolved in 40 ml of 1% citric acid. Mixed the solution and added glycerol with concentration 10% and 20% w/v aquadest into the solution and stirred by heating to 40 – 50°C. The edible film solution was stirred for 30 minutes, then the solution was poured on a mold and dried in oven at 50°C for 24 hours.

Gelatin Yield

The Yield of gelatin was calculated as the ratio of weight dried fish bone gelatin to the total weight of leached bone (ossein) on wet basis (Mahmoodani et al., 2014) using the formula that is shown in Eq (1):

$$\text{Yield (g)} = \frac{\text{weigh of dried fish bone (g)}}{\text{weigh of ossein (g)}} x\ 100 \quad (1)$$

Water Vapor Transmission Rate

Water vapor transmission rate was measured based on capacity edible films to hold water vapor. It was adopted from Rozanna et al (2021) which started by cutting samples in form of square to allowing them covering top side of glass, glass was weighed as the initial weight obtained before use. Then the edible film was glued to the surface of the glass which has been previously weighed and filled with 3 grams of silica gel. The samples stored in desiccator for 24 hours. The glass contained the sample was weighed as final weight. The water vapor transmission rate is expressed in g/m^2.day and calculated using the formula that shown in Eq (2):

$$WVTR = \frac{W}{A\ x\ t} \quad (2)$$

Thickness

Thickness of edible films was measured according to method which described by Amaliya & Putri (2014). Samples were measured their thickness using plastic digital thickness gauge (accuracy of 0.01 mm). There was measured in five different side of films. Thickness was expressed in mm.

Solubility

Solubility of edible films was measured according to method which described by Distantina et al (2018). Instant noodle seasoning was packaged using edible film then pour into the boiled water of instant noodles. The solubility of the edible film was evaluated based on the time it took for the seasoning to come out of the edible film into the boiled water of instan noodles.

Tensile Strength and Percent of Elongation

Tensile strength and percent of elongation were measured using texture analyzer adopted from Rusli et al (2017). The samples were cut in size of 7 x 2 cm. After that, the piece of samples was attached in the two tool holders, then set; one immobile (as a holder) and the another movable. The movable handle moved up samples slowly until the films were torn. The force value of movable handle to make films became torn is measured through software on the computer. The tensile strength of the films is calculated by dividing the maximum force to make the film torn (F) with the cross-sectional area of film (A). It is described by formula that shown in Eq (3).

$$\tau = \frac{F}{A} \quad (3)$$

Whilst, the percent of elongation was calculated by dividing the incremental length of the film when became torn at maximum force (b) with the initial length of the films before being pulled (a). Mathematically, it is present in Eq. (4):

$$\% \, elongation = \frac{b-a}{a} x \, 100\% \quad (4)$$

Scanning Electron Microscope

Scanning Electron Microscopy is a method to generate a macroscopic image of a specimen's Surface. The specimen is exposed to an electron beam with a diameter of 5 to 10 nm . The analysis was carried out on the cross section of the edible film using a SEM tool Microscopy) JEOL JSM-6360LA (Setiani et al, 2013).

RESULTS AND DISCUSSION

Gelatin Yield

In this research, the bone demineralization process of cuttlefish used an acid solution, namely citric acid ($C_6H_8O_7$) with a concentration 4%, the yield was 3.72%. Research conducted by Nurilmala et al (2006) on the manufacture of gelatin from tuna fish bones using 6% HCl for 3 days the resulted of the yield is 11.4%, while the results of Fatimah's research (2012) on making gelatin from milkfish bone using citric acid 9% for 48 hours the resulted of the yield is 9.74%. The result of Syahreni et al (2017) on making gelatin from snapper fish bone using citric acid 4% for 48 hours the resulted of the yield is 1.5%.

The difference in yield value was caused by the activity of strong acid solutions which more maximally loosened the collagen chain bonds and cross-links between collagen into simpler bonds than weak acid solutions. The higher the acid concentration, the more open the collagen structure will result in more hydrolyzed collagen so that more gelatin can be extracted during the boiling process (Ulfah, 2011). In addition to the acid concentration, it can be concluded that the type and condition of the raw materials affect the yield of gelatin produced.

Water Vapor Transmission Rate

The results of the average water vapor transmission rate in this reseach ranged from 9.716 – 11.725 g/m^2.day. The table of the results of the analysis of the water vapor transmission rate with the influence of the concentration of gelatin, chitosan, and glycerol is shown in Table 1. Table 1 shows the results of the analysis of the lowest water vapor transmission in the 8th experiment, which was 9.716 g/m^2day and the highest was in the 2nd experiment, which was 11.725 g/m^2day. The results of the analysis of the water vapor transmission rate have no significant difference. Allegedly, the results of taking numbers in the independent variables are not quite right. According to the Japanesse Industrial Standard (JIS) a good water vapor transmission rate is less than 10 g/m^2day. The results of the analysis show that the higher the concentration of glycerol causes the value of the water vapor transmission rate to increase. According to Nurdiani, et al. (2019) that the high value of water vapor transmission was due to the use of hydrophilic glycerol plasticizer so that the transfer of water vapor from the environment to the surface of the film sample became faster. However, in this research with a difference glycerol concentration, the results from the 8th experiment, the value of the water vapor transmission rate was smaller than the 7th experiment, this was influenced by the thickness of the edible film produced. According to Anugerah et al (2017) the more concentration of glycerol added will increase the total solids in the solution which will affect the thickness of the edible film where when the substance evaporates, the edible film formed becomes thicker along with the increasing number of total solids that settle as ingredients for the edible film. The value of the water vapor transmission rate decreased with increasing concentrations of chitosan and gelatin used. This is because the chitosan has hydrophobic properties which can cause water vapor to not easily penetrate or pass through the edible film, so the rate of water vapor transmission rate will be lower with increasing

Table 1. Result analysis water vapor transmission rate.

Run	Gliserol (%)	Gelatin (%)	Chitosan (%)	Result Analysis Water Vapor Transmission Rate (g/m^2.day)
1	10	6	0.5	11.408
2	20	6	0.5	11.725
3	10	8	0.5	9.916
4	20	8	0.5	9.924
5	10	6	1	10.075
6	20	6	1	10.305
7	10	8	1	9.88
8	20	8	1	9.716

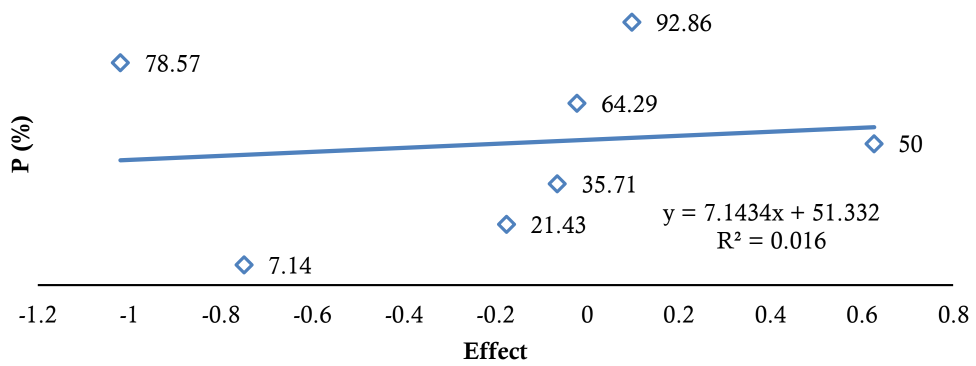


Figure 1. Normal probability relationship with effect (result of vapor transmission rate).

concentration of chitosan. Saragih (2018) states that chitosan has a negatively charged hydroxyl group (-OH) and a positively charged amine group (-NH_2) so that chitosan is able to have strong ionic bonds. The presence of negatively charged hydroxyl groups in chitosan causes chitosan to be hydrophobic, so that it can suppress the rate of water vapor transfer in edible films. According to Wulandari (2016) gelatin contains intermolecular (covalent disulfide) disulfide chains, ionic, and hydrogen bonds which if these chains are exposed will cause this protein to be more hydrophobic, similar to chitosan, gelatin has hydrophobic properties that the addition of gelatin causes a decrease in the value of the water vapor transmission rate. The lower the value of the vapor transmission rate, the better the characteristics of the edible film produced, because the amount of water left in the film network will be lower and the resistance of the edible film to water vapor is higher and better (Krochta et al, 1994).

Calculation of Variable Effects on Water Vapor Transmission Rate

Processing of data from the extraction of cuttlefish bone gelatin which is processed into edible film in this research using a factorial design experimental method with three independent variables, namely glycerol, gelatin, and chitosan. The use of the factorial design method is to determine the experimental variables and optimum operating conditions. With the factorial design method, the most influential variables on the manufacture of edible films from cuttlefish bone gelatin will be obtained. The main effects and interaction effects on the process of making edible films on the results of the vapor transmission rate can be seen in the table. 1 as follows From Figure 1 it can be seen that the Gel effect (Gelatin concentration) is the farthest point with a value of -1.01925. Thus the concentration of gelatin is the most influential variable on the water vaper transmission rate. This is in accordance with the statement according to Wulandari (2016) that gelatin contains intermolecular (covalent disulfide) disulfide chains, ionic, and hydrogen bonds which if these chains are exposed will cause this protein to be more hydrophobic, so that the addition of gelatin causes a decrease in the value of the water vapor transmission rate. . The larger the polymer that composes the film matrix, the lower the amount of water left in the film network, so that the value of the water vapor transmission rate is lower or better (Liu & Han, 2005).

Thickness of the edible film

The thickness of the packaging will affect the shelf life of a product, if the edible film is thicker, the rate of water vapor and gas transmission will be lower, but if the edible film is too thick it will give colors that are less transparent and will reduce consumer acceptance (Khotimah & Diana, 2006). The research shows in Table 2 that the results of the analysis are not significant and in accordance with the Japanesse Industrial Standard (JIS) which is ≤ 0.25 mm with the average thickness of 0.22 – 0.25 mm. The results of the analysis in table 2 show that the thickness of the edible film increases as the levels of glycerol, gelatin, and chitosan increase. This is because the addition of chitosan and gelatin causes an increase in the number of polymers or materials that make up the edible film. According to Poeloengasih (2002) the greater the concentration of solids, the thicker the edible film produced. The thicker film is caused by the more protein content used, so that the total solids contained in the film after drying is greater, it will produce a thicker film (Setianingrum, 2005). The addition of plasticizer concentration will increase the polymer making up the film matrix along with the increase in solutes in the film solution, causing the film thickness to increase (Sitompul & Zuabaidah 2017).

Table 2. The results thickness analysis of the edible film.

Glyserol (%)	Gelatin (%)	Chitosan (%)	Thickness (mm)
10	8	0.5	0.22
10	8	1	0.23
20	8	1	0.25

Solubility of the edible film

The solubility of the edible film was evaluated based on the time it took for the seasoning to come out of the edible film into the instant noodle stew. The solubility value of spices in edible films can be seen in Table 3 The results of the analysis show that the result are not significant and the seasoning can came out of the instant noodles for ± 1 minute, based on the analysis, the edible film is soluble in water but there is a little edible film that is less soluble and clumps this is due to the addition of chitosan which causes the edible film to be difficult to dissolve. According to Widodo et al. (2019), chitosan with free amino groups cannot be dissolved in water with a neutral pH and also chitosan is hydrophobic so it is more difficult to absorb water and does not dissolve in water. The addition of gelatin causes the edible film to be easily soluble in water because according to Wulandari (2016) the hydrophilic amino acid content in gelatin is relatively high, so the more levels of gelatin, the more hydrophilic amino acid content which causes the edible film to dissolve in water. The addition of glycerol causes the edible film to easily dissolve in water because according to Febianti et al. (2020) the hydrophilic characteristics of glycerol result in an increase in the degree of swelling along with the increase in the volume of glycerol added, causing the edible film to dissolve easily in water.

Table 3. The results solubility analysis of the edible film.

Glyserol (%)	Gelatin (%)	Chitosan (%)	Solubility
10	8	0.5	1 minute 22 seconds
10	8	1	1 minute 17 seconds
20	8	1	1 minute 20 seconds

Tensile Strength of the edible film

The results of the analysis shown in Tabel 4 that the tensile strength value is in accordance with the Japanesse Industrial Standard (JIS) which is ≥ 0.39 MPa. According to Wulandari (2016) that the glycerol plasticizer has hydrophilic properties, which causes flexibility in edible films due to the formation of cavities that can interfere with intermolecular attractive forces, the more flexible the film causes the force needed to pull the film small, so the tensile strength is also small. Gontard et al. (1994) explained that the glycerol plasticizer is a relatively small hydrophilic molecule and can enter between protein chains and form hydrogen bonds between amide groups on gluten proteins, when glycerol joins the gluten protein network, there is a direct interaction between protein chains and their proximity. becomes reduced, so that if the film is subjected to pressure the movement of the protein chains will be facilitated by the presence of glycerol which acts as a plasticizer. According to Ikhsan et al. (2021) the tensile strength value of edible film increases with the addition of chitosan concentration, then it will decrease again after passing the maximum point. Chitosan as a mixing biopolymer tends to increase the tensile strength value in certain formulations, this is because chitosan can form hydrogen bonds between chains so that the film becomes denser, this strong intermolecular bond occurs between NH_2 from chitosan and OH from gelatin (Setiani, 2013). In this study, the best formulation was the ratio of gelatin and chitosan, which was 8 grams: 0.5 grams. The addition of gelatin causes the tensile strength value to increase, this is due to the more protein in the edible film, the more hydrophobic amino acids are available. According to Poeloengasih (2002), the more the number of amino acids, the more protein-protein interactions that occur. Jangchud and Chinnan (1999) also explained that a high protein concentration in the film solution will form a strong bond between the polymers, and a greater tensile force is needed to break the film, so that the tensile strength value will increase.

Table 4. The results tensile strength analysis of the edible film.

Glyserol (%)	Gelatin (%)	Chitosan (%)	Tensile Strength (MPa)
10	8	0.5	6.30
10	8	1	3.15
20	8	1	1.06

Elongation of the edible film

In Table 5 shown that the elongation in this study is in accordance with the Japanese Industrial Standard (JIS) which is elongation ≥ 70%. Isnawati (2008) stated that a high percentage of elongation means that the edible film is not easily broken because it is able to withstand the load and tensile force given. The percentage of elongation is inversely proportional to the tensile strength, the more chitosan added to the film, the elongation will decrease but the tensile strength will increase. The decrease in elongation is due to the strong interaction between the composite material mixture, namely gelatin molecules with chitosan. The hydrogen bonds that occur between the gelatin and chitosan chains are getting tighter and more compact so that it will cause the edible film to become stronger so that the film is increasingly difficult to stretch or lengthen this of course will reduce the percentage of film elongation (Setiani et al., 2013). According to (Lismawati, 2017) chitosan is hydrophobic so that it reduces the percent elongation value. Increasing the level of glycerol plasticizer causes the bonds between polymers to decrease and make the film more flexible (Wirawan et al., 2012). The glycerol as a plasticizer can increase plastic flexibility. Plasticizer molecules will disrupt the compactness of the polymer thereby reducing intermolecular interactions and increasing the mobility of the polymer resulting in an increase in the percent elongation (Gontard et al., 1993).

Table 5. The results elongation analysis of the edible film.

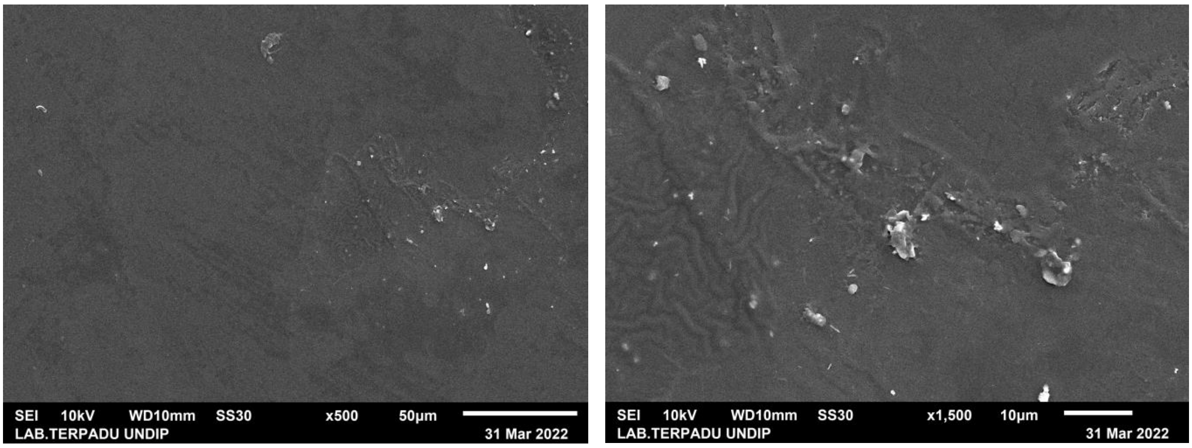
Glyserol (%)	Gelatin (%)	Chitosan (%)	Elongation (%)
10	8	0.5	166.3
10	8	1	140.8
20	8	1	166.5

SEM (Scanning Electron Microscope) of The Chitosan Gelatin Edible Film

Based on the results of the SEM (Scanning Electron Microscopy) test, the morphological shape of the chitosan gelatin edible film on the Figure 2 with a magnification of 500x the surface of the edible film looks smooth and flat, while with a magnification of 1500x the surface of the edible film looks less smooth and flat. This is because the mixing of gelatin, glycerol and chitosan cannot be mixed perfectly. According to Saragih (2018), the chitosan molecules hold intermolecular hydrogen interactions, which are stronger than intra-molecular hydrogen interactions. This causes the phase separation between the gelatin, glycerol and chitosan phases, which causes the components in the edible film to not mix perfectly. In addition, the factors that affect the surface unevenness are caused by the stirring process in the less homogeneous edible film solution.

CONCLUSION

The yield value of cuttlefish bone gelatin produced from the study was3.72%. The optimum concentration to get the water vapor transmission rate with the best value was obtained in the 8th experiment where the value of the vapor transmission rate was 9.716 g/m^2.day. The best thickness; tensile strength; value is the 4th experiment with a concentration of 10% glycerol, 8% gelatin and 0.5% chitosan, which is 0.22 mm; 6.30 MPa. The best solubility value was in the 7th experiment with a concentration of 8% gelatin, 20% glycerol, and 1% chitosan was 1 minute 17 seconds. The elongation results obtained from the best sample, namely the 8th experiment with a



(a) (b)

Figure 2. SEM analysis 8% gelatin concentration; 1% chitosan; glycerol 20% at (a) 500x (b) 1500x

concentration of 20% glycerol, 8% gelatin and 1% chitosan, obtained an elongation result of 166.5%, 7. The results of SEM analysis show that the surface of the edible film molecular structure in the image with a magnification of 500x the surface of the edible film looks smooth and flat, while with a magnification of 1500x the surface of the edible film looks less smooth and flat. So the research on making edible film from cuttlefish bone gelatin is in accordance with the Japanesse Industrial Standard (JIS) and is suitable for use as packaging for instant noodle seasoning

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