

Physical Properties of Edible Film from Tilapia Bones (*Oreochromis niloticus*) with Addition of Carragenan (*Kappaphycus alvarezii*)

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Abstract: Tilapia Fish (*Oreochromis niloticus*) bone gelatin was potential material for edible film manufacture. However, it needs some modifications to improve barrier properties. One of the modification is by adding a cross linking agent. Carragenan (*Kappapycus alvarezii*) containing phenol which is expected to form cross linking with gelatin. The purpose of this study was to determine the effect of various weight gelatin by adding carragenan (*Kappapycus alvarezii*) toward mechanic, hidrofibility properties and functional groups on bone tilapia (*Oreochromis niloticus*) edible film. Edible film solution was made by extraction of gelatin and addition of carragenan (*Kappapycus alvarezii*) at concentration 0%, 3%, 6% for each gelatin were 10 gram, 13 gram, 16 gram into 250 ml distilled water containing 30% glycerol (w/w) of gelatin. The addition of carragenan (*Kappapycus alvarezii*) effect on the mechanical properties of edible film include increasing the thickness of edible fim with the best value of 0.227 mm with a concentration of 10 grams gelatin and 6% (w / w) carrageenan, reducing tensile strength with the best value of 21.5 MPa which is shown by the treatment of 10 grams of gelatin was heavy and carrageenan concentration was 6% (w / w), increasing the elongation value (elongation) with the best value of 39.9% as indicated by the treatment of 16 grams of gelatin and carrageenan concentration of 0% (w / w), and decreasing elasticity with the best value of 0.547 MPa indicated by the treatment of 10 grams of heavy gelatin and carrageenan concentration of 6% (w / w). The nature of hydrofibility with the addition of carrageenan (*Kappapycusalvarezii*) has an effect on decreased solubility and water vapor permeability (WVP) of edible film. The lowest solubility and water vapor permeability shown by gelatin 16 gram and carragenan concentration 6% with value 62,85% and $1,31 \times 10^{-12}$ g/m.s.Pa. Observation FTIR spectra showed indication of cross linking formation at edible film with addition of carragenan (*Kappapycus alvarezii*).

1 INTRODUCTION

The high fisheries resources have an impact on the growth of the fish industry in Indonesia. One type of fish with the largest population is tilapia. In 2005 the export of tilapia to America in form of fillets amounted to 1.146.331 tons of the total export of tilapia by 37.554.537 tons (Prayitno, 2012). This indicates the large number of fish processing industries in Indonesia which cause abundant waste produced (Julianto, 2011). One of the fishery waste products is fish bones that have the potential as an alternative to collagen (Nagai and Suzuki, 2000). One of the uses of collagen in the food sector is as an ingredient in making gelatin (Maryani, 2010). This gelatin can become a solution to replace gelatin

from mammals such as pigs or cattle. The product that uses gelatin is edible film. So that it is expected that tilapia bone waste can be used as material for making edible films. Edible films are plastic or packaging that are biodegradable so they can reduce plastic waste and also environmentally friendly (Fardhyanti, 2015). Some of the advantages of edible film as a food packaging materials, namely the film will be stronger, denser, elastic, low steam transmission rate (Santoso, 2015).

Pranoto (2013) states that edible films from gelatin fish bones have lower functional properties than gelatin sourced from mammals (pigs and cattle), so chemical and physical treatments are often applied to modify polymeric tissue through cross-linking on chains. Natural ingredients that can be

added to edible film from gelatin to form cross linking are carrageenan (Pranoto, 2006). According to Athukorala (2003) carrageenan contains phenol compounds that can bind to the side chains of polypeptides from gelatin, so they can form denser and stronger matrices. It also can improve the mechanical and hydrofibility properties of edible films and maintain the quality of the packaged material. Research on edible film synthesis from tilapia bone with the addition of carrageenan is expected to improve the mechanical properties and hydrofibility of edible films.

2 EXPERIMENTAL

2.1 Materials

In this study, raw materials of tilapia bones were obtained from tilapia fish ponds in Kayen subdistrict, Pati, Indonesia. Other materials are 99.9% purity technical glycerol that were obtained from Merck, carrageenan (κ) that obtained from Pesona Green, Depok, Indonesia, also aquades from the Indrasari chemical store, and 5% hydrochloric acid obtained from the Integrated Chemical Engineering Laboratory, Universitas Negeri Semarang.

2.2 Experimental Procedures

2.2.1 Gelatin Extraction of Tilapia Bones

Gelatin extraction of tilapia bones refers to the method of Rahayu (2015) by using hydrochloric acid. Tilapia bones are cleaned using clean water until the dirt is removed, then the bones are boiled for 10 minutes using water at a temperature of 70°C and then left to dry. The stew bone is cut to a uniform size then soaked for 36 hours using 5% hydrochloric acid with a ratio of 1: 5 (w / v) until ossein is formed. After that the softened fish bone is washed until the pH becomes 4-5. The fish bones that have reached pH are then extracted using distilled water with a ratio of 1: 3 (w / v) at 55°C for 5 hours. Collagen converted into gelatin by hydrolysis will dissolve in distilled water. Then the gelatin solution was dried with a rotary evaporator then in the oven at 55°C for 24 hours until a gelatin sheet was formed. The gelatin sheet is blended to become gelatin powder.

2.2.2 Edible Film Synthesis

The edible film synthesis was made by using the method of Pranoto (2013). Fish gelatin with a variable of 10, 13 and 16 g was added with carrageenan at several concentration (0%, 3%, 6% w / w gelatin) and dissolved with distilled water. The solution was added by glycerol as much as 30% w / w gelatin and stirred by heating to 55°C and distilled water was added to a volume of 150 ml. The edible film solution was stirred for 30 minutes. Edible film solution was poured on a 20x20 cm glass plate and then the solution was dried in oven at 55°C for 24 hours to form a stable layer.

2.2.3 Thickness Test

Film thickness was measured using a micrometer with accuracy of 0.001 mm at 5 different points. Then the measurement results averaged as the result of film thickness (Nofianti et al., 2016). According to Japanese Industrial standard for edible film, the maximum thickness is 0.25mm (Ariska and Suyatno, 2015).

2.2.4 Tensile Strength Test

Tensile strength is the maximum stress that an object can hold when it is stretched or pulled before the film breaks or tears (Fatma et al., 2016). The tensile strength testing process is carried out in the Diponegoro University Integrated labororium using texture analyzer Brookfield CT 03 4500.

$$\text{Tensile strength (Mpa)} = \frac{F}{A}$$

where: F = Maximum stress (N)
A = Cross-sectional area (mm²)
(Rusli et al., 2017)

2.2.5 Elongation Test

Elongation is a measure of the ductility of a material as determined by a tension test. It is the increase of the gauge-length of a test specimen after fracture is divided by its original gauge-length. Higher elongation means higher ductility. Elongation is expressed as a percentage, and it is calculated by:

Elongation (%) = (b - a) / (a) × 100%
Description: a = Initial length of edible film
b = The final length of the edible film

2.2.6 Modulus Young

Lengthening values indicate the elasticity of a material. The higher the elongation value of the material, the more elastic the material will be. The standard value of the minimum lengthening of edible films is 0.35 Mpa (Ariska and Suyatno, 2015). The amount of elasticity is obtained from the ratio of tensile strength to elongation of a material (Setiani et al., 2013).

2.2.7 Solubility Test

The solubility test follows the procedure of Ghanbarzadeh (2011). The film is stored in a desiccators containing silica gel until it reaches a constant weight. After that, around 500 mg of film is soaked in a glass containing 50 ml of distilled water at 23°C for 24 hours by gentle agitation periodically. Next, the film was taken and placed in the desiccators to get the final dry weight of the film.

2.2.8 Water Vapor Permeability Test

Water vapor permeability test is carried out by following the ASTM (1996) E96 procedure with several modifications. The film is stretched over the permeation cell tube in a circle with a diameter of 4 cm. In this permeation cell, silica gel is added (0% RH). After being covered with film, the permeation cell is inserted into the desiccators which has been filled with saturated NaCl solution (70% RH) at 30°C. The rate of water vapor transfer can be determined from the weight of the permeation cell weight every 1 hour until several points are obtained. Then film thickness measurements were carried out at several points with a micrometer (Wirawan, 2012).

2.2.9 FTIR analysis

Functional group analysis with FTIR aims to determine whether the process of synthesizing edible film runs physically or chemically. To find out about this, one sample of edible film was taken to be analyzed using FTIR. Firstly the sample is placed in the set holder, then the appropriate spectrum is searched. The result will be obtained by diffractogram relationship between wave number and intensity. The FTIR spectrum was recorded using a spectrophotometer at room temperature (Setani, 2013).

3 RESULT AND DISCUSSION

In this study, the effect of gelatin and carrageenan concentration on the addition of glycerol as a plasticizer as much as 30% v / w with coding as follows:

G1 : 10 g gelatin ; C1 : 0% carrageenan w/w gelatin
G2 : 13 g gelatin ; C2 : 3% carrageenan w/w gelatin
G3 : 16 g gelatin ; C3 : 6% carrageenan w/w gelatin

3.1 Thickness

Film thickness is an important characteristic in determining the feasibility of edible film as a food product packaging. It is because thickness greatly influences the physical and mechanical properties of other edible films, for example tensile strength, elongation (Ariska and Suyatno 2015).

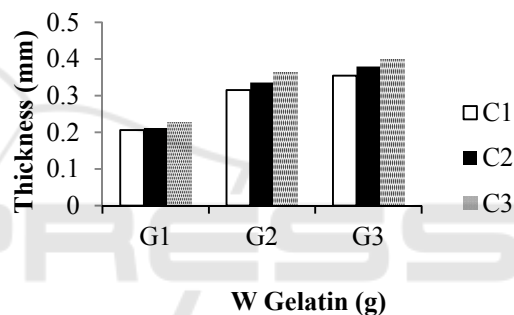


Figure 1: This Effect of gelatin and carrageenan concentration on the thickness of edible film

As seen in Figure 1, it shows that along with the increase in gelatin and carrageenan concentration, the result of the thickness is also increased. The Increasing thickness of edible film is also related to the nature of colloidal compounds that are unique as thickener and suspending, as well as the interaction between the constituent components of edible films (Handito, 2011). In addition thickness is also influenced by the concentration of glycerol that occupies the cavity in the edible film matrix and interacts with carrageenan molecules to form polymers which cause an increase in the distance between the carrageenan molecule polymers thereby increasing the thickness of the edible film (Rusli et al., 2017). The best thickness value for tested edible film from the gelatin of tilapia bone is 0.227 mm with a concentration of 10 grams of gelatin, 6% carrageenan w / w, and 30% glycerol v / w which

according to Industrial Javanese Standard maximum thickness of edible film is 0.25mm (Ariska and Suyatno, 2015).

3.2 Tensile Strength

Tensile strength is one of the important mechanical properties of edible film, because it is related to the ability of edible film to protect coated products. Edible films that have high tensile strength will be able to protect the products they pack from mechanical disturbances (Rusli et al., 2017).

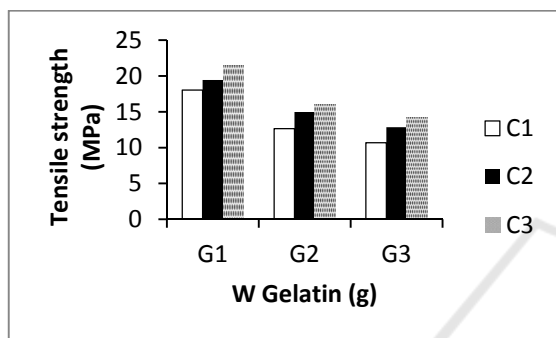


Figure 2: Effect of gelatin and carrageenan concentration on the tensile strength of edible films

Figure 2. shows that an increase in carrageenan concentration tends to increase the tensile strength of edible films. This shows that the increase in the amount of carrageenan in the synthesis of edible film causes the bond between the constituent molecules of edible film to increase resulting in an increasingly compact edible film (Rusli et al., 2017). These results are also supported in the study of Ariska and Suyatno (2015) stating that the more the concentration of carrageenan added in the synthesis of edible films will form a stronger matrix of films, so the force needed to decide edible film is also getting bigger.

The tensile strength of edible films that get additional glycerol has a tendency to decrease, this is due to a decrease in the interaction between molecules of edible film constituents. This decrease is caused by glycerol which can reduce internal hydrogen bonds which causes the weakening of the intermolecular polymer chain forces that are close together, thereby reducing breaking strength (Putra et al., 2017). This is consistent with research from Sinaga et al. (2013) which states that the higher the concentration of glycerol will cause a decrease in the

tensile strength of edible film. This is due to the reduction of intermolecular interactions in the protein chain so that the film matrix formed will be less.

3.3 Elongation

Elongation is the maximum increase percentage of film length when obtaining tensile forces until the film breaks compared to the initial length (Fardhyanti and Julianur, 2015). Elongation test is done by comparing the length increments that occur with the length of the material before the tensile test is carried out (Arini et al., 2017).

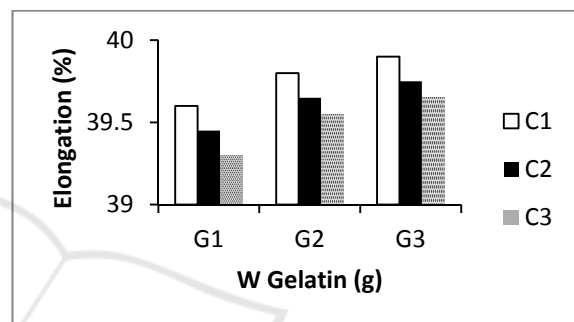


Figure 3: Effect of gelatin and carrageenan concentration on elongation of edible film

In figure 3, it can be seen that the result of the extension show that with the addition of the concentration of gelatin and carrageenan does not show a significant difference. The extension value produced in this study ranged from 39.3 - 39.9%. This value tends to increase due to the addition of glycerol in the manufacture of edible films. This is because glycerol can increase the stretch of intermolecular space in the matrix structure of edible film and increase flexibility, and reduce the number of hydrogen bonds so that it can reduce fragility and not break easily (Ningsih, 2015). The extension value of edible film produced in this study is quite good because it is above the Japanese Industrial Standards which stipulates that percent elongation is categorized as bad if it is less than 10% and categorized very well if more than 50% (Ariska and Suyanto, 2015).

3.4 Modulus Young

Young modulus is done to determine the size of the stiffness of the material produced. Modulus young can be known by comparing the tensile strength value with the extension value (elongation) (Febianti et al., 2015).

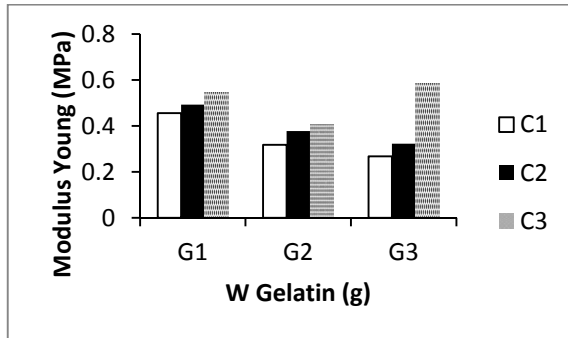


Figure 4: Effect of gelatin and carrageenan concentration on Modulus Young of edible film

In Figure 4 the value of elasticity produced tends to decrease with increasing concentration of gelatin and carrageenan. This decrease in elasticity (modulus young) is caused by increasing the concentration of gelatin and glycerol. The greater the concentration of gelatin and glycerol added, the greater the number of polymers making up the film matrix, which increases the value of tensile strength and decreases the value of elongation (Ariska and Suyatno, 2015). The elasticity value (modulus young) is directly proportional to tensile strength and inversely proportional to elongation (Nahwi, 2016). Figure 4 showed that there were several edible films from tilapia bone gelatin with the addition of carrageenan and gelatin were still relatively good because they were above the minimum standard value of elasticity (modulus young) edible film. According to Japanese Industrial Standard (1975), the minimum value of elasticity (modulus young) of edible films is 0.35 MPa (Ariska and Suyatno, 2015). Edible film that has a value of elasticity (modulus young) of less than 0.35 MPa can be caused by several factors, including the stirring process carried out manually which only uses a glass stirrer so that the mixture is not evenly distributed in the solution (Arini et al., 2017). The results showed that the best elasticity (modulus young) was 0.547 MPa with a concentration of 10

grams of gelatin, 6% carrageenan w / w, and 30% glycerol v / w.

3.5 Solubility

High solubility shows that edible films are easily degraded in nature and can be used as packaging for ready-to-eat products. They are also easily dissolved when consumed directly (Pitak, 2011). However, low solubility is an important requirement for edible film to become a packaging for wet and semi-wet food products (Atef, 2015).

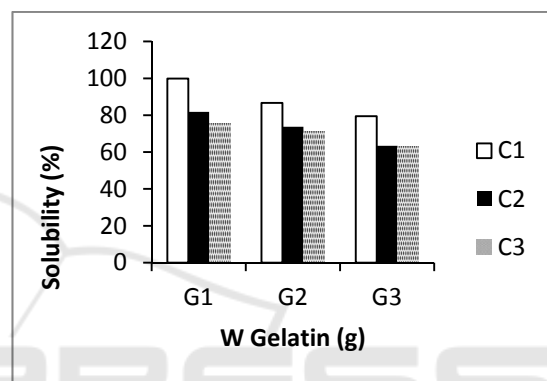


Figure 5: Effect of gelatin and carrageenan concentration on solubility of edible film

As seen in Figure 5, edible film synthesis with variations in gelatin weight and carrageenan concentration showed solubility results ranging from 99.851 to 62.841%. The highest solubility results based on Figure 4.1 contained 10 grams of gelatin with a solubility value of 0% carrageenan concentration was 99,851. Meanwhile, the lowest solubility results based on Figure 5 showed on 16 grams of gelatin weight and 6% (w / w) carrageenan concentration with a solubility value of 62.841%. The lowest value of edible film solubility from this study is still relatively high compared to Darni and Utami's study (2010) which made from sorghum based. It produce the lowest solubility value of 36.825%, and Santoso, et al., (2015) research which based on starch produce solubility values the lowest is 41%, thus showing edible film from tilapia bone gelatin is more suitable as a food packaging that can be consumed directly (Diova et al., 2013). One type of food coated with edible film with high solubility

value and can be consumed directly is beef sausage (Estiningtyas, 2010).

There is a decreasing in solubility due to an increase of carrageenan concentration tends to be inversely proportional to the solubility of edible film caused by the increased content of dissolved solids derived from edible film making materials and the increasing number of inter-molecular bonds in edible films (Rusli et al., 2017). Bonds between molecules in edible film can be increased due to the side chain of polypeptides in gelatin which binds to phenol compounds in carrageenan (Pranoto and Sutono, 2013).

Based on the Negara and Simpen research (2014), the greater the concentration of gelatin with the same plasticizer ratio will produce smaller solubility. This is due to the nature of the gelatin which forms a gel if it is mixed with water so that more gelatin in the mixture produces low solubility and requires heating to dissolve it (GMIA, 2012). Solubility is also determined by the thickness of the edible film. Based on Figure 4.1 edible film with a weight of 10 grams of gelatin and carrageenan concentration of 0% (w / w) produced the highest solubility of 99.851%. This is due to edible films produced from the above variations having the smallest thickness so that they can dissolve almost 100% in water before 24 hours (Santoso et al., 2013). From the results of the solubility test it can be indicated that the increasing of gelatin weight and carrageenan concentration tends to provide low solubility in the edible film.

3.6 Water Vapor Permeability

Water vapor permeability is the ability of the film to resist the rate of water vapor that penetrates it (Wirawan, 2012). Gontard (1993) states, that the value of water vapor permeability in edible films must be as low as possible. Low water vapor permeability values indicate a better barrier to water vapor (Pranoto, 2013). Observation of the permeability of edible film water vapor from tilapia bone gelatin with the addition of carrageenan is presented in Figure 6. The highest water vapor permeability value is at 10 grams of gelatin and 0% carrageenan (w / w) of $1,994 \times 10^{-12}$ g / msPa, while the lowest water vapor permeability value is 13 grams of gelatin and 6% carrageenan (w / w) of $1,305 \times 10^{-12}$ g / msPa.

According to Junianto (2013), decreasing water vapor permeability due to the increasing weight of gelatin, this is caused by increasing molecular

weight so that the film layer gets denser and reduces water vapor permeability. Increasing molecular weight is influenced by the increase of amino acids in edible films (Cao, 2007). Pranoto (2013) also stated that films with the addition of carrageenan can significantly reduce water vapor permeability compared to films without the addition of carrageenan. Film derived from gelatin when combined with carrageenan will significantly reduce WVP value compared to films without the addition of carrageenan (Rattaya et al., 2009). The decrease in WVP value of edible film may be caused by a decrease in free volume in the edible film matrix as a result of increased cross linking through covalent bonds formed due to the addition of herbal extracts on gelatin-based films (Hoque et al., 2011).

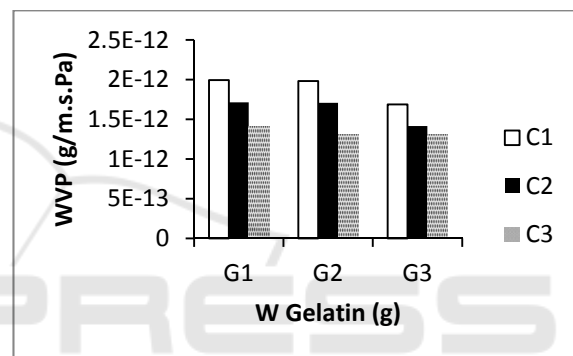


Figure 6: Effect of gelatin and carrageenan concentration on Water Vapor Permeability of edible film

Cross linking can cause increasing density in the polymer matrix and also in winding pathways for water molecules to pass through the tissue. This causes the free volume in the matrix to decrease so that the amount of diffusion of water through the film also decreases (Cao et al., 2007). The inhibition of the diffusion process of water molecules to pass through the complex film network results in decreasing WVP values (Pranoto and Sutono, 2013). From this it can be indicated that the increasing in gelatin weight and carrageenan concentration tends to decrease the WVP value.

3.7 FTIR Analysis

Analysis of functional groups with FTIR aims to compare the presence of CN (carbon-nitrogen) groups from edible gelatin films of tilapia with and without the addition of carrageenan. The shape of

the absorption peak and wave number can be seen in Table 1. We can see that the edible film with the addition of carrageenan has a wave number of 1644.38 cm^{-1} while the edible film without the addition of carrageenan has a wave number of 1642.16 cm^{-1} . According to Pranoto (2013) the wave number peak with the absorption area of $1600\text{-}1700 \text{ cm}^{-1}$ is the absorption peak which indicates the presence of CN (carbon-nitrogen) structure and peak which can identify the presence of cross linking. So that it can be indicated that a cross bond between gelatin and carrageenan is formed.

Table 1: Wave Numbers of FTIR analysis.

Absorption Area	Wave Length (cm^{-1})	
	(G1)	(G2)
amide A	3429,82	3409,54
amide I	1642,16	1644,38

Cross-linking occurs between the side chains of polypeptides in gelatin with phenol compounds in carrageenan (Pranoto, 2013). This is also reinforced by the decrease of the free NH group on edible film with the addition of carrageenan which is indicated by wave number $3409, 54 \text{ cm}^{-1}$ while for edible film without addition of carrageenan has a wave number $3429, 82 \text{ cm}^{-1}$.

4 CONCLUSIONS

Increasing the weight of gelatin and the addition of carrageenan concentrations can affect the mechanical properties of the edible film. The greater the concentration of gelatin and carrageenan, the thickness increases, the tensile strength decreases, the elongation value tends to increase, and the elasticity (modulus young) tends to decrease, and influences the hydrofibility properties of edible film which decreases solubility and permeability moisture on the edible film from the gelatin of the tilapia bone. This is reinforced by the presence of cross linking as indicated by the results of FTIR analysis. The more cross-linking that is formed, the edible film matrix will also be more tight so that it is not easy for water to pass through.

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