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The Quality of Edible Film Made from Nile Tilapia (Oreochromis niloticus) Skin Gelatin with Addition of Different Type Seaweed Hydrocolloid

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Abstract: The functional properties of fish skin’s gelatin lower than mammals, hence the gelatin proteins needed a polysaccharides hydrocolloids to form a continuous and more cohesive network of edible film. Polysaccharides hydrocolloid (carrageenan, agar and alginate) containing phenol compounds was oxidized to be converted into quinone and it was expected to act as a cross linking agent. The purpose of this study was to determine the characteristics (thickness, tensile strength, elongation, solubility and water vapor transmission rate) of edible film made from nile tilapia skin gelatin by adding different type polysaccharide hydrocolloid. Edible film was made by mixture of gelatin 5 g and addition of carrageenan (C1), agar (C2), alginate (C3) concentration ; 0.5% (v/w), all the materials were poured into 100 ml distilled water that containing 10% glycerol (w/w). The solution was then heated on a hot plate stirer at 40°C for 30 min and dehydrated in a oven at 50°C. All data were analysed using ANAVA. Based on the result it can be seen that the addition of oxidized polysaccharides hydrocolloid have a significant effect on tensile strength (TS), water vapor transmission rate, solubility and elongation at break properties, but did not in thickness. Edible film gelatin with the addition of alginate has better characteristics viewed by tensile strength (23.05 Kgf/cm²), water vapor transmission rate (0.61 gram/m²/hour) and thickness (0.16 mm) than carrageenan and agar.

Keywords: Edible Film, Nile Tilapia, Gelatin, Hydrocolloid, Quality

1. Introduction
Packaging with edible coating or film is one of the new relatively preservation techniques of a food. Research undergone on coating of food products with edible coating has been extensively done and proved to be able to lengthen the saving life and improve the quality of food products. Edible film is an organic packaging material made from hydrocolloid and fatty compounds, or their combinations. The compounds of hydrocolloid that can be used are protein and carbohydrate, while fat that enables
to be used is candle or wax, glycerol and fatty acids. Starch as a hydrocolloid compound, is a naturally formed polymer in varieties botanical/vegetable sources such as wheat, corn, potatoes, and tapioca. Starch as a renewable natural source is broadly available and easy to gain.

Generally, Edible film is an easily renewable substance. A constantly used biopolymer material is consisting of fat, polysaccharide and protein. Gelatin is a derivative of the amino acid glycine, proline and hydroxy proline. Gelatin can be made from the extraction of fish skin extraction. Edible film resulted from fish skin will have lower functional character if compared with mamals cartilage (cow and pig), so that the chemical and physical treatments are often applied to modify tissue through crosslinking of the polymer chains [1]. Chemicals commonly used as cross linking agents are generally toxic. So it is required the existance of cheap, natural, safe, renewable, and being able to increase the barrier of physical fish gelatin, especially in edible film.

Additional ingredients used in this study were carrageenan, agar, and alginate and glycerol. Carrageenan agar and alginate have the potential as an additional cheap elastic material that can be consumed, and renewable. The addition of the refined glycerol to make the film better becomes flexible and elastic. additionally, the addition of hydrocolloids is also rich because it is cert, safe, biodegradable and its presence is abundant in Indonesia.

The addition of seaweed as cross-linking on edible film will affect in the original properties of the protein. According to Athukorala et al. [2], seaweed extract contains of phenol compounds that can be converted to quinone when oxidized and potentially become cross-clearing agent on the formation of edible film. The existence of these crosslinks will make the pores of the film from the smaller and smaller protein, so that the water absorption of water vapor will be lower.

2. MATERIALS AND METHODS OF RESEARCH

2.1. Material
The sample used in this research was the skin of Nile tilapia fish (Oreochromis niloticus) gained from industrial waste. Fish skin obtained was under handling conditions using ice-cooling. The additional substances used in this study were acetic acid (CH$_3$COOH), glycerol, agar carrageenan and alginate.

2.2. Method of Research
The Nila’s fish skin was gained from the processing of waste of fish fillets of Nile tilapia PT Aquafarm Semarang. The early process was separating fish skin from the meat and sticking dirts. Then, the seperated fish skin was being cleaned and soaked in hot water for 1-2 minutes. After that, the immersing process of cleaned fish skin was done in CH$_3$COOH 3% for 12 hours. The fish skin was re-cleaned to netralize the pH. Thereafter, it was done the extraction process with aquades at 60-70 celsius degree for 2 hours. The extraction was filtered and dried in the oven at 60 celsius degree. After the dried extraction turned to gelatin sheets. They were being milled until they turned to gelatin powder.

The making of gelatin refers to Martianingsih and Atmaja [3] with the modification of the use of acetic acid as a solution of immersion and fish skin that is used as a sample.

2.2.1. The Procedure Making of Edible Film
Edible film was made refers to Junianto et al. [4] with the modification of the use of sample using 3 different type of hydrolysed polysaccharides. Glycerol as plasticizer was added of 10% from the total components. The edible film making process of gelatin skin of tilapia fish, 5% (5gram of the weight of the solution) was added to 100mL aquades and heated at 60-70 celsius degree. Then it was added 0,5% (0,5 gram of solution weight) of carrageenan, alginate, agar, and glycerol and heated for 30 minutes. The solution is then printed out in glass plate with the size of 20x20 m and ovened for 24 hours in temperature of 50 celsius degree. Control was made from gelatin skin of tilapia fish without cross linking using
2.2.2. **Testing Parameters**
Edible film testing includes thickness, tensile strength, percent elongation, water vapor transmission rate (WVTR) and solubility.

2.2.3. **The Design of Experiments and Data Analysis**
The design of Experiments used in this research was complete random designs where every treatments was replicated 3 times. Normality and homogeneity testing was done earlierly before ANOVA analysed in order to recognize the data characteristic. The analytical method used was ANOVA variance with Tukey's advanced test to determine if there were any differences between samples.

3. **RESULT AND DISCUSSION**

3.1. **The Characteristics of Edible Film**
The result of thickness test and the rate of water vapor transmission can be seen on Table 1.

| Table 1. The Thickness (mm) and The Rate of Water Vapor Transmission (g/m²/hour) Test |
| Sample       | Thickness (mm) | The rate of water vapor transmission (g/m²/hour) |
|--------------|----------------|-----------------------------------------------|
| Control      | 0.12 ± 0.0045  | 1,002 ± 0.031                                |
| Agar         | 0.13 ± 0.0035  | 0,748 ± 0.020                                |
| Carrageenan  | 0.14 ± 0.037   | 0,714 ± 0.100                                |
| Alginat      | 0,16±0.0055    | 0,610 ± 0.074                                |

Note: Value was average of 3 times replications
The same lowercase letters notation showed there was no significant difference between sample (P<0.05)

3.1.1. **The Thickness**
The results of this study indicated that the different types of using hydrocolloid polysaccharides did not give significant effect to the thickness. The average value of the thickness of the ranged from 0,12-0,16mm. According to Warkoyo [5], this could be occurring because the addition of active material can cause decrease of molekul density, free space formed on bigger film matrixes. As the result, the formed film is getting thicker. Japannese Industrial Standart (1975) in Santosa et al. [6], recommend the thickness of maximum edible film is 0,25 mm. So, it can be concluded that edible film of research result meet the standart.

3.1.2. **The Water Vapor Transmission Rate**
The results of this study indicated that the different types of the used hydrocolloid polysaccharides have not an effect on the rate of produced moisture transmission between samples. The further test results show that the valid difference to the value of water vapor transmission rate. The average value of water vapor transmission rate of research results to be ranged from 0,61-1,002 g/m²/hour. The thickness of the lowest edible film is on the control treatment that is with the value by 1,002 mm and the highest is in edible alginate film of 0,61 mm.

Gunawan [7] stated a good edible film has low water vapor transmission rate. It means low water vapor transmission rate can inhibit the loss of water from the product, so the freshness of product was maintained. **Japannese Industrial Standart (1975)** in Santosa et al. [6], stated that the maximum water vapor transmission rate is 10 g/m²/ day, so that it can be concluded that the water vapor transmission rate does not meet the standart.
Water vapor transmission rate affects on the ability of the edible film to held down water vapor. Edible film had low water vapor transmission rate that is suitable for wraps products with high humidity. Narendra et al. [8] added the value of high water vapor transmission rate will affect on storing endurance of a product that will be packed. The higher the value of water vapor transmission rate of the used edible film is, the more durable is the packed product.

The rate of water vapor transmission of edible film agar, carrageenan were lower than the control. Agar, carrageenan, and alginate had hydrophilic characteristic. Fatma et al. [9] Agar is a hydrophilic polymer of sulfate Polysaccharide which can be extracted from red seaweed (Rhodophyceae). According to Anward et al. [10] films made of alginate has weak mechanical strength, because alginites has only a negatively charged hydroxyl group so the bonds between molecules are weak. Meanwhile, films of alginate had high hydrophilic characteristic. The high hydrophilic characteristic of alginate causes film deriving from alginate enable to absorb water. This statement is made strengthened by Bourtoom [11] carrageenan is used as an edible film raw materials which is included in hydrocollic and generally a poor material for water vapor resistance. This was caused characteristic of carrageenan are hydrophilic which cause the edible film produced could be easily to absorb water vapor.

In addition Pranoto et al. [12] states from his research that on the effect of addition of seaweed extraction to edible film black tilapia fish skin. Decreased WVP (Water Vapor Permeability) in edible films is caused by the free volume of matrix polymers as a result of increased cross-linking through hydrogen bonding and hydrophobic interactions due to the addition of herbal extracts to gelatin films. Gelatin combined with seaweed extract significantly decreases WVP values, compared with control films (without the addition of seaweed).

Water Vapor Transmission Rate The rate of water vapor transmission affects on the ability of edible film to hold down water vapor. Edible film which has a small water vapor transmission rate is suitable for packaging products that have high humidity. Edible film will inhibit the amount of water vapor released out of the environment so that the product does not dry quickly. Narendra et al. [8] added a high water vapor transmission rate will affect on storing endurance of the product to be packaged. The higher the velocity value of the edible film water vapor is used, the less durable the packaged product is.

3.1.3. The Tensile Strength and Percent of Edible Film Extension
The result of thickness test and the rate of water vapor transmission can be seen on Table 2.

| Sample       | The Tensile Strenght (Kgf/cm²) | Percent of Extension (%) |
|--------------|---------------------------------|--------------------------|
| Control      | 12,51 ± 1,634^a                 | 20,545 ± 1,395^a         |
| Agar         | 13,89 ± 0,152^b                 | 49,905 ± 1,335^b         |
| Carrageenan  | 18,83 ± 1,067^c                 | 119,725 ± 1,71^c         |
| Alginate     | 23,05 ± 0,485^d                 | 81,571 ± 0,621^d         |

Note: Value is average of 3 times reiteration
The value of notation in the same lowercase letters shows invalid difference (P<0.05)

3.1.3.1. The Tensile Strength
The results of this study indicated that the different kinds of the used hydrocolloid polysaccharides have an effect on the resulted tensile strength. The further test results showed that the valid difference to the value of tensile strength. The average value of tensile strength of research results to be ranged from 12,51 to 23,05. The lowest tensile strength of the lowest edible film is on the control treatment with the value by 12,51 Kgf/cm² and the highest is in alginate of edible film by 23,05 Kgf/cm².
Katili et al. [13] state that edible film with high tensile strength is used for products that require high protection, while edible film with low tensile strength can be used for food products. The value of the edible tensile strength of the film has a standard according to Krochta and Johnson [14] of 10 kgf/cm². So it can be concluded that the tensile strength of edible film research results was meet the standard.

The tensile strength decreased because of reduction of intermolecular interactions of the protein chain so that the matrix of the formed film will be less. The reduction of intermolecular interactions of protein chains occurs to be caused by the addition of glycerol. Plasticizer molecules will disrupt the cohesiveness of starch, the decrease of intermolecular interactions and the increase of polymer mobility.

3.1.3.2. The Percent of Extension
The results of this study indicated that the different kinds of the used hydrocolloid polysaccharides affect on the produced percent elongation. The further test results show that the valid difference to the value of tensile strength. The average percentage of elongation of the results of study outcome to be ranged from 20.545 to 195.725%. The lowest percentage of edible film extension is on the control treatment with the value by 20.545% and the highest on the edible film of carrageenan was 195.725%.

Murdinah et al. [15], stated that edible film is categorized less well if the value of percentage of extension is less than 10%. Based on the standard, it can be claimed that percentage of edible film extension is quite good.

The component of edible film composition affected the percentage of elongation. The protein contained of gelatin, carrageenan and glycerol as plasticizer gave significant effect to the characteristic of edible film. The higher the percent value of elongation of edible film could be observed the better quality. However, it is not the treatment with the addition of carrageenan concentration on the control (without gelatin addition). This was due to the concentration of carrageenan added to the making of edible film will affect on the percent elongation of edible film. The more components of edible film filler will be difficult in the printing process in the glass plate, this is because the edible film will quickly block and when edible surface will be easily damaged.

The addition of an increasingly active ingredient may result in stretching the intermolecular spaces of the film matrix network and decreasing the amount of internal hydrogen bonds, thereby reducing the film's fragility and increasing the elongation percentage.

3.1.4. Solubility
The results of solubility test can be seen on Table 3.

| Sample        | Solubility        |
|---------------|-------------------|
| Control       | 99.3 ± 0.608c     |
| Agar          | 97.98 ± 0.275c    |
| Carrageenan   | 92.36 ± 0.865c    |
| Alginate      | 95.44 ± 1.025b    |

The results indicated that the different kinds of the application of hydrocolloid polysaccharides affected on the solubility. Furthermore the test results showed that there were any significant different to the solubility value. The average value of solubility of the research results to be ranged from 92.36 to 99.3%. The lowest edible film solubility was on the carrageenan addition treatment by 92.36% and the highest is in the control of film edible by 99.3%.

The Edible film made of carrageenan and gelatin mixture was lower than other treatments. This is because carrageenan is a polysaccharide made from hydrophobic seaweed. The addition of carrageenan to the edible film affected the solubility nature of the edible film. Santosa et al. [6], the
most common type of carrageenan in food is kappa carrageenan. The hydroxyl and sulphate groups on carrageenan are hydrophilic while the 3,6-anhydro-D-Galactose group is more hydrophobic.

The edible film packaging material that depends on the source of the used raw materials. The lower the solubility value of edible film is, the better it is used as packaging material and biodegradable. Perez-Gazo and Krochta [16] suggest that water solubility is an important feature of edible film. Water film insolubility is needed to improve the product integrity and the resistance to water.

Edible film with a high degree of solubility will be easily degraded in the soil. Zulferryenni et al. [17] suggests that the higher the solubility, the easier the components degraded. It can be stated there are hydrophilic compounds hence it can be easier degraded in water and soil, so the ability of the biodegradable film is very well used as packaging material.

The value of edible film solubility is very important to know because it will affect the packed product and packaging function. This was supported by Diova [18], if the application of a film is required as a feasible packing material, high solubility is desired otherwise if the application of edible film is in foods with high water content then it is used insoluble film.

4. Conclusion
Edible gelatin film with the addition of alginate and glycerol has better characteristic when tested from tensile strength (23.05 Kg/cm²), vapor transmission rate (0.61 gram/m²/day) and thickness (0.16 mm) than carrageenan and agar addition. However, the solubility (92.36%) and elongation percentage (119.725%) was the best edible gelatin film with the addition of carrageenan and glycerol.

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