THE EFFECT OF USING KAPPA-CARRAGEENAN: IOTA-CARRAGEENAN: MANGROVE FLOUR (*Avicennia marina*) IN RATIO AS EDIBLE FILM MATERIAL TOWARD ITS QUALITY

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Abstract

This study aims to determine the effect of using kappa carrageenan: iota carrageenan: mangrove flour (*Avicennia marina*) in ratio as a material edible films towards on its quality. The research method was an experimental laboratory design, with kappa-carrageenan: iota-carrageenan: mangrove fruit *Avicennia marina* flour treatment in variation ratios. The results were analyze using simple CRD which was repeated 3 times, further tests were carried out using the Duncan test to look for differences. The results showed that the thickness value was obtained in the use of iota-carrageenan and mangrove *Avicennia marina* fruit flour with a 1:3 ratio of 108.5 µm, the highest Tensile Strength was in iota-carrageenan and mangrove *Avicennia marina* fruit flour with the 3: 1 ratio of 5.95 N/mm2, Elongation value to the highest of 12.33% in the mixture of kappa: iota: mangrove (1: 2: 1). The lowest water vapor transmission value was found in the mixture of kappa: iota: mangrove (1: 2: 1) of 38.54 g /m^2 Hour). The use of kappa-carrageenan: iota-carrageenan: mangrove fruit flour of *Avicennia marina* had a significant effect on the quality of the edible film. The use of carrageenan iota: mangrove fruit *Avicennia marina* flour affects the quality of Tensile Strength, Water Vapour Transport Rate due to the presence of the -OH functional group in its intermolecular bonds. The research suggestion is to do further testing of the solubility and swelling power and water content of the edible film.

Keywords: *Eucheuma cottonii, Eucheuma spinosum, Mangrove Api-Api*

INTRODUCTION

One of the efforts to improve food products from environmental influences is packaging or coating food products, which is commonly known as edible film or edible coating. The use of synthetic materials with natural ingredients is a research effort currently being carried out [1]. The edible film is a thin layer made of edible material, used as a surface coating for food components which functions to inhibit the migration of water vapor, oxygen, carbon dioxide, aroma, and lipids. This edible film is biodegradable and edible so that it can reduce the use of non-degradable packaging [2]. Another advantage of using edible film according to [3] is that it can be eaten together with the packaged product, can be recycled, can improve the organoleptic properties of packaged food, can function as a nutritional

supplement, and antimicrobial and antioxidant agents.

Composite or multicomponent edible film is to increase the superiority of the properties and the complementarity of each material or component. Most of the composite films or coatings are associated with hydrophilic matrix structures and hydrophobic lipids to provide better functional properties [4]. Edible film constituents directly affect the morphological shape and characteristics of the film produced. The main components of the edible film are divided into three categories, namely hydrocolloids, composites, and lipids [5]. One of the edible film materials from the hydrocolloid group is a polysaccharide which has several advantages, including being selective to oxygen and carbon dioxide, non-greasy appearance, and low-calorie content [6]. Edible film constituents directly affect the morphological shape and characteristics of the film produced. carrageenan is a collective term for polysaccharides extracted from certain species of red seaweed of the family, Rodhophyceae.

Carrageenan is the most widely used form of edible film and the demand for it is also increasing. Carrageenan is a sulfated polysaccharide extracted from several species of red seaweed (Rhodophyceae). Based on the sulfate content, carrageenan is classified into kappa-carrageenan, iota-carrageenan, and lambda-carrageenan with the amount of sulfate respectively 20%, 33%, and 42% [7]. The source of carrageenan is from the *Kappaphycus alvarezii* species which produces kappa-carrageenan, while *Echeumma spinosum* produces iota carrageenan. Kappa-carrageenan and iota-carrageenan can be mixed and used as materials for making edible films because they can form good physical characteristics. Kappa-carrageenan has strong gelling properties but tends to be brittle and susceptible to syneresis, while iota-carrageenan has weak properties in gelling but is more flexible and not susceptible to syneresis [8]. Caragenan and mangrove fruit starch are carbohydrate groups, both of them which can be used as edible film materials and can improve the properties of edible film. According to [9] that the mechanical strength and gas retention are good in edible film, when using carbohydrate as material.

Mangrove fruit flour species api-api (*Avicennia marina*) can be used as raw material for making edible films mixed with kappa-iota carrageenan because it contains high carbohydrates. The constituent component of carbohydrates is starch. Starch is often used in the food industry as a biodegradable film to replace plastic polymers because it is economical, renewable, and provides good physical characteristics [10]. The levels of carbohydrates and starch of Api-api fruit flour (*Avicennia marina*) were 80.76% and 38%. It can be seen that the carbohydrate content of the api-api fruit flour is higher than the carbohydrate content of rice, which is 78.9%, and corn by 63.3% [11]. Physical properties that determine the quality and use of edible films include thickness, elongation, and tensile strength [6]. According to [12], chemical testing, namely water vapor transmission rate and moisture content test. Research on the manufacture of biofilms made from carrageenan and starch from mangrove fruit has not been widely conducted. Research conducted by [11], showed that giving 4% of lindur fruit starch, 1.5% glycerol, and 2.5% carrageenan produced the best physical characteristics of edible film. The problem is whether the use of kappa-carrageenan: iota-carrageenan: mangrove *Avicennia marina* fruit flour in the ratio as an edible film material affects the characteristics of the edible film.

The research objective was to find the effect of the treatment of using kappa-carrageenan (KC): iota-carrageenan (IC): mangrove fruit *Avicennia marina* flour (AMF) in the ratio to the quality of the edible film.

METHOD

The research method used is a laboratorium experimental research design, using Completely Randomized Design (CRD) as experimental research design, three replicates. The treatment was (kappa-carrageenan: iota-carrageenan: mangrove *Avicennia marina* fruit flour) in ratio, A1 = 0 : 1 : 3 ; A2 = 0 : 2 : 2 ;A3 = 0 : 3 : 1;A4 = 1 : 0 : 3 ;A5 = 1 : 1 : 2 ;A6 = 1 : 2 : 1; A7 = 2 : 1 : 1;A8 = 2 : 0 : 2; A9 = 3 : 0 : 1. The result research that was analyzed using SPSS 17 software programe.

Procedure of Research

Making Kappa and Iota-Carrageenan

Making kappa-carrageenan from the *Eucheuma cottonii* species in the form of SRC (Semi Refined Carrageenan) based on the gel press method according to [8], which has been modified as follows, first, seaweed from the type of *E. cottonii* is weighed as much as 25 g then washed. Then add water with a ratio of 1:20 (w/v) and immersion for ± 4 hours. Then blend for 1 minute until it becomes a paste and followed by extraction. Seaweed is heated at 85°C for 2 hours with the addition of 6% KOH. The extraction results are cooled then filtered using filter paper and the residue is taken. The residue was added with KCl 1.5% and then aerated. After being frozen, it is dried and ground until it becomes a powder and obtained kappa carrageenan powder. By using the same manufacturing method, the iota-carrageenan uses *Eucheuma spinosum* species. The extraction iota carrageenan process use $Ca(OH)_2$ and $Ca(Cl)_2$.

Making of Fruit Mangrove Flour

The manufacture of api-api fruit flour (*Avicennia marina*) using the method of [13] which has been modified, namely fruit that has been peeled and cleaned of the flower petals is boiled with water for 20 minutes. Then the boiled fruit is mashed in a blender. The next step is that the fruit is soaked in water for 2 days and every 6 hours the water is changed. Then the drying process is carried out for approximately 12 hours. The dried fruit is then carried out by a powdering process using a blender and sieving with a 100 mesh.

Making of Edible Film [14]

Making edible film begins with dissolving kappa carrageenan with aquadest, heated at a temperature of 85°C. The next step is to dissolve iota carrageenan with aquadest, then heat it at 80°C until it dissolves completely. Then the next step is to dissolve the mangrove fruit flour with aquadest, heat it to 90°C and the flour is completely dissolved. Weighed according to the treatment ratio. After that, mix the three ingredients again until they are completely dissolved water until 100% (*w/v*) at a temperature of 80°C. Then the hot solution is printed in a Petri dish, then dried. After drying, the edible film is ready to be removed.

Moisture Content [15]

Water content can be determined by heating methods. The principle of this method is that the sample is heated at a temperature of 100-105°C for 3-5 hours depending on the material being tested until a constant weight is obtained. At this temperature, all free water (which is not bound to other substances) can easily be evaporated, but this is not the case with bonded water. Weight loss is the amount of water in food.

$$\%WB = \frac{(A+B)-C}{B} \times 100\%$$

Where:
A = weigh bottle weigh (g)
B = sample weight (g)
C = weigh bottle weigh and sample that has been oven (g)

Water Transport Vapor Rate *(WVTR*) [16]

The edible film to be tested is cut. Then container 1 is filled with 15 mL of distilled water and placed in container 2 which contains silica gel. Before that, the silica gel was dried at 180°C for 3 hours. Then container 2 is stored at 25°C. measurements were made after storage at 0, 5, 10, and 24 hours. Water vapor transmission is calculated by the formula:

$$WVP = = \frac{\Delta W}{t \times A}$$

Where:
W = change in weight of the edible film after 24 hours
t = Time (24 hr)
A = film surface area (m^2)

Thickness [16]

The thickness test was performed using a micrometer at 3 different places then the measurement results were averaged as a result of the thickness of the film. The thickness is expressed in μm while the micrometer used has an accuracy of 0.01 mm.

Elongation [17]

The elongation percentage is calculated by comparing the length of the edible film at the break and the length of the edible film before being pulled by the tool. The calculation of the percent elongation can be written as follows:

$$\%\ elongation = \frac{(\text{change in length (up to the breaking point)})}{(\text{original length})} \times 100\%$$

Tensile Strength [17]

Tensile strength and elongation were measured using Tensile Strength and Elongation Tester Industries model SSB 0500. Before measurement, the film was conditioned in a desiccator with RH 75% for 24 hours. The maximum force value for cutting film can be seen on the tool display. Tensile strength is determined based on the maximum load when the film breaks and the percentage elongation is based on the length of the film when the film breaks. Mathematically, the relationship between the two can be written as follows:

$$\text{Tensile strength} = = \frac{F}{A}$$

Where:
F = Tensile Force (N)
A = Surface Area (mm^2)

RESULT AND DISCUSSION

Red Seaweed as Sources

Extraction of *Eucheuma cottonii* and *Eucheuma spinosum* seaweed produced kappa-carrageenan (KC) and iota-carrageenan (IC). Each carrageenan content has a percentage of water content, namely kappa-carrageenan at 9.38% and iota-carrageenan iota 10.24%. According to FAO, the water content of carrageenan should not be more than 12%.

Testing of Functional Groups using FTIR on Kappa and Iota-Carrageenan

This shows that the kappa and iota carrageenan produced from this study meet FAO standards. Both types of carrageenan were tested by FTIR to see the clusters in the carrageenan. The FTIR test results of kappa-carrageenan and iota carrageenan can be seen in Figure 1 and Figure 2.

Based on Figure 1, it can be seen that the 1st peak (927.76 cm^{-1}), is the 3,6 anhydrous-D-galactose group (3,6 AG), the 4th peak (1234,44 cm^{-1}) is a sulfate ester group. However, for the galactose 4-sulfate (G4S) group, the wavenumber is not readable. This is probably because the peaks produced in the G4S wave range are too small. By this analysis, it can be stated that *Eucheumma cottonii* will produce kappa type carrageenan. According to [18] stated that the standard identification of kappa-carrageenan includes sulfate esters with a wavenumber range of 1240-1260 cm^{-1}, 3.6 anhydrous-D-galactose (3.6 AG) with a wavenumber range of 925-935 cm^{-1} and 1075-1085 cm^{-1}, and galactose 4-sulfate (G4S) with a wavenumber range of 845-850 cm^{-1}.

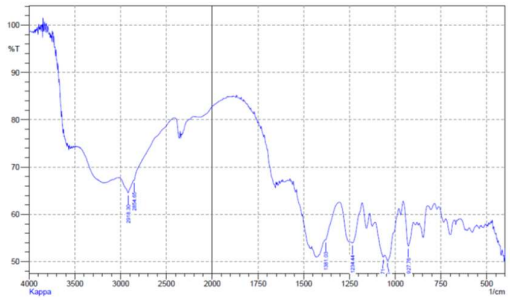

Figure 1. Functional number of spectra KC using FTIR

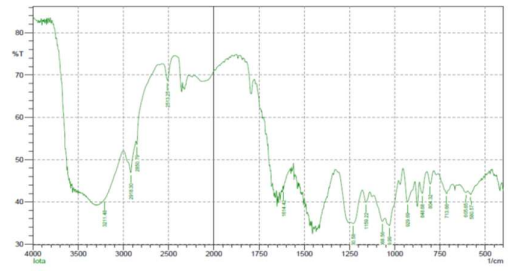

Figure 2. Functional number of spectra IC using FTIR

By Figure 2, it can be seen that the 4th peak (804.32 cm^{-1}), is the D-anhydrogalactose 2 sulfate (DA2S) group, the 5th peak (848.68 cm^{-1}) is the 4-sulfate galactose group. (G4S), the 6th peak (929.69 cm^{-1}) was the 3,6 anhydrogalactose group (3,6 AG) and the 10th peak (1230,58 cm^{-1}) was the sulfate ester group. Based on the data analysis, it can be said that *Eucheumma spinosum* will produce iota type carrageenan. According to [18] stated that the standard identification of carrageenan iota includes sulfate esters with a wavenumber range of 1240-1260 cm^{-1}, 3.6 anhydrous-D-galactose (3.6 AG) with a wavenumber range of 925-935 cm^{-1} and 1075-1085 cm^{-1}, galactose 4-sulfate (G4S) with a wavenumber range of 845-850 cm^{-1} and DA2S with a wavenumber range of 905-907 cm^{-1} and 804-808 cm^{-1}. The free hydroxyl group absorbs energy in the range 3200-3600 cm^{-1}, so the band that appears in the range 3091.89-3130.47 cm^{-1} in the spectra of the iota-carrageenan is a marker for the -OH group. Iota-carrageenan has 3211,48 cm^{-1} wavenumber.

Testing of Functional Groups using FTIR on the Fruit of Mangrove *Avicennia marina* Species

Raw material mangrove fruit of *Avicennia marina* flour species obtained from the area of Probolinggo. Water content was 62.8%, after drying the water content decreased to 8.77%. The moisture content of the material after drying is affected by the temperature and drying rate. Mangrove fruit functional groups using FTIR can be seen in Figure 3.

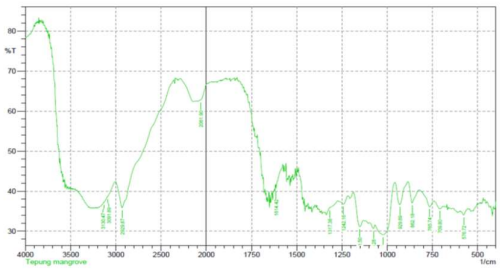

Figure 3. Functional groups of AMF using FTIR

Analysis of functional groups contained in mangrove flour was carried out using Fourier Transform Infra-Red Spectrophotometry (FTIR). The results of the analysis are presented in Figure 3. The free hydroxyl group absorbs energy in the range 3200-3600 cm^{-1}, so the band that appears in the range 3091.89-3130.47 cm^{-1} in the spectra of the mangrove flour is a marker for the -OH group. The band that appears at the wave number 2929.87 cm^{-1} in spectra is a marker for the presence of the C-H alkane group. The band that appears at wave number 1614.42 cm^{-1} indicates the presence of the C = C group. The bands that appear at the wavenumbers 1242.16 cm^{-1} and 1317.38 cm^{-1} indicate the presence of the C-N group. The bands that appear at the wavenumbers 1076.28 cm-1 and 1151.5 cm^{-1} indicate the presence of the C-O group. The bands that appear at the wavenumbers 709.8 cm-1, 765.74 cm^{-1}, and 862.18 cm^{-1} indicate the presence of the C-H group of aromatic structures.

Effect of Treatment on Edible Film Quality

Based on the results of the Analysis OF Variance test, it was found that the treatment affected the quality of the edible film. The effect of treatment on the quality of the edible film can be seen in Table 1.

Table 1. Effect of Treatment on Quality of Edible Film

Treatment (KC : IC: AMF)	Thickness (μ m)	Tensile Strenght (N/mm^2)	Elongation (%)	Water Vapor Transpor Rate (g/m^2. jam)
A_1 (0 : 1 : 3)	108,5±0,79^{g}	2,83±0,73^{a}	6,31±0,76^{cd}	100±7,40^{f}
A_2 (0 : 2 : 2)	54,83±0,60^{a}	4,19±1,26^{a}	10,91±0,65^{d}	94,79±0,41^{f}
A_3 (0 : 3 : 1)	76,33±0,45^{cf}	5,95±0,64^{ab}	1,83±0,35^{a}	76,04±0,56^{d}
A_4 (1 : 0 : 3)	64,3±1,23^{c}	2,32±0,99^{a}	5,41±0,46^{bc}	69,79±1,47^{c}
A_5 (1 : 1 : 2)	70,73±2,02^{d}	3,30±1,16^{a}	6,82±0,28^{cd}	68,75±3,13^{c}
A_6 (1 : 2 : 1)	75,67±0,78^{e}	4,38±0,78^{ab}	12,33±0,42^{e}	38,54±1,33^{a}
A_7 (2 : 1 : 1)	74,5±0,79^{e}	4,65±1,17^{ab}	5,38±0,49^{b}	57,29±5,41^{b}
A_8 (2 : 0 : 2)	58,87±0,31^{b}	3,71±0,60^{a}	5,68±0,48^{bc}	83,33±0,78^{e}
A_9 (3 : 0 : 1)	85,77±0,67^{f}	2,78±0,81^{a}	4,5±0,62^{b}	106,25±0,88^{g}

Note:
Different notations indicated a significant difference between treatments ($p < 0.05$)

The Thickness of Edible Film

The lowest thickness of the edible film was obtained in treatment KC:IC:AMF (0:2:2) and the largest thickness was obtained in treatment KC:IC:AMF (0:1:3). The lowest and the largest values for thickness were obtained in a mixture of ingredients containing IC and AMF. The greater the use of mangrove fruit flour, the thicker the edible film is formed. A thick edible film will provide better protection for packaged food products, but the water vapor permeability will be greater. The thick edible film will increase the tensile strength, but the elongation value and water solubility will decrease. The increase in the thickness of the edible film is also related to the unique properties of colloid compounds as a thickener and suspension, and the interaction between

the components of the edible film. IC and AMF are colloid-forming and thickening agents. IC is a gelling hydrocolloid with elastic properties. It is reinforced by [19] that IC has film-forming properties, namely thickens cold but requires - 65°C or higher to fully hydrate, dissolved up to 8%, moderately strong film.

Tensile Strength of Edible Film

The results of the diversity analysis presented in Table 1 show that the treatment of KC:IC:AFM with different ratios significantly affected the thickness of the edible film (P <0.05). The highest tensile strength value was obtained in the KC:IC:AMF treatment with a ratio (0: 3 : 1) of 5.95 N/mm^2, while the lowest value was obtained in KC:IC:AMF treatment with a ratio (1: 0: 3). From the ratio, it can be seen that an increase in IC will give the highest tensile strength value, while the presence of a mixture of KC: AMF gives the lowest tensile strength value of 2.32 N / mm^2. The increase in carrageenan concentration tends to increase the tensile strength of the edible film. This shows that the increase in the number of carrageenan in the solution for making edible films causes the bonds between the molecules, resulting in a more compact edible film. The higher the concentration of carrageenan that is added in making the edible film, it will form a stronger film matrix, so that the force needed to decide on an edible film is also greater. The difference in the properties of KC and IC is that KC forms a strong and brittle gel, while IC forms a soft and elastic gel. Thus the difference in the elasticity of the gelling agent is thought to affect the tensile strength value of the edible film. According to [19] that the polysaccharide structure of several gums that is related to the properties of film formation is the presence of a linear and neutral arrangement of polysaccharides which will form the greatest film strength. This is due to the linearity and non-ionic charge, the polymer bonds which can be more closely associated (by hydrogen intermolecular bonds) to produce a stronger film. From the FTIR observations in Figure 2 and Figure 3, it is found that IC and AMF have functional groups at wavenumbers with -OH group bond markers. Besides, it is confirmed by [19] that IC has a composition of L-galactose sugar units, 3,6 Anhydrogalactose, with linear structural features, sulfate substitution, and anionic charge as well as natural class.

Elongation of the Edible Film

The results of the diversity analysis presented in Table 1 show that the treatment of KC: IC:AMF with different ratios significantly affected the elongation value of edible film (P <0.05). The highest elongation value was obtained in treatment KC:IC:AMF with a ratio (1: 2: 1) with a value of 12.33% while the lowest elongation value was obtained in treatment KC:IC:AMF with a ratio (0: 3: 1). By this research results, it was found that the elongation value was strongly influenced by the KC and IC ingredients. Elongation is the percentage increase in the length of the film when it is pulled until it breaks. The mixture of ingredients between KC: IC and AMF is an interaction that can affect the elongation value. KC gels are strong, stiff, break easily, while IC has soft gel properties, elasticity, and doesn't break easily. The mixture of KC and IC ingredients will form a strong, soft, elastic, non-breaking, and non-synergetic gel. This is in line with the opinion of [20] that the interaction of KC and IC ingredients will form strong, elastic, and not easy syneresis gel properties. The higher the carrageenan concentration used in making edible films, the more carrageenan molecules will form a stronger film matrix so that the film is more inelastic or brittle, and consequently the elongation decreases [3].

Water Vapour Transport Rate (*WVTR*)

The results of the diversity analysis presented in Table 1 show that the treatment of KC: IC:AMF with different ratios significantly affected *WVTR* edible film (P<0.05). The lowest value was obtained in treatment KC:IC:AMF with a ratio (1: 2: 1) of 38.54 gr/m2.Hour, while the highest *WVTR* value was obtained in treatment KC:IC:AMF with a ratio (3: 0: 1) of 106.25 gr/ m2 .hour. The quality of the edible film is associated with the lowest *WVTR* value. The lowest *WVTR* value from observations found that the use of a mixture of KC:IC:AMF with a greater than IC ratio gave the lowest *WVTR* value. While the use of a mixture of KC and AMF gave the highest value to WVRT. The Hydrophilic nature of Polysaccharide, polysaccharides-

based films exhibit limited water vapor barrier ability. According to [21] that water vapor permeability (*WVP*) can be directly related to the quantity of -OH group on the molecules. Also, environmental conditions can significantly affect the *WVP*. Through Figure 2 and Figure 3 it is found that IC and AMF have an -OH group functional group.

Conclusion

The use of KC: IC: AMF in ratio different had a significant effect on the quality of the edible film. The use of IC:AMF affects the quality of Tensile Strength, *WVTR* due to the presence of the -OH group functional group in its intermolecular bonds.

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