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Analysis of Tensile Strength and Percent Elongation of Edible Composite Film from Milkfish Bone Gelatin and Red Algae Carrageenan

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Abstract: This research aims to develop an innovative solution to reduce the problem of plastic waste by designing and optimizing an edible film formula using milkfish bone gelatin and red algae as edible film composite materials. Milkfish gelatin was chosen because it can form an elastic and strong film, while red algae provides softness and resistance to microorganism activity. This research consists of several stages, including gelatin extraction, carrageenan extraction from Eucheuma cottonii and formulating composite edible films. Based on the results of the analysis carried out, the average yield of milkfish bone gelatin was 10.55% and the average yield of E.cottonii carrageenan extract was 20.62%. The thickness of composite edible film ranges from 0.15-0.25 mm, the highest tensile strength value is 6.3 MPa and the lowest is 3 MPa, and the highest percent elongation of edible film is 80% and the lowest is 36.67%. The values obtained are in accordance with the JIS 2-1707 (Japan Industrial Standard) (1946) standard.

Keyword: Tensile Strength, Percent Elongation, Edible Composite Film.

INTRODUCTION

The problem of environmental pollution caused by plastic-based packaging encourages sustainable alternative solutions so that natural biopolymer materials in food packaging applications are increasing to meet consumer demand and overcome environmental problems caused by petroleum-based plastic packaging in recent years (Wang *et al.*, 2021). Edible film has received attention as a promising food packaging material due to its biodegradability and potential to extend the shelf life of food products. Edible film is defined as a thin layer of edible material (safe for consumption) that is used as packaging to provide a barrier against external factors that stimulate food spoilage such as moisture, oxygen, light and contaminants (Santoso and Atma, 2020). Edible film uses biodegradable and renewable materials such as polysaccharides, proteins and lipids which are considered a replacement solution for synthetic plastics (Kumar *et al.*, 2022).

Various types of fishery waste can be used to make edible film, natural biopolymers such as proteins can be used to make biodegradable films (Gautam, Kakatkar and Karani, 2016). The protein used can be sourced from gelatin, where the potential of fish gelatin from fish bones as a material for making edible films is increasingly supported by the finding that fish bones are a viable alternative source for gelatin production (Atma *et al.*, 2018). Milkfish bones are a source of halal gelatin (Hasan and Dwijayanti, 2022). Issues regarding gelatin sources, fish gelatin offers a halal and environmentally friendly alternative to traditional gelatin sources (Faridah and Susanti, 2018). The physical properties of edible films formulated from fish bone gelatin reportedly meet important criteria for packaging materials, including appropriate thickness, moisture content, tensile strength, and elongation (Santoso and Atma, 2020).

Gelatin has high digestibility, making it potential as a raw material for making edible films. Gelatin is an ideal material for food packaging due to its versatile advantages such as low price, polymerization, biodegradability, antibacterial properties, good antioxidants, etc. However, gelatin film has poor water resistance and mechanical properties, which limits its development and application in food packaging. Previous studies have shown that pure gelatin can be modified by adding active ingredients and combining it with bio-polymers to improve its mechanical properties, aiming to achieve the desired preservation effect (Lu *et al.*, 2022). Combining two different components of polysaccharide and protein offers the possibility to create composite films with better properties to meet consumer expectations. The interaction of the components that make up the composite film will determine its structure and properties (Herrera-Vázquez *et al.*, 2022).

Edible films made from protein have good mechanical strength but poor water vapor barrier properties. Polysaccharide-based edible films have good gas barrier properties, but their water vapor barrier properties are also poor. However, the function of edible films with one component is often limited. Therefore, composite films containing a mixture of proteins, polysaccharides and/or lipids are often used to make edible films with desired properties. In addition, the functional properties of edible films can be adjusted by chemically modifying the biopolymer, using cross-linking agents or adding plasticizers (Chen *et al.*, 2021).

Carrageenan, a polysaccharide found in seaweed, has long been used in the food industry and has potential that has not yet been fully explored in making edible films. Its unique characteristics, such as gelling ability and viscosity, make it a suitable material for developing edible films. Carrageenan has been used in food packaging because of its ability to form films. However, films based on carrageenan have limitations due to their hydrophilicity and brittleness. Gelatin, one of the most popular edible biopolymers for developing high-performance films. When carrageenan and gelatin are used together to make active packaging films, it is expected to improve the properties of the films by highlighting their advantages. It has been reported that carrageenan combined with gelatin improves the mechanical and barrier properties of films (Alizadeh Sani *et al.*, 2022).

METHOD

Materials

The raw material used in making gelatin is milkfish bones with specifications of the large back bone, length ± 18 cm, diameter ± 5 mm, weight ± 7 grams. The chemicals used in making gelatin are technical HCl and Aquades. The seaweed raw material used for carrageenan is the Eucheuma cottonii type. The chemicals used for carrageenan extraction are KOH, KCl, and distilled water. Meanwhile, the materials used to make composite edible films are milkfish bone gelatin, extracted carrageenan flour, distilled water, glycerol.

Research Stages

Gelatin Extraction

Gelatin extraction from milkfish bones, referring to the best results from research (Masirah, Widjanarko and Yuwono, 2017), as follows: Making gelatin begins with a degreasing process on milkfish bones. This process is carried out by soaking the bones in water at a temperature of 80 0C for 30 minutes. Next, the bones are washed, drained and dried. The bones were then weighed as much as 100 grams and then demineralized with 4.65% HCl acid, for 26.89 hours. The process continued with extraction with distilled water (1:3) at an extraction temperature of 89.92 0C for 5 hours using a water bath. After that, the gelatin is filtered and then dried using an oven dryer and the final product obtained is dry gelatin (gelatin granules).

Carrageenan Extraction from Eucheuma cottonii

Fresh Eucheuma cottonii algae was washed using distilled water and dried at room temperature (air dried) for 7 days. The dried algae were then weighed first and extracted with 8% KOH for 2 hours. After extraction, the Eucheuma cottonii algae is cleaned by washing it with running water until the pH drops and then heating it again with distilled water for 2 hours. Each heating temperature is 90°C using a water bath shaker. After that, it is filtered using a blacu (filter cloth) to get the filtrate. Then the filtrate was poured into a 1.5% KCl solution for a settling process for 30 minutes. The precipitate was then washed again with distilled water and dried using a drying oven with a temperature setting of 60°C until dry. Next, the dried carrageenan fiber is then crushed until carrageenan powder is obtained.

Composite Edible Film Formulation

The composite edible film formulation was made using the Response Surface Methodology Box Behnken Design (RSM-BBD) method. At this stage, optimization of the production process of milkfish bone gelatin and carrageenan composite edible film was carried out using the Response Surface Methodology (RSM) method by varying the gelatin concentration (X1.%) by 3 levels, the carrageenan concentration (X2.%) by 3 levels and the glycerol concentration (X3, %) 3 levels, while the response parameters observed were thickness (Y1,, mm), tensile strength (Y2, MPa), elongation (Y3,%).

The combination of three treatment factors, namely gelatin concentration (X1,%), carrageenan concentration (X2,%) and glycerol concentration (X3,%) which are independent variables and thickness (Y1,,mm), tensile strength (Y2,MPa), elongation (Y3,%) which is a controlled variable (response). The midpoint (code = 0) was obtained from data on gelatin concentration, carrageenan concentration and glycerol concentration based on previous research results. Determination of the minimum limit (code = – 1) and maximum limit (code = +1) of the independent variable is determined based on preliminary research. Optimization of the carrageenan gelatin composite edible film production process with RSM was carried out to find the optimum level of gelatin concentration, carrageenan concentration and glycerol concentration needed for the production of milkfish bone gelatin composite edible film and carrageenan.

After the optimum conditions for gelatin concentration, carrageenan concentration and glycerol concentration are obtained, verification is carried out. Verification of the formulation is carried out using the composite edible film production process with optimum conditions.

Gelatin-Carrageenan Composite Film Edible Test Method

Thickness Analysis

Samples were measured using a micrometer at 5 different places then the measurement results were averaged as a result of the film thickness. Thickness is expressed in mm while the micrometer used has an accuracy of 0.01 mm.

Tensile Strength and Percent Elongation Analysis

To determine the tensile strength and elongation of edible film, use the Imada Force Measurement tool type ZP-200N. By following the tool's working procedures, data will be obtained for the tensile strength and elongation of the edible film. From this tool, data will be obtained for the force required to break the edible film and the extension of the edible film until the edible film breaks.

RESULTS AND DISCUSSION

E. cottonii Carrageenan Yield

The yield for making E.cottonii carrageenan begins by soaking 3 kg of fresh E.cottonii seaweed in 60 L of 5% KOH solution to obtain 646 grams of Alkali Treated Carrageenan (ATC). ATC was then dissolved in distilled water (1:60) and soaked in 2.5% KCl solution and obtained 614 grams of Semi Refined Carrageenan (SRC). The yield from carrageenan extraction can be seen in Table 1.

Table 1. Yield of E.cottonii Carrageenan Extract

Algae Species	Fresh Algae Weight (g)	ATC weight (g)	SRC weight (g)	Yield (%)	Average Yield
Eucheuma	3000	646	614	20,47%	
cottonii	3000	650	620	20,67%	20,62±0,11%
	3000	653	622	20,73%	

Source: Research data

The yield of kappa-carrageenan from Eucheuma cottonii extracted using the KOH solvent above was greater than the yield of carrageenan extracted using distilled water, namely 5.969 ± 4.51%. This is because extraction using alkali causes ion exchange between cations in the solvent (K+) and sulfate ions in seaweed so that sulfate levels are reduced and the formation of the 3,6-anhydrogalactose group is more optimal. The higher the 3,6-anhydrogalactose group formed in carrageenan, the higher the carrageenan fiber obtained so that the yield of carrageenan increases (Noor *et al*., 2021).

Milkfish Bone Gelatin Yield

The yield of milkfish bone gelatin begins by soaking 400 grams of milkfish bones that have been boiled and washed clean of fat in a 4.65% HCl solution for 27 hours, then the fish bones are neutralized by washing until pH 7. The fish bones are added with distilled water in the ratio (1:3) and hot extracted at 89.9°C for 5 hours. The gelatin solution was filtered and dried in an oven at 60°C, then the yield was calculated. The gelatin yield can be seen in Table 2.

Table 2. Milkfish Bone Gelatin Yield

Fish Species	Fish Bone Weight (g)	Gelatin Weight (g)	Average Gelatin Weight (g)	Average Yield (%)
Chenos	2000	210		
chanos	2000	214	211	10,55±0,11%
	2000	209		

Source: Research data

Table 2 shows that the average yield of milkfish bone gelatin was 10.55 ± 0.11%. The yield value is higher compared to research Syahputra *et al.* (2022) regarding the manufacture of milkfish bone gelatin using the citric acid solvent extraction method with a concentration of 13% with a soaking time of 48 hours, the yield value was 5.09%. This is influenced by the process of bone demineralization into ossein. The higher the level of soluble calcium in the bone demineralization process into ossein, the faster the collagen extraction process from ossein and the higher the yield of gelatin produced. Factors that influence demineralization are the concentration of the acid solution and the time the bones are soaked in acid.

Edible Film Parameter Test Results based on RSM

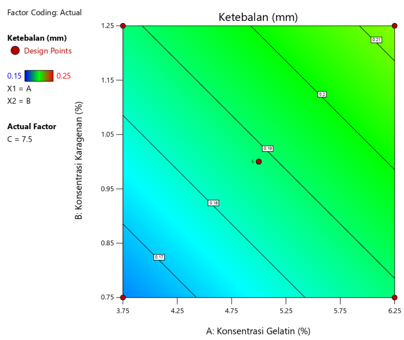
The test results for edible film parameters based on RSM can be seen in Table 3.

Table 3. Edible Film Parameters based on RSM

No	Parameter Levels			Extraction Parameters			Response					
	x1	x2	x3	Gelatin (%)	Carrageenan (%)	Glycerol (%)	Thickness (mm)	TS (MPa)	EAB (%)	WVTR (g/m^2)	S (%)
1	0	0	0	5	1	7,5	0.19	5.7	45	0.00028	100
2	-1	1	0	3,75	1,25	7,5	0.20	6.3	43.33	0.00061	100
3	1	0	1	6,25	1	8,75	0.25	4.4	56.57	0.000136	100
4	1	1	0	6,25	1,25	7,5	0.20	7.4	63.33	0.00029	85.93
5	-1	0	-1	3,75	1	6,25	0.15	4.2	46.67	0.000374	100
6	-1	-1	0	3,75	0,75	7,5	0.17	3	43.33	0.00029	100
7	0	0	0	5	1	7,5	0.20	5.5	43.33	0.000483	100
8	0	1	-1	5	1,25	6,25	0.19	7.5	46.67	0.000245	85.93
9	0	0	0	5	1	7,5	0.19	5.8	45	0.000459	100
10	0	1	1	5	1,25	8,75	0.20	7.1	80	0.000577	100
11	0	0	0	5	1	7,5	0.19	5.6	44	0.001759	100
12	0	-1	-1	5	0,75	6,25	0.17	5.4	46.67	0.000383	100
13	0	-1	1	5	0,75	8,75	0.20	3.7	43.33	0.000347	100
14	-1	0	1	3,75	1	8,75	0.17	3.4	36.67	0.00028	100
15	1	0	-1	6,25	1	6,25	0.19	6.1	70	0.00029	100
16	0	0	0	5	1	7,5	0.19	5.8	46	0.000257	100
17	1	-1	0	6,25	0,75	7,5	0.15	4.6	53.33	0.000468	100

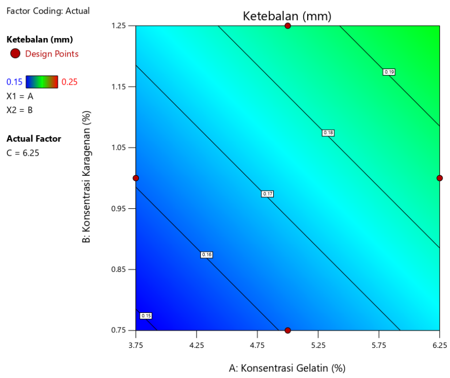
Source: Research data

Edible Film Thickness



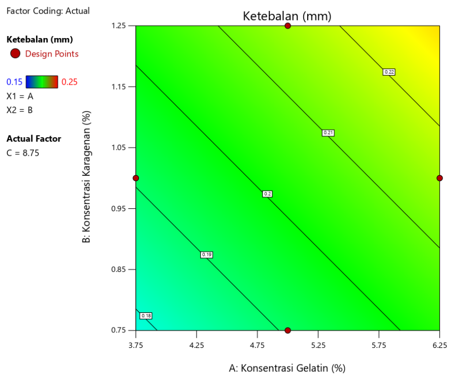
Source: Research Results

Figure 1. Edible Film Thickness with 7.5% Glycerol concentration



Source: Research Results

Figure 2. Edible Film Thickness with 6.25% Glycerol Concentration

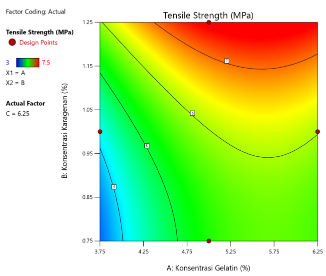


Source: Research Results

Figure 3. Edible Film Thickness with 8.75% Glycerol Concentration

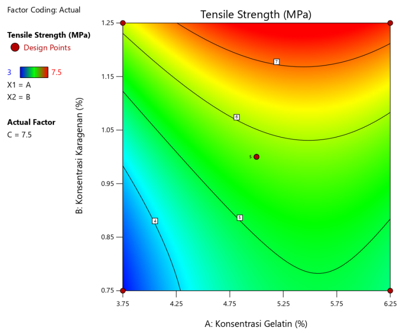
Film thickness greatly influences other physical and mechanical properties of edible film, such as tensile strength, elongation, solubility and water vapor permeability. Thick edible film will increase the tensile strength, but the elongation value and water solubility decrease (Racmayani and Husni, 2020). Table 3 shows that the thickness of the edible film for all treatments ranged from 0.15-0.25 mm. This value is in accordance with the JIS 2-1707 (Japan Industrial Standard) (1946) standard, which states that the maximum ideal edible film thickness is 0.25 mm. This is in line with research Rahmawati *et al*. (2019) which states that carrageenan edible film with the addition of 6% sorbitol plasticizer produces an edible film thickness of 0.222 mm. The thickness of edible film varies depending on its composition and preparation method, ranging from 0.085-0.23 mm for carrageenan-based films (Rusli *et al.*, 2017), 0.227 mm for alginate based films (Racmayani and Husni, 2020), and 0.12-0.147 mm for films made from seaweed fucoidan (Pouralkhas *et al.*, 2023).

Tensile Strength and Percent Elongation of Edible Film



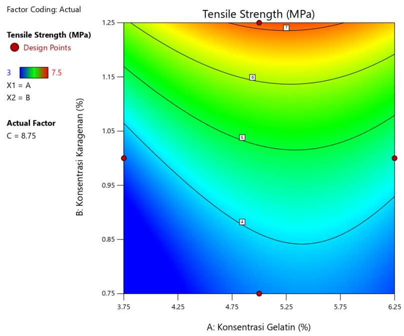
Source: Research Results

Figure 4. Tensile Strength of Edible Film with the Addition of 6.25% Glycerol



Source: Research Results

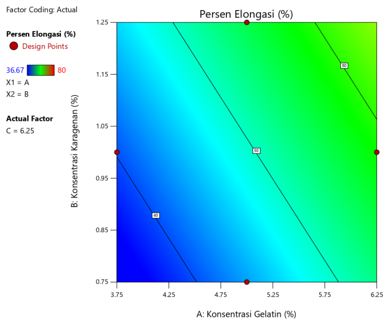
Figure 5. Tensile Strength of Edible Film with the Addition of 7.5% Glycerol



Source: Research Results

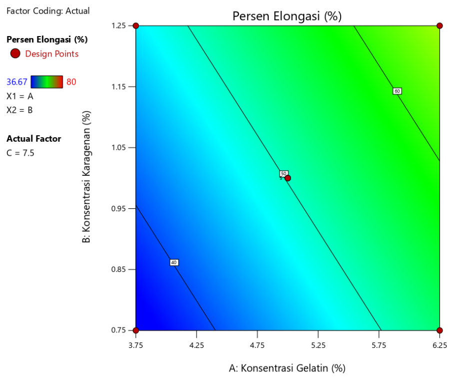
Figure 6. Tensile Strength of Edible Film with the Addition of 8.75% Glycerol

Tensile strength is one of the important mechanical properties of edible film which is related to the ability of the edible film to protect the product it coats. Edible film with high tensile strength is needed to protect food in packaging during handling, transportation and marketing (Poonia and Dhewa, 2022). Minimum tensile strength based on JIS standards is 0.39 MPa (Warkoyo *et al*., 2022). The tensile strength values for all treatments are in accordance with JIS standards. The highest tensile strength value was found in the edible film treatment with a gelatin concentration (-1) of 3.75%, carrageenan (+1) of 1.25% and glycerol (0) of 7.5% with a value of 6.3 MPa. The lowest tensile strength value was found in the edible film treatment with a gelatin concentration (-1) of 3.75%, carrageenan (-1) of 0.75% and glycerol (0) of 7.5% with a value of 3 MPa. Increasing the concentration of carrageenan tends to increase the tensile strength value of edible film, because increasing the amount of carrageenan in the edible film making solution causes the intermolecular bonds that make up the edible film to increase, resulting in an increasingly compact edible film. The higher the concentration of carrageenan added in making edible film, the stronger the film matrix formed, so the force required to break the edible film is greater (Tavassoli-Kafrani *et al*., 2016).



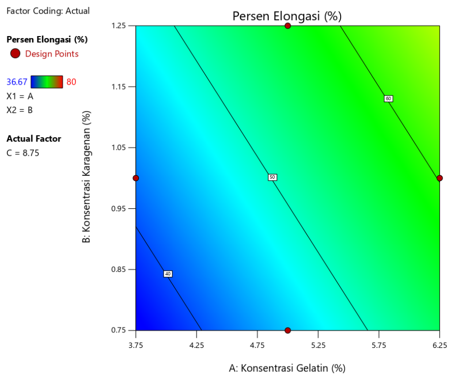
Source: Research Results

Figure 6. Percent Elongation of Edible Film with the Addition of 6.25% Glycerol



Source: Research Results

Figure 7. Percent Elongation of Edible Film with the Addition of 7.5% Glycerol



Source: Research Results

Figure 8. Percent Elongation of Edible Film with the Addition of 8.75% Glycerol

Percent elongation is the percentage increase in the maximum length of an edible film when it is pulled until it tears or breaks (Tulamandi *et al.*, 2016). Table 3 shows that the highest percent elongation was 80% in the edible film treatment with a gelatin concentration (0) of 5%, carrageenan (+1) of 1.25% and glycerol (+1) of 8.75%. The lowest percent elongation value was 36.67% in the edible film treatment with a gelatin concentration (-1) of 3.75%, carrageenan (0) of 1% and glycerol (+1) of 8.75%. All treatments are still within the JIS standard range, namely edible film is said to be very poor when the elongation percentage is <10%, and the quality is very good when the elongation percentage is >50%. The combination of increasing concentrations of carrageenan and glycerol had a positive effect on the elongation percentage. Carrageenan is hygroscopic, so water absorption can cause plasticization behavior (increased elongation at break) which accompanies increased addition of carrageenan. (Abdou and Sorour, 2014).

CONCLUSION

Based on the results of the analysis carried out, the average yield of milkfish bone gelatin was 10.55% and the average yield of E.cottonii carrageenan extract was 20.62%. The thickness of composite edible film ranges from 0.15-0.25 mm, the highest tensile strength value is 6.3 MPa and the lowest is 3 MPa, and the highest percent elongation of edible film is 80% and the lowest is 36.67%. The values obtained are in accordance with the JIS 2-1707 (Japan Industrial Standard) (1946) standard.

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