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CHARACTERISTICS OF CARRAGEENAN FROM SEAWEED HYDROLYSIS USING MARINE FUNGI AS HARD-SHELL CAPSULE MATERIAL

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Abstract

Carrageenan is a polysaccharide extracted from red algae and can be used as a raw material for hard-shell capsules. Carrageenan can be produced by biological hydrolysis of marine fungi. The viscosity of carrageenan resulting from hydrolysis using marine fungi is lower than that of commercial carrageenan. Gelatine can be used to modify the characteristics of polysaccharide-based materials. The characteristics and types of carrageenan and plasticizers influence the interactions between carrageenan and gelatin. This study aimed to determine the characteristics of carrageenan produced by seaweed hydrolysis of a hard-shell capsule material. The physical characteristics of the carrageenan produced by hydrolysis were determined, including yield, viscosity, and gel strength. The properties of the hard-shell capsules, including dimensions, capsule weight, disintegration time, and moisture content, were analyzed. The yield was 25%, and the viscosity and gel strength of carrageenan were 45 cP and 175 gf, respectively. Carrageenan contains 13% moisture, 8% ash, and 8% cellulose. Semi-refined carrageenan produced by this treatment was used to prepare hard-shell capsules. The capsule made from semi-refined carrageenan had a body length of 18 mm, capsule length of 10 mm, capsule weight of 0.9 grams, disintegration time of 10 min, and moisture content of 12%.

Keywords: cultivation, FTIR, *Kappaphycus alvarezii*, semi refined carrageenan

Karakteristik Karagenan dari Hidrolisis Rumput Laut menggunakan Kapang Laut sebagai Bahan Cangkang Kapsul Keras

Abstrak

Karagenan adalah polisakarida dari alga merah yang dapat digunakan sebagai bahan baku cangkang kapsul keras. Karagenan dapat diproduksi melalui metode hidrolisis biologis menggunakan cendawan laut. Bahan yang digunakan untuk memodifikasi karakteristik produk berbasis polisakarida salah satunya adalah gelatin. Interaksi karagenan dan gelatin dipengaruhi oleh karakteristik dan jenis karagenan, serta pemlastis (*plasticizer*). Penelitian ini bertujuan untuk menentukan karakteristik karagenan hasil hidrolisis rumput laut untuk cangkang kapsul keras. Karakteristik fisik dari karagenan yang diproduksi dengan hidrolisis, yaitu rendemen, viskositas, dan kekuatan gel. Cangkang kapsul keras dianalisis dengan menentukan berat kapsul, waktu pemecahan, dan kadar air. Hasil menunjukkan rendemen karagenan 25%, viskositas 45 cP, dan kekuatan gel 175 gf. Karakteristik kimia karagenan, yaitu kadar air 13%, abu 8%, dan tingkat selulosa 8%. *Semi refined carrageenan* yang diperoleh dari hidrolisis rumput laut yang selanjutnya dipilih dalam pembuatan cangkang kapsul keras. Cangkang kapsul keras yang dihasilkan memiliki karakteristik panjang total 18 mm, panjang kapsul 10 mm, berat 0,9 g, waktu desintegrasi 10 menit, dan kadar air 12%.

Kata kunci: FTIR, *Kappaphycus alvarezii*, kultivasi, *semi refined carrageenan*

INTRODUCTION

The annual production of wet seaweed in Indonesia experienced a significant increase from 205,000 metric tons in 2,000 to 3.9 million metric tons in 2010, and further rose to 11.27 million metric tons in 2015. However, it steadily declined to 9.78 million metric tons in 2019 (KKP, 2021). According to Parenrengi *et al*. (2020), the usage of low-quality seed (cuttings) has caused seaweed to grow more slowly, which has resulted in a recent decline in production. Heijden *et al*. (2022) noted the difference between official production data on one side and informed industry consensus that estimate the total annual dried seaweed production at 300,000 to 360,000 tons or 365,000 tons on the other side. A total production of 10 million mt of fresh seaweed would be equivalent to an annual production of approximately 1 to 1.6 million mt of dried seaweed, depending on the moisture content which can range from 42 to 35%.

Kappaphycus alvarezii, commonly referred as *Eucheuma cottonii*, belongs to the Rhodophyceae class. This alga species exhibits variations in coloration, ranging from reddish, yellowish, brown, to green, which are determined by the concentration of the phycoerythrin pigment. It may be readily cultivated and exhibits rapid growth, with a daily rise of approximately 4.5% (Gereniu *et al*., 2017). The hydrothermal hydrolysis of carbohydrates yielded limited outcomes due to the harmful impact of high temperatures on the structural integrity of the carbohydrates (Kim *et al*., 2014). The enzymatic hydrolysis resulted in producing environmentally friendly oligosaccharides with low levels of hazardous chemicals. However, this process was time-consuming and expensive (Wang *et al*., 2020). Utilizing microbes with enzymatic activity to accelerate the hydrolysis of seaweed carbohydrates is a highly successful alternative technique.

Carrageenan is a widely used ingredient derived from certain types of red seaweed. It is commonly extracted through chemical or biological methods. When carrageenan is obtained through biological hydrolysis, enzymes rather than chemical processes break it down. This method involves using enzymes to break down the carrageenan molecules into smaller components, which can have various applications in different industries (Pacheco-Quito *et al*., 2020). Biological methods rely on enzymatic action to extract carrageenan with lower environmental impact but potentially higher costs and slower processing times.

On the other hand, chemical methods involve alkaline and acid treatments for faster and more cost-effective extraction but may produce more waste and require careful handling of chemicals. The choice between these methods often depends on factors such as production scale, cost considerations, and environmental sustainability goals (Lomartire & Gonçalves, 2022). The production process through biological hydrolysis involves using enzymes to decompose red algae cell walls and release toxins. It is more environmentally friendly and can produce toxins with desirable properties for a particular application. Chemical extractions use chemicals such as alkalis or acids to extract toxins. Although faster and more efficient, it can hurt the environment and may result in a multiplication of less desirable properties for capsule applications. This analysis's main differences lie in product quality, environmental impact, and suitability for capsule applications. Biological hydrolysis outperforms product quality and environmental sustainability, while chemical extraction is faster and more cost-efficient. The two choices will depend on specific priorities, such as product superiority versus production efficiency (Ademola *et al*., 2013).

Carrageenan is a substance derived from the fisheries industry that is becoming increasingly popular in the culinary, cosmetics, and pharmaceutical industries. It is utilized in the pharmaceutical industry as a primary ingredient for manufacturing capsules. Capsules made from carrageenan have a longer period of disintegration compared to capsules made from gelatin (Gullapalli & Mazzitelli, 2017). The viscosity of the raw materials influences the disintegration time of a hard-shell capsule (Junianto *et al*., 2013). The viscosity of carrageenan resulting from hydrolysis using marine fungi has a lower value than commercial carrageenan (Rahman,

2016). Hence, the objective of this study is to to determine the characteristics of carrageenan produced by seaweed hydrolysis for hard shell capsule material.

MATERIALS AND METHODS

Raw Material Preparation

The initial step in preparing the raw materials involves immersing and bleaching *K. alvarezii*, with a quantity of up to 1 kg. The specimen was submerged in distilled water for 24 hours. The bleaching process involved utilizing a 0.5% calcium oxide (CaO) solution for 5 mins. The sample was pulverized using a blender until a uniform consistency was achieved. The finely pulverized samples were subsequently desiccated under direct sunlight for three days. The desiccated specimens were pulverized using a blender and strained through a 40/60 mesh screen (allowing particles smaller than 40 mesh to pass through while retaining those more significant than 60 mesh) to acquire seaweed powder. The seaweed was weighed, measuring 3.75 g, and then subjected to boiling at 60°C for 20 mins to create a gel. The gel was subsequently delignified physically by subjecting it to sterilization in an autoclave at a temperature of 121°C and a pressure of 1 atm for 2 hours.

Inoculum Preparation

The inoculum preparation entailed utilizing a novel substrate, specifically *K. alvarezii,* for acclimatization. The acclimatization begins by measuring a sample weighing 3.75 g and then covering it with aluminum foil. The inoculum preparation entailed using purified water, a solution comprising 1.5% *K. alvarezii*, and a solution containing 10% marine fungal EN. Moreover, the medium should be prepared by meticulously measuring and modifying the weight using distilled water until it attains a capacity of 250 mL. The media underwent sterilization through an autoclave and decelerated to ambient temperature. In the concluding phase, the isolates are introduced into the prepared PDB media using the process of inoculation into a 25 mL sample. The inoculation process was conducted at room temperature using a shaker operating at 120 rpm for six days. The inoculum preparation was based on the method described by Obata *et al*. (2015).

Hydrolysis

Obata *et al*. (2015) described the hydrolysis process. Hydrolyzing *K. alvarezii* was carried out using EN marine fungi, which consisted of a mixture of 1.5% *K. alvarezii* media, 10% EN fungi, and distilled water. The media preparation entails measuring 3.75 grams of *K. alvarezii*, followed by its appropriate encapsulation within the aluminum foil. The media was adjusted to a volume of 250 mL using distilled water and then sterilized using an autoclave. After sterilization, the samples are cooled to the surrounding ambient temperature. The generated fungus was then added to the sample in a volume of 25 mL (10%). The hydrolysis process was carried out at a shaking speed of 120 rpm for six days.

Carrageenan Precipitation

In the context of hydrolysis, precipitation refers to the process of isolating carrageenan from other molecules. A coagulant, such as 2-propanol, is introduced in a 1:1 ratio to produce carrageenan fibers to initiate this stage. Following this, centrifugation will be conducted at a velocity of 7,000 rpm at a temperature of 4^{o}C for 30 minutes. Carrageenan is obtained in a wet state through the process of centrifugation. The dehydration process of the wet carrageenan was conducted by subjecting it to an oven set at a temperature of 45^{o}C for 20 hours. The technique produced a desiccated carrageenan product, which was used for further experiments. This approach was based on Distantina (2007).

Capsule Preparation

The initial step in producing the hard shell capsule involves the preparation of a solution containing Carrageenan. In this procedure, a measuring cup combines 0.18 grams of carrageenan flour with 100 mL of distilled water. Subsequently, the mixture was agitated utilizing a magnetic stirrer and subjected to thermal treatment at 60^{o}C, employing a hot plate. Following this, a plasticizer comprising 0.5% (v/v) glycerol was

added to the mixture under continuous stirring until the temperature reached 80°C, which was subsequently sustained for 5 minutes. The generated carrageenan solution was subsequently administered onto the capsule using printing. The initial step in the capsule printing process involves the preparation of a capsule for the printing procedure. According to Obata *et al*. (2015), the capsules were immersed, rotated, or inverted to prevent leakage. They were then dried in an oven at 60°C for 3-4 hours.

RESULTS AND DISCUSSIONS

The marine endophytic fungus (EN) used in this study was isolated from a seagrass *Enhalus* sp. This fungus inhabits the internal parts of plants, such as leaves, twigs, short branches, or roots (Rahman, 2016). Marine fungus consists of hyphae, which are filamentous structures. EN exhibits the traits of septate hyphae and is classified as a member of the higher fungi/ascomycetes (Riquelme *et al.,* 2018). The investigations revealed that the EN exhibited both macroscopic and microscopic morphology. The macroscopic examinations revealed that the surface exhibited a white, velvety appearance, while the color behind the colonies appeared as a purplish-white shade. Additionally, the texture was seen to be soft. Rugose topography refers to irregularly grooved colonies that display radial lines. In addition, microscopic investigations revealed that the conidia had a spherical shape, and conidiophores, conidia, and hyphae surrounded the hyphae.

The growth medium used in this study was PDA media. PDA can encourage the growth of mycelium and sporulation and inhibit the growth of bacteria because it has a pH (4.5-5.6). In addition, PDA is one of the most used culture media because of its simple formulation and is the best medium because of its ability to support the growth of various fungi (Saha *et al.*, 2008). Mold has six growth phases: lag, acceleration, exponential, deceleration, stationary, and accelerated death (Rahman, 2016). Mold cells that are inoculated on the media and incubated at optimal physical growth conditions will produce growth consisting of a lag phase, an exponential phase, and a stationary phase (Kavanagh, 2011).

In the lag phase, cells adapt to their surroundings (Rahman, 2016). During this stage, enzymes are synthesized to facilitate the decomposition of the substrate. The lag phase occurs during days 0-3; the exponential phase refers to a time of fungal growth where cells proliferate rapidly by using enzymes created by the fungus and making use of existing components in the environment, such as nutrients (Andhikawati *et al*., 2014). The exponential phase occurs between day three and day 12; the stationary phase refers to a stage in which cell activity is at its peak and produces secondary metabolites that serve the purpose of self-defense (Munandar *et al*., 2014). The stationary phase occurs from day 12 to day 27 (Andhikawati *et al*., 2014).

The fungus grown on PDA media was then transferred to PDB media. PDB is a growth medium derived from potato and dextrose carbon sources and is the most critical component because microbial cells are mainly composed of carbon and nitrogen elements (Kusumaningtas *et al*., 2010). On the seventh day, the endophytic fungal culture on the PDB medium revealed a round endophytic mold stuck to the Erlenmeyer; some looked like small brownish granules. This suggested that endophytic molds could grow on a PDB medium because it gives them enough nutrients.

Potato Dextrose Broth (PDB) is a medium made from potato powder and dextrose, a food source for molds. PDB is used to cultivate molds and has the same composition as PDA, except that it does not contain agar. PDB is used to grow and identify molds because it contains a sufficient source of carbohydrates, consisting of 20% potato extract and 2% glucose, so it is suitable for mold growth and not good for bacterial growth. The mold from PDB media was then used for carrageenan hydrolysis. The hydrolysis process can be carried out by utilizing the fungal cellulase enzyme, which has the potential to hydrolyze complex polysaccharides into simple components (Henares *et al*., 2010). The

fungus will hydrolyze the 1,4-d-galactose-4-sulfate so that carrageenan turns into a more straightforward component.

Carrageenan Yield

Yield is a crucial factor in the production of carrageenan. Increasing the yield of a product will enhance production efficiency. The carrageenan yield obtained via hydrolysis in this study was determined by comparing the dry weight of hydrolyzed carrageenan to the dry weight of seaweed, given as a percentage. The carrageenan hydrolysis was conducted using a 10% concentration of EN fungus and a 1.5% concentration of *K. alvarezii* as substrates. The substrates were treated with refined carrageenan gelatinization (KB) and semi-refined carrageenan without gelatinization (KK). The hydrolysis process was carried out utilizing a shaker for six days. The carrageenan yield from hydrolysis was 10% in the KK treatment and 66% in the KB treatment. The elevated yield in the KB treatment can be attributed to the abundant presence of cellulose, pigments, and other compounds. Similar research conducted using *K. alvarezii* seaweed and the addition of KOH solution, was able to produce a yield on a carrageenan semi-refined treatment of as much as 6% (Arzani *et al*., 2020). According to Heriyanto *et al.* (2018), using high extraction temperatures over long periods can improve the yield of Carrageenan. The longer the extraction time results in the sulfate cluster on the sixth chain, the longer it takes to form 3,6 -anhydrous-d-galactose.

Chemical Characteristics of Carrageenan

Carrageenan is divided into two types based on the purity level: semi-refined and refined carrageenan. Semi-refined carrageenan is a polysaccharide with hydrocolloid properties consisting of carrageenan and a certain amount of cellulose, which is produced using an alkaline solution in a short time and at high temperature (FAO, 2014). Determination of carrageenan properties will be used to manufacture capsule preparations, including carrageenan's physical and chemical properties. The analysis was carried out to determine carrageenan's chemical properties, including moisture, ash, sulfate, cellulose, and physical characteristics of carrageenan, including whiteness, functional groups, gel strength, and viscosity. The results of determining the characteristics of carrageenan are listed in *Table 1*.

Moisture content is closely related to the product's shelf life because of the microbiological activity associated with the growth of bacteria and fungi. A low water content will inhibit bacterial activity because the less water available in the product, the lower the microbial activity. The resulting water content ranges from 13%-26%. The water content of carrageenan obtained by KK, KB, and KO was 26%, 13%, and 17%,

Table 1 Characteristics of carrageenan
Tabel 1 Karakteristik karagenan

Specification	Semi refined carrageenan (KK)	Semi refined carrageenan (KB)	Commercial carrageenan (KO)
Yield (%)	10	66	-
Water (%)	26	13	17
Ash (%)	20	17	19
Sulfate (%)	12	12	8
Cellulose (%)	0.14	8.5	0.10
Whiteness (%)	-	37	96
Viscosity(cP)	10	45	400
Gel strength (g/cm^2)	98.3	174.7	1,517.5

respectively. The value of K.B. carrageenan was lower than that of K.K. and K.O. Carrageenan. The water content obtained exceeds the standard set by FAO, which is a maximum of 12% (FAO, 2014). The high water content in this study was caused by drying seaweed and carrageenan raw materials that could have been more optimal, resulting in relatively high water content being tested.

The ash content shows the minerals contained in these foodstuffs (Akhyar *et al.*, 2009). Carrageenan ash content values obtained by KK and KB were 20% and 17%, respectively. This value is different from the ash content of knockout carrageenan but still meets the standards set by FAO. The ash content of KO carrageenan is 19%, while the standard value of carrageenan based on FAO is a maximum of 40% (FAO, 2014).

The cellulose content of KK and KB carrageenan treatment was 0.14% and 8.5%, respectively. The cellulose content is related to the cellulase activity produced by the EN fungus. The higher the cellulase activity produced, the lower the cellulose content in the carrageenan. The value of cellulose content in the KK carrageenan treatment is included in the refined carrageenan type because the cellulose content value is in accordance with the FAO which should not be more than 2% (FAO, 2014). Meanwhile, the cellulose content of the KB treatment was classified as semi-refined carrageenan because the value of the cellulose content was in accordance with that determined by FAO which was around 8-15% (Habib *et al.*, 2008).

The sulfate content is a metric utilized to measure the presence of polysaccharides in red algae of different varieties. Hydrolyzed and commercial carrageenan sulfate levels obtained in this investigation were 12% and 8%, respectively. The obtained sulfate content value did not comply with FAO's 15-40% criteria (FAO, 2014). The sulfate level of Carrageenan is directly related to its viscosity and inversely related to the strength of the carrageenan gel (Doh *et al.*, 2020). A decrease in the sulfate concentration of Carrageenan leads to an increase in its gelling characteristics (Campo *et al.*, 2009).

Gel strength refers to the highest amount of force needed to fracture the polymer matrix within the area under stress. As the weight increases, the gel strength also increases. Carrageenan's gel strength is a fundamental attribute that underlies its application in several industries. The gel strength was affected by the levels of sulphate and 3,6-anhydro-d-galactose presents. The gel strength values of carrageenan measured by KK and KB were 98.3 g/cm^2 and 174.7 g/cm^2, respectively (McHugh, 2003). The values differed from the strength value of the knockout carrageenan gel, but they nevertheless met the guidelines established by FAO. The gel strength of carrageenan is measured at 1,517.5 g/cm^2, which exceeds the typical gel strength range recommended by FAO of 20-500 g/cm^2 (FAO, 2014). The gel strength is affected by ionic interaction between the negative ions of the sulphate ester and certain cations (Fardhyanti & Julianur, 2015). The electrostatic repulsion among the negatively charged particles throughout the polymer chain, specifically the sulphate group, leads to the rigidification of the molecular chain. The carrageenan polymer is enveloped by mobile water molecules due to its hydrophilic character, resulting in the viscosity of the carrageenan solution.

Viscosity refers to the resistance of a fluid to flow, and it is quantified as the ratio of shear rate to shear stress (Glicksman, 1969). The presence of carrageenan solution, a polyelectrolyte, will impact the viscosity of carrageenan. The carrageenan solution's hydrophilicity is attributed to immobilized water molecules surrounding the polymer, increasing viscosity (Heriyanto *et al.*, 2018). As the viscosity value of carrageenan increases, so does its viscosity. The carrageenan viscosity values produced by KK and KB were 10 cP and 45 cP, respectively. This number differed from the viscosity value of KO carrageenan, although it nevertheless adhered to the guidelines established by FAO (FAO, 2014). The viscosity of KO carrageenan is 400 cP, which exceeds the minimum required viscosity value of 5 cP set by FAO (FAO, 2014). The viscosity of KB carrageenan exhibited a greater magnitude than that of KK carrageenan. The

distinction between KB carrageenan and KK carrageenan lies in their refinement levels. KB carrageenan is a highly refined form, whilst KK carrageenan is only partially processed. K.B. carrageenan is highly refined, while K.K. carrageenan is only partially processed. The viscosity of carrageenan is regulated by various factors, such as carrageenan concentration, temperature, type of carrageenan, molecular weight, and the presence of other molecules (Diharmi *et al.*, 2020). Cellulose is another molecule in semi-refined or semi-pure carrageenan (FAO, 2014).

Functional Groups of Carrageenan

The chemical properties of hydrolyzed and commercial carrageenan analyzed in this study were identification of carrageenan functional groups using Fourier Transform Infrared (FTIR) analysis. The results of the identification of carrageenan functional groups by FTIR can be seen in *Figure 1*.

The hydrolyzed and commercial carrageenan spectra showed a graph that is similar. Spectra of carrageenan KK, KB and KO were found at absorption 3,435.83–849.60 cm^{-1}; 3,429.79-890.51 cm^{-1}; 3,601.49-846.77 cm^{-1}. This value was not significantly different from the uptake value of KO carrageenan. An explanation of the hydrolyzed and commercial carrageenan functional groups identified by FTIR can be seen in *Table 2*.

The results of the identification of functional groups on K., KB, and KO carrageenan with FTIR analysis showed that sulfate groups were detected at a wavelength of 1,375.05 cm^{-1}; 1,372.29 cm^{-1}; 1,376.03 cm^{-1} and galactose groups 969.35 cm^{-1}; 970.57 cm^{-1}; 969.75 cm^{-1}. The characteristics of carrageenan are the presence of sulfate ester groups, galactose groups, and 3,6-anhydrogalactose groups (Pereira *et al.*, 2009). The functional groups of hydrolyzed carrageenan and commercial carrageenan obtained in this study were carrageenan.

Characteristics of Gelatin Capsule with Carrageenan Substitutes

Capsules are solid preparations consisting of drugs in soluble hard or soft capsules. Capsule characteristics are intact capsules, which means that the body and the seal are perfectly attached so that the medicinal ingredients do not come out (Qualicaps, 2011). Other characteristics include a flat surface, no holes, and the fact that the ingredients used do not react with the drug ingredients subsequently inserted into the capsule. The characteristics of the capsules resulting can be seen in *Figure 2*.

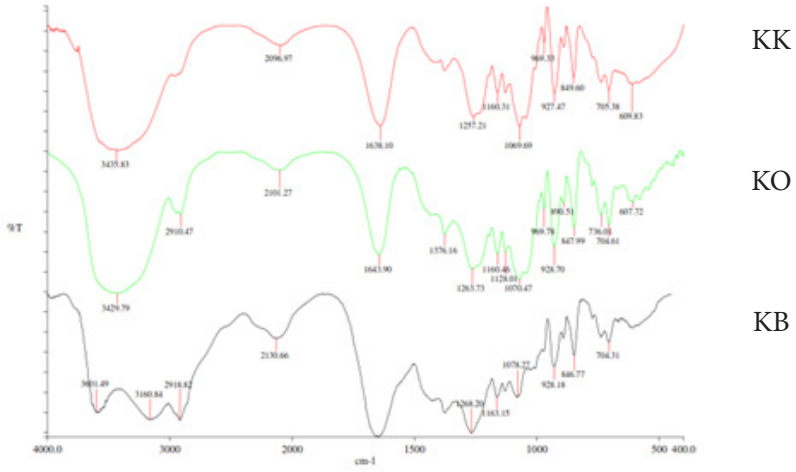
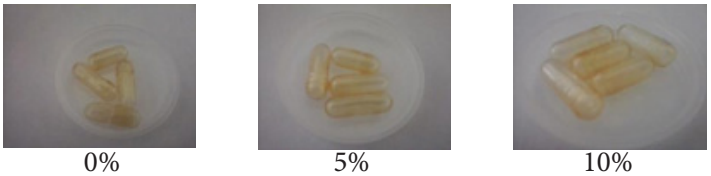


Figure 1 Carrageenan functional groups by FTIR, KK (semi refined carrageenan without gelatinization), KB (semi refined carrageenan with gelatinization), KO (commercial carrageenan)

Gambar 1 Gugus fungsional karagenan dengan FTIR, KK (karagenan *semi refined* tanpa gelatinisasi), KB (karagenan *semi refined* dengan gelatinisasi), KO (karagenan komersial)

Table 2 Multiple absorption data with FTIR
Tabel 2 Data serapan karagenan dengan FTIR

	Wave number (cm^{-1})				
Standard	-	-	1,259.43	1,069.33	970.43
KK	3,436.14	1,638.01	1,375.05	969.35	849.63
KB	3,517.89	1,645.99	1,372.29	970.57	890.92
KO	3,429.80	1,643.62	1,376.03	969.75	847.90
Bond type	-	-	S=O	C-O-C	-
Groups	-	-	Sulphate Ester	Galactose groups	-



0% 5% 10%

Figure 2 Capsules with different carrageenan substitutes
Gambar 2 Kapsul dengan substitusi karagenan berbeda

Capsules with different substitutes produce different characteristics. Capsule 5% carrageenan substitutes has thin characteristics with a perfectly mounted body and seal, but the surface of the capsule is not even. The characteristics are supposed to be thin because the variance has a low solution viscosity, so when printing, the solution that sticks to the capsule printer is only tiny. In contrast, the uneven capsule surface is presumed to be due to an incomplete drying process. With 10% commercial substitution, capsule has the characteristics of a thick capsule, a perfectly mounted body and seal, and a flat capsule surface. Different capsule thickness is presumed to be due to different viscosity. The viscosity of the solution is an important indicator to bear in mind in the manufacture of capsules, as it can affect the thickness of the capsule (Srividya & Reddy, 2014). Capsules 0, 5, and 10% carrageenan substitutes are then analyzed for physical and chemical characteristics, including dimensions, weight, breakdown time, and water content.

Mallik *et al.* (2013 stated that the size of the hard capsule in the industry consists of 8 types, ranging from the size 000 (most large) to the size 5 (smallest). The capsule printed on this study is a capsule with a 0-size stamp. The capsule dimensional measurement results can be seen in *Table 3*.

Scale analysis showed an influence on the level of substituting in gelatin on the size of the capsule produced (p<0.05). Duncan's further test results show that the length and diameter of commercial gelatin capsules have no fundamental differences with hydrolysis and commercial substitution capsules. Commercial gelatin capsule body length has no real difference in value from commercial gelatin substitution capsules but is different from hydrolysis gelatin substitution capsules. The thick body and commercial gelatin capsules have distinct values from commercial substitution and hydrolysis capsules. Manual and irregular reproduction of capsule prints can result in unequal prints and capsule body thicknesses.

The capsule weight is measured on the capsule with the body part and capsule cap attached, and it stated that the weight measurement on the capsule preparation was intended to determine the thickness of a capsule. Capsule weight is one of the parameters for meeting commercial capsule standards. The result of the capsule weight measurement can be seen in Figure 3. The scale analysis results show no influence on the substitution

Table 3 Characteristics of capsule dimension with different carrageenan substitutes
Tabel 3 Karakteristik dimensi kapsul dengan substitusi karagenan berbeda

Characteristics (mm)	Specification	0%	5%	10%
Cap	Length	6.30±1.12[a]	6.37±0.80[a]	8.10±0.90[a]
	Diameter	7.11±0.00[a]	6.85±0.36[a]	6.85±0.36[a]
	Thick	28.00±2.82[b]	25.50±2.12[c]	41.00±1.41[a]
Body	Length	16.99±0.15[b]	17.88±0.02[a]	17.35±0.07[b]
	Diameter	7.11±0.00[a]	6.85±0.36[a]	6.85±0.36[a]
	Thick	25.50±2.12[b]	23.00±1.41[c]	38.50±2.12[a]

Values with different superscript (a-c) indicate a significant difference at $p<0.05$

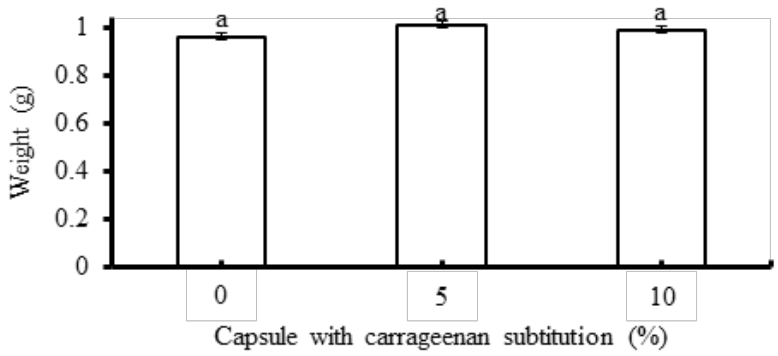


Figure 3 Capsule weight with a different substitution; Values with different superscript indicate a significant difference at $p<0.05$
Gambar 3 Bobot kapsul dengan substitusi karagenan berbeda; Huruf superskrip yang berbeda menunjukkan perbedaan nyata ($p<0,05$)

level of the substituted capsule ($p<0.05$). Duncan's further tests showed that capsule weights 0, 5, and 10% carrageenan substitutes do not differ significantly. The weight of the capsules produced in sequence is 0.96±0.09 g, 1.01±0.00 g, 0.99±0.12 g. The high weight of capsules is assumed to be due to manual dying and printing, which causes capsules to have a high weight. (Christi *et al*., 2016) stated that the capsule thickness is affected by the process of immersion and reproduction of the mold after immersion. Capsule printing processes performed manually and irregularly can result in unequal capsule density.

The body should easily absorb capsules as a packaging preparation medication. Disintegration time in the matrix (testing is intended to establish the compatibility of the crushing time limit of the capsule into aggregates or more fine particles (Qualicaps, 2011). The time test results on the capsule can be seen in Figure 4. The disintegration time of the obtained capsule ranges from 3.51-11.94 minutes (BPOM, 2014). Based on scale analysis, it is known that the substituted substitutes level has a real influence on capsule destruction time ($p<0.05$). Dissolving the drug in a water medium on a biological system is essential before systemic absorption. The selection of solvent media is critical in measuring the time of destruction. Different media are meant to determine how far the capsules can be destroyed in each digestive tract that the capsule passes. Capsules B and C have a faster disintegration time at acid pH. It suggests that the time the capsule is destroyed is determined by the agent's substitution level, the agent's material, and the medium used.

Water content play an important role in product stability due to microbiological activity. (Wenno *et al*., 2012). Capsule water levels can be seen in *Figure 4*. Capsule water content ranges from 15.53-16.37%. Based on the scale analysis, it is known that the level of

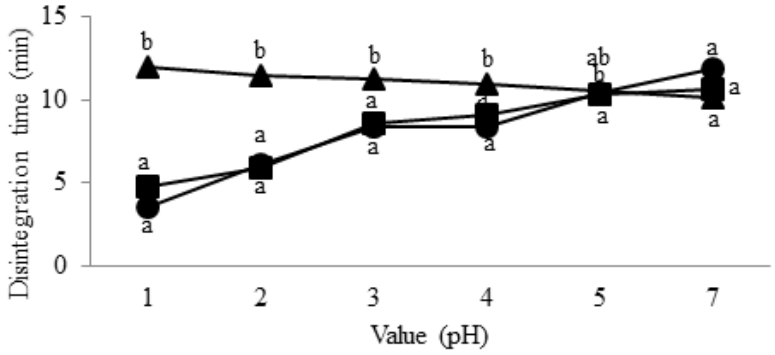


Figure 4 Disintegration time capsule with different carrageenan substitutes ▲ [0%], ■ [5%], ●[10%];Values with different superscript indicate a significant difference at $p<0.05$

Gambar 4 Waktu hancur kapsul dengan substitusi karagenan berbeda; ▲ [0%], ■ [5%],● [10%]; Huruf superskrip yang berbeda menunjukkan perbedaan nyata ($p<0,05$)

substitution has a significant influence on the capsule water level ($p<0.05$). Capsule 1 water content is in line with a study by (Junianto *et al*., 2013) which states that commercial capsules should have a water content of 13-16%. The water level of the drying capsule is strongly influenced by the temperature, humidity, drying time, and the physical viscosity properties of the solution of the material (Junianto *et al*., 2013). The higher the quantity added, the more water in the environment is bound by the hydrophilic quantity (Sulistyo *et al*., 2018).

Based on the results of the manufacture of the hard-shell capsule carrageenan, the capsule was subsequently analyzed in terms of its properties, including capsule dimensions, capsule weight, capsules disintegration time, and water content. The physical characteristics of the hydrolysis resulting from the product produce 37% white degrees; 45 cP viscosity; gel strength 174.7 g/cm^2, while the chemical characteristics produce 13% water content; 17% ash content; 8% sulphate content; cellulose content 8%. Semi refined carrageenan is the treatment chosen as the best result in capsule manufacturing.

CONCLUSION

Carrageenan can be synthesized via a process of enzymatic hydrolysis using marine fungus. The manufactured capsules were subsequently examined to determine their characteristics, encompassing the measurement of capsule size, weight, disintegration time, and water content. The hydrolysis of carrageenan led to specific physical properties, including whiteness level, viscosity, and gel strength. Semi-refined carrageenan has been identified as the optimal method for producing hard shell capsules, and its properties, including dimension, capsule weight, disintegration time, and moisture content, have been analyzed. According to the result, the hydrolyzed carrageenan capsules produced adhere to the standard features of capsules.

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