Biological Process to Valorise Marine Algae

To cite this article: K Tarman et al 2020 IOP Conf. Ser.: Earth Environ. Sci. 414 012026

View the article online for updates and enhancements.
Biological Process to Valorise Marine Algae

K Tarman¹2, N H Ain¹, S Sulistiawati¹, L Hardjito¹ and U Sadi²³

¹Department of Aquatic Product Technology IPB University, Kampus IPB Dramaga, Bogor 16680, Indonesia
²Center for Coastal and Marine Resources Studies, IPB University, Kampus IPB Baranangsiang, Bogor 16144, Indonesia
³Surfactant and Bioenergy Research Center (SBRC), IPB University, Kampus IPB Baranangsiang, Bogor 16144, Indonesia

E-mail: kustiaz@apps.ipb.ac.id

Abstract. Seaweeds are main sources of marine polysaccharides. Marine polysaccharides, such as agar, alginates and carrageenans are economically the most important products from macroalgae or seaweeds. Carrageenans, marine polysaccharides extracted from red algae are widely used in food, pharmaceutical, cosmetic, textile and printing industries as coagulate agent, stabilizer and gelling agent. Extraction of carrageenan is usually carried out by alkali treatment, but it may have negative impact to the environment. Carrageenan extraction can be processed biologically using microorganisms, such as marine fungi. The aim of this research was to obtain carrageenan from red alga (Kappaphycus alvarezii) using marine fungi and to evaluate the effect of pretreatment on the quality of carrageenan. The study was conducted in three steps including pretreatment of algae, hydrolysis of algae using marine endophytic fungi, and characterization of the carrageenan. Two marine fungi were used to hydrolyze the seaweeds. The best pretreatment was hydrolysis of dried seaweed using fungus RS6A and gelatinization by heating for 20 minutes (RK20). The yield of carrageenan was 71.28%. The gel strength of the carrageenan was 43.35 gf. The chemical and physical characteristics of carrageenan (RK20) met the standard of carrageenan according to FAO.

Keywords: Amorphophallus; carrageenan; hydrolysis; marine fungi; polysaccharide; seaweed.

1. Introduction

Seafood Carrageenans are hydrocolloids obtained from red algae. These hydrocolloids consist of the ammonium, calcium, magnesium, potassium, and sodium sulfate ester from galactose and polysaccharide 3,6-anhidrogalaktose [1]. Carrageenans have been used widely for functional ability as thickening, gelling, stabilizing, improve cheese texture, controlling the viscosity and texture of puddings, also as a filler and stabilizer in meat processing [2]. Based on the sulfate content, carrageenan is classified into several types such as λ, κ, ι, ε, and μ carrageenan which all contain sulfate 22-35% [3]. The most used type of carrageenan in various industrial fields was kappa carrageenan (κ-carrageenan), which is mostly obtained from seaweed Eucheuma cottonii [4].

Cellulolytic enzymes produced by various types of microorganisms, such as fungi. There have been many explorations of cellulolytic enzymes produced by fungi. Aspergillus sp. and Trichoderma sp. are known to have ability to produce cellulolytic enzymes with high activity [5, 6]. Carrageenan extraction can be done by utilizing fungi as a cellulolytic enzyme producer. The ability of fungi to produce cellulolytic enzymes is very important in the extraction process, but it is also necessary to use...
cellulolytic fungi that safe to use. Varadarajan et al. [7] reported that enzymatic carrageenan extraction using *Aspergillus niger* produced more carrageenan yield than the conventional extraction. Muthezilan et al. [8] has also performed carrageenan extraction using pure enzyme produced from coastal plant endophytic shoots and obtained a higher yield until 52%.

An endophytic fungus from sea grass (strain EN) has been known to produce extracellular cellulase enzyme [9]. In previous research, EN fungus has the ability to produce cellulase with high activity. Irma [10] reported that EN fungus can be used in carrageenan extraction from *Eucheuma cottonii* by utilizing EN fungus culture directly. Based on information about EN capability from previous research, further research is needed to know the ability of cellulase enzyme isolated from EN fungus in the carrageenan extraction process. The aims of this research were to obtain carrageenan from *Kappaphycus alvarezi* using marine fungi, and determine the effect of seaweed form as raw material and pretreatment on the carrageenan characteristics.

2. Materials and Method

2.1. Preparation of Seaweeds and Pretreatment

Fresh marine alga *K. alvarezi* (1 kg) was washed and cleaned of impurities under running water. Seaweeds were cut after cleaning. Seaweed samples were divided into two treatments namely 500 g for fresh *K. alvarezi* (S) and 500 g was sun dried for 3 days for the dried sample (K). After drying the seaweeds were minced using blender and filtered using a sieve with a mesh size of 40/60 to obtain seaweed powder. The obtained biomass was weighed to determine the proportion of dried and fresh materials. Dried and fresh seaweeds were then analyzed for water, cellulose, and lignin contents for the calculation of yield, degree of cellulose hydrolysis and degree of hydrolysis of lignin. The seaweeds were then heated (gelatinized) at 60 °C for 15 and 20 minutes to make algal cells expand and cell walls break so that carrageenan more easily comes out of the material. The alga was then physically delignified by autoclaving at 121 °C at 1 atm for 2 hours to remove lignin.

2.2. Fungal Cultivation and Seaweed Hydrolysis

Eight treatments of seaweed were prepared and then hydrolyzed using marine fungi as microbial producing cellulase enzymes. The cultivation was carried out by inoculation 10% of marine fungi (strain EN and RS6A). The fungi were cultivated on 500 mL Erlenmeyer flask with 200 mL of PDB. The culture was incubated for seven days at room temperature with 120 rpm [11]. The cultures were then acclimatized with new substrate using four media, namely fresh seaweed 20 minutes heating treatment (S20), 15 minutes heating (S15), dried *K. alvarezi* 20 minutes heating (K20), and 15 minutes heating (K15). Each treatment was made in two media for two marine fungi. The media culture was added with 0.5% peptone and 3% NaCl then sterilized by autoclaving. The incubation was carried out for 7 days and shaking at room temperature.

2.3. Carrageenan Precipitation

The precipitation process was done by adding coagulants. The coagulant used was 2-Propanol with a ratio of 1: 1 then centrifuged 7000 rpm at 4 °C for 30 minutes. The carrageenan fraction was then centrifuged and dried using an oven at a temperature of 45 °C for 20 hours and the product, a dried refined carrageenan was then used for further analyses.

2.4. Analysis of Carrageenan Extract

2.4.1. Yield

The yield of carrageenan extraction was calculated based on the ratio between carrageenan weight produced and weight of dried seaweed that was processed respectively.
2.4.2. Sulfate content
The carrageenan was weighed one g and put into the Erlenmeyer flask 50 mL of HCl 0.2 N then refluxed for 6 h until the solvent became clear. This solvent was transferred into a cup glass and heated to boiling. Furthermore, 10 mL of BaCl$_2$ solution was added over the water bath for 2 h. The precipitate formed was filtered with a non-gray filter paper and washed with boiling water to chloride free. The filter paper was dried into the dryer oven at 1000 °C until white ash was obtained. Ash was cooled in a desiccator and then weighed [12].

2.4.3. Viscosity
Viscosity measurement was done by dissolving 1.5% carrageenan in water then heated in a boiling water bath until the solvent temperature reached 75 °C. Viscosity was measured using viscometer. The hot solvent was adjusted to a precise, viscometer was turned on and the temperature of the solvent was measured. When the solvent temperature reached 75 °C and the viscosity value was measured on a scale of 1 to 100. The measurement was done after one minute of a full rotation for spindle number three [12].

2.4.4. Gel strength
The 1.6 percent carrageenan solution and 0.16 percent KCl were heated in a bath of boiling water with regular stirring to 80 °C. The volume of the solution was 50 mL. Hot solution was then put into the 4 cm diameter mold and kept at 10 °C for two hours. The gel in the mold was inserted into measuring instrument (Rheoner RE-3305). The plunger that will be in contact with the gel was in the middle. The plunger was activated and the samples were measured. Evaluation of measurement results was done by reading the resulting graph. The maximum force (gel strength) can be read on the recorder [12].

2.4.5. Acid-insoluble ash content
The carrageenan was boiled with 25 mL of 10% HCl for 5 minutes. The unsolved ingredients were filtered using a non-gray filter paper, then cooled in a desiccator for further weight [12].

2.4.6. Functional group test by FTIR
An amount of 0.1 g potassium bromide (KBr) powder was finely grounded and then added with 0.01 g carrageenan, crushed until homogeneous. The water content was removed by heating in an oven 60 °C for 48 h. Further FTIR spectrophotometric analysis was performed to determine the functional group [13].

3. Result and Discussion

3.1. Yield of carrageenan
Yield is the ratio of the amount of carrageenan produced by the amount of seaweed extracted. The results of carrageenan extraction can be seen in Figure 1.

The yield of carrageenan was about 45.40-71.28 %. Based on the yield results, it is known that the differences in fungal strain, raw material forms, and the length of heating (pretreatment) showed a significant effect with a P value <0.05. The use of RS 6A strain on each material and the heating period increased the hydrolysis yield. The treatment of dried seaweed and the heating period for 20 minutes resulted in a higher yield. This shows that the amount of hydrolysis yield is determined by fungal strain, raw materials and pretreatment.

RS 6A strain exhibited a greater cellulolytic index than that of EN strain. Sulistiawati [13] stated that the higher the cellulase enzyme produced, the more the cellulose component is hydrolyzed will increase the amount of carrageenan yield extracted from seaweed. This study shows that longer heating period of the seaweeds in the pretreatment increased the carrageenan obtained. Yulius et al. [14] stated that the longer seaweed comes in contact with heat and with the extracting solution, the more carrageenan is released from the cell wall and results the carrageenan yield to be higher.
3.2. Sulfate content

Sulfate content is a parameter used for various types of polysaccharides contained in red algae [15]. The value of carrageenan sulfate content in this study can be seen in Figure 2.

The carrageenan sulfate content ranges from 15.20-16.64%. The sulfate content of the carrageenan met the quality standard for carrageenan according to FAO [1] which ranges from 15-40%. Based on the analysis of sulfate content, it is known that differences in fungal strain, raw material form and heating period significantly affected the sulfate content of the carrageenan. The use of RS 6A strain on each form of material and the heating period decreased the sulfate content. The heating time of 20 minutes in the treatment of fungi EN and RS 6 decreased the sulfate content. This shows that the amount of sulfate content was determined by fungal strain as enzyme producer used in the hydrolysis process and heating period in the pretreatment.

This result indicates that fungus RS 6A produced higher enzymes than EN fungi. The enzymes produced was preferably break the α 1.4 D-galactose-6-sulfate bond so that the sulfate groups were
released. Boiling breaks the seaweed cell wall and releases the sulfate group. The relationship between sulfate content with viscosity and gel strength is that the greater the value of sulfate levels the viscosity increases, while the strength of the gel decreases. Doty [15] stated that the carrageenan with sulfate content less than 28% is categorized as kappa carrageenan.

3.3. Viscosity
Viscosity is the power of molecular flow in the solution system. Viscosity test was done to determine the degree of viscosity of carrageenan solution at a certain concentration and temperature. In this study, viscosity measurements were performed using 1.5% carrageenan solution at 75°C and measured by a Brookfield viscometer. The result of viscosity analysis can be seen in Figure 3.

The carrageenan viscosity in this study ranged from 22.22 - 28.30 cP. The highest viscosity value was found in carrageenan RK15 and the lowest was in carrageenan ES20. The carrageenan viscosity values met FAO standard [1] where at a concentration of 1.5% and the temperature of 75 °C the carrageenan viscosity value is at least 5 cP. Based on the results of carrageenan viscosity, it can be concluded that the differences of fungal strain, raw material form and heating period in the pretreatment significantly affected the viscosity of the obtained carrageenan. The use of fungus RS 6A on each form of alga and the heating time increased the hydrolysis yield. The use of dried seaweed in the hydrolysis using both fungi EN and RS 6A increased the yield, as well as heating time 20 minutes. This result shows that the carrageenan viscosity is affected by the fungus strain, raw material form and heating period in the pretreatment.

The viscosity of carrageenan is influenced by the sulfate content. Sulfate content in the carrageenan obtained in this study was low. Campo et al. [16] stated that the high sulfate content can increase the repulsion interaction between negatively charged sulfate groups so that the carrageenan polymer chains are weaker and elastic and can increase the viscosity of the solution. The viscosity of carrageenan solution is also influenced by the particle size, the smaller the particle size in the solution, the flow rate or the viscosity increases.

3.4. Gel strength
The gel strength value of carrageenan produced in this study can be seen in Figure 4.

The carrageenan gel strength ranges from 40.50-43.35 gf. The highest gel strength was carrageenan obtained from dried seaweed heated for 20 minutes (RK20) and hydrolyzed using fungus RS 6A. The lowest gel strength was carrageenan resulted from fresh seaweed treated for 15 and 20 minutes and hydrolyzed both using the two fungi (ES20 and RS15). Based on this result the carrageenan strength was mainly determined by the seaweed form as raw material. Wenno et al. [16] stated that the gel
strength is influenced by the sulfate content and 3.6-anhydro-D-galactose content. The lower the sulfate content, the higher the gel strength but the lower the viscosity.

![Figure 4](image-url)

**Figure 4.** Effect of fungal strain, raw material form and pretreatment on carrageenan gel strength.

The gel strength of carrageenan produced in this study was relatively low when compared to commercial carrageenan which mostly extracted conventionally. Conventionally, carrageenan is extracted using alkaline solvents which can increase the gel strength of the carrageenan. In the chemical treatment, alkaline solutions can convert 3.6 anhydrogalactose to form a double helix resulting in a gelation process [17]. The alkaline solution will form ions which will bind to the sulfate group, so the sulfate group transforms to form salts in solution. The salt will secrete a water molecule (dehydration) to form an anhydrous galactose polymer, in which the OH-alkaline solution reacts with carrageenan-shaped H-group bonds and water [18].

### 4. Conclusion

Form of raw material, fungal strain used in hydrolysis process and heating period in the pretreatment of seaweed determined the yield, sulfate content, viscosity and gel strength of the carrageenan. The highest yield of carrageenan was 71.28% with strongest gel strength 43.35 gf was obtained from dried seaweed heated for 20 minutes and hydrolyzed using marine fungus EN.

### Acknowledgements

This Acknowledgments are given to Ministry of Research, Technology and Higher Education of the Republic of Indonesia who has funded this research through Competency-Based Research Scheme to Dr. Kustiaryyah Tarman with contract number 1608/IT3.11/PN/2018 and 4135/IT3.L1/PN/2019.

### References

[1] [FAO] Food and Agricultural Organization 2007 *Compendium of food additive specifications* (Rome [IT]: FAO Fisheries and Aquaculture Technical Paper)

[2] Campo V L, Kawano D F, Silva Júnior D B and Carvalho I 2009 Carrageenan: biological properties, chemical modifications and structural analysis *Carbohydr Polym.* 77 167-180

[3] Necas J and Bartosikova L 2013 Carrageenan: a review *Vtr Medic.* 58(4) 187-205

[4] Webber V, Carvalho S M, Ogliari P J, Hayashi L and Barreto M P L 2012 Optimization of the extraction of carrageenan from *Kappaphycus alvarezii* using response surface methodology *Ciênc Tech Alim.* 32(4) 812-818

[5] Sivaramanan S 2014 Isolation of cellulolytic fungi and their degradation on cellulosic agricultural wastes *JAIR* 2(8) 458-463
[6] Reddy P L N, Babu B S, Radhaian A and Sreeramulu A 2014 Screening, identification and isolation ofcellulolytic fungi from soils of chittoor district India Int J Cur Microbiol Appl Scienc. 3(7) 762-771

[7] Varadarajan S, Ramli N, Ariff A, Saaid M and Yasir S M 2009 Development of high yielding carragenan extraction method from Eucheuma Cottonii using cellulase and Aspergillus niger Prosiding Seminar Kimia Bersama UKM-ITB 462-469

[8] Muthezilam, Jayaprakash K, Karthik R and Hussain A J 2014 Endophytic fungal cellulose for extraction of carrageenan and its use in antibiotics amended film preparation Bios Biotech Res As. 11 307-312

[9] Oktavia Y, Andhikawati A, Nurhayati T and Tarman K 2014 Karakterisasi enzim kasar selulase kapang endofit dari lamun JITKT 6(1) 209-218

[10] Irma A D 2018 Hidrolisis rumput laut menggunakan kapang endofit laut dengan media yang berbeda [skripsi] (Bogor [ID]: Institut Pertanian Bogor)

[11] Tarman K, Lindequist U, Wende K, Porzel A, Arnold N and Wessjohann L A 2011 Isolation of a new natural product and cytotoxic and antimicrobial activities of extracts from fungi of Indonesian marine habitats Marine Drugs 9(3) 294-306

[12] FMC Corp. 1977 Carrageenan Marine Colloid Monograph Number One (New Jersey [US]: Marine Colloids Division FMC Corporation) p 23-29

[13] Smith B C 2011 Fundamentals of Fourier Infrared Spectroscopy (Boca Raton [US]: C Press)

[14] Yulius F, Kusumaningrum I and Hasanah R 2016 Pengaruh lama perebusan terhadap mutu karaginan dari rumput laut (Kappaphycus alvarezzii) Jurnal Ilmu Perikanan Tropis 21(2) 41-47

[15] Doty M S, Santos G A 1987 The Production and Uses of Eucheuma: Studies of Seven Commercial Seaweeds Resources (Rome [IT]: FAO Fish. Tech Paper)

[16] Wenno M R, Thenu J L and Lopulalan C G C 2012 Karakteristik kappa karaginan dari Kappaphycus alvarezzii pada berbagai umur panen JPB 7(1) 61-67

[17] Pelegrin Y F, Robledo D and Azamar J A 2006 Carragheenan of Eucheuma isiforme (Solieraceae, Rhodophyta) from Yucatanm Mexico, effect of extraction conditions Botanica Marina 49(1) 65-71

[18] Uy F S, Easteal A J and Fard M M 2005 Seaweed processing using industrial single-mode cavity microwave heating: A preliminary investigation Carbohydrate Research 3401357-1364