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# CHARACTERISTICS OF CARAGENAN FROM RED SEAWEED (RHODOPHYTA) *Eucheuma spinosum* AND *Eucheuma cottonii* ORIGINATING FROM MORO ISLAND

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## ABSTRACT

Red seaweeds *Eucheuma spinosum* and *E. cottonii* are raw materials for producing carrageenan. Carrageenan is a group of polysaccharides widely used as thickening, stabilizing, and gelling agents across various industries. This study aims to determine the physicochemical characteristics of carrageenan from *E. spinosum* and *E.cottonii* seaweed. This research uses an experimental method: direct extraction with hot alkali, with three repetitions. The physicochemical parameters analyzed include yield, gel strength, viscosity, water content, ash content, and sulfate content. The results of the study of the physicochemical characteristics of carrageenan from *E. spinosum* raw materials produced a yield of 34.41%, gel strength 22.17 g/cm$^2$, viscosity 1450 cP, water content 11.57%, ash content 24.41%, and sulfate content 24.89%. The results of the study of the physico-chemical characteristics of *E. cottonii* produced a yield of 29.54%, gel strength of 488.58 g/cm$^2$, viscosity of 30.05 cP, water content of 9.95%, ash of 36.42%, and sulfate content of 21.42%. The FTIR profiles of *E. spinosum* carrageenan compounds show iota carrageenan, and those of *E. cottonii* carrageenan compounds show kappa carrageenan. The physicochemical characteristics of *E. spinosum* and *E.cottonii* carrageenan raw materials have met FAO standards, except for gel strength and ash content in *E. cottonii* carrageenan.

**Keywords:** Sulfate Ester, Viscosity, Kappa Carrageenan, Iota Carrageenan, Functional Groups

## 1. INTRODUCTION

Seaweed is a leading commodity in Indonesia's fisheries sector, possessing high economic value and export potential. Indonesia is known as a major global producer of seaweed, with the most widely cultivated species belonging to the Rhodophyta (red seaweed) family, such as *Eucheuma cottonii* and *E. spinosum*[1]. These two species are the primary sources of carrageenan, a compound widely used across industries such as food, pharmaceuticals, cosmetics, and biotechnology[2].

Carrageenan is a sulfated polysaccharide extracted from the cell walls of red seaweed, consisting of galactose and anhydrogalactose units, with variations in sulfate groups determining its type and properties[3]. In general, *E. cottonii* produces κ-type carrageenan (kappa-carrageenan) with the ability to form strong gels, while *E. spinosum* produces ι-type carrageenan (iota-carrageenan), which has high elasticity and good stability against calcium ions4. Environmental factors such as temperature, salinity, light intensity, and water nutrient content greatly influence the quality and characteristics of the carrageenan produced[5].

Moro Island, in the Riau Islands, has unique oceanographic conditions and the potential to become a site for seaweed

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cultivation. However, scientific data regarding the characteristics of carrageenan produced from *E. cottonii* and *E. spinosum* grown in this region is still very limited.

Therefore, an in-depth study is needed on the characteristics of carrageenan from these two red seaweed species originating from Moro Island, including physical-chemical properties such as yield, viscosity, sulfate content, and functional group structure as determined by FTIR analysis. This information will provide an overview of the potential and quality of local carrageenan as an industrial raw material and support efforts to develop a sustainable coastal resource-based economy. Based on the above description, the author is interested in researching the physical and chemical properties of carrageenan from *E. spinosum* and *E. cottonii*.

## 2. RESEARCH METHOD

### Time and Place

This research was conducted from September to December 2024 at the Fisheries Product Chemistry Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau, and the Food Technology and Agricultural Product Testing Laboratory, Faculty of Agricultural Technology, Gadjah Mada University.

### Procedures

### Media Preparation

The samples used in this study were tropical red seaweeds *E. spinosum* and *E. cottonii* obtained from the waters of Jang Island, Moro District, Karimun Regency, Riau Islands Province. Sample preparation began by washing *E. spinosum* and *E. cottonii* with running water to remove dirt adhering to the seaweed, then draining and drying them at room temperature.

### Carrageenan Extraction of *E. spinosum*

Dried *E. spinosum* was extracted in alkaline conditions using $Ca(OH)_2$ under hot conditions for 3 hours at 90-95°C. The first stage of dried *E. spinosum* was soaked in 1:50 distilled water for 16 hours at room temperature. Then, it was heated for 1 hour at 60°C, and $Ca(OH)_2$ was added with a ratio of 0.2 g/g seaweed, and the temperature was raised to 95°C. The extraction time of 3 hours was calculated after the solution temperature reached 95°C. The extraction temperature was lowered from 95°C to 80°C, and the mixture was stirred for 30 minutes; the filtrate was obtained. The filtrate was left at room temperature for 16 hours, after which its pH was lowered to 9 with 5% HCl. The filtrate was precipitated with 96% ethanol at a 1:3 volume ratio for the first precipitation, until fibers formed, and then filtered.

The second precipitation with a 1:2 ratio, then the fibers were filtered again. The third precipitation with a ratio of Dried *E. spinosum* was extracted in alkaline conditions using $Ca(OH)_2$ under hot conditions for 3 hours at 90-95°C. The first stage of dried *E. spinosum* was soaked in 1:50 distilled water for 16 hours at room temperature. Then, it was heated for 1 hour at 60°C, and $Ca(OH)_2$ was added with a ratio of 0.2 g/g seaweed, and the temperature was raised to 95°C. The extraction time of 3 hours was calculated after the solution temperature reached 95°C.

The extraction temperature was lowered from 95°C to 80°C, and the mixture was stirred for 30 minutes; the filtrate was obtained. The filtrate was left at room temperature for 16 hours, after which its pH was lowered to 9 with 5% HCl. The filtrate was precipitated with 96% ethanol at a 1:3 volume ratio for the first precipitation, until fibers formed, and then filtered. The second precipitation with a ratio of 1:2, then the fibers were filtered again the third precipitation with a ratio of 1:1, then filtered. The obtained fibers are then dried at room temperature and then milled to obtain *E. spinosum* carrageenan powder[6].

### *Eucheuma cottonii* Carrageenan Extraction

*Eucheuma cottonii* is extracted using the hot alkali extraction method. First, weigh 100 g of dried *E. cottonii* and extract it with

a hot 12% KOH solution at a 1:20 ratio for 2 hours at 80°C. The seaweed is then washed to remove the KOH and adjust the pH to 8-9. The seaweed is then heated in distilled water at a 1:20 ratio for 30 minutes at 80°C, or until it disintegrates. After the extraction process is complete, the seaweed is filtered through a filter cloth. The resulting filtrate is collected and then added to a 2% KCl solution (1:2) at 30°C. Finally, the carrageenan fibers are dried at 60°C, ground to a fine powder that passes through an 80-mesh sieve. The carrageenan powder was subjected to a physical-chemical analysis[7].

**Yield Analysis**

The yield of carrageenan as an extract was calculated based on the ratio of the weight of the resulting carrageenan to the weight of the dried seaweed before extraction[8].

$$\text{Yield (\%)} = \frac{\text{weight of dried carrageenan}}{\text{weight of dried seaweed}}\ x\ 100\%$$

**Gel Strength**

Gel strength was measured using an Instron Universal Testing Instrument (Zwick Z.05 texture analyzer). A 3 g sample of carrageenan was weighed, dissolved in distilled water, heated to 80°C, and then weighed to a carrageenan concentration of 1.5%. The hot carrageenan solution was poured into a container until it was full and immediately sealed. After cooling, the gel was stored at 10°C in a refrigerator for 16 hours, and then the gel strength was measured[9].

**Viscosity (Rozi, 2018)**

A 2.7g sample was taken and dissolved in 170ml of hot water. After dissolving, the weight was adjusted to 180g, resulting in a concentration of 1.5% (w/w). The solution was heated in a water bath with stirring until it reached 80°C and transferred to a 100ml beaker. Viscosity was analyzed using a Brookfield DV-2T viscometer. The sample viscosity was measured using a 64-bit spindle at 60 rpm. The measurement was performed for 2 minutes until the needle reading stabilized. The rotor rotated, and the needle moved until the sample viscosity was obtained. The viscosity reading was taken when the needle stabilized. The sample viscosity is reported in cP (centipoise)[10].

**Moisture Content**

Water content was measured using the oven method. The cup is dried in an oven at 100-105°C for 30 minutes, or until a constant weight is obtained. After that, it is cooled in a desiccator for 30 minutes and then weighed. The sample is weighed as much as five salts (B1) in the cup and then dried in an oven at 100-105 °C until a constant weight is achieved (8-12 hours). The sample is cooled in a desiccator for 30 minutes and then weighed (B2). The calculation of water content is done as follows[11]:

$$\text{Moisture content} = \frac{B-C}{B-A} \times 100$$

Description:
A = weight of empty cup (g)
B = weight of cup with sample (g)
C = weight of cup with dry sample (g)

**Ash Content**

The crucible was dried for 30 minutes in an oven at 105ºC, then cooled in a desiccator before being weighed. A 3 g sample was weighed into a crucible of known constant weight. The crucible containing the sample was ashed in an electric furnace at 550ºC for 6 hours, until the ash turned white. The crucible was placed in a desiccator for 30 minutes and then weighed. The ash content was calculated using the formula[11]:

$$\text{Ash Content} = \frac{C-A}{B-A} \times 100\%$$

Description:
A = Weight of empty crucible (g)
B = Weight of crucible with sample (g)
C = weight of crucible with dried sample (g)

**Sulfate Content**

Sulfate content was determined by hydrolyzing carrageenan and precipitating the sulfate as $BASO_4$. A 1 g sample was placed in an Erlenmeyer flask, then 50 mL of 0.2 N HCl was added. The mixture was

heated to boiling for 1 hour. After 1 hour, 25 mL of $H_2SO_4$ was added, and the mixture was heated for 5 hours until the solution became clear. This solution was transferred to a beaker and heated to boiling. Next, 10 mL of 10% $BaCl_2$ was added, and the mixture was heated on a hot plate for 2 hours. The precipitate formed was filtered through ash-free filter paper (Whatman No. 42) and washed with distilled water until chloride-free. The filter paper was then dried and ashed at 700°C until a white ash was obtained. The ash was cooled in a desiccator and weighed until it reached a constant weight. The sulfate content was calculated using the following equation.

$$\text{Sulfate Content (\%)} = \frac{\text{p x 0,4116)}}{\text{Weight of sampel}} \times 100\%$$

**FTIR Functional Group Analysis**

The functional groups of carrageenan were analyzed using a Bruker Tensor 37 Fourier-transform infrared (FTIR) spectrometer. A 0.02 g sample was mixed with KBr and pressed to form a thin film. Spectra were captured over the 4000-500 cm-1 range with four scans at a resolution of 4 cm-1

## 3. RESULT AND DISCUSSION

**Morphology of Seaweed *E. cottonii* and *E. spinosum***

The raw materials for this research were red seaweeds (Rhodophyta), namely *E. cottonii* and *E. spinosum*, obtained from the waters of Jang Island, Moro District, Karimun Regency, Riau Province. The morphological characteristics of the two types of seaweed are shown in Figure 1.

Fresh *E.cottonii*

Dried *E.cottonii*

Fresh *E. spinosum*

Dried *E.spinosum*

**Figure 1**. Morphology of seaweed

Based on Figure 1, the observations of *E.cottonii* and *E.spinosum* harvested at 40 days showed relatively fresh, brownish-yellow morphology. This seaweed has a cylindrical thallus, is hard, and has dense branches. Harvest age is an important factor that affects the quality and quantity of carrageenan produced. Harvesting too early can lead to low yields because the biomass is not yet optimal, while harvesting too late can degrade active compounds and reduce gel quality. Several studies reported that harvest age affects carrageenan yield in *E. cottonii* seaweed.

**Seaweed Carrageenan Extraction *E.cottonii* and *E. spinosum***

Extraction was carried out using heat treatment to break down the seaweed cell walls. The resulting filtrate was precipitated using KCl to separate the carrageenan fibers from the water. The dried fibers were then ground to obtain a powdery carrageenan with a particle size of 80 mesh. The results

of the carrageenan extraction are shown in Figure 2.

**Figure 2.** *E. cottonii* carrageenan

The resulting carrageenan is white and smooth. The white color indicates an optimal extraction process that removes chlorophyll and other natural pigments. The smooth texture allows the carrageenan to dissolve easily in hot water, forming a homogeneous solution, which is essential for producing edible films with a smooth surface and uniform thickness[12].

The extraction process for *E. spinosum* seaweed involves maceration for 16 hours to facilitate carrageenan release, followed by heating and an alkali solution for 4 hours and 30 minutes. The resulting filtrate is extracted. To obtain the carrageenan fibers, precipitation is performed using an ethanol solution to draw out the fibers and remove water. The resulting fibers are dried and ground to produce carrageenan powder with a particle size of 80 mesh. The results of the carrageenan extraction from *E. spinosum* seaweed are shown in Figure 3.

**Figure 3.** *E. spinosum* carrageenan

Based on Figure 3, the carrageenan powder extracted from *E. spinosum* appears yellowish-cream in color with a smooth texture. Other factors that affect the color of carrageenan include high extraction temperatures and strong alkalis, which can cause mild oxidation reactions among organic compounds in the material, resulting in a slightly yellowish color. The smooth texture of the powder indicates good grinding and is easily.

**Characteristics of the Physical and Chemical Properties of *E. cottonii* and *E. spinosum* Carrageenan**

The physical-chemical characteristics of the carrageenan analyzed consisted of yield, gel strength, viscosity, water content, ash content, and sulfate content. The physical and chemical characteristics of *E. cottonii* and *E. spinosum* carrageenan are presented in Table 1.

The carrageenan yield of *E. spinosum* is 34.41% higher than that of *E. cottonii*, which only reaches 29.54%, and both are still above the FAO standard of >25%. The research by Diharmi[13] showed a range of *E. spinosum* yields of 25.81–37.16%. In addition, Momo et al.[14] also reported that the carrageenan yield from *E. cottonii* originating from Tablolong waters reached 43.75%. The high yield indicates good extraction process efficiency and the availability of adequate raw materials for making edible films.

Differences in carrageenan yields in seaweed may be due to the use of high concentrations of alkali, which increase extraction capacity and result in faster formation of carrageenan or 3,6-anhydrogalactose. Furthermore, carrageenan yield is influenced by the age of seaweed harvest, as the polysaccharide content varies across growth stages[15]. Gerung et al.[16] stated that high extraction duration and temperature can affect the interaction between the alkali solvent and the carrageenan polymer chain, potentially leading to the formation of new bonds or the breaking of the polymer chain.

Based on test results, the gel strength of *E. cottonii* carrageenan (488.58 g/cm²) is significantly higher than that of *E.spinosum* carrageenan (22.17 g/cm²). However, both

values still fall short of the FAO standard of >500 g/cm². Research by Setyorini et al.[17] reported that the gel strength of *E. cottonii* carrageenan reached 575 g/cm².

**Table 1**. Characteristics of the physical and chemical properties of *E. cottonii* and *E. spinosum* Carrageenan

| Parameter | Average value of *E.cottonii* Carrageenan | Average value of *E. spinosum* carrageenan | Carrageenan Standard (FAO)* |
|---|---|---|---|
| Yield | 29.54 % | 34.41% | > 25% |
| gel strength | 488.58 g/cm$^2$ | 22.17 g/cm$^2$ | > 500 g/cm$^2$ |
| Viscosity | 30.05 cP | 1450 cP | ≥ 5 cP |
| Moisture | 9.95% | 11.57% | Max. 12% |
| Ash | 36.42% | 24.41% | 15 – 30% |
| Sulfate | 21.42 % | 24.89 % | 14-40% |

The viscosity of *E. spinosum* carrageenan (1450 cP) is much higher than that of *E.cottonii* carrageenan (30.05 cP). Both types of carrageenan are above the FAO standard (≥ 5 cP). Several studies also report varying carrageenan viscosity values. Setyorini et al.[17] reported a viscosity of *E.cottonii* of 227 cP. Meanwhile, Diharmi[13] reported carrageenan viscosities of 650 cP, 1080 cP, and 1200 cP from Nusa Penida, Sumenep, and Takalar, respectively. According to Oliveira et al.[18], high viscosity is thought to be due to the hydrophilic nature of the material. The polymer is surrounded by carrageenan. Viscosity is caused by carrageenan concentration, temperature, sulfate content, and carrageenan molecular weight.

The water content of *E.spinosum* carrageenan (11.57%) is close to the FAO maximum limit (12%), while *E.cottonii* carrageenan has a lower water content (9.95%). Several studies show similar results; for example, Diharmi[13] reported that the water content of *Eucheuma spinosum* reached 11.02%. Arfini[19] reported that the water content of *E.cottonii* ranged from 6.76 to 9.73%. In addition, Bunga et al.[20] reported that the water content ranged from 13.76 to 19.46%. The high-water content is thought to be caused by differences in drying methods, resulting in variations in the final results[21]. In addition, according to Wenno et al.[22], seaweed's harvesting time increases due to its hydrophilic properties. High water content can shorten the film's shelf life by increasing its susceptibility to microbial contamination, but edible films can offer greater flexibility[23].

The ash content of *E. spinosum* carrageenan (24.41%) is within the FAO standard range (15–30%), while that of *E.cottonii* (36.42%) exceeds this standard limit. Diharmi[13] reported that the ash content of *E. spinosum* carrageenan from three locations: Nusa Penida, Sumenep, and Takalar, was 29.03, 29.57, and 28.26%, respectively. Meanwhile, Asikin et al.[15] reported that the ash content of *E. cottonii* carrageenan ranged from 23.33 to 23.39%. The main causes of high ash content in carrageenan flour are minerals and salts inherent in the algal polymer, such as K, Mg, Ca, Na, and ammonium galactose, as well as its sulfate content[24].

The sulfate content of *E.spinosum* carrageenan was 24.89% higher than that of *E.cottonii* (21.42%), and both comply with FAO standards (14–40%). Diharmi[13] reported that *E. spinosum* seaweed in three locations: the waters of Nusa Penida, Sumenep, and Takalar, ranged from 30.74% to 32.27%. Wenno et al.[22] reported a sulfate content of 14.75% for *E.cottonii* carrageenan from Lemukutan, West Kalimantan. The higher sulfate content in *E. spinosum* makes the film more elastic but less moisture-resistant.

In contrast, *E. cottonii* carrageenan with lower sulfate content produces a stronger film and is more resistant to water vapor[25]. The high sulfate content obtained

may be influenced by the growing environment of the seaweed, as reported by Diharmi[13], who found that the sulfate content of *E.spinosum* is influenced by environmental differences in each seaweed.

**FTIR Analysis of *E. cottonii* anda *E. spinosum* Carrageenan**

Infrared spectroscopy is a technique used to identify functional groups present in a compound. The results of the FTIR analysis of *E. cottonii* carrageenan are presented in Figure 4.

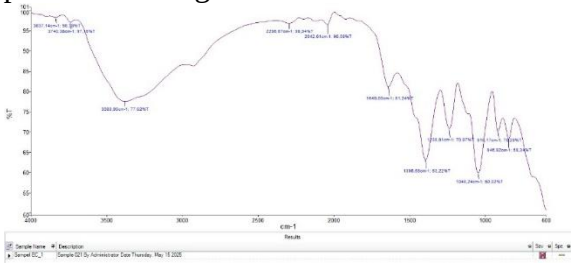

**Figure 4**. *E. cottonii* carrageenan spectra

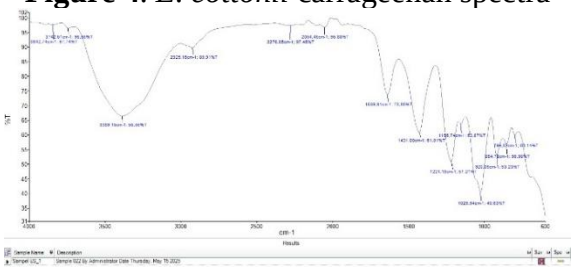

**Figure 5.** *E. spinosum* carrageenan spectra

FTIR of *E. cottonii* carrageenan. The figure shows broad absorptions at 3380.95 cm-1 and 1640.00 cm-1, and narrow absorptions at 1395.68 cm-1, 1238.81 cm-1, and 918.17 cm-1. Sharp absorption at wave number 1048.24 cm-1 (Table 2).

Fourier transform infrared spectroscopy (FTIR) analysis was performed to identify the main functional groups in the carrageenan structure (Figure 5).

The spectroscopic spectrum in Figure 15 shows a broad absorption band at 3389.16 cm-1 and a sharp absorption band at 1221.18 cm-1. Furthermore, at wavelengths of 920.05 cm-1, 854.78 cm-1, and 799.5 cm-1, the absorption bands are sharp and narrow (Table 3).

Table 3 shows the results of infrared spectroscopy analysis, which show the presence of sulfate ester functional groups, glycosidic bonds, C-O-C-O (3,6-anhydrogalactose), C-O-$SO_3$ at C4 of 3,6-anhydrogalactose-4-sulfate, and C-O-$SO_3$ at $C_2$ of 3,6-anhydrogalactose-2-sulfate. The sulfate ester peak in *E. spinosum* carrageenan at 1221.18 cm-1 differs from the peak in iota sigma at 1377.17 cm-1.

**Table 2.** Wave numbers and functional groups of *E. cottoni* carrageenan

| Wave Number ($cm^{-1}$) | Kappa ($cm^{-1}$) | Functional Group |
|---|---|---|
| 3380.95 | 3000-3600 | O-H |
| 1640.00 | 1640-1645 | C-H |
| 1238.81 | 1230-1270 | S=O (ester sulfate) |
| 1048.24 | 1040-1080 | C-O-C (glycosidic bond) |
| 918.17 | 928-933 | 3,6 anhydro-D-galactosa |
| 846.92 | 840-850 | 3,6 anhydro-D-galactose-4-sulfate |

**Table 3**. Wave Numbers and Functional Groups of *Eucheuma spinosum* Carrageenan

| Wave number | | Functional Group |
|---|---|---|
| *E.cottonii* carrageenan | Iota ($cm^{-1}$) | |
| 1221.18 | 1377.17 | Sulfate ester |
| - | 1083.99 | C-O (3,6 anhydrogalactose) |
| 1025.54 | 1026.13 | Glycosidic bond |
| - | 968.41 | Galactose |
| 920.05 | 933.55 | C-O-C-O (3,6- anhydrogalactose) |
| 854.78 | 852.54 | C-O-$SO_3$ on C4 3,6 anhydrogalactose-4-sulfate |
| 799.5 | 806.25 | C-O-$SO_3$ on $C_2$ 3,6- anhydrogalactose-2-sulfate |

According to Yermak et al.[26], the sulfate ester peak is at 1220–1250 $cm^{-1}$. Correa & Diaz et al.[27] reported that the sulfate ester group was detected at wave numbers 1250 and 1370 $cm^{-1}$, while The FTIR spectrum of the *E.spinosum* sample showed agreement with iota-sigma[13], as the peaks produced by the *E.spinosum* carrageenan sample were close to those of pure iota, particularly at 1025.54 cm-1, 799.5 cm-1, and 854.78 cm-1. According to Diharmi[13], iota carrageenan is characterized by the presence of two sulfates. Other undetected peaks indicate a less pure sample.

## 4. CONCLUSION

The average value of *E. cottonii* and *E. spinosum* carrageenan characteristics meets FAO standards except for the viscosity characteristics of both carrageenans and ash content in *E. cottonii* carrageenan. The wave numbers and functional groups detected in the *E.cottonii* carrageenan spectrum indicate kappa carrageenan, whereas *E.spinosum* shows iota carrageenan.

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