Characteristic of carrageenan *Eucheuma cottonii* collected from the coast of Tanjung Medang Village and Jaga Island, Riau

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**Abstract.** Carrageenan is a group of polysaccharides extracted from red seaweed. Carrageenan extraction can be done by several methods, one of which is alkaline solution (KOH) because KOH produces a stronger gel than other alkalis. Carrageenan was extracted using hot alkali followed by precipitation, drying, and milling. The purpose of this study was to determine the chemical composition of dried *E. cottonii* from North Tanjung Medang Village and Jaga Island coastal sea regions and physico-chemical characteristics of carrageenan extracted with KOH solutions 4, 8 and 12%. The results of analysis showed that the chemical composition of dried *E. cottonii* of both coastal sea regions not significant. The chemical characteristics of carrageenan from Tanjung Medang Village and Jaga Island using KOH 4, 8, and 12%, were moisture (11-18%), ash (27-36%), acid-insoluble ash (1.66-2.0%), and sulphate content (24-29%), those not significant all carrageenan except ash content. Whereas the physical characteristics of both carrageenan were significantly different. The results of this study indicated that the carrageenan extracted with KOH (4.8, and 12%) has similar physico-chemical characteristics except for gel strength, viscosity, and ash content.

**Keywords:** carrageenan, *E. cottonii*, extraction, gel strength, KOH

1. **Introduction**

Seaweed is an important marine product in Indonesia. One of widely cultivated seaweed is *E. cottonii* or *Kappaphycus alvarezi* (Wijaya 2006). This seaweed is a member of red seaweed (Rhodophyceae) which produces kappa carrageenan. Carrageenan is a group of polysaccharides extracted from seaweed (Diharmi *et al* 2011). Carrageenan can be used in food and non-food products. The function of carrageenan in the food industry is as a stabilizer and improves the texture of products such as cheese, pudding, and sauces (Campo *et al* 2009).

Extraction is a method of separating a solid (liquid) component from a mixture using a number of solvents to separate a component (Aprilia 2006). Carrageenan processing is carried out by several extraction methods, one of them is extraction using alkali. Alkali can be obtained by adding the base solution e.g NaOH or KOH solution.
The alkali helps the extraction of polysaccharides become more perfect and accelerate the elimination of 6-sulphate from the monomer unit to 3.6-anhydro-D-galactose so as to increase gel strength and product reactivity to proteins (Ega et al. 2016). The rate of extraction depends on temperature, alkaline concentration, ionic strength, and the specific alkaline media (Viana et al. 2004). KOH was chosen because it has a cationic effect on kappa carrageenan and produces a stronger gel than other types, such as NaOH and Ca(OH)$_2$.

*E. cottonii* collected from the coastal areas of Riau (Utara Tanjung Medang Village and Jaga island) is still rarely performed, so that the necessary research on the characteristics of carrageenan. This study was aimed to determine the physico-chemical properties (yield, viscosity, gel strength, moisture content, ash content, carbohydrate content, protein content, fat content, sulphate content, acid insoluble ash content) of *E. cottonii* extracted with solution KOH (4, 8 and 12%).

### 2. Materials and methods

#### 2.1. Materials

The *E. cottonii* was collected from Tanjung Medang, Kawasan Tanjung Medang, Bengkalis, Provinsi Riau, Pula Jaga, Kawasan Moro, Provinsi Pulau Jaga. Seaweed between 40-45 days old was harvested by farmers. The harvested seaweed was first cleaned with freshwater to remove dirt and excess of salt and then dried at sunlight until moisture content of the seaweed was around 20%, while other parts were used for carrageenan extraction and analysis.

#### 2.2. Methods

The research activities consisted of several stages as follows: preparation of seaweed raw materials by sorting, washing and drying, analysis of the chemical composition of dried seaweed, extraction, and characterization of carrageenan.

##### 2.2.1. Extraction of Carrageenan

Carrageenan was extracted from the seaweed using hot alkaline extraction method according to Nasrudin et al. (2006) with some modification. One hundred gram of dried seaweed was extracted using hot KOH solution (4, 8, and 12%) for 2 hours at 80°C. Then the seaweed was washed to remove KOH and the seaweed pH becomes 8-9. The seaweed was further soaked in water with a ratio 1:20 for 30 minutes at 80°C or until the seaweed was destroyed. After the extraction process was complete, the seaweed was filtered with calico cloth. The resulting filtrate was collected, then adding with 2% KCl (1:2) solution at 30°C. Finally, carrageenan fiber was dried at 60°C milling and into fine powder to pass 60 mesh.

##### 2.2.2. Impurities analysis (SNI 8168: 2015)

Fifty grams of dried seaweed was weighed in a glass cup and recorded weight (Wo). Then the dirt (other types of seaweed, plastic, shells, coral, and other foreign objects) was separated from seaweed. The dirt then were weighed and recorded as the weight (Wd). The impurities was calculated following equation 1.

\[
\text{Crude Impurities} = \frac{\text{Wd}}{\text{Wo}} \times 100\% 
\]

Wd= the weight of the dirt collected

Wo= the weight of dried seaweed and the container

##### 2.2.3. Clean anhydrous weed (CAW) (SNI 8168: 2015)

Fifty grams of dried seaweed were soaked for 30 minutes and stirring every 5 minutes. Furthermore, the water was removed and repeated adding water and stirring again. Seaweed was filtered and drained for 2 minutes. Meanwhile prepared aluminum foil for seaweed containers, dried in an oven at 60°C for 60 minutes, then weighed (Wa). After draining the
seaweed was placed on aluminum and dried in an oven at 60°C for 20-22 hours to a constant weight. After dry weighed seaweed and recorded the weight (Wd). The CAW content was calculated used equation 2.

\[
\text{CAW} \% = \left( \frac{\text{Wa} - \text{Wd}}{\text{Wo}} \right) \times 100\% \quad (2)
\]

Wo: weight of seaweed (g)
Wa: dry aluminum foil container weight (g)
WD: Weight of dried seaweed and container (g)

2.2.4. Moisture content (AOAC 2006). Three grams of sample were weighed, then dried in an oven at 105°C for 14 hours. The drying was done until constant weight was achieved. Moisture content was measured according to equation 3.

\[
\text{Moisture content} = \frac{\text{weight of seaweed lost}}{\text{weight of initial seaweed}} \times 100\% \quad (3)
\]

A = Weight of empty propel ash cup (g)
B = Weight of the propel ash tray with sample (g)
C = Weight of the propel ash tray with sample after drying (g)

2.2.5. Ash content (AOAC 1995). Five grams of carrageenan were burned in a furnace with a temperature of around 105°C, followed at 600°C for 2-3 hours. Ashing process was carried out until the ash into white ash after the cup was cooled in a desiccator for 30 minutes, then weighed. Ash content was calculated equation 4.

\[
\text{Ash content} = \frac{\text{C} - \text{A}}{\text{B} - \text{A}} \times 100\% \quad (4)
\]

A = Weight of empty propel cup (g)
B = Weight of the propel ash tray with sample (g)
C = Weight of the propel ash tray with sample after drying (g)

2.2.6. Sulphate. One gram of carrageenan was weighed in an erlenmeyer and 50 mL 0.2 N HCl was added and heated to boiling for 1 h. After 1 h in boiling, 25 mL H2O2 was added and heated for 5 h. This solution was transferred into a beaker and further heated to boiling. Furthermore, 10 mL 10% BaCl2 was added and for 2 h. Precipitate formed upon boiling was filtered through an ashless filter (No. 42, Whatman, Munich, Germany) filter and washed with hot distilled water to remove the residual chloride. Filter paper containing precipitate was then dried and burned at a temperature of 700°C in a furnace. the cup containing ash was cooled in a desiccator and weighed until a constant weight was obtained. Ash was put in a desiccator and weighed to a constant weight. Sulphate content was calculated based on equation 5 (JECFA 2007).

\[
\text{Sulphate content} (%) = \left( \frac{\text{P} \times 0.4116}{\text{weight of sample}} \right) \times 100\% \quad (5)
\]

where; 0.4116 is a relative atomic mass of SO4 divided by the relative atomic mass of BaSO4, and P is the weight of BaSO4 precipitate (g).

2.2.7. Viscosity (FMC 1979). About 2.7 g of carrageenan sample was dissolved in 170 mL and heated. After dissolving the weight was set to 180 g, so the concentration becomes 1.5% (w/w). The solution was heated in a water bath while stirring until it reached a temperature of 80°C. Viscosity was measured by Viscometer Brookfield.
2.2.8. Gel strength (FMC 1977). Three grams of sample was dissolved in 197 g of water and heated. The solution was heated with regular stirring until the temperature reached 80°C. Hot solution was poured into a plastic container (diameter 6 cm, height 6.5 cm) and stored in a refrigerator at 10°C for 16 h before analysis. The gel strength of carrageenan was determined using a Texture Analyzer (TA-XT plus, Stable Microsystems, Goldaming, UK).

2.2.9. Yield (AOAC 1995). Yield of carrageenan as a result of extraction was calculated based on the percentage between carrageenan weight produced and the weight of dried seaweed.

\[
\text{Yield} = \frac{\text{weight carrageenan}}{\text{weight seaweed}} \times 100\% \tag{6}
\]

2.2.10. Statistical Analysis. Data were analyzed using analysis of variance (ANOVA) in SPSS version 16 (SPSS, Chicago, IL).

3. Results and discussion

3.1. Chemical characteristics of E. cottonii

E. cottonii seaweed has cylindrical thallus, slippery, surface and there are sharp spines on the thallus (figure 1). Seaweed from Tanjung Medang Village dan Jaga island had a CAW value of 15.2-26.1% and impurities of 1.04-3.79%.

![Figure 1. Seaweed E. cottonii (a). North Tanjung Medang Village (b). Jaga island.](image)

The chemical compositions of seaweed were summarized in table 1. The moisture content of dried E. cottonii from Tanjung Medang Village and Jaga Island was lower than SNI 2354.2: 2015 where the maximum moisture content of seaweed E. cottonii 30%.

| Table 1. Proximate composition of Eucheuma cottonii. |
|---------------------------------|----------------|----------------|
| Composition (%)                 | Tanjung Medang | Jaga Island    |
| Ash                             | 14.22±0.07     | 17.7±0.14      |
| Fat                             | 0.47±.00       | 0.38±0.00      |
| Protein                         | 7.21±0.07      | 5.3±0.14       |
| Carbohydrate (by difference)    | 78.16±0.14     | 76.59±0.28     |
| Crude fiber                     | 8.52±0.32      | 5.55±0.37      |
3.2. Carrageenan Extraction
The extraction was carried using KOH at 80°C. High temperatures resulting greater carrageenan yield compare to low temperature. The longer the seaweed interacts with heat and aqueous extraction, the more the carrageenan released of the cell wall. The long extraction may lead to the structure damage reducing carrageenan yield. The resulting carrageenans were presented in figure 2.

![Carrageenan from E. cottonii](image1)

**Figure 2.** Carrageenan from *E. cottonii* (A1B1 (KOH 4%), A1B2 (KOH 8%), A1B3 (KOH 12%), A2B1 (KOH 4%), A2B2 (KOH 8%), A2B3 (KOH 12%).

3.3. Chemical characteristics of carrageenan
Chemical characteristics of carrageenan include moisture, ash, insoluble acid, and sulphate is showed in table 2.

| Composition      | A1B1          | A1B2          | A1B3          | A2B1          | A2B2          | A2B3          |
|------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Moisture         | 10.25±1.86    | 9.21±1.26     | 7.99±0.51     | 11.45±0.44    | 9.96±0.49     | 9.89±0.18     |
| Ash              | 30.85±1.19    | 38.16±0.08    | 33.00±2.51    | 22.84±5.40    | 36.52±5.40    | 36.21±1.14    |
| Acid - insoluble | 1.93±0.22     | 1.83±0.44     | 1.83±0.13     | 1.89±0.24     | 2.25±0.24     | 2.4±1.47      |
| Sulphate         | 30.08±0.16    | 30.41±0.44    | 30.4±0.39     | 20.03±2.05    | 22.46±5.69    | 23.79±5.69    |

A1B1 (Tanjung Medang with KOH 4%), A1B2 (Tanjung Medang with KOH 8%), A1B3 (Tanjung Medang with KOH 12%), A2B1 (Jaga island with KOH 4%), A2B2 (Jaga island with KOH 8%), A2B3 (Jaga island with KOH 12%). *wet weight, dw (dry weight). Letters indicate statistical significance (p < 0.05) for KOH treatment.

3.4. Moisture content
Moisture content is one of the most important characteristics in food, because moisture can affect the appearance, texture of foodstuffs (Martinez et al 2007). Table 2 shows that the highest moisture content of Tanjung Medang Village seaweed in KOH treatment (A1B1) with a moisture content of 10.25%, while the lowest moisture content was found at a concentration of 12% (A1B3) and seaweed moisture content. The highest Jaga Island in KOH treatment was 4% (A2B3) with a moisture content of 11.45%, while the lowest moisture content was at a concentration of 8% (A2B2) of 9.96%. The results of this
study are higher than the moisture content of Arfini (2013) with a range of moisture content of 6.76-9.73% and lower than the research of Bunga et al (2013), namely 13.76-19.46.

The moisture content meets the requirements issued by FAO, which is 12%. The results of variance analysis on carrageenan showed the results of each treatment on the origin of seaweed and the concentration did not have a significant effect (P> 0.05) on the value of carrageenan moisture content.

3.5. Ash content
The ash content of carrageenan from Tanjung Medang with 8% KOH had ash content of 34.58%, while the lowest ash content was at 4% (A1B1) of 27.65%. The highest ash content seaweed from Pulau Jaga with KOH 8% (A2B2) was 36.51% while the lowest was at a concentration of 4% (A2B3) 22.84%. Analysis of variance of KOH concentration factors on ash content showed significant, the higher the KOH concentration, the higher the carrageenan ash content.

3.6. Acid-insoluble ash content
Acid-insoluble ash content is one of the criteria in determining the level of cleanliness in the processing process (Basmal et al 2003). The average content of carrageenan acid insoluble ash from this study ranged from 1.66 to 2.25%. The value of carrageenan acid insoluble ash content of the highest extraction of Tanjung Medang Village in KOH treatment 12% (A1B3) of 2.02% and the lowest in the treatment of 8% (A1B2) of 1.66%, while the content of insoluble ash of the highest acid of Jaga Island in treatment 8 % (A2B2) of 2.25% and the lowest at 4% (A2B1) of 1.89%

The low content of acid-insoluble ash indicated uncontaminated carrageenan during the process of handling raw materials and processing (Wennno et al 2012). Acid insoluble ash content in this study is not different from that determined by the EEC which is a maximum of 2%, FAO and FCC maximum 1%. The results of variance analysis on carrageenan showed the results of each treatment on the origin of seaweed and the given KOH concentration had no significant effect (P> 0.05) on the value of insoluble ash content of carrageenan acid.

3.7. Sulphate content
Carrageenan sulphate levels with different KOH treatments are presented in table 3. The highest of sulphate content of carrageenan was from Villagent Tanjung Medang in the treatment of 4% KOH (A1B1) with sulphate content of 29.64%, and the lowest treatment of 12% (A1B3) at 25.38%, while the highest sulphate content in Pulau Jaga was at a concentration of 4% (A2B3) of 26.03%, and the lowest treatment of 12% (A2B3) of 21.47%. The sulphate content was produced in this study meets carrageenan standards (FAO). The results of the analysis of variants of sulphate levels in carrageenan showed the treatment of seaweed origin and KOH concentration were not significantly different (P<0.05).

3.8. Physical characteristics of carrageenan
3.8.1. Gel strength. The gel strength is the main physical properties of carrageenan because the strength of the gel shows the ability of carrageenan in gel formation (Wennno 2009). Table 3 shows that the highest gel strength was found in carrageenan from 12% KOH both from Tanjung Medang Village and Jaga Island, while the lowest strength of gel was found at 4% KOH concentration. The highest values of Tanjung Medang Village and Jaga Island gel strengths were 2005.24 (gcm$^{-2}$) and 837.91 (gcm$^{-2}$) and the lowest values of Tanjung Medang Village and Jaga Island gel strength were 22.40 (gcm$^{-2}$) and 0 detected (undetection).

The high strength of gel may be influenced by the extraction time and KOH concentration, the use of KOH in the extraction process is also able to increase the gel strength carrageenan. According Murdinah et al (2008) the gel strength of carrageenan was strongly influenced by KOH concentration, temperature,
and extraction time. The results of analysis of variance showed that seaweed origin and KOH concentration had a very significant effect on the strength of the gel.

Table 3. Physical characteristic of carrageenan.

| Composition   | Treatments |
|---------------|------------|
|               | A₁B₁       | A₁B₂       | A₂B₁       | A₂B₂       | A₂B₃       |
| Gel Strength g.cm⁻² | 22.4 ±1.42ᵃ | 289.88 ±17.03ᵇ | 2005.24 ±175.61ᶜ | nd           | 805.21 ±81.91ᵇ | 837.91 ±74.50ᶜ |
| Viscosity (cP) | 13.5 ±0.70ᵇ | 12.75 ±0.35ᵇ | 3.00 ±0.70ᵇ | 16.5 ±0.70ᵇ | 15.5 ±0.70ᵇ | 2.50 ±0ᵇ |

A₁B₁ (Tanjung Medang with KOH 4%), A₁B₂ (Tanjung Medang with KOH 8%), A₂B₁ (Tanjung Medang with KOH 12%), A₂B₂ (Jaga island with KOH 4%), A₂B₃ (Jaga island with KOH 8%), A₂B₄ (Jaga island with KOH 12%), ww (wet weight), dw (dry weight). nd (not detected). Letter a, b, c indicate statistical significance (p < 0.05) for KOH treatment.

3.8.2. Viscosity. Viscosity is one of the important physical characteristics of carrageenan. Viscosity testing was carried out to determine the viscosity level of carrageenan as a solution at a certain concentration and temperature, the viscosity of carrageenan is usually measured at a temperature of 75°C with a concentration of 1.5% (Wenno 2009). The viscosity of carrageenan ranged between 2.50-16.50 cP (table 3). The viscosity of the carrageenan from Tanjung Medang Village for treatment of 4% KOH (A₁B₂) was 13.50 cP while for treatment of 12% (A₁B₃) was 3.00 cP. The viscosity of carrageenan from Jaga Island for treatment of 4% (A₂B₃) was 16.50 cP) while for the 12% treatment (A₂B₁) was 2.50 cP. These viscosity values from this study were within the range values recommended by FAO, i.e. 5-800 cP. The addition of KOH affects the viscosity value of carrageenan. According to Wenno et al (2012) the lower the sulphate content, the lower the viscosity although the gel strength is higher. The analysis of variance showed that the seaweed without treatment and the seaweed with KOH treatment were difference in viscosity level.

3.8.3. Yield carrageenan. The highest yield of carrageenan produced was obtained from using 4% KOH concentration both from Tanjung Medang Village and Jaga Island, while the was lowest at 8% KOH concentration. The yield was highest for carrageenan from Tanjung Medang Village and Jaga Island. Rendemen were 20.97 and 30.77%, and yields were the lowest from Tanjung Medang Village and Jaga Island were 15.25 and 14.56%.

4. Conclusion

The main component of E. cottonii was fibre. KOH 12% treatment resulted in the highest effect on gel strength 2005.24 g.cm⁻² (Tanjung Medang) and 837.91g.cm⁻² (Jaga Island). The concentration 4% improved the viscosity while the concentration 8% had an impact on ash.

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