Characterization of semi-refined kappa-carrageenan from *Kappaphycus alvarezii* with different solvents in Tanjung Sumenep

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**Abstract.** The quantity and quality of carrageenan produced is influenced by the method of extraction and the solvent. Characterization of the carrageenan products necessary to get certain standards so the carrageenan product acceptable to consumers both locally and internationally. The purpose of this research was to determine the solvents that can produce carrageenan from *Kappaphycus alvarezii* with the best characteristics and to compare the results of the extraction of carrageenan with international standards in order to obtain a standardized carrageenan. The results showed that the solvent produce a kappa-carrageenan from *K. alvarezii* with the best characteristics is NaOH 0.5 N. Kappa-carrageenan has been able to compared FAO, FCC and Marine Science Co., Ltd. standards for parameters: Gel strength, viscosity, sulfate content and ash content. Alkali able to extract and modify mu-carrageenan (precursor) into a kappa-carrageenan. The extraction process uses alkali caused the event ion exchange between cations in the solvent (K⁺ or Na⁺) ions with sulphate in the sea grass that reduced sulfate content. Need to do more research on the characterization of carrageenan include microbiological tests as well as the shelf life of carrageenan test.

1. **Introduction**

The use of seaweed in Indonesia can be optimized by diversifying its products, one of which is carrageenan. Carrageenan is a polysaccharide consisting of linear galactose with a certain degree of sulfation [1]. Carrageenan has been used in various fields such as drug formulation, cosmetics, pharmaceutical, food industry and textile industry [2]. The main function of carrageenan in food ingredients is as a stabilizer and texture builder [3]. The three types of carrageenan that are mostly produced are kappa-carrageenan, iota-carrageenan and lambda-carrageenan, what distinguishes between the three carrageenan is their chemical composition and structure [4]. The quantity and quality of carrageenan that is produced is strongly influenced by the extraction method and the solvent used [5]. The carrageenan industry has used certain alkaline solvents to extract and modify carrageenan from its precursors [6].

Characterization of carrageenan products needs to be done to meet certain standards so that the carrageenan products can be accepted by consumers both locally and internationally. The physical characteristics of carrageenan include gel strength, viscosity and yield, while the chemical characteristics of carrageenan include sulfate content, moisture content and ash content [7]. The purpose of this study was to determine the solvent that can produce carrageenan from *Kappaphycus alvarezii* with the best characteristics and to compare the extracted carrageenan with international standards in order to obtain standardized carrageenan. The solvents used were distilled water and alkaline solvents, namely KOH 0.5 N and NaOH 0.5 N.

2. **Material and methods**

2.1. **Time and place of experiment**
This research was conducted from January to March 2016 at the Educational Laboratory of the Faculty of Fisheries and Marine Universitas Airlangga for the process of carrageenan extraction, testing of moisture content, ash content and yield. Research at the Food Quality and Safety Testing Laboratory, Faculty of Agricultural Technology, Brawijaya University for testing gel strength and viscosity, as well as at the Testing Service Unit, Faculty of Pharmacy, Universitas Airlangga for testing sulfate levels.

2.2. Tools and material
The tools used in this study include masks, gloves, trays, buckets, knives, plastic bags, volume pipettes, funnels, sample bottles, label paper, newsprint, stainless steel spoons, stainless steel filters, stirring rods, balance sheets, Beaker glass (three liters), measuring cup, Erlemeyer flask, glass mold, porcelain cup, crush pliers, Sartorius® BS 124 S analytical balance, thermometer, ash free filter paper, pH paper, pH meter, HH-6 waterbath thermostat DFS® KW-1000DB, thermolyne Cimarec® 2, magnet stirrer, brookfield viscosimeter, Imada® ZP-200 NT texture analyzer with a capacity of 0-200 N, desiccator, Thermo Scientific® heratherm oven, Thermo Scientific® Thermolyne furnace F48010-33 and spectrophotometry Fourier Transformed Infrared (FTIR) Thermo Fisher Scientific Inc.®

The material used was K. alvarezii seaweed aged 45 days [8] obtained from Tanjung, Sumenep, Madura. The solvents are distilled water, KOH 0.5 N and NaOH 0.5 N, as well as 90% technical ethanol, BaCl2, HCl and AgNO3 / HN03 solutions. Commercial carrageenan as a comparison.

2.3. Work procedure
K. alvarezii seaweed is cleansed by washing it with water, then chopping it into about 1 cm in length. Seaweed is dried under the sun to a constant weight characterized by no change in weight as a function of drying time. 50 grams of dried seaweed is immersed in 500 ml of distilled water for 15 minutes, then filtered and extracted. The solvent is first heated to a temperature of 80⁰C, the weight ratio of dry seaweed: the volume of the solvent is 1: 50 (g / mL) [7]. The extraction time starts when the seaweed is added to the solution. Solvent with a temperature of 80⁰C is added every time to replace the evaporating solution so that the ratio between the seaweed and the solvent remains constant.

The extraction process was carried out for 30 minutes, after which the filtrate was taken by filtering and then poured into 90% technical ethanol. The volume of ethanol is three times the volume of the filtrate, the mixture is stirred to form carrageenan fibers and left for about 30 minutes [7]. The carrageenan fibers were filtered and dried in an oven at 60⁰C until the weight of constant, marked with no change in weight as a function of drying time. All stages in the extraction process are carried out for all types of solvents, namely distilled water, KOH 0.5 N and NaOH 0.5 N with six repetitions.

2.4. Experiment procedure
The main parameters in this study were carrageenan gel strength, carrageenan viscosity, carrageenan yield, carrageenan sulfate content, carrageenan water content and carrageenan ash content. The supporting parameters in this study were pH, color, smell and appearance of carrageenan.

2.5. Testing for gel strength and yield
The carrageenan gel strength test refers to the method of [9] namely dry carrageenan is dissolved in distilled water by heating to obtain a solution of 1.5% (weight / volume). 10 ml of carrageenan solution was poured into a container with a diameter of 3 cm with a solution height ranging from 1.2 to 1.4 cm. The solution was left to stand for 12 hours at room temperature. A stainless steel cylinder rod with a cross-sectional area of 0.786 cm2 is placed on top of the sample and then pressed using a texture analyzer tool to a depth of one cm. The gel pressure value is recorded based on the value on the instrument monitor. The gel strength value is the average value of the same two samples. The yield produced is crude carrageenan and is calculated using the equation [10]:

\[
\text{Yield \%} = \frac{\text{weight (carrageenan)}}{\text{weight (sample)}} \times 100\%
\]
2.6. Viscosity testing

The carrageenan viscosity test refers to the method used by the Food Marine Colloids Corporation [11], namely that a 1.5% concentration of carrageenan solution is heated in a boiling water bath while stirring regularly until it reaches a temperature of 75⁰C. Viscosity is measured by means of a viscosimeter at a solution temperature of up to 75⁰C. The reading is made after one minute of full rotation for spindle no. 02 brookfield viscosimeter. The measured viscosity has units of poise (one poise = 100 centipoise). The viscosity value is the average value of the same two samples.

2.7. Sulfate level testing

Testing of carrageenan sulfate levels refers to the method used by the Food Marine Colloids Corporation [11] which has been modified, namely using 1 gram of carrageenan that has been ignited in the furnace. The ash was dissolved in HCl (1:1) and 50 mL of distilled water into the flask and then filtered. The filtrate is added with 100 mL H2O and 5 mL 2N HCl, then heated to boil. BaCl2 solution (10%) was added by dropping it while stirring until a white precipitate appeared. The sample was let to sit on a water bath for two hours then cooled and filtered with an ash-free filter paper. The precipitate was washed with hot water until the filtrate was chloride free (test with AgNO3 / HNO3). The sample was dried and transferred to a porcelain crucible, then annealed in the furnace. The sulfate level value is the average value of the same two samples. Calculation of sulfate levels [11] using the relative atomic mass of SO4 (0.4116) using the following equation:

\[
\text{Sulfate \%} = \frac{\text{Precipitation weight of BaSO}_4 \times 0.4116}{\text{Sample weight}} \times 100\%
\]

2.8. Water content testing

Testing of carrageenan water content refers to the method used by the Association of Official Analytical Chemist [12], namely one gram of carrageenan is mashed first and then dried in an oven at 105⁰C for three hours, then it is cooled in a desiccator and weighed. This treatment was repeated until a constant weight was reached, the difference in weighing was <0.2 mg, respectively. The water content value is the average value of the same two samples. Water content can be calculated by the equation:

\[
\text{Water Content \%} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100\%
\]

2.9. Ash content testing

The carrageenan ash content test refers to the method used by the Association of Official Analytical Chemists [12], which is to weigh the mineral residue as a result of burning organic matter at a temperature of around 550⁰C. The porcelain plate was dried in an oven for one hour at 105⁰C then cooled for 30 minutes in a desiccator and weighed until a fixed weight was obtained (A). A sample of 1 gram (B) is put into a porcelain dish and put in an electric furnace at a temperature of 600ºC for about six hours. Furthermore, the plate was cooled in a desiccator and then weighed to obtain a fixed weight (C). The value of the ash content is the average value of the same two samples, calculated by the equation [12]:

\[
\text{Ash content \%} = \frac{A + B - C}{B} \times 100\%
\]

2.10. Data analysis
This research was conducted experimentally with an experimental design in the form of a completely randomized design (CRD). The treatment in this study consisted of three treatments in the form of using different solvents, namely distilled water, KOH 0.5 N and NaOH 0.5 N with six repetitions. The research data were analyzed using ANOVA (Analysis of Variance) which was then tested further by Duncan. The research data were also analyzed descriptively by comparing with the standards used.

3. Results and discussion

3.1. Fourier transformed infrared (FTIR) spectrophotometric analysis

The most dominant spectrum for kappa-carrageenan is at the wave number (frequency) 1220-1260 cm\(^{-1}\) which is the S = O bond on the sulfate ester and at a frequency of 928-933 cm\(^{-1}\) is the CO bond at 3,6-anhydrogalactose as a typical region or fingerprints (finger print area) [13]. The results of FTIR spectrophotometric analysis can be seen in Figure 1., in order from top are commercial carrageenan, KOH 0.5 N solvent, 0.5 N NaOH solvent and distilled water.

![Figure 1. Results of FTIR spectrophotometric analysis](image)

The frequency marked with a black circle indicates that the functional group is characteristic of kappa-carrageenan. Aquades treatment has an absorption peak at a frequency of 1218.66 cm\(^{-1}\) and 923.55 cm\(^{-1}\), KOH treatment at a frequency of 1232.93 cm\(^{-1}\) and 922.99 cm\(^{-1}\), NaOH treatment 0.5 N at a frequency of 1225.22 cm\(^{-1}\) and 916.12 cm\(^{-1}\), while commercial carrageenan at a frequency of 1223.91 cm\(^{-1}\) and 923.59 cm\(^{-1}\). These results indicate the similarity in the fingerprint area (finger print area) between commercial carrageenan and extracted carrageenan with various solvents, so it can be concluded that the extraction result obtained is kappa-carrageenan.

3.2. Gel strength

The main characteristic of carrageenan is gel strength [7]. Gel strength is defined as the ability to withstand a force at a certain pressure to penetrate the sample as deep as 1 cm [9]. The compressive test in this study used a texture analyzer. Based on Table 1, it is known that the highest average gel strength value in 0.5 N NaOH treatment was not significantly different from 0.5 N KOH treatment but significantly different from distilled water treatment, distilled water treatment did not produce gel strength values. The observation process showed that the distilled water treatment sample formed a thick
liquid that could not form gelation at a concentration of 1.5% with a preheating of 75°C at the time of preparation of the gel strength test. This causes the aquades treatment sample to be unable to continue for the gel strength test. Samples in 0.5 N KOH and 0.5 N NaOH treatments can form a gel at a concentration of 1.5% by preheating at 75°C and allowed to stand for 12 hours during preparation of the gel strength test.

Table 1. Average value of carrageenan gel strength (g / cm²)

| Treatment  | Average of Gel Strength ± SD (g/cm²) |
|------------|---------------------------------------|
| Aquades    | 0.00b ± 0.00                          |
| KOH 0.5 N  | 421.48a ± 156.74                      |
| NaOH 0.5 N | 431.68a ± 262.02                      |

The test results use Newton (N) units and then converted to the standard of 1 N (N / cm²) = 101.97162 g / cm². The value of gel strength is influenced by the solvent used because the solvent affects the sulfate content in carrageenan, high sulfate levels can reduce the gel strength. The test results show that the aquades treatment sample is not able to form a gel, this is presumably because aquades is not able to reduce the sulfate content in seaweed [5]. The treatment of KOH 0.5 N and NaOH 0.5 N was able to form a gel because the solvent used was alkaline. Alkali is able to extract and modify mu-carrageenan (precursor) to kappa-carrageenan [6].

OH acts as a catalyst in cyclization reactions [14] and cations from alkalis can increase the ionic strength of carrageenan [15]. The extraction process using alkalis causes ion exchange between cations in the solvent (K⁺ or Na⁺) and sulfate ions in seaweed so that sulfate levels are reduced [7]. The gel formation mechanism consists of two stages, starting with a change in the intramolecular conformation that is not related to ions, then followed by the formation of cross-bonds which depend on the presence of specific ions (cations) that cause the gel structure to form [5].

Judging from the standards used in this study on the parameter of gel strength, the extracted carrageenan with KOH solvent 0.5 N reached an average of 421.48 g / cm² (Table 2). Carrageenan extracted with 0.5 N NaOH solvent with an average of 431.68 g / cm² has met the standards set by FAO (200-500 g / cm²), FCC (200-500 g / cm²) and Marine Science Co., Ltd. (200-300 g / cm²). Commercial carrageenan as a control in this study had a very large gel strength value compared to the results of distilled water solvent treatment, KOH 0.5 N and NaOH 0.5 N which was 1886.47 g / cm².

Table 2. Standardization of carrageenan

| Parameter                  | FAO                  | FCC                  | Marine Science Co., Ltd. |
|----------------------------|----------------------|----------------------|--------------------------|
| Gel strength               | 200-500 g/cm²        | 200-500 g/cm²        | 200-300 g/cm²            |
| Viscosity                  | ≥5 cp                | ≥5 cp                | ≥5 cp / 20-50 mPa·s      |
| Sulfate Level              | 15-40%               | 18-40%               | 10-30%                   |
| Water Content              | ≤12%                 | ≤12%                 | ≤12%                     |
| Ash Content                | 15-40%               | ≤35%                 | 15-35%                   |

Source: FCC (1978); FAO (2007); Marine Science Co., Ltd. (2011)

3.3. Viscosity

The viscosity property is closely related to the cyclization reaction of mu-carrageenan to kappa-carrageenan, whose viscosity tends to be higher [16]. The cyclization reaction is able to reduce the sulfate levels in carrageenan. The extraction process using alkalis causes ion exchange events between cations in the solvent (K⁺ or Na⁺) and sulfate ions in seaweed so that sulfate levels are reduced [7]. This mechanism proves that the high viscosity value of the 0.5 N KOH and 0.5 N NaOH treatment samples is caused by alkaline solvents.

Commercial carrageenan as a control has a very high viscosity value compared to aquades treatment, KOH 0.5 N and NaOH 0.5 N. On the viscosity parameter, the carrageenan extracted with KOH 0.5 N solvent had an average of 30.333 cP, the extracted carrageenan with 0.5 N NaOH solvent had an average
of 31.167 cP, and the extracted carrageenan with distilled water had an average of 31.167 cP. An average of 11,667 cP has met the standards set by FAO (≥5 cp), FCC (≥5 cp) and Marine Science Co., Ltd. (≥5 cp). Commercial carrageenan as control in this study is very different from aquades treatment, KOH 0.5 N and NaOH 0.5 N. The viscosity value of commercial carrageenan is 64 cP.

The water content in carrageenan binds more and more sulfate (hydrophilic) groups [5]. Incomplete drying and low homogenization results in high water content [17]. This is presumably due to the less than optimal heating process when drying the samples after extraction using an oven [17]. High sulfate groups result in decreased viscosity and non-uniform values.

3.4. Yield

The yield value of carrageenan is strongly influenced by the solvent used at the time of extraction [18]. The results in Table 3 show that the use of alkaline solvents (KOH 0.5 N and NaOH 0.5 N) is able to form carrageenan fibers and the yield of carrageenan is better than aquades solvent. This is because extraction using alkalis causes ion exchange between cations in the solvent (K$^+$ or Na$^+$) and sulfate ions in seaweed so that the sulfate levels are reduced [7] and the formation of the 3,6-anhydrogalactose group is more optimal. The higher the 3,6-anhydrogalactose group formed in carrageenan, the higher the carrageenan fiber obtained so that the carrageenan yield increases [5].

| Treatment  | Average of Yield ± SD (%) |
|------------|---------------------------|
| Aquades    | 5.969 ± 4.561             |
| KOH 0.5 N  | 42.394 ± 15.632           |
| NaOH 0.5 N | 68.955 ± 11.345           |

Observation (qualitative) descriptively shows that the resulting carrageenan (yield) has a less attractive appearance when compared to commercial carrageenan. Commercial carrageenan has the appearance of a pure white color, soft texture and odorless, while the extracted carrageenan is more brown-black in color, has an uneven texture and tends to be rancid. Carrageenan results from distilled water treatment have a higher brightness level than 0.5 N NaOH treatment, while carrageenan color results from KOH treatment 0.5 N are darker than 0.5 N NaOH treatment. Carrageenan fiber is a polysaccharide composed of galactose which is prone to browning reactions (Maillard reaction) [19]. The higher the polysaccharide (carrageenan fiber) possessed by kappa-carrageenan, the easier the Maillard reaction will occur, especially during the heating process (oven drying) [19].

3.5. Sulfate Level

Sulfate content is the main chemical characteristic of carrageenan. The sulfate level in the carrageenan sample test is a calculation of barium sulfate (BaSO$_4$) with the assumption that the sulfate level obtained is the sulfate bound in the bleaching reaction. Sulfate levels affect the gelling ability and viscosity of carrageenan, sulfate levels in carrageenan is influenced by the concentration and type of alkaline solvent used [7].

Samples in distilled water treatment extracted using distilled water tend to have high sulfate levels, this is because distilled water is not effective in reducing sulfate groups [5]. Hydrophilic sulfate groups tend to bind water (aquades) so that the water content in the aquades treatment sample is high without reducing the sulfate groups in seaweed [5]. This has a negative impact on carrageenan products with low values of gel strength and viscosity but high levels of sulfate and water content so that it also has an impact on appearance and odor [7].

Judging from the standards used in this study (Table 2.) on the parameters of sulfate levels, the extracted carrageenan with KOH solvent 0.5 N reached an average of 15.105%, the extracted carrageenan (Table 4.) with 0.5 N NaOH solvent with an average of 15,502% and carrageenan extracted with distilled water with an average of 27.64% have met the standards set by FAO (15-40%) and Marine Science Co., Ltd. (10-30%). Commercial carrageenan as a comparison (control) with a sulfate content of 12.04% only meets the standards of Marine Science Co., Ltd. with a range of 10-30% sulfate. The
results of this study indicate that KOH 0.5 N solvent is a solvent capable of producing kappa-carrageenan with the lowest average sulfate content and has met international standards for parameters of sulfate levels.

| Table 4. Average Value of Carrageenan Sulfate Content (%) |
|----------------------------------------------------------|
| Treatment                                              | Average of Sulfate Level ± SD (%) |
|---------------------------------------------------------|
| Aquades                                                 | 27.64° ± 1.243                   |
| KOH 0.5 N                                               | 15.105° ± 3.176                  |
| NaOH 0.5 N                                              | 15.502° ± 5.38                   |

3.6. Water Content

The water content tested in this study is bound water, while free water is thought to have evaporated during the drying process of the carrageenan sample at the end of the production process [18]. Water content testing is intended to determine how much water content is in carrageenan because water content greatly affects its shelf life [20]. Water content greatly affects microbial activity during carrageenan storage [21]. Moisture content is strongly influenced by drying conditions, packaging and storage methods [22].

Observations during the extraction and production process of carrageenan showed that the high water content in the distilled water treatment formed a softer texture and a brighter color in carrageenan compared to the KOH treatment 0.5 N and NaOH 0.5 N presumably due to high water content [21]. Extracted carrageenan (Table 5.) KOH 0.5 N treatment reached an average of 31.431% (pH 8), 0.5 N NaOH treatment averaged 33.084% (pH 8) and distilled water treatment reached an average of 58.749% (pH 8) has not been able to meet the water content standards set by FAO (≤12%), FCC (≤12%) and Marine Science Co., Ltd. (≤12%) but meets the pH standard of carrageenan at a solution concentration of 1.5%. These parameters are reviewed from the water content standard used in this study (Table 2) and the carrageenan standard pH [23] which ranges from 8-11.

| Table 5. Average value of carrageenan content (%) |
|---------------------------------------------------|
| Treatment                                         | Average of Water Content ± SD (%) |
|---------------------------------------------------|
| Aquades                                           | 58.74° ± 1.35                     |
| KOH 0.5 N                                         | 31.43° ± 6.64                     |
| NaOH 0.5 N                                        | 33.08° ± 11.58                    |

Commercial carrageenan as a comparison (control) has a water content that is much lower than the KOH treatment 0.5 N and NaOH 0.5 N even more so when compared to aquades treatment. Based on the test results, commercial carrageenan has a moisture content of 5.247% (pH 8) and has been able to meet the water content standards of the FAO, FCC and Marine Science Co., Ltd., as well as the pH standard of carrageenan based on FAO [23]. The low water content is thought to be obtained from the optimal heating process when drying the sample using a machine [17]. Water content affects the appearance, color and odor of the product, including its shelf life [21].

3.7. Ash content

Judging from the standard ash content used in this study (Table 2), the carrageenan extracted from the KOH treatment of 0.5 N averaged 34.96% and 0.5 N NaOH treatment averaged 34.43% has met the ash content standards set by FAO (15-40%), FCC (≤35%) and Marine Science Co., Ltd. (15-35%). Carrageenan extracted from distilled water treatment reached an average of 37.9% only able to meet the standards of the FAO (15-40%). Commercial carrageenan as a comparison (control) has a much lower ash content than the treatment of KOH 0.5 N, NaOH 0.5 N and aquades and has met the standards in this study. Test result data can be seen in Table 6.

| Table 6. Average value of carrageenan content (%) |
|---------------------------------------------------|
| Treatment                                         | Average of Ash Content ± SD (%) |
|---------------------------------------------------|
| Aquades                                           | 37.9° ± 1.155                   |
| KOH 0.5 N                                         | 34.9° ± 2.291                   |
NaOH 0.5 N  

| 34.4± ± 2.72 |

Ash is an inorganic substance left over from the combustion of an organic material and is closely related to the amount of mineral content of a material [18]. Analysis of the ash content in the carrageenan sample was carried out to determine in general the mineral content contained in carrageenan [18]. Judging from the raw material, seaweed is a food material that contains quite high minerals because of its ability to absorb minerals from its environment [20]. The relatively high ash content is thought to be due to the less than optimal precipitation process after extraction. The alkaline solvents used tend to contribute K⁺ and Na⁺ as minerals [24]. Precipitation is carried out using 90% technical ethanol to separate the solvent from carrageenan fibers [7].

4. Conclusion
The results of this study indicate that the solvent that can produce kappa-carrageenan from K. alvarezii with the best characteristics is 0.5 N NaOH solution. Kappa-carrageenan that is produced from extraction with 0.5 N NaOH solvent has been able to meet the FCC, FAO and Marine standards. Science Co., Ltd. for parameters of gel strength, viscosity, sulfate content and ash content. Further research is needed on carrageenan characterization including microbiological tests such as TPC, Salmonella sp. contamination, Escherichia coli, molds and fungi as well as carrageenan storage test. Kappa-carrageenan produced by extraction using 0.5 N NaOH solvent can be used as a food additive for various types of food according to the concentration used. The product is safe as long as it is in proper storage and remains dry.

5. References
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