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Carrageenan Properties Extracted From *Eucheuma cottonii*, Indonesia

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Abstract**—**The effect of extraction solvent upon properties of carrageenan from *Eucheuma cottonii* was studied. The distilled water and KOH solution (concentration 0.1- 0.5N) were used as the solvent. Extraction process was carried out in water bath equipped by stirrer with constant speed of 275 rpm with a constant ratio of seaweed weight to solvent volume (1:50 g/mL) at 86^{o}C for 45 minutes. The extract was then precipitated in 3 volume of 90% ethanol, oven dried at 60^{o}C. Based on experimental data, alkali significantly influenced yield and properties of extracted carrageenan. The extracted carrageenan was found to have essentially identical FTIR spectra to the reference samples of kappa-carrageenan. Increasing the KOH concentration led to carrageenan containing less sulfate content and intrinsic viscosity. The gel strength increased along with the increasing of KOH concentration. The decreasing of intrinsic viscosity value indicates that a polymer degradation occurs during alkali extraction.

Keywords**—** gel strength, sulfate, intrinsic viscosity, *Eucheuma cottonii*

I. Introduction

CARRAGEENANS are sulfated linear polysaccharides extracted from certain red seaweed of the Rhodophyceae class. They have been extensively used in the food industry as thickening, gelling agent and more recently used in the food industry as excepient in pill and tablets [1]. Since natural carrageenans are mixtures of different sulfated polysaccharides, their composition differs from batch to batch. Carrageenan from particular seaweed species and geographic district differ considerably in their structure and rheological properties of solutions and gels. Therefore, the quantitative analysis of carrageenan batches is of greatest importance for industry to deliver a standard product and to develop new application based on their unique intrinsic properties.

Kappa carrageenan which can form strong gel is highly valued in dairy application. A good source of kappa carrageenan is *Eucheuma cottonii*, which is mainly harvested in the Phillipines and Indonesia. The yield and physical properties of carrageenan such as gel strength, gelling and melting temperature as well as chemical properties, determine its values to the industry. The seaweeds are usually extracted with alkali at elevated temperature [16]. The alkaline treatment is an important and well-known reaction of carageenans, and is used to commercially enhance gelation behavior [10, 16].

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This reaction is showed in Fig. 1 [10]. The $^{1}C_{4}$ conformation of 3,6 Anhydro-D-galactose (DA) unit in kappa carrageenan allows a helical secondary structure, which is essential for the gel forming properties.

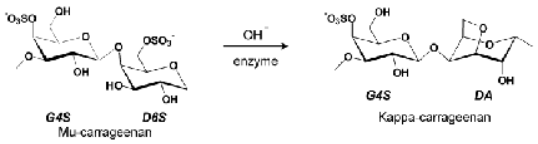

Fig 1 Cyclization reaction with hydroxide to generate kappa carrageenan

The rate of this reaction depends not only on temperature, alkali concentration, ionic strength of the medium but also the seaweed species [2, 3, 4, 17].

Refining of solubilized carrageenan by isopropyl alcohol precipitation, gel pressing and freeze drying resulted in only minor differences in chemical composition of carrageenan from *Eucheuma cottonii* [10]. According Montolalu [14], both the weight-average and the number-average molecular weight of *Eucheuma cottonii* extract decreased with the increasing extraction temperature and extraction time. However, various aspects of alkali modification, extraction, and quantitative functionalities concerning *Eucheuma cottonii* have not been studied so far.

Data are quite limited on intrinsic properties of carrageenan extracted under different solvent. The aim of this research is to investigate the effect of potassium hydroxide concentration on sulfate content, gel strength, and intrinsic viscosity of carrageenan extracted from *Eucheuma cottonii* cultivated at Makasar Sulawesi, Indonesia.

II. Materials and Methods

A. Material

Commercial carrageenans (Irish mosh) type I Sigma were supplied as the reference carrageenan. Predominantly kappa and lesser amounts of lambda carrageenan contained potassium 10.2%, calcium 2.7% and sodium 0.7%.

Dried seaweeds of *Eucheuma cottonii* were harvested in June 2010 from Makasar, South Sulawesi, Indonesia. All of the seaweeds were washed to eliminate the salt and sand using tap water, and then cut into ± 1 cm length, and finally sun dried to constant weight. The 'clean seaweed' sample was kept in a dry state until further processing was done.

Distilled water and solution from technical grade of

potassium hydroxide were used as the solvent.

B. Extraction

The 30 gram of clean seaweed was soaked in distilled water for 15 minutes. After soaking, the water was separated from the seaweed by filtration. Firstly, a known amount of solvent was heated in a beaker as an extractor which emerged in a water bath equipped by a stirrer. If the temperature of solvent reached 85°C, the seaweeds then were added into solvent, and the time of extraction started to be counted. The speed of stirrer was set constant at 275 rpm. The constant ratio of seaweed weight to solvent volume (1/50; g/mL) was maintained by adding hot water. The extraction was stopped after 45 minutes. Filtrate was separated from residue using filter cloth and immediately poured into 3 volumes of cold (5°C) technical ethanol (90% w) which caused precipitation of polysaccharides. The precipitation was done for 30 minutes with stirring gently by hand. The precipitated carrageenans were collected and oven dried at 50-60°C to a constant weight. The experiments were carried out with different solvent (distilled water and KOH of 0.1; 0.3; and 0.5 N) in triplicate. Some parameters of the extracted carrageenan were determined, namely the yield, chemical structure, sulfate content, and intrinsic viscosity.

C. Carrageenan Analysis

Yield is defined as ratio of dried carrageenan weight to dried seaweed weight.

Percent sulfate content was determined using the method of sulfate hydrolysis followed by precipitation of sulfate as barium sulfate [11]. A known amount of dried carrageenan (W1, g) was hydrolyzed with 50 mL 1N HCl for 30 minutes at its boiling temperature. Ten-mL $BaCl_2$ 0.25M was added dropwise within boiling for 5 minutes. After cooled at room temperature for 5 hours, the $BaSO_4$ precipitates were filtered using ashless paper filter (Whatman no.42) and then burned in a furnace for 1 hour at 700°C. The white ash was weighed (W2). Percent of sulfate content was calculated using equation below.

$$\% \text{ sulfate} = (W2/W1) \times 100 \times 0.4116 \quad (1)$$

Assessment of the extracted carrageenan chemical structure was performed by Fourier transform infrared spectroscopy (FTIR) Shimadzu. The spectra were recorded in the 4000 – 400 cm^{-1} region from thin film of extracted carrageenan. The extracted carrageenan was diluted in distilled water at its boiling. The solution was transferred into container to form thin film and dried.

The gel strength was determined using the method described by Falshaw [5] with minor modifications. The dried carrageenan was diluted by distilled water with heating to obtain a 1.5% (w/v) carrageenan solution. For determining gel strength (GS), ten-mL solution was poured into a container (diam. 3.2 cm) and the height of solution was approximately 1.1 – 1.3 cm. After cooling overnight at room temperature, the container was placed on balance. A stainless rod (surface area 1.2 cm^2) was pressed by hand into the gel surface until it collapsed, the maximum balance was noted. Gel strength is defined as ratio difference weight of before and after gel collapse to surface area of the rod. The calculated gel strength was an average of two determinations on the same sample.

Intrinsic viscosity was determined experimentally by measurement of the viscosity of dilute concentration (C) solution. Carrageenan was dissolved in a distilled water by heating. Samples were then filtered through paper filter prior to viscosity measurement. An Oswald glass capillary viscometer (Brand Germany, no.1) was used to measure the passage time of solution (t) and solvent (t_o) through the capillary at room temperature (24.5 – 27°C). The capillary was filled 10 mL of sample. Polymer concentrations were prepared by serial dilution in this work. Denoting solution and solvent viscosity as $\eta_{solution}$ and $\eta_{solvent}$ respectively, the intrinsic viscosity is defined by the following relationships:

$$\text{Relative viscosity: } \eta_{rel} = \frac{\eta_{solution}}{\eta_{solvent}} = \frac{t}{t_o} \quad (2)$$

$$\text{Specific viscosity: } \eta_{sp} = \eta_{Rel} - 1 \quad (3)$$

$$\text{Intrinsic viscosity: } [\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{C} \quad (4)$$

The intrinsic viscosity can be obtained by measuring specific viscosities at different concentration at the same shear rate. The intrinsic viscosity was obtained by extrapolating data from graphic relation between $\frac{\eta_{sp}}{C}$ and C to the value of zero solute concentration.

III. Result and Discussion

Based on our preliminary experiment, it was found that the yield resulted by extraction time of 30 minutes and 60 minutes were no different. Therefore, we run the extraction time for 45 minutes in this present work.

a. FTIR spectra

The FTIR spectra of *Eucheuma cottonii* carrageenan which extracted using 0.3N KOH were compared with those of commercial kappa- carrageenan (Sigma) in Fig. 2. According to JECFA [11] the study of carrageenan by FTIR spectroscopy shows the presence of very strong absorption band in 1210-1260 cm^{-1} region (due to the S=O of sulfate esters) and 1010-1080 cm^{-1} (ascribed to glycosidic lingkage) in all carrageenan type. The other chemical groups are characteristics of a given carrageenan type, namely 3,-6-anhydro-D-galactose at 928-933 cm^{-1}, D-galactose-4-sulfate at 840-850 $cm^{-1}$and 3,6-anhydro-D-galactose-2-sulfate at 800-805 cm^{-1}. Both kappa and iota carrageenan FTIR spectra show a band at 840-850 cm^{-1}, but the 800-805 cm^{-1} band is characteristic and distinctive of iota.

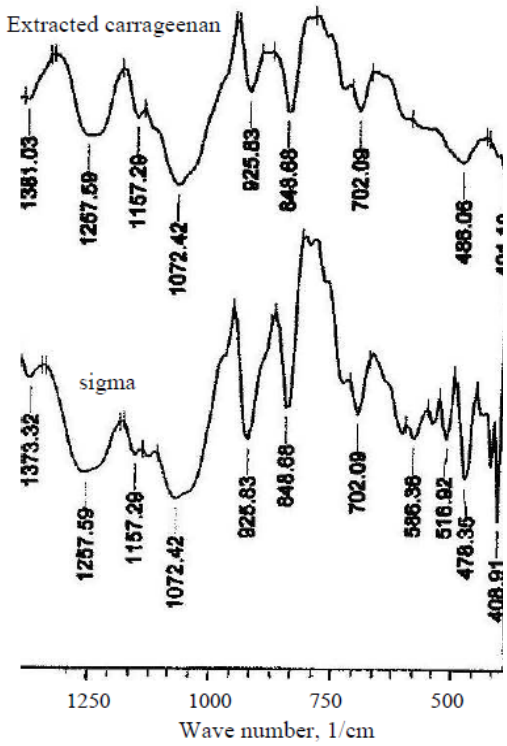

Fig 2 FTIR Spectra of extracted carrageenan and Sigma product

The spectra of extracted carrageenan in Fig. 2 show the main features of kappa carrageenan.

b. Effect KOH Concentration on Yield, Sulfate Content and Gels Strength

Table I displays some parameters evaluated based on experimental data under different solvent used in extraction process.

TABLE I
SOME PARAMETERS RESULT FROM UNDER DIFFERENT CARRAGEENAN TYPE

Carrageenan type	Yield, %	Sulfate, %	Gel strength, g/cm^2	[η], dL/g	M, g/gmol
Sigma	-	18.62	223.63	34.31	14.14 x10^5
Solvent :					
Distilled water	46.43	15.80	nd	nd	nd
0.1N KOH	44.43	16.15	69.8	26.84	10.76x10^5
0.3N KOH	38.22	14.24	73.89	14.61	5.48x10^5
0.5N KOH	37.02	11.45	127.3	14.88	5.59x10^5

nd=not determined

Distilled water resulted the highest yield in our work, but could not form gel in 1.5% solution. Compared to pure distilled water, the low yield on extraction using KOH may be due to polymer destruction. Polymer destruction produced the low molecular weight material which can not be precipitated by alcohol. This suggests that extraction using alkali causes degradation of polymer. The similar results in some degradation of the polisaccharide were also found by other researchers [6, 9, 12].

Concentration of potassium hydroxide strongly influenced both the yield and the gel strength. It seems that with increasing potassium hydroxide concentration the yield decreased but the gel strength increased significantly.

Table I also presents the effect of potassium hydroxide on sulfate content. Based on this result, the sulfate content also related with the value of gel strength. There is a trend where the increasing potassium hydroxide concentration caused the value of sulfate content decreased and the gel strength increased. The reduction of sulfate content by potassium hydroxide indicates that there was a carrageenan reaction which converted precursor carrageenan (mu carrageenan) into kappa carrageenan. The cyclization process (Fig.1) involves release of sulfate groups, thus after alkali treatment the sulfate content should be lower.

The structure kappa carrageenan drives to gel formation [16]. Although the value of sulfate content of carrageenan extracted by distilled water is slightly lower than that by KOH 0.1N, the solution of 1.5% could not gelify. This means that native carrageenan extracted by distilled water has a weak gel property in 1.5% solution. It may be caused by the lower cation in native carrageenan. Although the extraction using 0.1N KOH as the solvent would not reduce significantly the sulfate value in extracted carrageenan, it was found that the gel strength increased significantly compared with extraction using distilled water. According to Campo [1] the water solubility depends essentially on the level of sulfate groups (very hydrophilic) and on their associated cations. The main ionizable cations found in carrageenan are sodium, potassium, calsium and magnesium. Consequently, the proportion of sulfate content and the equilibrium of cations in the water solution determine the gel strength of carrageenan.

In our work, KOH 0.3N and 0.5N can reduce the sulfate content of native carrageenan about 9.8%, and 27.5%, respectively. Compared with 0.1N KOH, the KOH 0.3N and 0.5N increase the gel strength about 5% and 82%, respectively.

Comparison sulfate content and gel strength of extracted carrageenan in this work and sigma product are also showed in Table 1. The sulfate content of Sigma carrageenan was 18.62%, higher than that extracted carrageenan in our work, but it showed strong gel. This could be caused by the different of seaweed source and extraction condition used.

c. Effect KOH Concentration on Intrinsic Viscosity

Fig 3. described relation between $\frac{\eta_{sp}}{C}$ and concentration at different carrageenan type. The value of intrinsic viscosity ([η]) is generally estimated through a graphic double extrapolation procedure by plotting $\frac{\eta_{sp}}{C}$ or $(\ln \eta_{rel})/C$ as a function of concentration C. The analytical alternative is to correlate the same quantities with Huggins' or Kraemer's equation :

$$\frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C, \quad (5)$$

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k''[\eta]^2 C, \quad (6)$$

where k' is the Huggins constant and k" is the Kraemer constant.

For carrageenan resulted in our work, an increase in reduced viscosity was observed as the concentration decreased (Fig. 3). Attempts to determine the [η] by the Huggins's equation failed in this our present work. Other works showed the same trends, such as for hsian-tsao leaf gum [13], xanthan gum and the mixture xanthan gum-locust bean gum [8]. According to Higiro [8] non ionic polysaccharides exhibited linear plots of lower slope, whereas ionic polysaccharides displayed a sharp increase slope. All of the carrageenans type in our work exhibited behaviour of ionic polysaccharides.

Intrinsic viscosity, [η] is a measure of the hydrodynamic volume occupied by a macromolecule, which is closely related to the size and conformation of the macromolecular chains. In dilute solutions by definition, the polimer chains are separated and there is no interaction between them. Therefore, the [η] of polymer in solution depends only the dimension and the molecular weight of polymer chain [7]. In our work, intrinsic viscosity was evaluated by extrapolation of reduced viscosity to the value at zero carrageenan concentration for each different carrageenan type (Fig.3).

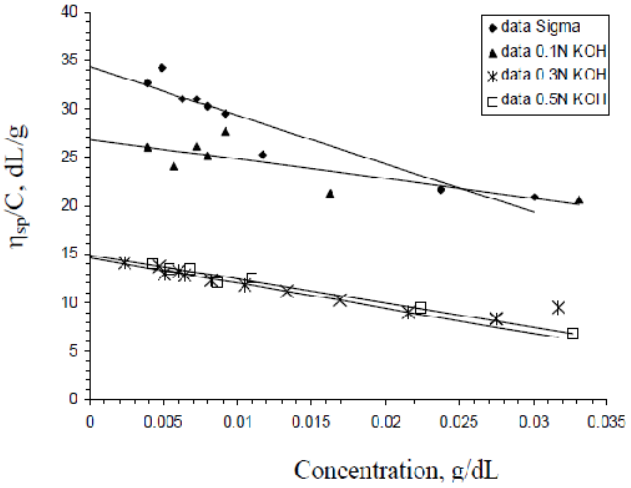

Fig 3 Spesific viscosity/concentration vs concentration for determining intrinsic viscosity at 24.5 – 27°C

It seems that intrinsic viscosity was 34.31 dL/g of Sigma carrageenan, whereas in extracted carrageenan resulted in present work varied. The values of intrinsic viscosity were affected by potassium hydroxide concentration used as the solvent in extraction process. Intrinsic viscosity of 26.84, 14.61, 14.88 dL/g for carrageenan resulted from 0.1N, 0.3N, and 0.5N KOH, respectively (Table I).

The molecular weight (M;g/mol) can be evaluated from obtained intrinsic viscosity ([η]; dL/g) using relation of Mark-Houwink for kappa carrageenan in water at 25°C [18] as the following:

$$[\eta] = 10^{-4}.M^{0.9} \quad (7)$$

The molecular weight of extracted carrageenan was shown in Table I. It was found that with the increasing potassium hydroxide concentration the molecular weight will be decreased. This reduction of molecular weight indicates that the polymer degradation occur when extraction using aqueous alkali. The polymer degradation can be explained by the lower yield obtained (Table I). Nickerson [15] investigated the effect of added ions (potassium and calsium ion) on solution properties of kappa carrageenan, and reported that no significant effect of ionic strength on intrinsic viscosity was found. These findings also support that the variations of intrinsic viscosity value are due to the difference of weight molecule. The decreasing weight molecule means that the polymer degradation occurs when alkali is used as the solvent in the present work.

IV. Conclusion

FTIR spectra showed that extracted carrageenan in this work and Sigma product were kappa carrageenan structure. Pure distilled water was the most efficient solvent with regard to yield but certainly not gel strength. Higher KOH concentration led lower sulfate content, higher gel strength of extracted carrageenan. There was found that alkali extraction caused polymer degradation.

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