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SYNTHESIS OF REFINED CARRAGEENAN FROM EUCHEUMA COTTONII WITH VARIATION OF PRECIPITATING SOLVENT

Ragilia Rahma Maulidia, Siti Nurkhamidah*, Fadlilatul Taufany, Yeni Rahmawati, Fahmi, Wahyu Meka

Chemical Engineering Department of Institut Teknologi Sepuluh Nopember, Kampus ITS Sukolilo, Surabaya 60121, Indonesia.

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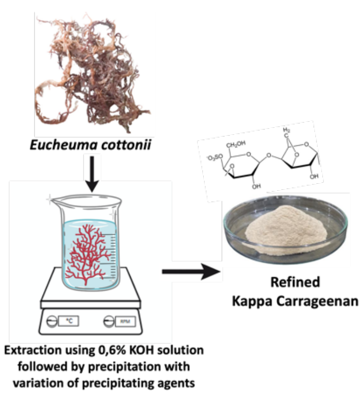
*Corresponding author
nurkhamidah@chem-eng.its.ac.id

Graphical abstract



Abstract

Red seaweed, Euchema cottonii, is a kappa carrageenan source that grows in Indonesia's coastal areas. Carrageenan is one of the polysaccharides composed entirely of D-galactopyranose units. Besides being used in the food industry, carrageenan is also used in the non-food industry, such as in pharmaceutical, cosmetic, printing, and textile formulations. Various studies have been carried out to produce high-quality Carrageenans. However, the extraction process of carrageenan is quite complicated, and relatively energy intensive. The primary step in carrageenan production is extraction, washing, filtration, precipitation, filtering, pressing, drying, and milling. This study aims to optimize the carrageenan extraction process from red seaweed and characterize the physicochemical of the extracted carrageenan. Red seaweed was extracted using 0.6% of KOH, and the ratio between seaweed and KOH solution was 1:20. Extraction temperature was varied at 30ºC and 80ºC, and several precipitants, i.e., 96% alcohol, isopropyl alcohol, and KCl were used. The results showed that the best carrageenan extraction process was at 80 ºC of extraction temperature and using KCl as a precipitating solvent. From these results, the yield, water content, ash content, viscosity, and L value were 21.24%, 12.56%, 22.69%, 310.96 cP, and 97.93, respectively. Based on the Food Agriculture Organization (FAO) standard, the physical properties of carrageenan produced in this study are under the range permitted by this standard. The study of carrageenans by FTIR spectroscopy shows the presence of S=O of sulfate ester; and C-O of 3,6-anhydrogalactose; galactose and D-galactose-4-sulfate and the results indicated that the carrageenan was κ-type.

Keywords: Extraction, precipitation, seaweed, *Eucheuma cottonii*, carrageenan.

1.0 INTRODUCTION

Seaweed is the primary commodity in the marine and fisheries industrialization policy of East Java Province, along with albacore tuna, mackerel tuna, skipjack tuna, shrimp, milkfish, and catfish [1]. According to data [2] total production of seaweed in East Java in 2021 is 688,020 tons, spreading across several districts including Pacitan Regency, Banyuwangi Regency, Situbondo Regency, Pasuruan Regency, Sidoarjo Regency, Sumenep Regency, and Pasuruan City. Sumenep district is the largest seaweed producer in East Java. Seaweed is a promising commodity because of several advantages: high economic value, complete industrial trees, easy cultivation technology, short planting period (only 45 days), and low production cost.

Seaweed is one of the most in-demand species for industrial raw materials both domestic and abroad. Due to high demand, it is necessary to increase the capacity of seaweed farming communities to increase production in the global market. Seaweed productivity continues to increase every year, especially for the international market. According to Badan Pusat Statistika [2], the total amount of exported seaweed in 2021 is 206,185 tons, a 7.2% increased from the previous year.

Seaweed is a member of the algae which is a chlorophyll plant. Seaweeds are grouped into four classes based on the pigments contained, namely *Chlorophyta* (green algae), *Rhodophyta* (red algae), and *Phaeophyta* (brown algae). Some of the high economic value seaweeds found in Indonesia are *Eucheuma spp., Glascillaria sp., Gelidium sp., Sargassum sp., and Hypnea sp*. *Eucheuma cottonii* and *Eucheuma spinosum* are two major species of red algae in Indonesia that have high carrageenan content [3]. The commodity value of seaweed about Rp 6.000/kg or 0.4 USD/kg while for carrageenan up to Rp 125.000/kg or 8.3 USD/kg, taking into account the potential for high profits, it is necessary to utilize seaweed as carrageenan products.

Carrageenan is a compound that belongs to the galactose polysaccharide group extracted from seaweed. Most carrageenan contains sodium, magnesium, and calcium, bound to sulfate ester groups of galactose and 3,6-anhydro-galactose copolymers [4]. Carrageenan is commonly used as a stabilizing, emulsifying, gelling, and thickening agent in food industries [5]. Carrageenan is obtained from several genus of red seaweed, including *Chodrus, Eucheuma,* and *Gigartina*. *Eucheuma cottonii* is the most common species that grows perfectly In Indonesia. Even though seaweed is abundant in Indonesia, the refined carrageenan industry is still rare, with only ± 10 factories across Indonesia. Carrageenan comprises up to 50% weight of dried seaweed. Carrageenan yields depend on seaweed cultivation methods, ages (harvest time), post-harvest handling, including storage and distribution, and extraction methods.

Preparation methods of good quality carrageenan have been developed. Some studies lead to process optimization and quality improvement in acquiring better-quality carrageenan. The optimum process of carrageenan preparation has been reported: Carrageenan can be extracted using strong alkalies or through enzymatic hydrolysis with cellulases [6]. Using 2% KCl solution as a precipitating solvent was optimum for carrageenan precipitation [7]. The best carrageenan quality was extracted using 12% KOH solution at 95°C for 30 minutes [8]. The use of a temperature of 80°C for 30 minutes and 10% KOH concentration is the best condition of the extraction of carrageenan [9].

The main drawback in the seaweed industry was that the carrageenan extraction process is complicated, time-consuming, and relatively energy-intensive. This has hampered the development of the carrageenan industry in Indonesia. Research on the best extraction process still needs to be done, particularly simpler methods aiming for efficient extraction time and low cost on material and energy.

2.0 MATERIALS AND METHODOLOGY

2.1 Materials

The equipment used were a glass beaker, analytical balance, filter press, hot plate, stirrer, erlenmeyer, grinder, thermometer, pH paper, measuring cup, oven, volumetric flask, and sieve. The main raw material used is dried seaweed, *Eucheuma cottonii*. The materials used for carrageenan extraction were KOH, KCl, isopropyl alcohol, 96% alcohol and distilled water. Other materials used for chemical analysis were required for laboratory analysis.

2.2 Methodology

2.1.1 Preparation Of Raw Seaweed

Raw seaweed was prepared by sun drying the fresh seaweed collected from the farm. Sun-dried seaweed was washed using distilled water for two purposes: to remove salt, dirtiness and contaminants. The Maluku seaweed, Indonesia, was used for this study.

2.3 Extraction Of Carrageenan

The 1:20 ratio of alkaline solution was utilized with various precipitation methods. Extraction was carried out with clean seaweed in 0.6% KOH solution for 2 hours at 30 or 80°C, and then it was washed until neutral pH. The extracted seaweed is then separated between the pulp and the filtrate. Then, the filtrate was precipitated using a 2% KCl solution, 100 ml of isopropyl alcohol, and 100 ml of 96% alcohol at 30°C stirred to form carrageenan fibres. The precipitated carrageenan fibers were filtered after soaking for ± 30 minutes. The precipitate formed was pressed. After being pressed, a carrageenan gel was formed. The gel was then dried using an oven. The optimum temperature for the carrageenan drying process is 60°C, with a drying time of 48-72 hours. The dried carrageenan gel is then ground using a grinder resulting in refined carrageenan.

2.4 Chemical And Physical Analysis

Chemical and physical analysis included yield [10], viscosity using NDJ-8S Viscometer [11], water content [10], ash content [10], Fourier Transform Infrared Spectroscopy (FTIR) using Agilent Cary 630 FTIR [12], and colorimetry test using colorimeter WR-10 QC Color Meter [13].

3.0 RESULTS AND DISCUSSION

Carrageenan is a group of galactose polysaccharides extracted from seaweed with an alkaline solution. According to [14], gel formation is a two-stage process. The first stage involves intramolecular conformational changes unaffected by ions. The second stage requires specific ions to create the gel structure by reducing solubility and forming cross-links. The specific cation that is able to influence the formation of the gel in kappa-caraginan is the K+ ion. This ion also acts as a binding agent between the polymer chains of carrageenan by reinforcing the three-dimensional structure, allowing the polymer to maintain its shape when pressure is applied. K+ ions can increase gel strength by increasing ionic strength in the carrageenan polymer chains, resulting in balanced interaction between dissolved ions and ions bound in the carrageenan structure, ultimately forming a gel.

3.1 Yields

Yield is a crucial factor in determining the effectiveness of carrageenan extract processing, as evidenced by comparing the yield of extracted seaweed with that of raw seaweed. The findings indicate that an increased temperature leads to a rise in carrageenan yield as the seaweed can be extracted completely.

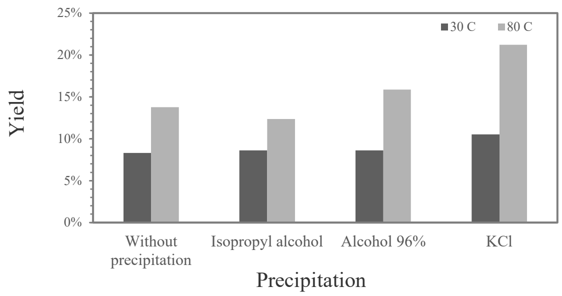


Figure 1 Yield of refined carrageenan with the variation of extraction temperatures and precipitants

The carrageenan yield in this study was determined via weighing carrageenan after the 100 mesh sifting process. The carrageenan yield reported by the FAO ranges between 18 and 35%, while this research reports a yield of 21.24% shown in Figure 1. The yield of carrageenan from KCl precipitation was higher than that of the other studies [15] and [16].

3.2 Viscosity

Viscosity testing determined the carrageenan solution's viscosity level at a particular concentration and temperature. Typically, viscosity is measured at 75°C with a concentration of 1.5% [11].

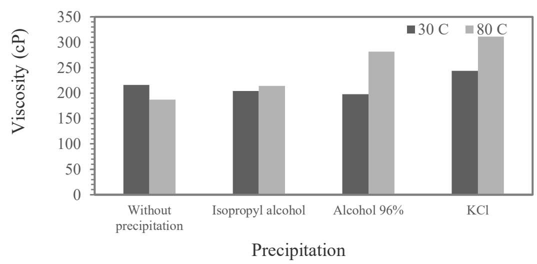


Figure 2 Viscosity of refined carrageenan with the variation of extraction temperatures and precipitants

The carrageenan obtained in this study exhibited a viscosity of 186.86 to 310.96 cP shown in Figure 2, thus meeting the minimum standard viscosity requirement of 5 cP set by FAO. Therefore, the produced carrageenan meets the FAO's suggested standard for viscosity qualification.

3.3 Water Content

The water content of carrageenan significantly impacts the product's shelf life. Water content in food affects the metabolism of enzymes, microbial activity, and chemical activity.

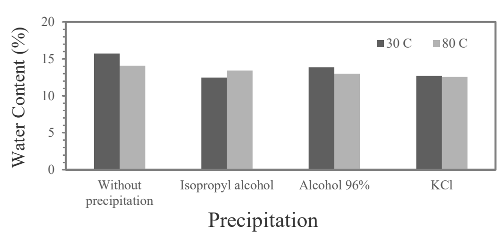


Figure 3 Water content of refined carrageenan with the variation of extraction temperatures and precipitants

According to the commercial quality requirements for carrageenan, the permitted water content should be below 15%. Based on the standard requirements for carrageenan, the water content obtained from Figure 3 was 12.48% to 14.72%. This range satisfies the criteria for standard water content.

3.4 Ash Content

The ash content of the carrageenan was analyzed to determine its mineral content. Minerals can be divided into two categories within materials: organic and inorganic minerals [17]. Seaweeds are known to contain high levels of minerals. The ash content of carrageenan is affected by the salinity of the seawater in which it is cultivated. Ash in carrageenan can be traced back to the hydrocolloid containing ester sulfate, potassium, sodium, calcium, magnesium, and ammonium from galactose and 3,6 anhydro-D-galactose [7]. According to international standards for carrageenan, the acceptable range for ash content is 14-40%. Based on this requirement, the ash content value of the carrageenan produced in this study, shown in Figure 4 is within the range allowed by international standards.

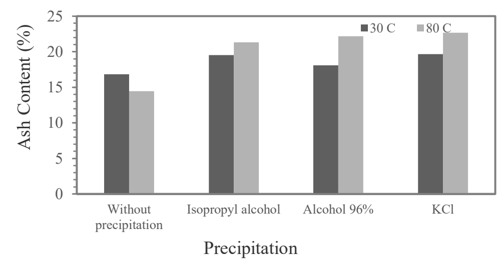
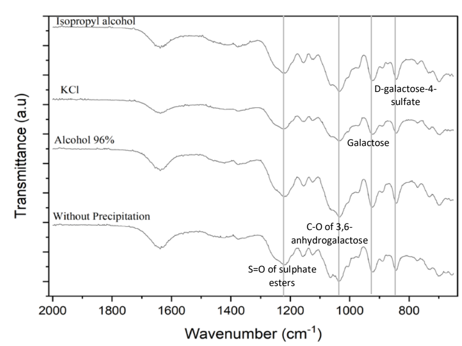


Figure 4 Ash content of refined carrageenan with the variation of extraction temperatures and precipitants

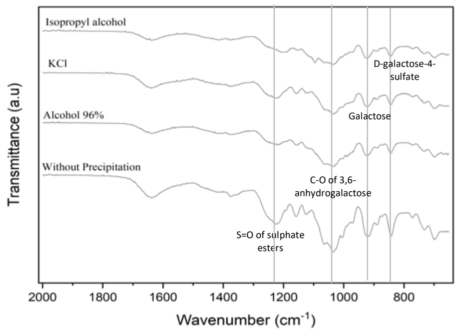
The presence of potassium ions in using KCl in the precipitation process is considered the high ash content of the carrageenan obtained in this study. *Potassium* is a mineral element that does not burn.

3.5 Ftir Analysis

To determine the variation of precipitant type between KCl, 96% alcohol, and isopropyl alcohol on carrageenan, FTIR analysis was conducted.



(a)



(b)

Figure 5 FT-IR analysis of refined carrageenan extracted at (a) 30°C and (b) 80°C with variation of precipitant

Figure 5(a)(b) shows the FTIR spectrum of carrageenan. The study of carrageenans by FTIR spectroscopy shows the presence of absorption bands in Figure 5(a) 1234 cm^{-1} region, Fig. 5(b) 1246 cm^{-1} region (due to the S=O of sulfate ester); higher sulfate ester levels mean lower gel strength. Higher sulfate content also means a higher risk for human consumption. Hence, the sulfate content of carrageenan must be reduced before it is applied to various foods. Based on Figure 5(a), it can be seen that the most negligible sulfate ester content is obtained in the precipitation variation using KCl; based on Figure 5(b), it can be seen that the most negligible sulfate ester content is obtained in the precipitation variation using isopropyl alcohol. Another signal was observed at Figure 5(a) 1069 cm^{-1} region Figure 5 (b) 1071 cm^{-1} region (ascribed to the C-O of 3,6-anhydrogalactose), from Figure 5(a) 970 cm^{-1} region, Figure 5(b) 972 cm^{-1} region (attributed to galactose) and Figure 5 (a) 840 cm^{-1} region, Figure 5 (b) 844 cm^{-1} region (due to the D-galactose-4-sulfate). The former peak, also observed in other studies [18], [19], indicated that the carrageenan was κ-type.

3.6 Colorimerty

The results of data analysis showed that the effect of variation of precipitation with a temperature of 30°C and 80°C significantly differed in the L* value of carrageenan (Table 1). Significant difference in the L* value of carrageenan (Table 1); the highest L value was 97.93 and the lowest L value was 76.64.

Table 1 Effect of variation of precipitation on L value

Temperature	Precipitation	L	a	b
30°C	Without	89.07	6.77	24.54
	KCl	83.78	7.91	27.03
	Alcohol 96%	85.2	7.95	27.48
	Isopropyl alcohol	76.64	9.12	26.43
80°C	Without	96.53	8.48	19.17
	KCl	97.93	8.69	16.98
	Alcohol 96%	96.55	8.38	19.75
	Isopropyl alcohol	95.94	7.74	19.67

Figure 6 Appearances of refined carrageenan

The L* value indicates the brightness of the color with a range of 0 (black) - 100 (white). The higher the L* value, the brighter the color [20]. Based on the results of the colorimetric test, the color of the carrageenan product that is close to the color of commercial carrageenan with an L of 100 is the sample using Precipitation with KCl at a temperature of 80°C and an L value of 97.93.

4.0 CONCLUSION

Precipitation with KCl in 80°C extraction increased carrageenan yield, viscosity, ash content, and water content compared to in 30°C. KCl is the promising precipitant to produced carrageenan compared to another precipitants using in this study. The best result is obtained when the extraction temperature is 80°C and using KCl for the precipitation resulting yield of carrageenan is 21.24 with the following properties: viscosity 310.96 cP, water content 12.56%, ash content 22.69%, L value 97.93. The carrageenan was confirmed to be κ-carrageenan based on the infrared spectrum.

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