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Physiochemical properties of semi refined carrageenan by bleaching pretreatment

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ABSTRACT

The semi-refined carrageenan (SRC) product is the result of extracting red algae from the palm plant. Semi refined carrageenan is one of the elements in Eucheuma cottonii. Carrageenan is a sulfated galactan derived from red algae (Rhodophyta), composed of D-galactose, which is deeply bonded 5-007-1.3 β -1.4 bonds. Extraction conditions affect the quality of semi pure carrageenan products. Extraction conditions such as pretreatment, time, and extraction temperature significantly affect the yield of SRC products. This study aimed to investigate the physicochemical properties of carrageenan extracted from red seaweed, which had been pretreated with physical bleaching. The best results on the variation of extraction time and solvent concentration were at 80°C at 2 hours. The best physicochemical properties of carrageenan are gel strength 715 gram/cm^2, water content 8%, ash content 18%, sulfate content 11%, viscosity seven cP, gel point 47.7°C, melting point 57.8°C, whiteness 61%, and the absence of heavy metals in SRC products. FTIR analysis showed that the extracted SRC consisted mainly of kappa-carrageenan. The atomic absorption spectrometry (AAS) method was used to determine the actual metal content in SRC, and the results met FAO standards.

ABSTRAK

Produk semi refined carrageenan (SRC) merupakan hasil pengekstrakan alga merah dari tumbuhan lontar. Semi refined carrageenan merupakan salah satu unsur dalam Eucheuma cottonii. Karaginan merupakan sulfat galaktan yang berasal dari alga merah (Rhodophyta), tersusun dari D-galaktosa yang terikat secara dalam 5-007-1.3 ikatan β-1.4. Kondisi ekstraksi mempengaruhi kualitas produk karaginan semi murni. Kondisi ekstraksi seperti perlakuan awal, waktu, dan suhu ekstraksi sangat berpengaruh terhadap hasil produk SRC. Penelitian ini bertujuan untuk menyelidiki sifat fisikokimia dari karaginan yang diekstrak dari rumput laut merah yang telah dilakukan perlakuan awal pemutihan fisik. Hasil terbaik pada variasi waktu ekstraksi dan konsentrasi pelarut adalah pada suhu 80°C pada 2 jam. Sifat fisikokimia karaginan yang paling baik adalah kekuatan gel 715 gram/cm^2, kadar air 8%, kadar abu 18%, kadar sulfat 11%, viskositas tujuh cP, titik gel 47.7°C, titik leleh 57.8°C, tingkat putih 61%, dan tidak adanya logam berat dalam produk SRC. Analisis FTIR menunjukkan bahwa SRC yang diekstraksi sebagian besar terdiri dari kappa-karaginan. Metode atomic absorption spectrometry (AAS) digunakan untuk menentukan kadar logam substansial dalam SRC, dan hasilnya memenuhi standar FAO.

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1. Introduction

Indonesia is a large producer of Eucheuma cottonii seaweed, known as red seaweed (Rhodophyceae). *Carrageenan* is a compound extracted from seaweed, one of which is red seaweed. The three main types of carrageenan used in the food industry are kappa, iota, and lambda. Carrageenan is graded according to its substitution pattern for sulfate and 3.6-anhydrous galactose [1]. Semi-fine carrageenan can be in the form of a gel to play a role in the food and medical industry as a stabilizer, ignition, and emulsifier. SRC is a hydrocolloid compound consisting of sodium-potassium ester, magnesium, and potassium sulfate. This study aims to determine the optimum conditions for SRC extraction with variations in temperature and extraction time. NaOH is used in this extraction process because Na^+ can increase viscosity, but the gel strength is low. Therefore, pretreatment and variations in temperature and extraction time are very influential in the extraction of SRC.

Soaking seaweed in pretreatment is very important to improve carrageenan quality and gel strength. Pretreatment is significant to improve carrageenan quality and gel strength. Hydrogen peroxide (H_2O_2) is a powerful oxidizer to degrade polysaccharides, reduce carrageenan molecular weight, and can bleach [2]. Therefore, hydrogen peroxide is also a health risk. Calcium hydroxide was used as a soak in a previous study, considering the reactivity of kappa-carrageenan to calcium (Ca). This spice is produced from calcium oxide and water reaction, which is thought to reduce sulfate levels in carrageenan. Molecules of CaO will immediately bind to water molecules (H_2O), which will form calcium hydroxide. However, only immersing in the process will not improve the color of the SRC product. Research on physicochemical properties with physical treatment combined with a chemical treatment to reduce product bleaching has not been carried out by many researchers.

2. Research Methodology

2.1. Alkaline Extraction of Seaweed

Seaweeds of E. cottonii type were obtained from Lontar Beach, Banten. Seaweed (200g) was extracted with extracting agents (1000 ml) KOH 9 %. The seaweed was then pretreated by washed under running tap water, placed in a closed container for three days, soaked with $Ca(OH)_3$ for 3 hours, clean with tap water, and dried. Seaweed was dried until a constant weight was reached. The seaweed was extracted using three different temperatures 60, 70, 80°C and extraction time for 1 and 2 hours. Extraction was performed at the specified temperature using a water bath, neutralized with washed using aquadest until PH 7 seaweed was dried in the oven at 60°C for 9 hours until the water content was below 12% [3]. Dried SRC was then milled to determine the quality of carrageenan content.

2.2. SRC Properties Analysis

The yield was calculated by dividing the carrageenan by the dry weight of the algae. Testing machine MPY (viscometer Brookfield measured PA-104-30) used for analyzed the strength of the gel. Viscosity and gel strength testing referring to FMC Corp (1977) and FTIR spectrometer (Perkin Elmer Spectrum 2000, USA) was used for the analysis of the SRC sample (wavelength region, 650-1400 cm^{-1}). The moisture, ash, and acid-insoluble ash content were measured using the gravimetric method (AOAC, 1990). Color measurements have been made using the Chromameter CR-300 (Minolta Camera, Co. Japan). The melting point used Marine Colloids (1984) methods. Heavy metals are measured using AAS (atomic absorption spectrophotometry) and scanning electron microscopy (SEM) Carl Zeiss Group German used to measured microstructural semi-refined carrageenan.

3. Results and Discussion

3.1. Initial Treatment

The initial treatment affects the color difference of the resulting product. The first treatment was soaking with $CaOH_2$ for 5 hours. The second treatment was the seaweed was put in a closed container for three days, followed by soaking with CaOH. The first treatment produces a dark color, and the second treatment produces a milky white color. The physical treatment reduced the pigment phycoerythrin, salt, and moss.

3.2. Moisture Content

Carrageenan moisture content is significant to extend the shelf life and durability of the material. Table 1 shows the average value of water content in the study ranging from 2-8%. The moisture content of all carrageenan samples met FAO standards. The moisture content of semi-fine carrageenan is affected by the extraction temperature, which can be seen in Table 1. The higher the extraction temperature, the more lumps in the SRC solution. Since kappa-carrageenan will secure the gelling in the potassium salt solution at a higher temperature [4-5] when agglomeration occurs, the process will reduce the surface area. As a result, there is still water trapped in the SRC. The long extraction time will reduce the water content due to the hydrolysis of the cellulose polymer in the carrageenan, which causes the molecular chains to be shorter. Shorter molecular chains will facilitate the drying process because shorter molecular chains will reduce the water content of SRC.

Table 1. Effect of temperature and time on moisture content.

Variation	Research result	Commercials standard
60°C 1 h	2.0	< 12
70°C 1 h	7.7	
80°C 1 h	8.2	
60°C 2 h	2.0	
70°C 2 h	4.9	
80°C 2 h	8.0	

Ash content is used to determine the mineral concentrations of residual inorganic components. The research showed in Table 2 that ash content affects temperature fluctuation. The ash concentration varies between 15% and 18%. According to the standardization of commercial quality, the maximum ash content required is 15-40% [6]. The average outcome of the study indicates that various temperature treatments have affected the ash content. As can be observed, the greater the temperature, the more ash is produced. Increased extraction time prolongs the contact duration between the K^+ cation in potassium hydroxide and carrageenan. It will enhance the cation K^+'s entry into seaweed through the cell wall. Ash content is indicated the amount of potassium salt contained in SRC that's important for gel strength in SRC [7].

Table 2. Effect of temperature and time on carrageenan yield.

Variation	Result	Commercial standard
60 ^{0}C 1 Hour	27,5	
70 ^{0}C 1 Hour	39,0	
80 ^{0}C 1 Hour	40,0	>25%
60 ^{0}C 2 Hour	24,5	
70 ^{0}C 2 Hour	35,0	
80 0C 2 Hour	39,0	

The yield was determined by dividing the carrageenan weight by the dry weight of the seaweed. The yield estimation of the SRC is based on the commodity's dry weight, which means that the yield reflects the efficiency value of the processing process. High temperature also increases solvent solubility and can expand the solids' pores so that when the KOH solvent passes through the orifice and dissolves, the sulfate is retained in the solids.

3.3. Sulfate Content

This research showed in Table 3 a higher temperature decreases in sulfate content. The decline in sulfate content has affected the properties of the product. The material's sulfate content is significant in the 3.6 anhydrous-galactose productions and raises the low sulfate content of 3.6 anhydrous-galactose. Enhanced anhydrous galactose 3.6 increases the gel strength value [8]. The sulfate content produced attractive forces between negative charges sulfate groups, such that the polymer chains were stiffened and taut, resulting in increased viscosity. Molecular chains are strains due to the hydrophilic properties surrounded by a water molecule that has caused an increase in thickness [9]. The sulfate content of all carrageenan samples met the FAO standard of 15-40 percent.

Table 3. Impact of temperature and time on carrageenan sulfate content.

Variation	Sulfate content (%)	Commercial standard
60ºC 1 h	28.3	Max 30
70ºC 1 h	13.3	
80ºC 1 h	11.2	
60ºC 2 h	29.6	
70ºC 2 h	19.2	
80ºC 2 h	13.3	

3.4. Gelling point

The gel's temperature is the solvent's temperature that starts to form the gel at a given concentration. Carrageenan may form a gel reversibly, which indicates that a gel is formed when it is cooled and melts back once it is heated. The findings showed in Table 4 that higher temperatures would make it more accessible for KOH to minimize sulfate in algae. The temperature gelling point is directly related to the 3.6-anhydrous-galactose content and inversely related to the sulfate content [10]. The existence of sulfate appears to cause a temperature-shaped polymer that causes the gelling point's high temperature.

Table 4. Effect of temperature and time on the carrageenan gelling point.

Variation	Result	Commercial standard
60ºC 1 h	30	
70ºC 1 h	47,7	
80ºC 1 h	47,0	Max 30
60ºC 2 h	23,0	
70ºC 2 h	24,0	
80ºC 2 h	26,0	

3.5. Melting Point

The melting point is the temperature of the aqueous solution, which melts at a specific concentration. This study's results have shown that the type of ion compound bonds affected the carrageenan's melting point. It is known that the ion compound properties have a high melting point, both stable and soluble in polar solvents. The form of seaweed structure without extraction is thick sheets with melting points higher than 80°C compared to Figure 1(b) that showed granulated structure whit melting point 60-80°C. The melting point of carrageenan samples met the commercial standard with a minimum melting point of 50,21°C can be seen in Table 5 [11].

Table 5. Effect of temperature and extraction time against melting point.

Variation	Research result	Commercial standard
60°C 1 h	59.3	50
70°C 1 h	61.2	
80°C 1 h	57.8	
60°C 2 h	57.8	
70°C 2 h	59.2	
80°C 2 h	60.4	

3.6. Viscosity

On the other hand, a 1-hour extraction procedure at 80°C results in the lowest viscosity. The longer the extraction period, the more damage is done. A saturated solution of KOH is used to remove sulfate from seaweed. Since Ion K+ may replace sulfate groups, the reduction in sulfate levels led to the formation of 3.6-anhydrous-D-galactose bonds [12].

Table 6. Effect of temperature and extraction time against viscosity.

Variation	Research result	Commercial standard
60°C 1 h	21.9	Min 5
70°C 1 h	11.4	
80°C 1 h	7.1	
60°C 2 h	73.8	
70°C 2 h	18.5	
80°C 2 h	36.9	

3.7. Gel Strength

Gel strength is the main physical characteristic of carrageenan. The gel strength demonstrates the ability of carrageenan to form a gel. The effectiveness of the gel is impaired by sulfate content and 3.6-anhydrous-D-galactose. According to [8], the sulfate content value is directly related to viscosity and inversely related to the gelling power. The lower the carrageenan sulfate content, the higher the gel strength. The use of KOH as a solvent in the extraction process can increase gel stress. Carrageenan can bind with ion K^+ that will increase iconic strength in polymer bonds. It could be dissolved inter-molecular force is increased, which causes the balance between the dissolved ions and the ions bound in the carrageenan structure to form a gel. Increasing extraction time will decrease gel strength because the longer time it will optimize the extraction showed in Table 7. Still, also it would lead to degradation of the carrageenan that will reduce the gel strength. The gel strength of carrageenan samples met the commercial standard with minimum gel strength of 685.5 gram/cm^2.

Table 7. Effect of temperature and extraction time against gel strength

Variation	Research result	Commercial standard
60°C 1 h	689.0	685.5
70°C 1 h	702.0	
80°C 1 h	715.0	
60°C 2 h	685.0	
70°C 2 h	687.0	
80°C 2 h	708.0	

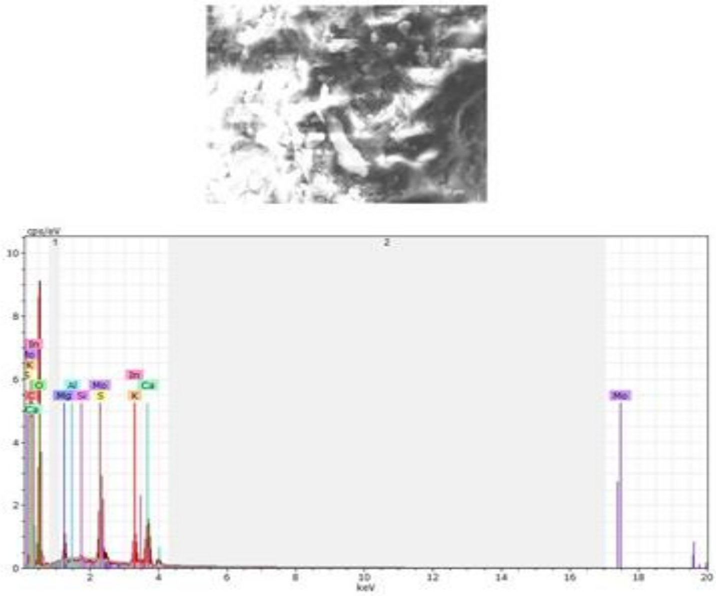
3.8. Color

Figure 1 shows the result of SRC after drying. The red algae have a red pigment dominated by phycoerythrin. In this study, we choose the bright color of SRC to measure the brightness with the chroma meter CR-300 (Minolta Camera, Co. Japan). The best result is at one-hour extraction at 80°C, whit with a degree of white is 63.3 % the standard of brightness in SRC is 60%.

Figure 1. Dried semi refined carrageenan.

3.9. Heavy Metal Content

SRC with the best quality in SRC was selected based on the previous process, namely SRC, which was extracted for 1 hour at 80°C for further testing. The results of AAS can be seen in Table 8 and Figure 2, in which AAS did not detect heavy metals.



Spectrum: Acquisition

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Carbon	K-series	31.55	31.67	41.02	4.89
Oxygen	K-series	55.63	55.85	54.30	7.80
Magnesium	K-series	0.92	0.92	0.59	0.09
Aluminium	K-series	0.00	0.00	0.00	0.00
Silicon	K-series	0.07	0.08	0.04	0.03
Sulfur	K-series	2.87	2.88	1.40	0.14
Potassium	K-series	1.65	1.66	0.66	0.09
Calcium	K-series	4.00	4.02	1.56	0.17
Molybdenum	L-series	1.49	1.50	0.24	0.10
Indium	L-series	1.42	1.42	0.19	0.09
	Total:	99.60	100.00	100.00	

Figure 2. AAS image of semi refined carrageenan.

3.10. Morphology of Microscopic Structures

SEM was used to observe the morphology of the microscopic structure of semi-fine carrageenan, and it can be seen in Figure 3. Figure 3(a) shows the results of the untreated process. The figure shows a sheet-like structure of seaweed and shows the product is less stable and the gel strength is small. Figure 3(b) shows the microstructure that looks rough and hard, while Figure 3(c) shows the SRC structure that is not sharp, and there are holes of 2-7 micrometers. Mineral calcium levels decreased where cottonii seaweed before treatment had Ca 29.9% then decreased 4% to 25.9%. While other compounds with details of 31.55% carbon content, 55.63% oxygen, 0.92% Mg, 0.07% silicon, 2.87% sulfur, 1.65% potassium, 1.49% molybdenum, and indium. 1.42%.

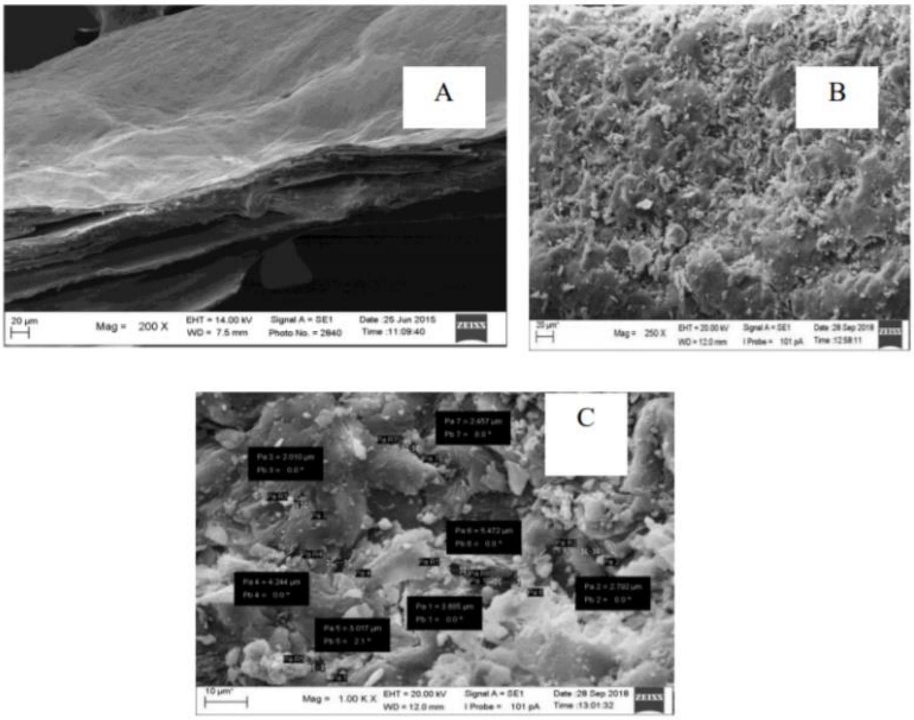


Figure 3. SEM images (a) Seaweed without extraction (b) Extraction 1 hour 80°C magnification 250x (c) Extraction 1 hour 80°C magnification 1000x.

3.11. FTIR Analysis

FTIR analysis is an analysis used to determine the functional groups of a chemical compound shown in Figure 4. Based on the FTIR test chart of Figure 4, the Kappa FTIR spectra are carrageenan commercial shows that there is a strong absorption at the number 1232.51cm^{-1} and 1068.56 cm^{-1}, narrow absorption at 929.69 cm^{-1} and 846.75 cm^{-1}. FTIR analysis shows the detection of sulfate esters, galactose-4-sulfate groups, 3.6-anhydrous galactose, and glycosidic. The presence of sulfate ester, galactose, and 3.6-anhydrous galactose group are characteristics of carrageenan kappa [13]. Based on that, it can be stated that the commercial carrageenan used in this study is a type of carrageenan kappa.

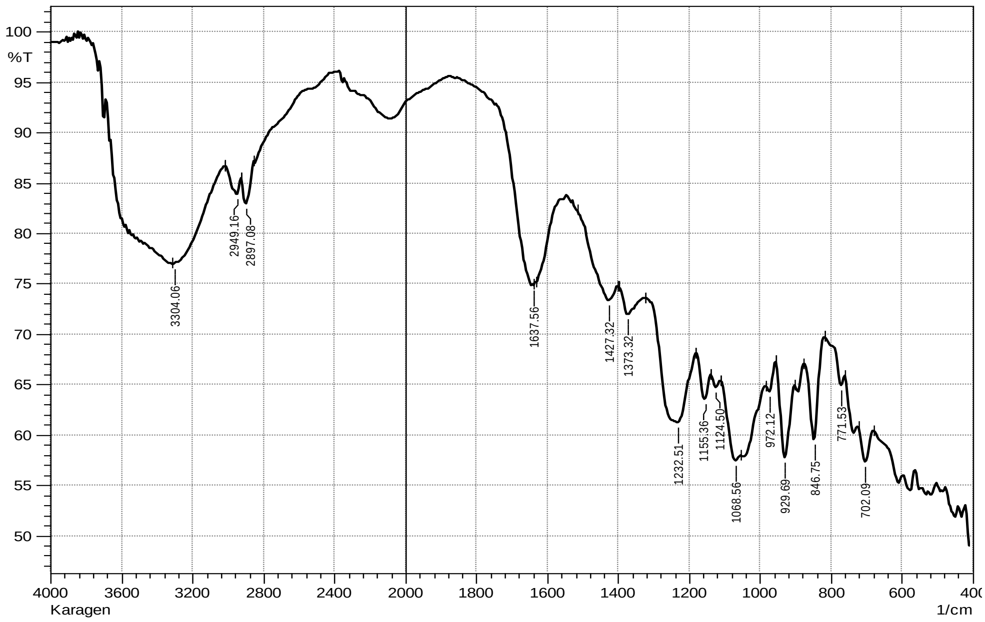


Figure 4. FTIR semi refined carrageenan.

4. Conclusions

Based on the study results, it can be concluded that the temperature of extraction is 80ºC and the time 1-hour extractions. The best quality of semi-refined carrageenan by producing a gel strength of 715 gram/cm^2, moisture content of 8%, ash content 18%, sulfate content 11%, a viscosity of 7 cP, gelling point 47.7ºC, a melting point of 57.8ºC, a white degree of 61%, and the absence of heavy metals in SRC products.

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