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CHARACTERISTICS OF REFINED CARRAGEENAN FROM *Eucheuma spinosum* WITH POTASSIUM HYDROXIDE SOLVENT USING MICROWAVE ASSISTED EXTRACTION (MAE) METHOD IN VARIOUS EXTRACTION TIMES

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Abstract

Extraction time with Microwave Assisted Extraction (MAE) influences the yield and quality of Carrageenan from *Eucheuma spinosum*. This research aims to determine the effect of extraction time using microwave-assisted extraction (MAE) on the yield value and quality parameters of *E. spinosum* carrageenan, including water content, ash content, sulfate content and viscosity. *E. spiosum* was extracted using 0.5% NaOH base with the microwave-assisted extraction (MAE) method at varying extraction times of 2 minutes, 5 minutes and 8 minutes. The filtrate was filtered and precipitated with ethanol, drying and grinding until a fine carrageenan powder was obtained. Extraction time affected the yield where an increase in the average yield was received along with the length of extraction time. The highest yield in the 8-minute extraction was 17.62% ±0.368. Extraction time also influences the water content and viscosity of the obtained carrageenan but does not affect the extracted carrageenan's ash or sulfate content.

Keywords: carrageenan, *eucheuma spinosum*, extraction time, *microwave-assisted extraction* (MAE)

Introduction

Carrageenan is a product derived from seaweed that increases market demand yearly. In 2018, demand for seaweed increased by 8.4 percent from the previous year, with global demand totalling US$1,131,820,000.[1] The world market value of carrageenan in 2019 increased to 864.3 million US dollars. The prevalence of carrageenan is estimated to increase by 5.45% per year (2019-2024) with an estimated transaction of $1.12 billion by 2024.[2] This numbers only placed Indonesia in 2014 as a supplier of carrageenan in the world market in the 9th position, defeated by China in first place and the Philippines in second place, followed by the Netherlands, Germany, Spain, United States, India, and France.[3]

Carrageenan is a complex polysaccharide that is extracted from certain red algae that are composed of the repetition of galactose and 3.6-anhydrous-galactose units that bind to sulphate groups.[4] This natural polymer can form a gel in a thermo-reversible or viscous solution if it is added to a saline solution, so it is widely utilized as a gel-forming, thickening, and stabilizing material in various industries such as food, pharmaceutical,

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cosmetics, printing, and textiles.[5] In addition, it also serves as the former film (thin-layer forming), syneresis inhibitor (prevention of water release), and flocculating agent (fastener agent).[6]

Based on the sulfate group, substituents in each monomer, carrageenan can be divided into several types, including those that have high economic value, namely the type kappa- (κ), Iota- (ι) and lambda- (λ).[5] Iota (ι) carrageenan is characterized by the presence of 4-sulfate esters in each D-glucose residue and 2-sulfate ester groups in each 3,6-anhydro-D-galactose group, this type of carrageenan is produced from red algae *E. spinosum* which has sulfate content more than 30% with the ability to form an elastic gel with calcium salt, clear in colour without releasing liquid, and stable in a frozen or heated state.[7]

Generally, carrageenan is categorized into two types: refined Carrageenan and semi-refined Carrageenan. In Indonesia, the semi-refined Carrageenan industry is more popular than refined carrageenan. The difference between these two types of carrageenan lies in the cellulose content and solubility.[8] Compared to semi-refined carrageenan with a cellulose content of 8-15%, Refined Carrageenan has a lower cellulose content of <2%, so it has the advantage of being a water-soluble linear polymer that is suitable as a drug delivery system in drug formulas. Manufacturing semi-refined carrageenan does not undergo a precipitation phase using alcohol or KCl, so the resulting gel or solution is more rugged than refined carrageenan.[9]

Separating carrageenan fibres with their solvents can be done with KCl or alcohol. In terms of economics, the method of blocking with KCl is cheaper, but in terms of quality, the deposition method with alcohol produces the best quality.[10] Carrageenan extraction is generally carried out by conventional heating, which takes 2-4 hours to achieve optimal extraction results. Nowadays, there are disadvantages to the traditional carrageenan extraction, such as time and high energy. Another disadvantage is that consuming large amounts of water and solvents is less economical.[11]

One of the applications of "green extraction methods" innovation is microwave-assisted extraction (MAE). Microwave-assisted extraction (MAE) utilizes the irradiation of microwaves in the heating process, which has advantages such as short extraction time, fewer solvent usage, higher extraction yield and lower costs compared to traditional extraction methods.[12] Extraction of *Kappaphycus alvarezii* and *E. spinosum* using microwave successfully acquired Kappa-and iota-Carrageenan without further purification procedures existing in conventional extraction procedures.[13] While research conducted by Navarro[14] has succeeded in extracting 3.6-anhydrogalactose from seaweed *Iridaea undulosa* and *Porphyra Columbina* at various times of microwave heating in a range of 15 seconds to 10 minutes and obtained from different 3.6-anhydrogalactose percentage of results.

Based on the description above, the researcher wants to know the effect of using microwave-assisted extraction (MAE) on carrageenan extraction using NaOH base by separating carrageenan fibre using ethanol in various extraction times, which are 2 minutes, 5 minutes and 8 minutes on carrageenan results and quality parameters of *E. spinosum* carrageenan. The results include the analysis of water content, ash content, sulfate content, and viscosity following the requirements of the Food Agriculture Organization (FAO), Food Chemicals Codex (FCC) and European Economic Community (EEC).

Method

Tool

The tools used were a microwave-special-glass container, Sharp low-wattage microwave R-230R (S), Severin SM3736 hand blender, beaker glass, erlenmeyer, measuring cup, filter, round-bottom flask, condenser, water bath, ash-free filter paper (Whatman No. 42), porcelain cup, oven, crucial pliers, electric furnace FB1410M-33 (Thermo), desiccator, BEL Engineering i-Thermo Touch 163M Moisture Analyzer, Brookfield Dial Reading Viscometers Model RVT.

Material

The materials used were seaweed *E. spinosum*, Aqua dest, Sodium hydroxide (NaOH), Ethanol (C2H5OH), Hydrogen chloride (HCl), Hydrogen peroxide (H2O2), Barium chloride (BaCl2).

Procedure

Sample Preparation

E. spinosum samples were obtained from Poteran, Sumenep, and Madura. Samples were cleaned using flowing fresh water to remove residual salt, dirt and sand, then drained and dried using an oven at 40^{o}C until a dry seaweed was obtained, classified by its constant weight.

E. spinosum dried seaweed that has been cleaned and weighed as 100g (each extraction treatment). Chop and soak it in aqua dest for 24 hours to soften the seaweed texture. The ratio of seaweed to aquadest used to soak seaweed is 1:50. *E. spinosum* marinade was then destroyed using a blender and carried out to the extraction stage.

Carrageenan Extraction

The sample preparation results were extracted using 0.5% NaOH base solution with a ratio of seaweed with a solvent of 1:10 placed in a special microwave-special-glass container. Extraction was carried out 3 times, with each having a different time, 2 minutes, 5 minutes, and 8 minutes, with 3 repetitions at each extraction time. The extraction results from each other were then filtered in a hot state to prevent gel formation. The filtrate obtained was then subjected to the deposition process.

Next was precipitation. This was done by adding ethanol in the filtrate ratio of ethanol (1: 2) and stirring for about 15 minutes until the carrageenan fibre precipitate is obtained. The precipitate obtained was then filtered and dried using an oven at 60^{o}C until a constant weight was achieved. After it dried, grind it until carrageenan powder was obtained and sieved with a mesh-60 sieve. The fine powder obtained was then weighed to find out the yield and tested to determine the quality of the carrageenan.

Carrageenan Yield

The yield analysis was done by comparing the weight of carrageenan flour obtained with the weight of the dried seaweed. The yield was calculated using the formula:

$$Yield = \frac{Weight\ of\ dried\ carrageenan}{Weight\ of\ dried\ seaweed} \text{ x } 100\ \%$$

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Parameters of Carrageenan Quality

Water Content

A total of 2 grams of carrageenan fine powder samples were weighed into an aluminium pan on the BEL Engineering i-Thermo Touch 163M Moisture Analyzer. The aluminium pan on the previous device had to be cleaned and automatically tasted. The temperature used for the analysis is 105 ^{o}C. The tool would automatically stop if a constant weight had been achieved. The valid water content value would be taken from the number shown in the instrument.[15]

Ash Content

A total of 2 grams of carrageenan samples were weighed in porcelain cups (with constant weights) and were put in an electric furnace at 550 ^{o}C until the ash was completely formed.[16] Ash content was calculated using the following formula:

$$\textit{Ash Content} = \frac{W1}{W2} \text{ x } 100\ \%$$

W1 = weight of the sample before ash formed (g)
W2 = weight of the sample after ash formed (g)

Sulfate Content

Sulfate contents were determined by hydrolyzing carrageenan and precipitating the sulfate as BaSO4. First, weigh accurately 1g of sample (W1). Transfer the sample to a 100ml long-neck-round-bottom flask, then add 50ml 0.2N HCl. Attach the condenser (preferably one with 5 condensation balls) to the flask and reflux for 1 hour. After 1 hour, add 25 ml of 10% H2O2, continue reflux for 5 hours, and get a clear solution.

Transfer the solution to the beaker glass, add 10 ml of BaCl2 10% dropwise and heat it in a water bath for 2 hours. Filter the precipitate formed using ash-free filter paper (Whatman No. 42) and wash it with distillate water until it is chloride-free. The filter paper was dried and grayed at 800 ^{o}C until white ash was obtained. Ash was then cooled in a desiccator and weighed until a constant weight (W2) was obtained.[17] Sulfate contents were calculated based on the following equation:

$$(W2/W1)\ X\ 100\ X\ 0.4116$$

W1 = Initial sample weight (g)
W2 = Weight of deposited BaSO4 ash result (g)
0.4116 = relative atomic mass of SO4 divided by the relative atomic mass of BaSO4

Viscosity

Carrageenan solution with a concentration of 1.5% was heated in a water bath while stirring constantly until it reached a temperature of 80°C. Add distillate water if volume loss occurs due to evaporation. Then, reduce the temperature of the solution to reach 76-77°C. Viscosity was measured using the Brookfield Dial Reading Viscometers Model RVT, and the temperature of the solution reached 75°C. Reading for the instrument would just be conducted after 6 spins out for Spindle LV no 01. At 30 rpm, read the scale on 0- 100. The measured viscosity had a poise value (1 poise = 100 centipoise).[17]

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Data Analysis

Data on carrageenan quality parameters, including water content, ash content, sulfate content and viscosity from the extraction results, were analyzed using Kolmogorov-Smirnov to determine data normality and the *Leveve test* to determine data homogeneity.

If the data were normal and homogeneous, the parametric test would be conducted using the ANOVA (Analysis of Variance) test to determine whether or not there were differences in the results of each treatment of the *E. spinosum* carrageenan extraction using microwave-assisted extraction (MAE) of the carrageenan quality parameters. If the data obtained were not normal and not homogeneous, then non-parametric statistical tests would be performed with the Kruskal-Wallis test.

Result

Table 1. Carrageenan Physical Quality

Organoleptic Observation	Extraction Time		
	2 Minutes	5 Minutes	8 Minutes
Shape	Soft powder	Soft powder	Soft powder
Colour	Brownish-yellow	Brownish-yellow	Brownish-yellow
Smell	No Smell	No Smell	No Smell
Taste	No Taste	No Taste	No Taste

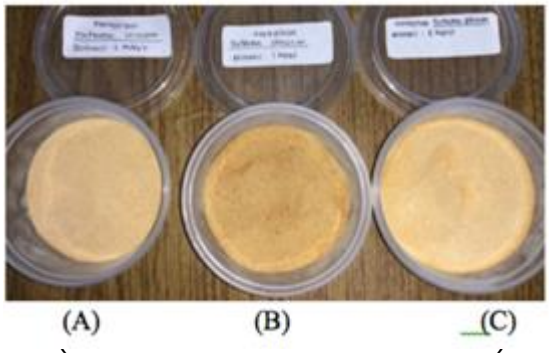

Figure 1. Carrageenan of extraction results
Information:
(A) Carrageenan of 2-Minute Extraction Results
(B) Carrageenan of 5-minute Extraction Results
(C) Carrageenan of 8 Minute Extraction Results

Yield

Table 2. Carrageenan Yield

Extraction Time	Repetition	Carrageenan Yield (%)	Average ± SD
2 Minutes	1	9,61	8,86% ± 1,06
	2	8,10	
5 Minutes	1	10,00	10,27% ± 0,38
	2	10,54	
8 Minutes	1	17,36	17.41% ± 0,07
	2	17,47	

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Parameters for the Quality of Carrageenan

Water Content

Table 3. Carrageenan Water Content

Extraction Time	Repetition	Water Content(%)	Average ± SD
2 Minutes	1	7,57	7,62% ± 0,064
	2	7,59	
	3	7,69	
5 Minutes	1	6,28	6,33% ± 0,278
	2	6,08	
	3	6,63	
8 Minutes	1	6,09	6,21% ± 0,185
	2	6,42	
	3	6,11	

Ash Contents

Table 4. Ash Carrageenan Content

Extraction Time	Repetition	Ash Contents (%)	Average ± SD
2 Minutes	1	27,93	27,61% ± 0,343
	2	27,66	
	3	27,25	
5 Minutes	1	30,04	30,10% ± 0,185
	2	30,31	
	3	29,95	
8 Minutes	1	26,48	27,62% ± 0,997
	2	28,31	
	3	28,07	

Sulfate Contents

Table 5. Data on Carrageenan Sulfate Contents

Extraction Time	Repetition	Sulfate Contents(%)	Average ± SD
2 Minutes	1	21,15	21,06% ± 0,449
	2	21,45	
	3	20,57	
5 Minutes	1	20,60	20.99% ± 0,432
	2	20,92	
	3	21,46	
8 Minutes	1	21,30	20,71% ± 0,686
	2	20,88	
	3	19,96	

Viscosity

Table 6. Carrageenan Viscosity Data

Extraction Time	Repetition	Viscosity (mPa˙s)	Average ± SD
2 Minutes	1	177	176,33 mPa˙s ± 1,15
	2	177	
	3	175	
5 Minutes	1	126	125,66 mPa˙s ± 2,51
	2	128	
	3	123	

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Table 6. (Extension)

Extraction Time	Repetition	Viscosity (mPa˙s)	Average ± SD
8 Minutes	1	121	120,66 mPa˙s ± 0,57
	2	120	
	3	121	

Discussion

Carrageenan physical quality was one of the initial standards for measuring the acceptance of a product. The acceptance quality parameters observed organoleptically include shape, colour, smell and taste.[18] Overall, as seen in Table 1 and Figure 1, from the extraction results in this study, good carrageenan had been obtained at the extraction time of 2 minutes, 5 minutes and 8 minutes, which gave results under the standard; fine powder form, brownish yellow, no smell and no taste.[17]

In this study, the average yield was 86% ± 1,06 at 2 minutes extraction, 10,27% ± 0,38 at 5 minutes extraction, and 17,41% ± 0,07 at 8 minutes extraction. There was an increase in the average value of yield as the extraction time increased. The highest average value of carrageenan was obtained at extraction in 8 minutes see Table 2. Anova test data analysis results obtained a significance value of 0.001 where $p < 0.05$ so that H0 was rejected and H1 was accepted, meaning there were significant differences from the three extraction times. The LSD follow-up test also showed that there was a difference in yield of 8-minute extraction from 5- and 2-minute extraction. It can be seen that the extraction time using microwave-assisted extraction (MAE) in this study had an effect where the more prolonged the extraction time, the higher the yield result, where the highest yield was obtained with an optimal extraction time of 8 minutes.

This can be caused by the longer extraction time, which causes the extraction process to be more perfect where the contact time of the solvent penetrates the cell wall and enters the seaweed cell cavity so that there is more carrageenan dissolved in the extraction process.[19]

The microwave heating factor also affected carrageenan yield according to an experiment conducted by Andhiarto[20] where the results of optimization of the E. spinosum carrageenan yield were higher in microwave extraction than conventional heating. Microwaves can cause molecules in seaweed to collide, push, and spin from within seaweed, making it easier for NaOH-extracting solvents to dissolve carrageenan. Research from Ega[21] also stated that the longer the extraction process, the greater the heating effect caused, so it can maximize the permeability of the *E. spinosum* cell wall, which was able to increase the rate of solvent diffusion towards the *E. spinosum* cells and in the end, the extraction process was speeded up.

The average value of water content obtained in this study ranged from 6.21% to 7.62% see Table 3. The value followed the standard quality specifications set by the Food Agriculture Organization (FAO), Food Chemicals Codex (FCC), and the European Economic Community (ECC) and was 12% at maximum.[22] The average value of the water content was smaller than the research conducted by Desiana[7], where the water content of carrageenan *E. spinosum* obtained from Sumenep waters extracted by conventional heating was 11.09%. The smaller the value of water content obtained, the better the quality of the Carrageenan.[23]

The decrease in the average value of carrageenan water content with increasing extraction time respectively was 7.62% at extraction time of 2 minutes, 6.33% at 5 minutes, and 6.21% at 8 minutes. The statistical analysis test found that the significance value of Anova was 0,000, which meant that there was a significant difference between the extraction time and carrageenan water content. The LSD follow-up test also showed a difference in water content of 2 minutes extraction time, 5 minutes and 8 minutes extraction time. It can be said that the extraction time using microwave-assisted

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extraction (MAE) in this study affected carrageenan water content, where the longer the extraction time, the bigger the decrease in the value of water content where the optimal water content value obtained at the time of 8 minutes extraction with a value of 6.21%.

This was likely to occur due to the tendency of more prolonged contact of samples with added NaOH base solvents, increasing the extraction process[7] because the alkaline atmosphere of NaOH solution can inhibit the binding of water in the seaweed molecule of *E. spinosum* so that the water content was reduced.[21]

Ash content was associated with minerals of a material. The value of ash content was based on weighing the remaining minerals due to the combustion of organic matter.[24] The value of ash content obtained from this study was around 27.61% to 30.10% see Table 4. These results indicated that the ash content obtained meets the carrageenan quality standards set by FAO, WHO[17] was 15 - 40% and a maximum FFC of 35%. The results of the Kruskal Wallis statistical test of ash content obtained a significance value of 0.061, which meant there was no significant difference in carrageenan extraction time from the value of ash content. Therefore, it can be seen that the extraction time using Microwave Assisted Extraction (MAE) in this study did not affect the value of carrageenan ash contents obtained.

The value of ash content obtained in this study approached the research conducted by Diharmi,[25] which was 26.32%, and 2017 amounted to 29.57%, where the *E. spinosum* samples obtained were from Sumenep, Madura waters as used in this study. High ash content in carrageenan can be caused by carrageenan containing minerals such as potassium, sodium, calcium, and magnesium.[20] The process of washing the initial sample was a factor that might affect the value of ash content because there were still mineral salts attached to the surface of *E. spinosum* and also possible by the mineral content of tap water used during the washing process.[26] Another factor that can affect the value of ash content is the location and age of seaweed harvest. Plantations were associated with salinity, where high salinity caused the seaweed to contain a lot of mineral salts.[10] While The older the harvest, the more the ash content increases because the longer seaweed is in the water, the more mineral salts are absorbed by seaweed.[27] Research conducted by Damayanti[28] stated that the physical and chemical properties of carrageenan *E. spinosum* met the export standards and quality standards set by FAO, FCC and EEC on seaweed samples 45 days old after planting.

Sulfate contents were parameters used for various polysaccharides found in red algae. It had been reported in previous studies that the acquisition of Iota-type carrageenan sulfate contents in the same seaweed species was 21.822%,[19] 27.76%,[29] 30.74%.[25] In this study, the value of sulfate contents obtained ranged from 20.71% to 21.06% see table 5 and followed the quality standard requirements of carrageenan sulfate contents of 15-40% set by the FAO, FFC and EEC.

Anova's statistical analysis of sulfate content in this study had a significance value of 0.718, which stated that there was no difference in the value of sulfate content with carrageenan extraction time. Still, a graph showed decreasing sequential sulfate content values of 21.06% at 2 minutes of extraction, 20.99% at 5 minutes and 20.71% at 8 minutes. Long extraction time can reduce carrageenan sulfate contents.[21] However, in this study, it was known that the extraction time using microwave-assisted extraction (MAE) did not affect the value of the sulfate contents obtained. Yet, there was only a decrease in the value of the average percentage of sulfate contents, where the lowest was 21.71% at 8 minutes of extraction.

There was no further research related to the kinetics of carrageenan sulfate reduction reactions or the length of time of extraction. However, the graph visually showed a decrease in the value, as the results obtained in a study conducted by Andhiarto,[24] where the longer the extraction time, the higher the sulfate contents decreased. In addition, according to Moses,[30] the process of "alkali treatment" given

during processing can affect the value of carrageenan sulfate contents. In this study, NaOH was used so Na+ would react with sulfate groups to form Na2SO4 salt dissolved in water so that sulfate contents in carrageenan would decrease. Sulfate contents were closely related to carrageenan viscosity, where viscosity was one of the main factors in carrageenan *E. spinosum*.

Viscosity affected gel formation and melting point. High viscosity results in higher melting and gel formation rates than low viscosity. The viscosity of carrageenan solution was affected by molecular weight, concentration, temperature, the presence of ions in carrageenan, the use of additional ions, carrageenan type, and sulfate content.[20] In this study, an average viscosity value of 120.66 mPa•s to 176.33 mPa•s was obtained see Table 6 and fulfilled the standard requirements for carrageenan quality by FAO, FFC and EEC, which was ≥ mPa•s. The results of the data analysis stated that there was a significant difference in the carrageenan viscosity and the extraction time. There was also a decrease in the graph of the mean value of carrageenan viscosity obtained sequentially, 176.33 mPa•s at extraction time of 2 minutes, 125.66 mPa•s at 5 minutes and 120.66 mPa•s at 8 minutes. This was in line with the decrease in the average value of carrageenan sulfate content in this study, where the sulfate content caused a repulsive force between negative sulfate groups, making the polymer chains stiff and tight, which caused an increase in viscosity.[4] The sulfate content was directly proportional to the viscosity value. The higher the sulfate value, the higher the viscosity, and the higher the viscosity, the lower the gel strength would decrease.[5]

Therefore, it was known that the extraction time using microwave-assisted extraction (MAE) in this study affected the value of carrageenan viscosity produced where the highest viscosity was obtained at 2 minutes extraction as 176.33 mPa•s and the lowest viscosity as 120.66 mPa•s at 8 minutes extraction. The 8-minute extraction was the optimal time in this study because the viscosity obtained was the lowest, so the expected strength of the gel increased.

Conclusion

The extraction time using the microwave-assisted extraction (MAE) method affects the yield of carrageenan produced. The highest yield was obtained at the extraction time of 8 minutes with an average value of the percentage of carrageenan as 17,41% ± 0,07. The extraction time by the microwave-assisted extraction (MAE) method affects the quality parameters of the carrageenan standard produced, which was the water content and viscosity, where the extraction time of 8 minutes had an optimal percentage of water content of 6.21% and viscosity at 120.66 mPa•s. The extraction time did not affect the standard quality parameters of ash and sulfate content.

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