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Optimization of carrageenan extraction from *Kappaphycus alvarezii* red algae using microwave assisted extraction method with variation of solvent concentration

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Abstract

Over time, new extraction methods have been developed, one of which is the Microwave Assisted Extraction (MAE) method. The mechanism of action of this method is to use microwave energy to heat the solvents that come into contact with the sample. The problem is whether carrageenan can be extracted from the red algae *Kappaphycus alvarezii* using the MAE method. For that reason, this research has been carried out with the aim of determining the optimum conditions of carrageenan extraction with the MAE method. The highest carrageenan yield was obtained by extracting with a 3% KOH solution with a ratio of ingredients and solvents of 1:20 and the extraction time of 30 minutes using a 30% power of 68.92%. Chemical identification results indicate carrageenan characterized by formation of

fibrous precipitate after addition of 1% methyl blue solution and FT-IR spectrophotometry results indicate that the type of carrageenan obtained is kappa type which is characterized by the similarity of sulfate ester groups identified at wave number 1230- 1265 cm^{-1}; C-O-C (glycosidic bond) at wave number 1033-1010 cm^{-1}; C-O (3,6 anhydrogalactose) at wave number 925 cm^{-1}, C-O-SO3 (D-galactose-4-sulfate) at wave number 844.82 cm^{-1} with the main functional groups in the carrageenan kappa standard.

Keywords: Carrageenan, Microwave Assisted Extraction, MAE, Kappaphycus alvarezii

1. Introduction

Carrageenan is a sap obtained from red algae by extraction using water or an alkaline solvent. Carrageenan is a linear or straight chain polysaccharide and consists of galactan molecules with the main units being galactose [1]. These polysaccharides are composed of a number of galactose units with (1,3) D-galactose and (1,4) 3,6-anhydrogalactose bonds alternately, either containing sulfate esters or without sulfates [2].

Based on the stereotype of the molecular structure and the position of the sulfate ions, carrageenan can be divided into three types, iota-carrageenan, kappa-carrageenan, and lambda-carrageenan. All three differ in the nature of their gel to protein reaction. Kappa-carrageenan produces a strong gel, while iota-carrageenan forms a smooth and easily formed gel. In addition, each carrageenan is also produced by different types of red algae. The solubility of carrageenan in water is influenced by several factors, namely temperature, the presence of other organic compounds, salt that is soluble in water, and the type of carrageenan itself [3].

Carrageenan can be obtained from red algae (*Rhodopyta*) genus of *Chondrus, Gigartena, Eucheuma, Kappaphycus, Hypnea, Laurencia, Solenia, Agardihiella* and *Sarconema*. Red algae that contain carrageenan only *Eucheuma denticulatum* and *Kappaphycus alvarezii* that have been cultivated [4]. Carrageenan extraction procedures from various red algae have been developed. Generally, this procedure consists of three stages of work namely; extraction, screening and deposition. At the extraction stage, the speed and solubility of carrageenan in water is affected by the temperature and time of the process of joining all the carrageenan fractions from red algae with the water fraction used as a solvent medium [5].

The success of the carrageenan extraction process from red algae is highly dependent on several factors such as age and post-harvest processing, purity of the organic solvent used and the extraction method chosen. Separation of carrageenan from extracting material is carried out by means of screening and deposition. Filtering carrageenan extract generally still uses conventional filtering, namely filter cloth or filter press, in a hot state which is intended to avoid gel formation. Carrageenan deposition can be done, among others, by the method of gel press, KCl press, or precipitation with alcohol. Wet carrageenan drying can be done by using an oven or sun drying. Drying using an oven is carried out at 60ºC. Carrageenan is dried on the ground, sifted, standardized and mixed, then packaged in a tightly closed container [6].

Since the last two decades, carrageenan production has increased rapidly because of increasing demand. Based on data released by FAO [7], Indonesia became the highest producer of carrageenan with a production of 8.3 million tons compared to the Philippines which only produced 6000 tons in 2013. The increase in carrageenan production was due to increased consumer demand and most of it was exported to other countries.

Carrageenan production in the industry still uses conventional methods that require higher energy and costs during the extraction process, the amount of solvent used is large, and the raw materials needed are also large. In recent years, a new extraction method has been developed, the Microwave-Assisted Exctraction (MAE) method. This method uses microwave energy to heat the solvents that come into contact with the sample, the required time is short, and a high extraction rate using a small amount of solvent [8].

Vazques-Delfin, et al. [9], extracted carrageenan from *Hypnea muciformis* using MAE method with 3% KOH solvent and distilled water with extraction time of 10 minutes, yields 16.6% and 21.5% respectively. Syaharuddin [6], succeeded in extracting carrageenan from *Kappaphycus alvarezii* red algae using conventional methods with a yield of 48.04%.

Based on the above problems, an optimization study of carrageenan extraction conditions from *Kappaphycus alvarezii* was carried out using MAE method with variations in solvent concentration in order to determine the optimum extraction conditions with this method to obtain the highest carrageenan yield and determine the type of carrageenan produced.

2. Methods

2.1. Preparation of red algae sample

The sample used in this study was *Kappaphycus alvarezii* red algae taken from Punaga Village, Takalar Regency, South Sulawesi [6]. Fresh red algae *Kappaphycus alvarezii* samples were collected from the cultivation site, selected and washed with water until clean. Furthermore, the sample was weighed and soaked in rice washing water with a ratio of 1: 20 for 12 hours then removed and washed thoroughly with water then continued soaking with water for 12 hours. After it is removed and cleaned with water, cut into small pieces (2 cm in size) to facilitate drying. Samples that have been cut into small pieces were dried in direct sunlight for 3 days. Dried red algae *Kappaphycus alvarezii* samples were then packed in plastic.

2.2. Carrageenan extraction

Carrageenan extraction was carried out by the Vazques method [9] which was modified using the Microwave Modena MV 3002. *Kappaphycus alvarezii* red algae was weighed as much as 10 g then immersed for 1 hour with 100 mL of distilled water, 3% KOH and NaOH 0.1 N each. Then the solvent was added according to the specified ratio, namely 1:10, 1:15 and 1:20. Furthermore, it was extracted using a microwave for 30 minutes. The extraction results were filtered in a hot condition (± 45^{o}C) using filter cloth. The filtrate obtained was added with 96% ethanol little by little while stirring until a carrageenan fiber precipitate was formed and left for 30 minutes. Then the precipitate was filtered, added with H_2O_2 (bleach) and dried in the oven at 40^{o}C for 3 hours. Dry carrageenan fibers were blended until carrageenan powder was obtained and packaged in plastic.

2.3. Carrageenan identification

2.3.1. Chemical identification. (a) Add 2 g of carrageenan powder to 100 ml of water, heat the mixture in a water bath at a temperature of approximately 80^{o}C while stirring until a thick solution was formed. Replace the loss of water due to evaporation, leave it to room temperature, the mass becomes thick and can form a gel. (b) To 5 ml of the solution or gel obtained in the identification test (a), add 1 drop of 1% w/v of methyl blue solution, a fibrous precipitate was formed.

2.3.2. Spectrophotometric identification. Carrageenan identification was obtained by comparing the main functional groups obtained from the spectrum of Fourier Transform

Infrared Spectrophotometry (FTIR) carrageenan reference materials (Merck). Carrageenan powder weighed as much as 0.02 g was mixed with KBr powder and pressed to form a thin film. Spectrum analysis was performed using infrared spectrophotometry in the wave range of 4000-500 cm^{-1}.

2.4. Analysis of carrageenan quality

Analysis of carrageenan quality was carried out by measuring the yield of carrageenan produced, water content, viscosity and pH.

2.4.1. Yield measurement. Carrageenan yield was determined by comparing the weight of carrageenan obtained in this study with the weight of the dried red algae sample used. Calculation of yield used the formula:

$$\%Yield = \frac{Weight\ of\ extract}{Weight\ of\ dried\ sample}\ x\ 100\%$$

2.4.2. Water content measurement using moisture balance. The carrageenan sample weighed carefully as much as 1.00 g was inserted into the pan then analyzed by pressing the start button of the moisture balance and waited until the figure appears then recorded the results of percent water content.

2.4.3. Viscosity measurement. The carrageenan sample was carefully weighed as much as 1.5% and dispersed in 100 ml distilled water at 75°C. The viscosity was measured using Brookfield viscometer with spindle number 2 at 50 rpm. Calculation of carrageenan viscosity used the formula:

Viscosity = scale reading x correction factor

2.4.4. pH measurement. The carrageenan sample was carefully weighed as much as 0.2 g then dissolved with 20 mL of distilled water at 75°C. The pH electrode was removed from the container, dipped in distilled water and dried with a tissue. Then the pH electrode was dipped into the sample until the reading results were stable.

3. Results and Discussion

3.1. Carrageenan identification

The chemical identification of carrageenan showed thick solution after heating at 80°C and formed gel at room temperature and after addition of 1 drop of methyl blue solution, it formed fibrous precipitate. These results indicate that the sample examined contains carrageenan. The determination of the main functional group of carrageenan using infrared spectrophotometer showed that the carrageenan obtained from *Kappaphycus alvarezii* is a kappa type carrageenan characterized by the presence of sulphate esters identified at wave number 1230-1265 cm^{-1}; C-O-C (glycosidic bond) at wave number 1033-1010 cm^{-1}; C-O (3,6 anhydrogalactose) at wave number 925 cm^{-1}, C-O-SO3 group (D-galactose-4-sulfate) at wave number 844.82 cm^{-1}. Carrageenan spectra using FT-IR spectrophotometry can be seen in Figure 1.

Van de Velde [10] reported that the main functional group of carrageenan would be seen at wave number 1210 - 1260 cm^{-1} detecting sulfate ester groups (there is S = O bond in sulfate esters); 1010 - 1080 cm^{-1} for glycosidic bonds; 928 - 933 cm^{-1} for 3,6-anhydrogalactose; 840 - 850 cm^{-1} for galactose-4-sulfate; 825-830 cm^{-1} for galactose-20sulfate; 810 - 820 cm-1 for galactose-6-sulfate and wave number 800-805 cm^{-1} for 3,6 anhydrogalactose-2-sulfate [11].

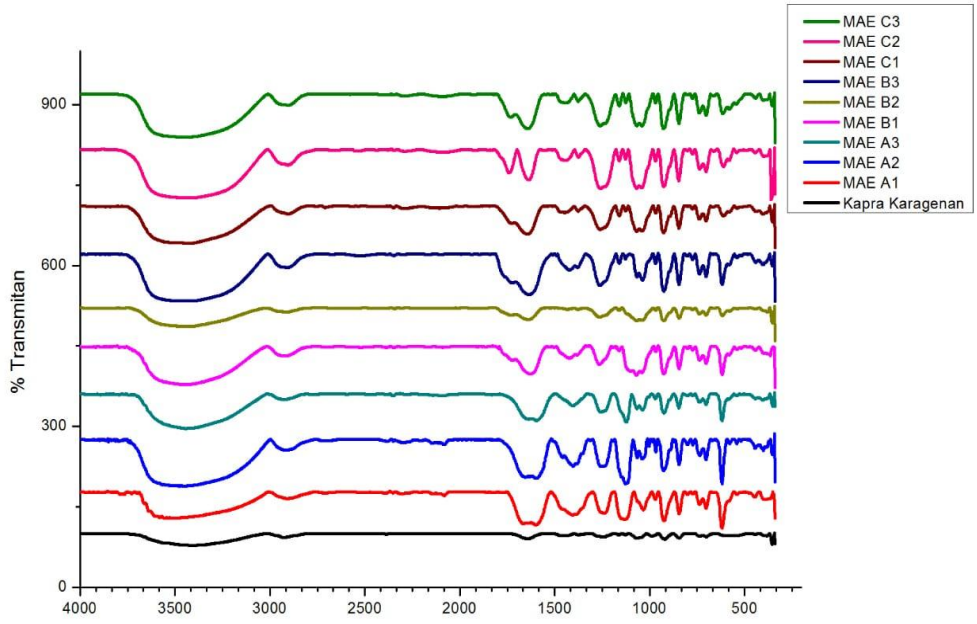

Figure 1. Carrageenan spectra using FT-IR spectrophotometry

3.2. Analysis of carrageenan quality

3.2.1. Carrageenan yield. The method used for carrageenan extraction from *Kappaphycus alvarezii* red algae was the microwave-assisted Extraction (MAE) according to Vasques [9] with slight modification. This method is used because the extraction time is very short and uses little amount of solvent. The results of carrageenan extraction from *Kappaphycus alvarezii* red algae with several variations of treatment using 3% KOH solvent, 0.1N NaOH and distilled water showed different yields which can be seen in Table 1 and Figure 2.

Table 1. Yield of extracted carrageenan from *Kappaphycus alvarezii* red algae

Variation of Concentration	**Yield (%)**	**Standard (FAO)**
MAE_{A1}	68.90± 0.75	>25 %
MAE_{A2}	49.89±1.61	
MAE_{A3}	25.61±1.18	
MAE_{B1}	52.87±0.63	
MAE_{B2}	40.74±0.65	
MAE_{B3}	37.32±1.00	
MAE_{C1}	52.88±1.07	
MAE_{C2}	49.04±0.87	
MAE_{C3}	39.50 ±0.89	

Note:

MAE : Extraction method used

SD : Standard deviation

Solvent : A = KOH 3% B = NaOH 0,1N C = distilled water

Solvent ratio : 1 = (1 : 20); 2 = (1:15); 3 = (1 : 10)

The highest yield was obtained from extraction using 3% KOH with a ratio of solvent samples (1: 20) which is 68.40%. The amount of yield obtained from extraction using a strong alkaline solvent (KOH) can help extract polysaccharides to be more perfectly and accelerate the elimination of 6-sulfate from the monomer unit forming 3,6-anhydro-D-galactose so that the yield obtained increases [12].

The yield is an indicator of the efficiency of an extraction process, the higher the yield the greater the output produced. According to Distantina *et al*. [13], during the extraction process it is estimated that a reaction involving ion exchange occurs, i.e. cations in the solvent diffuse

into the red algae tissue then react to release sulfates and replace sulfate ions in the carrageenan. The heat generated from microwaves causes the diffusion process faster so that the breakdown of red algae cells also becomes faster. Extraction using KOH solvent results in a higher yield than NaOH because potassium cations have greater molecular weight than sodium cations. Besides that, the extraction process is also influenced by the ratio of the volume and concentration of the alkaline solvent. The greater the volume and concentration of the alkaline solvent used, the greater the yield of carrageenan produced.

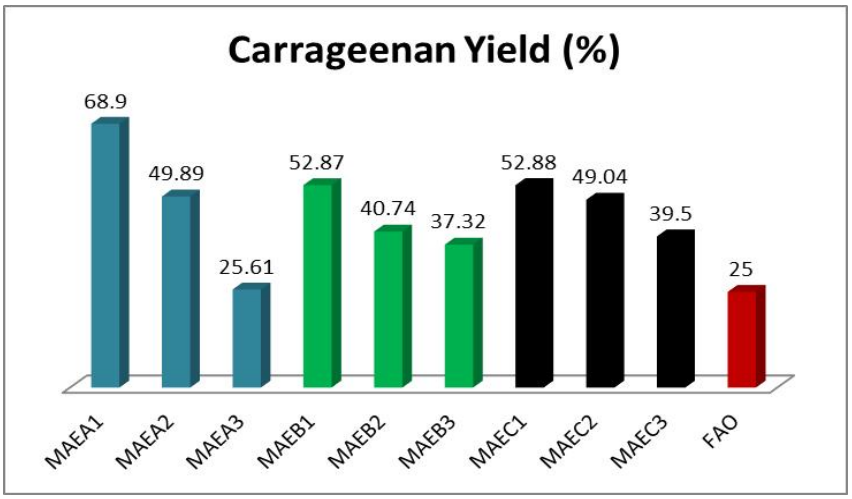

Figure 2. Percent yield of carrageenan based on extraction solvents used compared to FAO standard

3.2.2. Water content analysis. Water content is one of the parameters that affect the quality of a raw material because it affects the shelf life and the presence of microbiological activity [14]. The standard water content determined by FAO [7] is a maximum of 12% while the water content obtained in this study can be seen in Table 2 and Figure 3.

Table 2. Water content of the extraction from *Kappaphycus alvarezii* red algae

Variation of Concentration	Water Content (%)	Standard (FAO)
MAE_{A1}	14.16±0.03	Not more than 12%
MAE_{A2}	8.62±0.08	
MAE_{A3}	7.91±0.11	
MAE_{B1}	6.86±0.57	
MAE_{B2}	7.75±0.19	
MAE_{B3}	8.32±2.60	

MAE_{C1}	12.95±0.27
MAE_{C2}	11.74±0.16
MAE_{C3}	11.70±0.24

The results obtained in Table 2 show that the highest water content in the variation of MAE_{A1} treatment is 14.16%. This might be due to the fact that there is still enough water in the carrageenan to make it difficult to evaporate. Carrageenan water content is directly proportional to the yield obtained. The highest yield was obtained at MAE_{A1} which has the highest water content. This result is in accordance with the statement of Oviantari and Parwata [15] which states that the higher the yield obtained, the higher the water content.

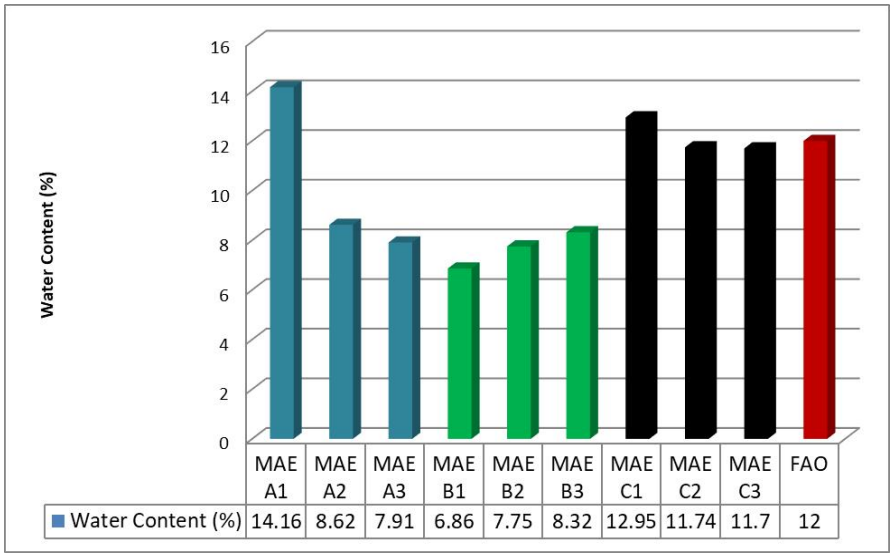

Figure 3. Relation between solvent variation and water content of carrageenan produced

3.2.3. pH of carrageenan produced. The solvent used in the extraction process can affect the pH of the carrageenan produced. pH is one of carrageenan quality parameters that need to be considered both in the extraction process and during storage. According to FAO [7], carrageenan is stable at pH 8-11, at pH lower than 8, carrageenan stability will decrease. Full results can be seen in Table 3 and Figure 4.

Table 3. pH of the carrageenan produced from *Kappaphycus alvarezii* red algae

Variation of Concentration	**pH**	**Standard (FAO)**
MAE_{A1}	11.17±0.03	8 – 11

MAE_{A2}	11.16±0.02
MAE_{A3}	10.94±0.01
MAE_{B1}	4.82±0.01
MAE_{B2}	4.12±0.05
MAE_{B3}	4.75±0.02
MAE_{C1}	4.22±0.05
MAE_{C2}	3.75±0.01
MAE_{C3}	3.90±0.02

The results of the analysis in Table 3 show that the lowest carrageenan pH was obtained from solvent MAE_{C2} which was 3.75 which indicates that the carrageenan was acidic, as was the case with MAE_{C1}, MAE_{C3}, MAE_{B1}, MAE_{B2} and MAE_{B3}. This may occur because of the reaction during the extraction process especially during neutralization of sulfuric acid formed by the release of a portion of the sulfate ester group or an exfoliating reaction that is catalyzed by the alkali causing the formation of saccharic acid which can reduce the pH of carrageenan [16].

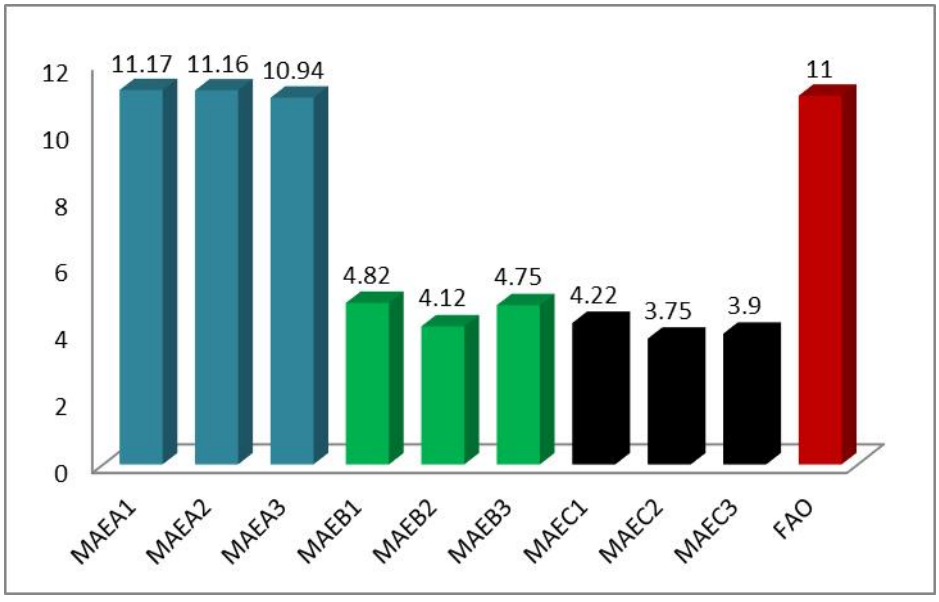

Figure 4. pH of the carrageenan produced using variations of extraction solvents

3.2.4. Viscosity measurement. Viscosity is one of the parameters of carrageenan quality standards. Viscosity testing is conducted to determine the level of viscosity of carrageenan at certain concentration and temperature [18]. The consistency of the viscosity is affected by the

solvent used in the extraction and its temperature. Full results can be seen in Table 4 and Figure 5.

Table 4. Viscosity of carrageenan at concentration of 1.5% measured at 75°C

Variation of Concentration	Viscosity (cPs)	Standard (FAO)
MAE_{A1}	516.00±1.25	Min. 5 cPs
MAE_{A2}	625.33±1.93	
MAE_{A3}	669.33±1.49	
MAE_{B1}	632.00±2.51	
MAE_{B2}	536.66±0.28	
MAE_{B3}	546.66±1.10	
MAE_{C1}	606.66±3.25	
MAE_{C2}	661.33±1.38	
MAE_{C3}	569.33±0.58	

According to Anwar *et al.* [19] and Syaharuddin [6, 20], the higher the KOH concentration the higher the viscosity, because KOH dissolves the salts found in red algae. Wenno *et al.* [17] suggested that the viscosity value is influenced by sulfate levels. The less the sulfate content, the smaller the viscosity value but the consistency of the gel increases. According to the standard of commercial carrageenan quality requirements issued by FAO [7], the minimum viscosity value is 5 cPs, so it is concluded that the carrageenan produced meets the quality standards.

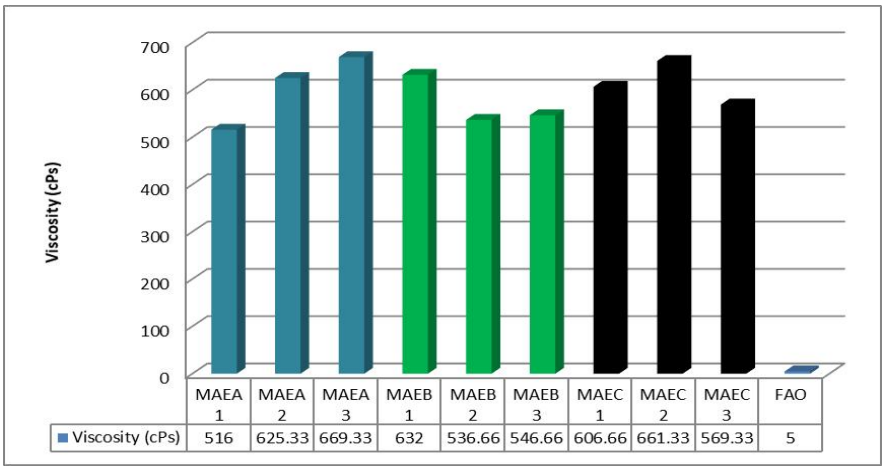

Figure 5. Relation between variation of solvents used and the pH of carrageenan produced

4. Conclusion

The Microwave-Assisted Extraction (MAE) method can be used to extract carrageenan from *Kappaphycus alvarezii* red algae. Optimal extraction conditions to obtain the highest yield of carrageenan is to use alkaline KOH at a concentration of 3% with the ratio of ingredients and solvents is 1:20 and the extraction time is 30 minutes. The highest carrageenan yield was obtained by extracting with a 3% KOH solution (1:20) for 30 minutes using a 30% power of 68.92%. Chemical identification results indicate carrageenan characterized by formation of fibrous precipitate after addition of 1% methyl blue solution and FT-IR spectrophotometry results show that the type of carrageenan obtained is kappa type characterized by the similarity of sulfate ester groups identified at wave number 1230- 1265 cm^{-1}; C-O-C (glycosidic bond) at wave number 1033-1010 cm^{-1}; C-O (3,6 anhydrogalactose) at wave number 925 cm^{-1}, C-O-SO_3 (D-galactose-4-sulfate) at wave number 844.82 cm^{-1}, with the main functional groups in the carrageenan kappa standard.

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