

Extraction and Physicochemical Properties of Refined Kappa-Carrageenan from *Kappaphycus alvarezii* Originated from Semporna, Sabah

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Received: December 06, 2020; Accepted: June 16, 2021

In this research, the influence of different solvents (1.0 M KOH and 1.0 M NaOH) and different drying methods (freeze dryer and oven) towards the chemical and physical properties of refined carrageenan by using *kappaphycus alvarezii* collected from Semporna, Sabah were determined. The physical characterizations involved were viscosity, surface morphology and functional group while, the chemical characterization was sulphate content. The result showed that the carrageenan yield were 12% (carrageenan extracted using 1.0 M KOH) and 18% (carrageenan extracted using 1.0 M NaOH) respectively for oven as a drying method. The carrageenan yield using freeze dryer were 10% (carrageenan extracted using 1.0 M KOH) and 12% (carrageenan extracted using 1.0 M NaOH). The functional groups identification for sulphate ester, anhydro-galactose and galactose were analyzed by using FT-IR. The surface morphology of carrageenan was analyzed by using SEM for different drying method and carrageenan extracted using 1.0 M KOH (Oven as drying method) showed a smooth surface compared with other sample. The sulphate content was analyzed by using UV-Vis spectrophotometer. The concentration of sulphate were 22.5 mg/L (carrageenan extracted using 1.0 M KOH) and 69.4mg/L (carrageenan extracted using 1.0 M NaOH) for oven as drying method. The result for freeze dryer method were 33.8 mg/L (carrageenan extracted using KOH) and 44.9 mg/L (carrageenan extracted using NaOH), respectively.

Keywords: Extraction; refined kappa-carrageenan; *Kappaphycus alvarezii*

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[http://dx.doi.org/10.6180/jase.202206_25\(3\).0006](http://dx.doi.org/10.6180/jase.202206_25(3).0006)

1. Introduction

The important of seaweed polysaccharide are alginate, agar, agaroses and carrageenan which are commonly used in food production. The alginate is an anionic polymers which were isolated from brown seaweed and it has been extensively utilized in food application to maintain the structure in frozen food [1]. Carrageenan is sulfated polysaccharides,

commonly isolated from red seaweed. It has been widely utilized in food industry as thickening, gelling, stabilizing agent and air freshener gel [2-8]. The solvent and drying method must be highlighted in order to gives more advantage to carrageenan because it can remove the moisture content which can give a good quality and produce product with higher concentration of nutritionally chemical.

1.1. Carrageenan Sources

Carrageenan is a sulfated linear galactan which contain ester sulfate about 15-40% that consists 3,6-anhydro-galactose and D-galactose and bound by β -1,4 and α -1,3 linkage [9]. There are many types of carrageenan such as λ , μ , ι , κ , ϵ which are containing 22 to 35% sulphate groups and the classification of carrageenan is based on its solubility in the potassium chloride [10]. The properties of carrageenan is influenced by its position and number of ester sulfate groups as well as the content of 3,6-anhydro-galactose.

κ -Carrageenan has one sulfate ester, while ι - and λ -carrageenan contain two and three sulfates per dimer respectively [11]. The higher level of ester sulphate in the carrageenan, the lower its solubility temperature and gel strength [12]. Kappa carrageenan has 25-30% ester sulfate and 28-35% 3,6-anhydro-galactose. Lambda carrageenan has 32-39% ester sulfate and has no 3,6-anhydro-galactose. Meanwhile, iota carrageenan has 28-38% ester sulfate and 25-30% 3,6-anhydro-galactose [12, 13].

1.2. Type of Carrageenan

Carrageenan can be classified into three main structure, called kappa, iota and lambda which are different in position and number of sulphate group and 3,6-anhydro-galactose rings [2]. The 3,6 anhydro group helps α helix formation which is very important for gelling characteristics in the carrageenan [14]. carrageenan is a high molecular mass material with a high degree of polydispersity. Generally, different species of seaweed can produce variety types of carrageenan. Their structures are varying according to species, seasons, geographic location and age of population [15].

Fig. 1 show chemical structure of different type carrageenan and kappa carrageenan will be focused in this project.

In this study, carrageenan was extracted from *Kappaphycus* species through different solvent and drying method. This method was used to determine the effect of parameter towards the viscosity, functional group, surface morphology and sulphate content.

2. Methodology

2.1. Materials and Sample Preparation

The sample seaweed of *Kappaphycus alvarezii* species was obtained from Semporna, Sabah. Before extraction process begin, the pre-treatment was carry out on seaweed to remove foreign particle such as sand, dried sea animals and sodium chloride [16]. All solvents and reagents including NaOH (QREC) and KOH (Sigma) were obtained from

commercial vendors and used without further purification. The drying method of is one of the procedures to recover carrageenan. There are two methods of drying the sample including freeze dryer (Labconco Freezone 4.5 L) and oven drying.

The drying process gives more advantage to carrageenan because it can remove the moisture content which can be preventing from decay the carrageenan and give a good quality and produce product with higher concentration of nutritionally chemical [2, 13–17]. The refined carrageenan was consist of two following process which were pre-treatment of dried seaweed and carrageenan preparation. The extraction procedure is modified from [18, 19].

2.2. Characterization

Viscosity was measure using Brookfield DV-E Low Cost Digital Viscometer using spindle No. 5 (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 50 rpm. The Surface morphology and pore size of carrageenan was determine using SEM, Carl Ziess AG. FTIR spectra were obtained with Perkin Elmer Spectrum 100. The sulphate content of carrageenan was analyzed by using an Agilent Cary 60 UV-Vis spectrophotometer and repeat in triplicates.

3. Result and discussions

3.1. Refined Carrageenan Preparation

The dry seaweed of 5.0 g *Kappaphycus alvarezii* was added into 200 mL KOH and 200 mL distilled water. The temperature used to heat the carrageenan until it boil was 105°C with continuous stirred. The colour of solution before heated was colourless. The hot alkaline is used to extract the carrageenan was because the reaction of mu carrageenan and functional group of hydroxyl (OH⁻) will formed strong and rigid gels.

3.2. Analysis of Carrageenan Extraction

In this study, two different drying method are done with different solvent which are 1.0 M KOH and 1.0 M NaOH. Table 1 show the yield of each extracted sample. Based on Ilias (2017) [18], the percentage yield for each extracted are lower because fully refined carrageenan already removed residual cellulose from the cell walls in the filtration process by using vacuum filtration. 1 below shows the percentage of carrageenan yield based on the drying method. Based on Table 1 shows 1.0 M NaOH gives higher yield for both drying methods compared to 1.0 M KOH.

Percent yield was higher once NaOH was used as solvent compare to KOH due to the hydroxide part in the sodium hydroxide reagent penetrate into the carrageenan

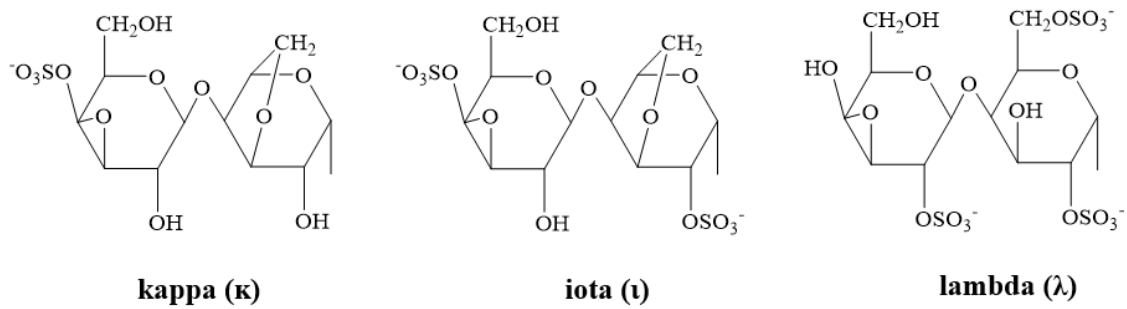


Fig. 1. Chemical structure of different type of carrageenan.

Table 1. Yield of extracted sample using oven and freeze dryer as a drying method.

Dry Method	1M KOH solvent Experiment (5.0 g)	1M NaOH solvent Experiment (5.0 g)
Oven	0.5934 ± 0.16 (12%)	0.8890 ± 0.24 (18%)
freeze dryer	0.4868 ± 0.13 (10%)	0.5809 ± 0.06 (12%)

and remove the sulphate group in the carrageenan through the desulphation at 6-position of galactose unit of carrageenan to recurring 3,6-anhydro-galactose while the Na⁺ is able to neutralize the charge to removed sulphate group by combining together to form sodium sulphate Ahmad et al. [19]. Like all strong bases, the reaction of both NaOH and KOH with water is strongly exothermic, in other words, they generate heat and give off hydrogen. But the reaction with NaOH is slightly more exothermic which can make up for other more positive factors that KOH possess.

3.3. Physical Characterization

3.3.1. Viscosity

Based on Table 2, the viscosity for carrageenan extracted using 1.0 M NaOH was higher for both drying method which were 49.7 cP (oven) and 47.4 cP (freeze dryer) while, the viscosity for carrageenan extracted using 1.0 M KOH was slightly lower which were 34.4 cP (oven) and 33.5 cP (freeze dryer).

Table 2. Viscosity reading for sample replicates.

Viscosity(cP)	1M KOH	1M NaOH
Oven	34.4	49.7
Freeze dryer	33.5	47.4

This was due to the repulsion force between the negative charges along the polymer chain of the sulphate group cause the molecules chains to tighten. Then, the hydrophilic nature which was the carrageenan polymer was covered by immobilized water molecule that cause the solution to become viscous.

3.3.2. Surface Morphology

Based on Fig. 2, the magnification of 5000x shows all of the sample was regular in their shape but not for carrageenan extracted with 1.0 M KOH using freeze dryer as a drying method (b). The different drying method of refined carrageenan have different diameter size. The (carrageenan extracted with 1.0 M KOH and 1.0 M NaOH) using oven drying method (a and c) was giving different morphology shape which were more regular and homogenous compared to (b and d). The carrageenan dried using freeze dryer gives different particle size, which more regular, smooth and homogenous compared to carrageenan dried using oven (a and c) sample.

The semi refined carrageenan would show the rough heterogenic surface with the average size 56 to 75 μm. The semi refined carrageenan has granular form and well distributed [4, 5]. In this result showed, the refined carrageenan mostly has regular structure when using magnification of 5000x except for KOH freeze dryer (b).

3.4. Chemical Characterization

3.4.1. Functional Group of Carrageenan

The functional group of carrageenan are known by using FT-IR. The Table 3 showed the wave number of each extracted sample with the literature review from the previous study [4–6]. The carrageenan of each extracted sample did not show significant different of each extracted sample.

The result shows most prominent seaweed absorption bands at 1240 cm⁻¹ which predicted to be S=O sulphate ester bond. According to Dewi et al. [20], the absorption band which is presence at area 1210 cm⁻¹ to 1260 cm⁻¹ is a good indicator for the presence of S=O.

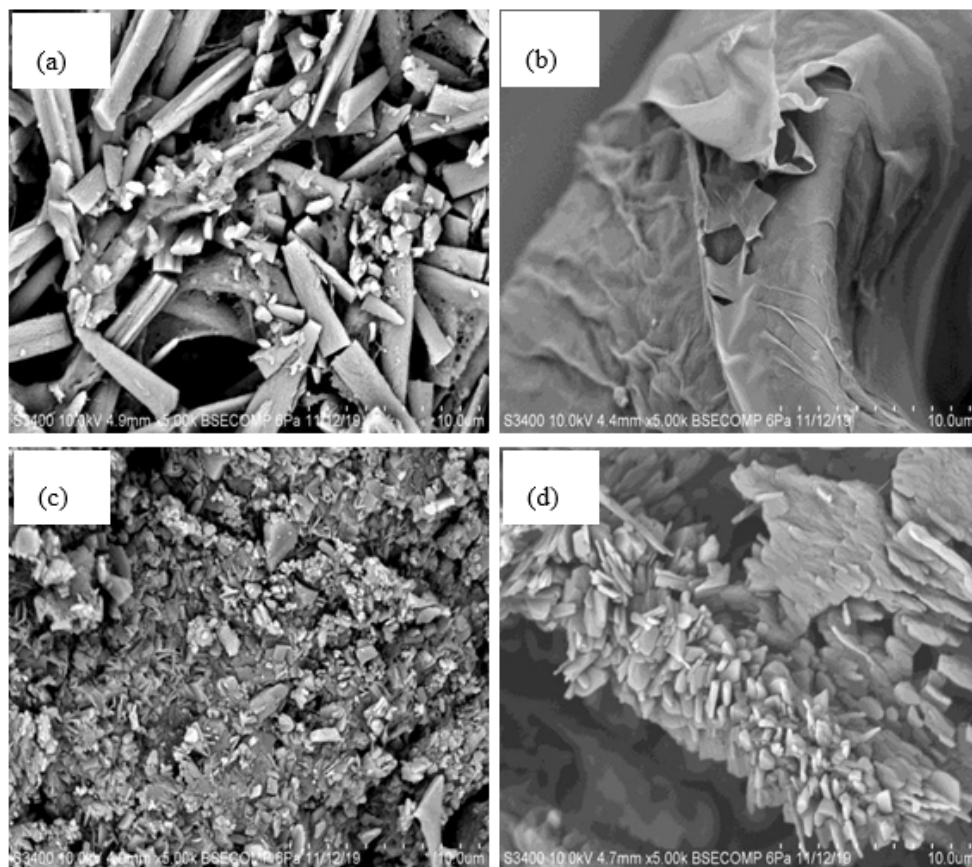


Fig. 2. Surface morphology of refined carrageenan with magnification 5000x (a) 1M KOH Oven, (b) 1M KOH Freeze dryer, (c) 1M NaOH Oven and (d) 1M NaOH freeze dryer.

Table 3. Functional group of carrageenan with wave number of each extracted sample

Samples	Wave number (cm^{-1})	Literature review [20]
1M KOH Oven	3460 (O-H strength)	
	1101 (3,6-anhydro-galactose)	
	1681 (C=C)	
	831 (C-O-SO ₃ ; C4 galactose)	
	831 (kappa type)	1240-1260 (S=O sulphate ester)
1M KOH Freeze dryer	3454.40 (O-H strength)	1070 (C-O; 3,6-anhydro- galactose)
	1681 (C=C)	1558 (C=C)
	1060 (C-O; 3,6-anhydro-galactose)	3461 (O-H)
	844 (kappa type)	1072 (aliphatic, carboxylic and glycosidic linkage)
	1072 (aliphatic, carboxylic and glycosidic linkage)	845 (C-O-SO ₃ ; C4 galactose)
1M NaOH Oven	3456 (O-H strength)	840-850 (kappa type)
	1060 (C-O; 3,6-anhydro-galactose)	
	844 (kappa type)	
	1219 (sulphate ester)	
	1685 (C=C)	
1M NaOH freeze dryer	3454 (O-H strength)	
	1681 (C=C)	
	1060 (aliphatic, carboxylic and glycosidic linkage)	
	878 (C-O-SO ₃ ; C4 galactose)	
	844 (kappa type)	

3.4.2. Sulphate Content of Carrageenan

Based on the absorbance readings that are measured by UV-Vis spectrophotometer, the concentration of sulphate is calculated by using the linear equation, $y = 0.001x + 0.034$, where y is the absorbance and x is the concentration. Table 4 shows the concentration sulphate in the sample carrageenan.

The sulphate ion in the seaweed solution was detected by using BaCl_2 solution and HCl. The HCl was used to prevent super saturated in the solution and to reduce turbidity. The reaction of sulphate soluble in the carrageenan with the BaCl_2 in the presence of HCl to form BaSO_4 . This will produce solid precipitate in the sample solution. K_2SO_4 acts as references and to increase the sensitivity of the test.

Table 4. Sulphate concentration of carrageenan sample.

Sulphate concentration (mg/L)	1M KOH	1M NaOH
Oven	22.5	69.4
Freeze dryer	33.8	444.9

Based on the result, the sulphate concentration for carrageenan extracted using 1.0 M KOH solvent was lower for oven and freeze dryer method which are 25.5mg/L and 33.8mg/L while the sulphate concentration for carrageenan extracted using 1.0 M NaOH solvent is higher which is 69.4mg/L and 444.9mg/L. This was because the hydroxide part of the reagent in KOH penetrates the seaweed higher which reduces the sulphate content and increases the 3,6-anhydro-galactose.

4. Conclusion

In conclusion, refined carrageenan extracted using different solvent extraction (1.0 M NaOH and 1.0 M KOH) under liquid-liquid extraction were achieved. The yield of carrageenan extracted using 1.0 M NaOH for both drying methods (18% oven and 12% freeze dryer) were higher compared with carrageenan extracted with 1.0 M KOH. The information generated here can be applied to food products and non-dairy food products such as pharmaceutical formulation and cosmetics. For the future recommendation, research such as Response Surface Methodology can be done to produce variety sample product and high yield.

Acknowledgements

The authors would like to thank UMS under Niche Fund Scheme Research Grant (DN20088) for the financial, facilities, support and assistance in this research project.

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