Jurnal Akta Kimia Indonesia

INDONESIA CHIMICA ACTA

PAPER

View Article Online

View Journal View Issue

Cite this: *Indo. Chim. Acta.*, 2023, **16**, **1**.

Received Date:
17th February, 2023
Accepted Date:
15th August, 2023

Keywords:
Complex compound;
Dithiocarbamate;
Mycobacterium tuberculosis

DOI:
http://dx.doi.org/10.20956/ica.v16i1.25487

Synthesis and Characterization of κ-Carrageenans from *Eucheuma cottonii* from Bantaeng Regency

Nurlaida[1]*, Saadatul Husna[1], and Aswar[1]

Abstract. The objectives of the study are to to produce κ-Carrageenan from seaweed *Eucheuma cottonii* which comes from Bantaeng Regency, South Sulawesi, and to determine the characteristics of the carrageenan. After received the best way to produce it in terms of concentration of NaOH and KCl that are used to extract carrageenan, extraction time, and precipitation time, then the next step is characterization of the produced carrageenan. The best combination found to extract the highest amount of carrageenan are NaOH 4%, extraction time of 1 hour, KCl 6%, and precipitation time of 1 hour as well (data not shown). For the characterization of ash, fiber, sulfate content, and viscosity are 2.24%; 0.65%; 0.35%; and 5 cP respectively.

Introduction

Out of all globally produced seaweed, there are only seven different species of seaweed of which amazingly contribute to around 95% of the total water plant production. Four of them are highly valued source of food while the remaining three which are *Gracilaria, Eucheuma* dan *Kappaphycus* are commercially cultivated as source of carrageenan and agar where Indonesia responsible for 66% of the total circulation of this hydrocolloid seaweed around the world. Within Indonesia, South Sulawesi becomes the largest producer of seaweed. It holds one third of nationally seaweed production or 20% of total carrageenan supply around the globe (Nuryartono et al., 2021; Simatupang et al., 2021).

Kappaphycus alvarezii is one of the members of red algae (Rhodophyta). As the main source of κ-Carrageenan, *Kappaphycus alvarezii*, known as *Eucheuma cottonii*, together with *Eucheuma denticulatum* as the main source of iota carrageenan, commit almost 90% of carrageenan production. Indonesia, along with Philippines, Malaysia, and some other countries contribute to the most of it (Hayashi et al., 2010; Liao et al., 2021; Martín-del-Campo et al., 2021; Prihastuti and Abdassah, 2019; Rasyid, 2003).

Carrageenan is utilized in many areas of human life. In pharmaceutical industry, carrageenan is used as one of the ingredients to make acetic acid. The combination of carrageenan, polylysine, and pectin also reported to possess the ability to form antimicrobial delivery system. Carrageenan is also reportedly can be used as an alternative to gelatin to produce hard capsule shell. While in Food and Agriculture industry, κ-Carrageenan is used in vinegar production, to modify the texture of food, to enhance the food stability and flavor, and as a suspending, gelling, and thickening agent (Carina et al., 2021; Prihastuti Abdassah, 2019; Simatupang et al., 2021; Suryanto et al., 2023; Zhou and Huang, 2021).

The capability of seaweed to produce various compounds has elevated the importance of this aquatic plant. They are regarded as the perfect candidates in attempt to develop biologically active compounds that can be used in pharmaceutical, food, and cosmetic industry. The increasing demand of naturally developed products also contributes to the rapid growing of seaweed cultivation (Cotas et al., 2020; Gomez-Zavaglia et al., 2019).

Indonesia has led the production of seaweed since it was first cultivated in 1980. Since then, the production of seaweed, especially *Kappaphycus* had spread to many regions in the country. Almost all island has their own provinces where seaweed becomes the main source of income of small farmers who live in coastal area (Simatupang et al., 2021).

[1]*Departement of Chemical Analysis, Manufacturing Industry Community Academy of Bantaeng, 92461, Bantaeng, Indonesia;* ***Email: nurlaida@akom-Bantaeng.co.id***

More than half of seaweed cultivated are for human consumption. However, in Indonesia, that kind of seaweed only produced in a fraction amount (around 1%) while the majority like *Eucheuma* and *Kappaphycus* are used as fertilizer and biofuel. Even though seaweed is the main source of Indonesia's aquaculture production, 80% of its export still consist of low-quality raw material (Saleh and Sebastian, 2020).

There are several factors that need to be carefully planned when carrageenan extraction is performed. According to indonesiaseaweed.com, there are six steps to perform in order to get redefined carrageenan, which is the carrageenan extracted from seaweed. The steps are cleaning and washing, alkali treatment and extraction, filtering, recovery, gel pressing process, and drying and milling (indonesiaseaweed.com, 2018). Several studied had been done to determine the best way to extract carrageenan from various species of seaweed. With the facts stated above and that South Sulawesi where this study took place is among the highest producer of seaweed, this study aims to produce carrageenan extract from seaweed *Eucheuma cottonii* obtained from Bantaeng regency using the best extraction method and to determine the characteristic of the carrageenan.

Experimental

Materials and Methods

The materials used in this study were seaweed *Eucheuma cottonii* obtained from Bantaeng Regency, South Sulawesi, filter paper, ethanol, NaOH, KCl, HCl 0.1 N, $BaCl_2$, H_2SO_4 0.3 N, pH universal, and aquadest.

Procedures

Carrageenan extraction

Carrageen extraction. Carrageen extraction was done using method published by Astuti et al., 2017. Seaweed *Euchoma cottonii* were washed until it's cleaned from foreign substrates, then cut into tiny pieces, and then soaked in aquadest for 30 minutes. After it was filtered, the seaweed was drowned in 4% (v/v) NaOH and 1 hour extraction time. The comparison between sample seaweed and NaOH solution was 1:20. Extraction was done in 95 °C. After all the steps above were done, the sample and NaOH were separated and the filtrate was collected.

Carrageen precipitation. Filtrate that was collected from previous step was mixed with 6% (v/v) KCl and 1 hour of the precipitation time. The mixture between filtrate and KCl was 2:1 v/v. The precipitation was done in room temperature and the fiber formed in this step was saved. After fiber was formed, it was dried in 60 °C for 24 hours, then it was crushed until become powder.

Characteristic of carrageenan

The All the characteristics stated below was done in Politeknik Negeri Ujung Pandang (PNUP).

Sulfate content. 0.5 g (W1) sample was hydrolyzed using 50 mL of HCl 0.1 N for 30 minutes. While hydrolysis was done in boiling condition, 10 mL of $BaCl_2$ 0.25 M was added to the solution little by little for 5 minutes. Sample then was put in room temperature for 5 hours until it is no longer hot. Sample was filtered and the precipitate was put in the furnace at 700 °C for 1 hour. The weight of Ba_2SO_4 (W2) is considered equal with the weight of white ash. The sulfate content was calculated using formulas as follow (Astuti et al., 2017):

$$\text{Sulfate content} = \frac{(W2 \times 0.4116)}{W2} \times 100\% \quad (1)$$

Fiber content. 1 g of carrageenan sample was put in a test tube and 30 mL of H_2SO_4 0.3 N was added into it. Fiber was extracted in boiling water for 30 minutes. 15 mL of NaOH 1.5 N was added and the extraction time was extended for 30 minutes longer in boiling water. The mixture solution was filtered using vacuum pump. Filtrate was collected and washed using hot water, H_2SO_4, and alcohol 50 mL for each. The precipitate was cooled in desiccator for 30 minutes and the weight was measured and noted as weight (a). the sample then put in furnace at 500 °C for 3 hours. The sample was cooled for a while before put in desiccator for another 30 minutes, and weighed as weight (b). To calculate fiber content, we used the formula below (Asikin et al., 2015):

$$\text{Fiber content} = \frac{(a-b)}{\text{Initial weight of sample}} \times 100\% \quad (2)$$

Ash content. A porcelain cup was dried in oven at 105 °C for 1 hour before cooled in desiccator. It was weighed until it is constant and noted as weight (a). 2 g of carrageenan as weight (b) was put in the cup and heated for some times using flame and then it was put in to furnace at 650 °C for around 12 hours. The sample and the cup were cooled in desiccator and weighed until constant weight is achieved as weight (c). the formula below was used to determine ash content (Astuti et al., 2017):

$$\text{Ash content} = \frac{(a+b)-c}{b} \times 100\% \quad (3)$$

Viscosity. Viscosity was measured using Brookfiled viscometer. Carrageenan sample with concentration of 1.5% was heated at 90 °C. The stirring process was continued until the temperature reached 77 °C. viscosity of the carrageenan was measured at 100 rpm after 1 minute of rotation cycle (Astuti et al., 2017).

Result and Discussion

Carrageenan extraction

Carrageen extraction and precipitation. In the carrageenan extraction and precipitation procedure, after carried out the several combinations, the best combination that produce the highest amount of carrageenan were NaOH 4% and KCl 6% with extraction and precipitation time were 1 hour for each (full data not shown).

Alkali solution, in this case was NaOH, was used due to its cation that will interact with polymer chain of carrageenan and form κ-Carrageenan, a special type of carrageenan that can only be found in certain species of seaweed (Asikin et al., 2015). After the best combination was found, that combination would be used to do the characteristic test. The same extraction and precipitation steps would be done, only this time, a much larger amount of seaweed would be used. According to (Astuti et al., 2017), the higher concentration of alkali used, the higher chance to carrageenan to get extracted. This is due to the ability of alkali to help the expansion process of the seaweed cells. As well as extraction time, it is understandable that the longer extraction time, the better result of extraction. This is because it will have more times to the heating effect to take place. Since the heating process will help to maximize the permeability of cell walls of carrageenan. This will cause the easier for compounds to diffuse between cells and solution.

Characteristic of carrageenan

Sulfate content. From this study, it was revealed that sulfate content was 0.35%. This result is way lower than some of reports such as ±30%; ±13%; 20-30%; 6-8% by (Astuti et al., 2017; Diharmi et al., 2020; Manuhara et al., 2016; Moses et al., 2015) respectively. The study's result is also lower than condition set by FAO which is 15-40% (Astuti et al., 2017). According to Moses et al. (2015), it was reported that there is an inversely proportional correlation between concentration of alkali solution and level of desulfation of carrageenan. However, they argued that there is strong correlation between those two factors. Instead, they believed the decrease of sulfate level could be due to the number of inorganic substrates that were not removed properly when washed prior to extraction and the alkali solution could play a role in rearrangement to create a stable structure.

Fiber content. This study found that the fiber content of the carrageenan was 0.65%. Other research like (Asikin et al., 2015) found higher number between 8-12%. Fiber content possesses strong correlation with viscosity. When fiber that is contained in food mixed with water which will cause gel formation. Although in this report, we do not study the correlation between them. (Nofreeana and Azril, 2022) concluded that seaweed Eucheuma cottonii contains approximately 24% and 56% of soluble fiber and insoluble fiber respectively. In dry weight of seaweed, fiber contributes to 60% of total weight (Dong et al., 2020), report the formation fiber in carrageenan using wet spinning method with the combination of ion Ba^{2+} and alcohol. They found a better fiber formation with addition of Ba^{2+}. This method could be used as comparison to get a better understanding of our result.

Ash content. Compare to other reports that showed ash content ranging from 7-70% depends on NaOH and KCl concentration used (Astuti et al., 2017); 22-36% depends on KOH concentration (Diharmi et al., 2020), this study revealed a very small number instead which is 2.24%. this number is also does not meet the requirement set by FAO which set between 15-40%. Astuti et al. (2017) explained in their report that alkali solution may play a role in ash formation in carrageenan. When the extraction process takes place, sodium from alkali solution will get attached to the seaweed, and this will cause increasing level of ash produced. Extraction time also give impact to ash content. The longer extraction time done, the more ash resulted. This probably caused by the longer sodium attached to seaweed which will affect the creation of ash. In report by Moses et al. (2015) stated that the reason behind increasing as content could be caused by high level of inorganic molecule in the sample.

Viscosity. This study revealed viscosity at 5 cP. Although this number has met the standard set by FAO which is also 5 cP, numerous reports showed a higher viscosity number. Depends on the various factors such as alkali and KCL concentration as well as extraction time, (Astuti et al., 2017) reported range between 9-19 cP. While (Diharmi et al., 2020) showed a similar number to this study (3-16.5 cP), lower viscosity came from low concentration of alkali, and this result is actually the opposite of what this study found out.

Table 1. Characteristics of carrageenan (NaOH 4%; extraction time 1 hour; KCl 6%; precipitation time 1 hour.

Properties	Value
Sulfate content	0.35%
Fiber content	0.65%
Ash content	2.24%
Viscosity	5 cP

Conclusion

The results of this study as follows: the best condition to produce the highest amount of carrageenan were NaOH 4%; extraction time of 1 hour; KCl 6%; and precipitation time of 1 hour. Using this condition, the characteristics were 0.35%; 0.65%; 2.24%; and 5 cP for sulfate content; fiber content; ash content; and viscosity respectively. The numbers are relatively different with another research that have been reported. One way that could be the cause was at the extraction step. After mixed with KCl solution, (Astuti et al., 2017) stated that carrageenan obtained at this step need to washed with water until the pH of the solution reached 7. This particular treatment probably could change this study's result if it's done.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgements

This project was funded by Akademi Komunitas Industri Manufaktur Bantaeng as part of yearly internal research program.

References

Asikin, A. N., Kusumaningrum, I., and Sutono, D. D. (2015). Extraction and Characterization of Functional Properties of Carrageenan *Kappaphycus Alvarezii* From Coast of Kutai Timur District. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, *7*(1). 49-58.

Astuti, K. W., Putu, N., Wijayanti, A. D., Ngurah, G., Dewantara Putra, A., and Laksmiani, L. (2017). Optimization of Isolation Method of Carrageenan from *Kappaphycuss Alvarezii* Doty Using Factorial Experimental Design. *Journal of Health Sciences and Medicine*, *1*(2), 4-7.

Carina, D., Sharma, S., Jaiswal, A. K., and Jaiswal, S. (2021). Seaweeds Polysaccharides in Active Food Packaging: A review of recent progress. *Trends in Food Science & Technology*, *110*, 559–572.

Cotas, J., Leandro, A., Pacheco, D., Gonçalves, A. M. M., and Pereira, L. (2020). A Comprehensive Review of The Nutraceutical and Therapeutic Applications of Red Seaweeds (Rhodophyta). *Life*, *10*(3), 1-23.

Diharmi, A., Rusnawati, and Irasari, N. (2020). Characteristic of carrageenan Eucheuma cottonii collected from the coast of Tanjung Medang Village and Jaga Island, Riau. *IOP Conference Series: Earth and Environmental Science*, *404*, 1-8.

Dong, M., Zhang, K., Wang, L., Han, J., Wang, Y., Xue, Z., and Xia, Y. (2020). High-Strength Carrageenan Fibers with Compactly Packed Chain Structure Induced by Combination of Ba^{2+} and Ethanol. *Carbohydrate Polymers, 236*, 1-8

Gomez-Zavaglia, A., Prieto Lage, M. A., Jimenez-Lopez, C., Mejuto, J. C., and Simal-Gandara, J. (2019). The Potential of Seaweeds as a Source of Functional Ingredients of Prebiotic and Antioxidant Value. *Antioxidants*, *8*(9), 1-30.

Hayashi, L., Hurtardo, A. Q., Msuya, F. E., Bleicher-Lhonneur, G., and Critchley, A. T. (2010). *Seaweeds and their Role in Globally Changing Environments* (J. Seckbach, R. Einav, and A. Israel, Eds.; Vol. 15). Springer Netherlands.

Liao, Y.-C., Chang, C.-C., Nagarajan, D., Chen, C.-Y., and Chang, J.-S. (2021). Algae-derived Hydrocolloids in Foods: Applications and Health-related Issues. *Bioengineered*, *12*(1), 3787–3801.

Manuhara, G. J., Praseptiangga, D., and Riyanto, R. A. (2016). Extraction and Characterization of Refined K-carrageenan of Red Algae [*Kappaphycus Alvarezii* (Doty ex P.C. Silva, 1996)] Originated from Karimun Jawa Islands. *Aquatic Procedia*, *7*, 106–111.

Martín-del-Campo, A., Fermín-Jiménez, J. A., Fernández-Escamilla, V. V., Escalante-García, Z. Y., Macías-Rodríguez, M. E., and Estrada-Girón, Y. (2021). Improved Extraction of Carrageenan from Red Seaweed (Chondracantus Canaliculatus) Using Ultrasound-assisted Methods and Evaluation of The Yield, Physicochemical Properties and Functional Groups. *Food Science and Biotechnology*, *30*(7), 901-910.

Nofreeana, A., and Azril, M. (2022). Seaweed as A Dietary Fiber to Prevent The Cardiovascular Disease. *Journal of Aquaculture Development and Environment, 5*(1), 314-318.

Nuryartono, N., Waldron, S., Tarman, K., Siregar, U. J., Pasaribu, S. H., Langford, A., Farid, M., and Sulfahri. (2021). *Analisis Diagnostik Industri Rumput Laut Sulawesi Selatan*. The Australia-Indonesia Centre.

Prihastuti, D., and Abdassah, M. (2019). Karagenan dan Aplikasinya di Bidang Farmasetika. *Farmasetika*, *4*(5). 146-154.

Rasyid, A. (2003). Algae Coklat (Phaeophyta) Sebagai Sumber Alginat. *Oseana*, *XXVIII*(1), 33–38.

Simatupang, N. F., Pong-Masak, P. R., Ratnawati, P., Agusman, Paul, N. A., and Rimmer, M. A. (2021). Growth and Product Quality of The Seaweed *Kappaphycus alvarezii* from Different Farming Locations in Indonesia. *Aquaculture Reports*, *20,* 1-8.

Suryanto, H., Yanuhar, U., Aminnudin, Pradana, Y. R. A., and Bintara, R. D. (2023). *Starch/Carrageenan Blend-Based Biocomposites as Packaging Materials* (pp. 139–161). Springer.

Zhou, S., and Huang, G. (2021). Preparation, Structure and Activity of Polysaccharide Phosphate Esters. *Biomedicine and Pharmacotherapy*, *144*. 1-6.