Optimization Of Isolation Method Of Carrageenan From *Kappaphycuss Alvarezii Doty* Using Factorial Experimental Design

Ketut Widyani Astuti[1] , Ni Putu Ayu Dewi Wijayanti[1], I Gusti Ngurah Agung Dewantara Putra[1],
Ni Putu Linda Laksmiani[1]

[1] Department of Pharmacy,
Faculty of Mathematics and Natural Science, Udayana University
Bali, Indonesia
E-mail : ketutwidyani@gmail.com

Abstract

Kappaphycus alvarezii Doty is a species of red algae (*Rhodophyceae*) producing kappa carrageenan. The value of carrageenan will increase if it is able to form thermoreversible gels. The quality of carrageenan is determined by the isolation method which includes several stages, namely soaking, extraction, purification, and drying. This study aims to obtain an optimum isolation method of carrageenan from seaweed *Kappaphycus alvarezii* Doty using the factorial experimental design software, Design Expert Version.7.0.0. with the characteristics that meet the standards set by FAO.

The seaweed was collected from the sea in Nusa Lembongan, Bali Province. The carrageenan isolation method was optimized by varying three factors, namely the concentration of NaOH (4% and 8%), extraction time (0.5 hour and 3 hours), and precipitation time (0.25 hour and 0.5 hour) in order to obtain 8 formulae which then went through an evaluation of the physical and chemical characteristics of the carrageenan. The physical and chemical characteristics include the yield, viscosity, sulfate content, and ash content, analyzed using Design Expert Version 7.0.0 program to determine the optimum method of isolation.

The analysis showed that the optimum method of carrageenan isolation was using NaOH with a concentration of 4.40%, the extraction time of 2.16 hours and the precipitation time of 0.29 hour. The physical and chemical characteristics of carrageenan with the optimum isolation method include the yield of 25.73%, the viscosity of 11.92 cPs, sulfate content of 31.95% and the ash content of 26.16% which have met the standards set by FAO.

Key words : **carrageenan, FAO, isolation method, *Kappaphycuss Alvarezii* Doty.**

I. Introduction

Carrageenan is a galactose polysaccharide compound present in seaweed cell walls or intracellular matrix which is the largest composition of seaweed dry weight [1]. Carrageenan will increase in value if it has the ability to form thermoreversible gels [2]. *Kappaphycus alvarezii* Doty is a type of seaweed producing kappa carrageenan widely cultivated in Indonesia.

According [3], most of Indonesia's seaweed is exported in the dry form and only 20% is processed by domestic industries. The main problem in the seaweed industry is the method of carrageenan isolation that is quite complicated, which takes a considerable amount of time and energy [4].

Distantina [5], conducted carrageenan isolation using 0.2 N NaOH solvent for 30 minutes and precipitation using 90% ethanol for 30 minutes. However, the carrageenan yield obtained was 22%. This value does not meet the standard set by the Ministry of Trade that is at least 25%. Carrageenan isolation using 0.2 N KOH for 30-minute extraction time and precipitation using KCl 3.5% for 30 minutes resulted in a higher carrageenan yield and stronger gel properties compared to the yield produced using ethanol. Different methods used in carrageenan isolation will have a different effect on the quality of carrageenan produced [6] [7]. Therefore, in order to obtain carrageenan products with a quality that meets the standard set by FAO, the isolation method of carrageenan was optimized by varying 3 factors, namely NaOH concentration (4% and 8%), extraction time (0.5 hours and 3 hours), and precipitation time (0.25 hour and 0.5 hour), using the factorial experimental design program, Design Expert Version 7.0.0. The data on the results of evaluation of the physical and chemical characteristics of carrageenan include the yield, viscosity, sulfate content and ash content analyzed using the one-way ANOVA. The optimum isolation method is determined by the desirability value closest to 1 in the Design Expert Version 7.0.0. program.

II. METHODS

2.1 Optimization of Isolation Method of Carrageenan

The isolation method of carrageenan from seaweed *Kappaphycus alvarezii* Doty was optimized by varying the NaOH concentration, the extraction time and the precipitation time with KCl 5% at the highest and lowest levels using factorial experimental design software, Design Expert Version 7.0.0.

Table 1. Optimization Formula for the Isolation Method using *Design Expert V. 7.0.0*

Formula	Run	NaOH Concentration (%)	Extraction Time (hour)	Precipitation Time (hour)
F1	7	4	0.50	0.25
F2	4	8	0.50	0.25
F3	8	4	3.00	0.25
F4	6	8	3.00	0.25
F5	2	4	0.50	0.50
F6	3	8	0.50	0.50
F7	5	4	3.00	0.50
F8	1	8	3.00	0.50

2.2 Carrageenan Extraction

Dried *Kappaphycus alvarezii* Doty seaweed was cut into small pieces (± 1 cm), and then weighed as much as 50 grams. It was then soaked in distilled water for 30 minutes. Then, it was extracted using NaOH with the ratio of seaweed to the solvent 1:20 (g/mL). The extraction was done using a hot plate at 90^{o} C. Furthermore, it was filtered using a gauze when it was still hot.

2.3 Precipitation

Carrageenan precipitation was done by adding KCl 5%. The ratio of the filtrate volume to the KCl 5% volume was 1:2 (v/v). It was allowed to stand at the room temperature until it formed fibers, with variations of precipitation time of 0.25 hour and 0.5 hour. After the fibers were formed, they were washed to reach the pH of ± 7, and filtered using a gauze. The carrageenan fibers were then dried in the oven at 60° C for 24 hours or until it reached a constant weight. The carrageenan was then mashed to form carrageenan powder.

2.4 Evaluation of the Carrageenan Physical and Chemical Characteristics

a. The yield

The carrageen yield was calculated based on the ratio of the weight of the carrageenan produced to the weight of dried seaweed used [8]

b. The viscosity

The 1.5% carrageenan solution was heated in a beaker at 90° C. The temperature was then lowered, and the solution was stirred regularly until the temperature reached 76-77° C. The viscosity was measured using a Brookfield Viscometer at 100 rpm. The viscosity was read after one minute of rotation [9]

c. Sulfate Content

A total of 0.5 g (W_1) of sample was hydrolyzed using 50 mL of 0.1 N HCl for 30 minutes at the boiling temperature. Then, 10 mL of 0.25 M $BaCl_2$ was added little by little while boiling for 5 minutes. The sample was cooled for 5 hours, and then the precipitate was filtered by Whatman filter paper (No. 42 ashless). Next, it was burned in the furnace at the temperature of 700^{o}C for 1 hour. The weight of white ash was the weight of barium sulfate (W_2). The sulfate content was calculated using the formula of ((W_2 x 0.4116)/W_1) x 100% [10].

d. Ash Content

The porcelain crucible was heated for one hour in an oven at the temperature of 105° C, and then cooled in a desiccator. Next, it was weighed until a constant weight (A) was obtained. The carrageenan powder was weighed as much as 2 g (B), and then put into the crucible and heated above the flame. After that, it was put into the furnace with a temperature of 650^{o} C for ± 12 hours. The crucible was then cooled in a desiccator, and subsequently weighed to a constant weight (C). The ash content was then calculated using the formula of ((A+B)-C)/B x 100% [8].

2.5 Data analysis

The data obtained by calculating the yield, viscosity, sulfate content and ash content of the carrageenan from *Kappaphycus alvarezii* Doty were statistically analyzed using the one-way analysis of variance (ANOVA) method, at the 95% confidence level, using the Design Expert Version 7.0.0 program. The optimum isolation method was determined by choosing a formula with a desirability value close to 1 in the Design Expert Version 7.0.0 program.

III. RESULTS AND DISCUSSION

Table 2. Results of Evaluation of the Carrageenan Physical and Chemical Characteristics

Formula	Results of Evaluation (x ± SD)			
	Yield (%)	Viscosity (cPs)	Sulfate Content (%)	Ash Content (%)
F1	15.317±0.001	13.1±0.833	32.402±0.570	27.551±3.190
F2	21.403±0.004	16.54±0.4	36.693±1.949	68.984±1.182
F3	30.683±0.001	9.733±0.462	29.243±1.034	26.036±3.389
F4	28.017±0.001	17.467±0.462	38.865±0.498	28.964±0.142
F5	12.830±0.001	10.533±0.231	32.209±1.218	12.807±0.782
F6	23.469±0.002	15.4±0.346	35.197±0.274	43.025±1.447
F7	32.701±0.001	13.8±0.21	34.002±0.687	7.897±0.572
F8	34.453±0.002	19.067±0.231	39.54±0.523	10.493±2.244

a. Yield

The percentage of yield based on the quality standard determined by the Ministry of Trade (1989) as quoted in [11] is at least 25%. The result of the analysis using the one-way analysis of variance (ANOVA) showed that NaOH concentration, extraction time, and precipitation time had a significant effect ($p < 0.05$) on the yield.

Using 8% NaOH concentration, the carrageenan yield produced was higher than that using 4% NaOH concentration. The higher the alkali concentration, the higher the pH of the solution. This will then cause more carrageenan to get extracted. In addition, alkaline solvents help the expansion process of the seaweed cell tissues, thus facilitating the carrageenan extraction from within the tissues. The carrageenan yield produced in the 3-hour extraction time was higher than the carrageenan yield produced in the 0.5-hour extraction time. According to [12], the longer the extraction time, the greater the heating effect caused to maximize the permeability of the cell walls. Increased cell wall permeabilization plays a role in accelerating the reaction process and increasing the rate of diffusion of compounds through the cell walls. Ary [13] mentioned that an addition of KCl in the precipitation process affects the yield of carrageenan produced. The longer the precipitation time the more the resulting yield. This is presumably because the longer the precipitation time the better the reaction of solid formation in the solution, thus producing more carrageenan yield.

b. Viscosity

FAO (2007) established a minimum viscosity standard of 5 cP. The result of the analysis using the one-way analysis of variance (ANOVA) showed that NaOH concentration, significantly affected ($p<0,05$) the viscosity. The viscosity value resulted from the formula using 8% NaOH concentration was higher compared with that using 4% NaOH concentration. An increase in the viscosity at an 8% concentration of NaOH is presumably caused by high sulfate levels. Moirano [14] suggests that the higher the sulfate content, the higher the viscosity, but the lower the gel strength. The repulsion force between negative charges along the polymer chain of the sulfate group causes the chain of the molecules to tighten. Due to its hydrophilic nature, the polymer is covered by immobilized water molecules, thus causing the solution to become viscous (the viscosity of the solution is high).

c. Sulfate Content

FAO set the standards for the carrageenan sulfate content ranging from 15% to 40%. The result of statistical analysis using the one-way analysis of variance (ANOVA) showed that NaOH concentration had a significant effect ($p <0,05$) on the sulfate content. The sulfate content in the formula using 8% NaOH concentration was higher than that in the formula using 4% NaOH concentration. This is because in the research there was an imbalance between the solvent and the extracted material. Extreme increase in the concentration of NaOH resulted in the imbalance between the solvent and the extracted material. This caused the extraction process to become not optimal [15]. Increased sulfate concentration resulted in the formula using 8% concentration was also due to high ash content. In the formula using 8% NaOH concentration, the yield was higher than that in the formula using 4% NaOH concentration. However, the high ash content indicated high content of minerals, which means that the carrageenan purity was lower. This is because in the combustion process, some of the sulfate evaporates into SO_2 and the rest becomes minerals or oxides which do not evaporate during the combustion [16].

d. Ash Content

FAO set a carrageenan ash content standard ranging from 15% to 40% [17]. The statistical analysis using the one-way analysis of variance (ANOVA) method showed that NaOH concentration, extraction time and precipitation time have a significant effect ($p<0,05$) on carrageenan ash content produced.

An addition of NaOH affects the percentage of ash content in the carrageenan produced. The 8% NaOH concentration results in higher ash content compared with the 4% NaOH concentration. In this case, sodium (Na) is attached to the seaweed during the extraction. The increasing amount of sodium and other minerals attached to the seaweed during the extraction results in increased ash levels in the resulting carrageenan[18]. The ash content produced in 3 hours of extraction time was lower than the ash content produced in 0.5-hour extraction time. This is supposedly because the longer the extraction time the longer the seaweed contacts with heat and the extracting solution. The presence of sodium ions from the use of NaOH as the extracting solution affects the content of carrageenan ash resulted [18]. The longer the extraction time the more the NaOH that reacts with the sulfate group to form Na_2SO_4 in the solution causing the lower ash content resulted. The ash content during the 0.25-hour precipitation time is higher than the ash content in 0.5-hour precipitation time. In the precipitation process, there is an addition of KCl as a settling material. Potassium ions from KCl cause high content of carrageenan ash [19]. The shorter the precipitation time is, the more potassium ions that have not reacted perfectly with carrageenan to form gel. This is presumably the cause of the high ash contents.

e. Determination of Optimum Carrageenan Isolation Method

Based on the result of the analysis using Design Expert Version 7.0.0, the optimum carrageenan isolation method was obtained by extracting the seaweed using 4.40 % NaOH, in 2.16-hour extraction time, and 0.29-hour precipitation time, which results in 25.73% yield, the viscosity of 11.92 cPs, the sulfate content of 31.95% and the ash content of 26.16% which have fulfilled the FAO standards.

IV. CONCLUSION

Seaweed extraction using 4.40% NaOH solution, in the extraction time of 2.16 hours, and 0.29-hour precipitation time is the optimum method of carrageenan isolation with chemical and physics characteristics which fulfil the standard set by FAO.

ACKNOWLEDGMENT

We would like to express our gratitude to the Center of Research and Community Service of Udayana University for its support, and to the Laboratory of Non-Sterile Dosage Formulation and Technology of Pharmacy Department in the Faculty of Mathematics and Natural Sciences of Udayana University for the facilities and infrastructure.

REFERENCES

[1] Hellebust, J. A., J. S. Cragie. 1978. *Handbook of Phycological Methods.* London: Cambridge University Press.

[2] Rowe, R.C., P. J. Sheskey, and J. W. Paul. 2009. *Handbook of Pharmaceutical Exipients. Sixth Edition.* London: Pharmaceutical Press.

[3] Desiana, E., and T. Y. Hendrawati. 2015. *Seminar Nasional Sains dan Teknologi*: *Pembuatan Karagenan dari Eucheuma cottonii dengn Ekstraksi KOH Mengggunakan Variabel Waktu Ekstraksi*. ISSN: 2407-1846.

[4] Anggadiredja, J., Z. Achmad., P. Heri, and S. Istani. 2006. *Rumput Laut: Pembudidayaan, Pengolahan dan Pemasaran Komoditas Perikanan Potensial.* Jakarta: Penebar Swadaya.

[5] Distantina, S., Fadilah, Y. C. Danarto, Wiratni, and M. Fahrurrozi. 2009. Efek Bahan Kimia pada Tahap Presipitasi terhadap Rendemen dan Sifat Karagenan dari Rumput laut *Eucheuma cottoni. Ekuilibrium*. Vol. 8, No. 2:47-53.

[6] Mtolera, M.S., and Buriyo, A.S., 2004, Studies on Tanzanian Hypneaceae: Seasonal Variation in Content and Quality of Kappa-Carrageenan from Hynea musciformis, *Western Indian Ocean J. Mar. Sci*., 3, pp. 43-49

[7] Pelegrin, Y.F., Robledo, D., and Azamar, J.A.,2006, Carrageenan of *Eucheuma isiforme* (Solieriaceae, Rhodophyta) from Yucatanm Mexico. I. Effect of Extraction Conditions, *Botanica Marina*, 49, pp. 65-71.

[8] AOAC. 1995. *Official Methods of Analysis of the Association of Official Analytical Chemist.* Virginia: AOAC Inc. Arlington.

[9] Andriani D. 2006. *Pengolahan Rumput Laut (Eucheuma cottonii) Menjadi Tepung ATC (Alkali Treated Cottonii) dengan Jenis dan Konsentrasi Larutan Alkali yang Berbeda*. (undergraduate thesis). Makassar: Faculty of Agriculture and Forestry, Universitas Hasanudin.

[10] Distantina, S., Fadhilah, Rochmadi, Wiratni, M. Fahrurozi. 2010. Proses Ekstraksi Karagenan dari *Eucheuma cottonii*. *Seminar Rekayasa Kimia dan Proses*. Department E. H. Miller, “A note on reflector arrays (Periodical style—Accepted for publication),” *IEEE Trans. Antennas Propagat.*, to be published.

[11] Syamsuar. 2006. *Karakteristik Karaginan Rumput Laut Eucheuma Cottonii pada Berbagai Umur Panen, Konsentrasi KOH dan Lama Ekstraksi.* (Undergraduate thesis). Bogor: Graduate School of IPB.

[12] Wang, W. C. 1995. *Ohmic Heating of Foods: Physical Properties and Applications.* (Dissertation). Colombus: The Ohio State University.

[13] Ary, A. P. 2015. *Studi Pengaruh Metode Presipitasi terhadap Rendemen yang dihasilkan dari Proses Ekstraksi Rumput Laut Eucheuma cottonii Melalui Pemanasan OHMIC*. (Skripsi). Makassar: Universitas Hassanudin.

[14] Moirano, A. L. 1977. *Sulfated Seaweed Polysacharides* in *Food Colloids*. Westport, Connecticut: The AVI Publishing Company.

[15] Ningsih, F. L. 2014. *Jenis dan Konsentrasi Alkali dengan Presipitasi KCl yang Berbeda terhadap Mutu Karaginan dari Rumput Laut Kappaphycus alvarezii Asal Pulo Panjang Serang Banten.* (Undergraduate thesis). Banten: Universitas Sultan Ageng Tirtayasa.

[16] Romenda, A. P., R. Pramesti, A. B. Susanto. 2013. Pengaruh Perbedaan Jenis dan Konsentrasi Larutan Alkali terhadap Kekuatan Gel dan Viskositas Karaginan *Kappaphycus alvarezii* Doty. *Journal of Marine Research.* Vol. 2, No. 1:127-133

[17] Food and Agriculture Organization (FAO). 2007. *Carrageenan.* FAO JECFA Monogaphs 4

[18] Winarno. F. G. 1990. Kimia Pangan dan Gizi. Jakarta: Gramedia Pustaka Utama.

[19] Winarno, F. G., S. Fardiaz, and D. Fardiaz. 1996. *Teknologi Pengolahan Rumput Laut.* Jakarta: Putaka Sinar Harapan.