International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 12-08-2022, Accepted: 29-08-2022, Published: 15-09-2022
Volume 7, Issue 3, 2022, Page No. 69-73

Quality control of drying stages of carrageenan form red algae (*Eucheuma spinosum)* using freeze-drying method

Yanu Andhiarto, Bambang Widjaja, Ahad Urifatul Ulumia
Study Program of Pharmacy, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia

Abstract
Carrageenan is a polysaccharide extracted with water or alkaline solvents from red algae species (Rhodophyceae). Carrageenan has a variety of uses in the pharmaceutical industry, such as stabilizers, thickening, gelling agents, emulsifiers, and binders. Every year, the Indonesian carrageenan industry has good growth, and it necessitates efforts to establish efficient manufacturing procedures to produce high-quality carrageenan. This study aimed to determine the characteristics of *Eucheuma spinosum* carrageenan resulting from the drying process using the Freeze Drying method with different freezing temperatures. The sample of red algae (*Eucheuma spinosum*) came from the Sumenep, Madura sea. The quality characteristics tested include yield, moisture content, sulfate content, gel strength, viscosity, and ash content. Based on the results, the different freezing temperatures in the freeze-drying method did not affect the yield of carrageenan and the quality parameters of the gel strength standard. The ash content results were in the range of 35.90% - 38.70%, where the results met the carrageenan quality standard set by FAO of 15-40%.

Keywords: iota arrageenan, red algae, freeze dryer, drying, E. spinosum

Introduction

Carrageenophyte is a red seaweed (Rhodophyceae) that contains a lot of carrageenan polysaccharides, including Euceuma spinosum, which produces iota (ι) carrageenan. (Anggadiredja *et al*., 2010) [2]. Iota (ι) carrageenan is characterized by the presence of 4-sulfate ester in each D-glucose group and 2-sulfate ester in each 3,6-anhydrous-D-galactose group (Blakemore and Harpell, 2010) [4]. Carrageenan has various benefits across a wide range of industrial sectors, including food and non-food, pharmaceuticals, and cosmetics. Besides, Carrageenan can also function as an emulsifier (Velde *et al*., 2002) [16], gelling (Campo *et al*., 2009) [5], stabilizer, colloid, or gum.

Every year, there is an increase in the industrial need for carrageenan. But up to this point, the domestic industry has continued to produce carrageenan of poor quality. This is demonstrated by the fact that the resulting carrageenan is brown (browning), and it contains a significant amount of water (more than 20%) (Prasetyaningrum *et al*., 2007). The poor quality of carrageenan may result from the drying system's inaccuracy as the final handling unit (finishing product), such as the temperature is too high, causing browning, and the viscosity and strength of the gel produced do not meet the quality requirements of carrageenan.

Many other drying techniques have been developed, including the Freeze-Drying technique to reduce the browning. Since the temperature used to dry the extract is relatively low, the Freeze-Drying process has the advantages of improving porosity, density, and gel strength while reducing the danger of compound degradation (Kim HW, 2003) [12]. Because of the differences in temperature, humidity, and airflow velocity between the freeze-dryer and the oven, the extract dried using a freeze-dryer had a lower moisture content than an oven drier (Sembiring, 2009) [15].

Research Method

The tools used in this research were Sharp low wattage microwave R-230R(S), Freeze-dryer set (Daihan Scientific Unifreez FD), ash-free filter paper (Whatman No 42), porcelain dish, oven, crucible pliers, electric furnace, and desiccator. *Eucheuma spinosum* seaweed was the main ingredient used in this study. Other materials included: Aquadest, Sodium hydroxide (NaOH), Ethanol (C_2H_5OH), Hydrogen chloride (HCl), Hydrogen peroxide (H_2O_2), and Barium chloride ($BaCl_2$).

The red algae (*Eucheuma spinosum*) was the starting point for sample preparation. It was produced using an extraction procedure involving adding 10% KOH solvent at a ratio of 1:10 and an extraction period of 8 minutes. Additionally, the Microwave Assisted Extraction (MAE) technique was used to perform the extraction. The filtrate obtained was then neutralized by pH using HCl. The next step was to precipitate carrageenan fibers using a ratio of 1:2 96% ethanol. By reacting to bind the water in the filtrate, ethanol can precipitate carrageenan, causing the heavier component of the polymer to settle and create carrageenan fibers (Distantina *et al*., 2009) [7]. The wet carrageenan fibers were then subjected to a drying process using freeze-drying with different freezing

temperatures: -20◦C, -40◦C, and -60◦C for 24 hours. From this process, refined carrageenan powder was obtained.
The refined carrageenan powder was then analyzed for yield, gel strength, and ash content.

Carrageenan Yield
Yield analysis was carried out by comparing the weight of carrageenan powder obtained with the weight of dried seaweed using the FMC Corp method.

$$Yield\ (\%) = \frac{Weight\ of\ dry\ carrageenan}{\text{Weight of dry seaweed}} x\ 100\% \quad (1)$$

Carrageenan Gel Strength
Carrageenan was dissolved in distilled water at a concentration of 1.5%, then added KCl solution with a concentration of 0.2%. The gel strength of the carrageenan solution was measured at 20°C using a texture analyzer TA-XT2i (Hayashi *et al*., 2007) [11].

Ash Content
Two grams of seaweed sample were put in a porcelain dish with known constant weight in an electric furnace at 550ºC until complete ashing. After completing the ashing process, the furnace was turned off at a temperature below 250ºC; the cup was cooled in a desiccator and weighed. The weighing was repeated until a constant weight was obtained (AOAC, 2005) [1]. Ash content was then calculated using the following formula:

$$Ash\ Content\ (\%) = \frac{\text{W2}}{\text{W1}} x\ 100 \quad (2)$$

Where:
W1 = weight before ashing (g)
W2 = weight after ashing (g)

Statistic Analysis
The research method was a completely randomized design (CRD) with 3 different freezing temperature treatments: -20◦C, -40◦C, and -60◦C. The SPSS statistical test will also be used to examine the collected data.

Results and Discussion
Based on organoleptic observations such as form, color, odor, and taste, Extracted-refined Cargenan based on variations in freezing temperature throughout the freeze-drying method was identified. The results are listed in Figure 1 and Table 1.

(A) (B) (C)

Fig 1: Refined Carrageenan Results of Drying Process

Description
A. Carrageenan from Extraction Result of Freezing Temperature -20 ºC
B. Carrageenan from Extraction Result of Freezing Temperature -40 ºC
C. Carrageenan from Extraction Result of Freezing Temperature -60 ºC

Table 1: Physical Quality of Refined Carrageenan

Organoleptic Observations	Freezing Temperature		
	-20 ºC	-40 ºC	-60 ºC
Form	Fine powder	Fine powder	Fine powder
Color	Blurry white	White	White
Odor	odorless	odorless	odorless
Taste	Tasteless	Tasteless	Tasteless

Carrageenan Yield

Yield is one of the important parameters in assessing the effectiveness of the carrageenan extraction process. In the study, the average yield value was 13.48% at a freezing temperature of -20◦C, 13.55% at a freezing temperature of -40◦C, and 13.63% at a freezing temperature of -60◦C. According to the findings of the ANOVA test analysis, which had a significance value of 0.211 where the results of $p > 0.05$, there was no significant change in the yield of refined carrageenan at the freezing temperatures of 20 ºC, -40 ºC, and -60 ºC.

Table 2: Data of Refined Carrageenan Yield from Drying Process

Freezing Temperature	Repetition	Carrageenan Yield (%)	Mean ± SD
-20 ºC	1	13,36 %	13,48%± 0,109
	2	13,52%	
	3	13,57%	
-40 ºC	1	13,64%	13,55%± 0,808
	2	13,48%	
	3	13,54%	
-60 ºC	1	13,7%	13,63% ± 0,064
	2	13,58%	
	3	13,6%	

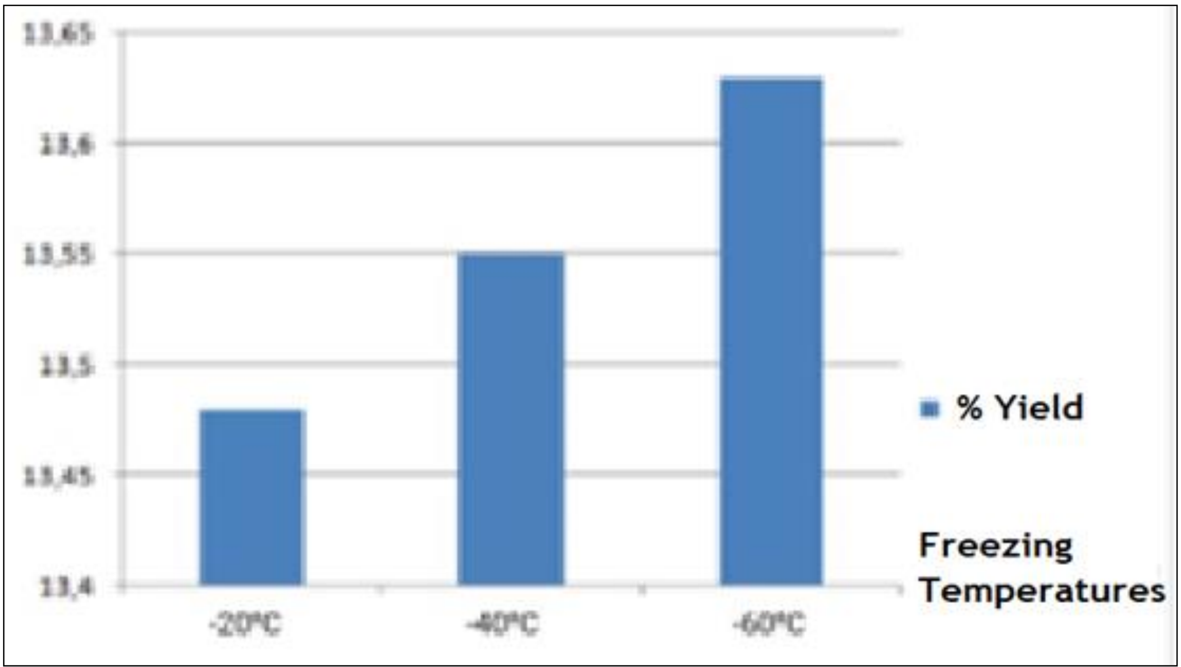

Fig 2: Chart of Carrageenan Yield

This demonstrates that drying has no impact on the yield. The yield of carrageenan is influenced by the age of harvest, extraction method, extraction time, and concentration of alkaline solvent used (Asikin *et al*., 2019; Ega *et al*., 2015) [3]. The average yield value found in this study did not meet the 25% quality threshold established by the Indonesian Ministry of Trade for the efficacy of the manufacturing process.

Gel Strength

Gel strength is the primary physical property of carrageenan because gel strength indicates the ability of carrageenan in gel formation (Asikin *et al*., 2019) [3]. The gel strength in this study at freezing temperatures of -20◦C, -40◦C, and -60◦C obtained the same average result of 0.001g/cm^2, where the results are still very far from the quality standard set by FAO, which is equal to 20-500 g/cm^2

Table 3: Data on the strength of the dried carrageenan gel

Freezing Temperature	Repetition	Gel Strength		Mean ± SD
		N/M²	g/cm²	
-20ºC	1	0,1	0,001	0.0010± 0,00000
	2	0,1	0,001	
	3	0,1	0,001	
-40ºC	1	0,1	0,001	0,0010± 0,00000
	2	0,1	0,001	
	3	0,1	0,001	
-60ºC	1	0,2	0,002	0.0017±0.00057
	2	0,1	0,001	
	3	0,2	0,002	

The findings of statistical tests conducted on the results of carrageenan viscosity revealed that the data were not homogenous and not normally distributed, making the ANOVA test invalid for examining this connection. The Kruskal-Wallis test was then used to conduct the non-parametric statistical test. The results of the Kruskal-Wallis gel strength test obtained a significance value of 0.368 where the results of $p>0.05$; therefore, there was no significant difference in the results of gel strength with freezing temperatures of -20 ºC, -40 ºC, -60 ºC.

The formation of a carrageenan gel from a liquid to a solid form involves the incorporation of polymer bonds to form a double helical structure enabling the creation of a three-dimensional network. A gel's consistency is influenced by several factors, including the type of carrageenan, concentration, and presence of ions. Excess trapped in a three-dimensional structure can cause the structure to be irregular and affect the low strength of carrageenan gel (Blakemore and Harpel 2010) [4].

KOH concentration, pH, temperature, and extraction time are other variables that may impact the carrageenan gel strength value. The higher the sulfate content, the lower the gel strength, but the higher the viscosity. The high sulfate content of carrageenan causes the three-dimensional structure formed to absorb a lot of water. Such a carrageenan gel will find it difficult to keep its structure under pressure, resulting in a low gel strength value (Wenno *et al*., 2012) [17].

Ash Content

The ash content value shows the mineral content contained in carrageenan (Prasetyowati, 2008) [14]. Ash content is the residue from the combustion of an organic material which is closely related to the amount of mineral content of a material (Prasetyowati, 2008) [14]. Due to its capacity to absorb minerals from its surroundings, seaweed is a dietary element that contains sufficient minerals (Wenno *et al*., 2012) [17]. Minerals contained in carrageenan include potassium, sodium, calcium, and magnesium (Dirhami *et al*., 2011) [6].

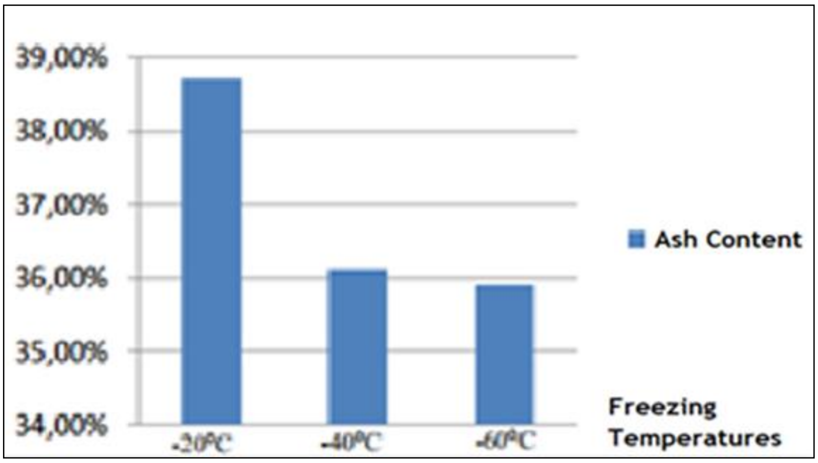

Fig 3: Chart of Carrageenan Ash Content

The carrageenan ash content analysis results in this study ranged from 35.90% to 38.70% (Figure 2). The highest ash content was found in the -20ºC sample, which was 38.70%. Meanwhile, the lowest ash content was found in the -60ºC sample, which was 35.90%. These findings show that the ash content achieved satisfies the FAO's quality criteria for carrageenan, 15–40%, whereas the FCC specifies a maximum of 35%.

Conclusion

Based on the research on the effect of different freezing temperatures on the drying process using the freeze-drying method on the quality parameters of carrageenan from Eucheuma spinosum, the conclusion is that the difference in freezing temperature affects the quality parameters of the standard ash content. Still, it does not affect the yield and strength of the carrageenan gel.

References

1. [AOAC] Association of Official Analytical and Chemistry. Official Method of Analysis of The Association of Official of Chemist. Arlington, Virginia (US): Association of Official Analytical and Chemist, Inc. International Journal of Molecular Sciences,2005:18:708.
2. Anggadiredja JT, Zatnika A, Purwoto H, Istini S. Rumput Laut: Pembudidayaan, Pengolahan, dan Pemasaran Komoditas Perikanan Potensial. Penebar Swadaya. Jakarta, 2010.
3. Asikin AN, Kusumaningrum I. Karakteristik fisikokimia karagenan berdasarkan umur panen yang berbeda dari perairan Bontang, Kalimantan Timur. Jurnal Pengolahan Hasil Perikanan Indonesia,2019:22(1):136-142.
4. Blakemore William R, Alan R Harpell. Carrageenan: Food Stabilisers, Thickeners and Gelling Agents In A. Imeson Ed. Blackwell Publishing Ltd. Singapore, 2010.
5. Campo VL, Kawano DF, da Silva Jr, DB dan Carvalho I. Carrageenans: Biological Properties, chemical modifications and structural analysis-A review. Elsevier Carbohydrate Polymers,2009:77:167-180.
6. Dirhami A, Fardiaz D, Andrawulan N, Heruwati Sri E. Karakteristik Karagenan Hasil Isolasi Eucheuma spinosum (Alga Merah) Dari Perairan Sumenep Madura. Jurnal Perikanan dan Kelautan,2011:16(1):117-124.
7. Distantina S, Fadilah, Danarto YC, Wiratni dan Fahrurrozi M. Efek Bahan Kimia pada Tahap Presipitasi terhadap Rendemen dan Sifat Karagenan dari Rumput Laut Eucheuma cottoni. *Ekuilibrium*,2009:8(2):47-53.
8. Ega L, Gracia Cynthia LC, Meiyasa F. Kajian Mutu Karagenan Rumput Laut Eucheuma cottoni Berdasarkan Sifat Fisiko – Kimia pada Tingkat Konsentrasi Kalium Hidroksida (KOH) yang Berbeda. Indonesian Food Technologists: Jurnal Aplikasi Teknologi Pangan,2016:5(2):38-44.
9. FAO (Food and Agriculture Organization). JECFA Monograph 16, Compendium Of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives. 79th meeting 2014 Food and Agriculture Organization of The United Nations World Health Organization. Rome, 2014, 7-12.
10. Food Marine Colloids Corp (FMC Corp). Carrageenan Marine Colloid Monograph Number One Springfield New Jersey. USA: Marine Colloid Division FMC Corporation, 1977, 23-29.
11. Hayashi L, de Paula EJ, Chow F. Growth rate and carrageenan analyses in four strains of Kappaphycus alvarezii (Rhodophyta, Gigartinales) farmed in the subtropical waters of Sao Paulo State, Brazil. Journal of Applied Phycology,2007:19:393-399.
12. Kim HW. Knowles CJ. Kim HE. Hydroxyapatite and gelatin composites foams processed via novel freeze-drying and crosslinking for use as temporary hard tissue scaffold. University College London and Seoul National University, 2003.
13. KKP (Kementrian Kelautan Perikanan). Peta Lalulintas Rumput Laut Nasional. Kementrian Kelautan dan Perikanan Republik Indonesia, 2018.
14. Prasetyowati, Jasmine C, dan D Agustiawan. Pembuatan Tepung Karagenan dari Rumput Laut (Eucheumacottonii) Berdasarkan Perbedaan Metode Pengendapan. Jurnal Teknik Kimia,2008:15(2):27-33.
15. Sembiring B. Pengaruh Konsentrasi Bahan Pengisi Dan Cara Pengeringan Terhadap Mutu Ekstrak Kering Sambiloto. Bulletin Littro,2009:20(2):173-181.
16. Velde F, van de, Lourenco ND, Pinheiro HM, Bakker M. Carrageenan: a Food-grade and Biocompatible Support for Immobilisation Techniques. Advanced Synthesis and Catalysis,2002:344(8):815-835.
17. Wenno MR, Thenudan JL, Lopulalan CGC. Karakterisasi Kappa Karagenan dari Kappaphycus alvarezii pada Berbagai Umur Panen. Jurnal Pasca panen dan Bioteknologi Perikanan,2012:7(1):61-67.