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Research Article

LOCALLY CARRAGEENAN TESTED FOR ITS FOOD GRADE BY FOOD AND AGRICULTURE ORGANIZATION METHOD

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ABSTRACT

Objective: This study aims to determine whether seagrass lalamakan (*Dictyota dichotoma*), ades (*Gelidium* sp.), agar (*Gracilaria* sp.), and saribuhu (*Sargassum* sp.) obtained from the Sindangkerta beach, Tasikmalaya, can produce carrageenan food grade according to the Food and Agriculture Organization (FAO), Food Chemical Codex (FCC), and European Economic Community (EEC).

Methods: Seaweed was extracted with distilled water and 0.1N KOH at high temperature (90°C). The extract was tested for its characteristics such as solubility test, test sulfate, gel formation, and its infrared (IR) spectrum.

Results: The yield obtained from solvent extraction with distilled water ranged from 21% to 27%, whereas for 0.1N KOH solvent between 10% and 11% by weight. The results of the study indicated that the fiber obtained from both extractions was carrageenan, as shown by the results of the solubility test, the formation of gel and the infrared spectrum showed a sulfate ester, 3,6-anhydrogalactose, galactose-4-sulfate, and 3,6-anhydrogalactose-2-sulfate which was characteristic of carrageenan. IR spectrum confirmed the availability of carrageenan. Results of the sulfate test content in all sample fibers using either distilled water or 0.1N KOH were below 15.0%.

Conclusion: These results indicated that the generated carrageenan did not meet food grade standards set by the FAO, FCC, and EEC which was 15-40%. However, in fact, the carrageenan from the Sindangkerta beach has been produced and marketed in the local market.

Keywords: Seaweed, Carrageenan, Extraction, Infrared spectrophotometer, Distilled water, KOH.

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INTRODUCTION

Diversity of Indonesian marine resource potential to produce a wide variety of bioactive compounds derived either from plants or animals. This is an opportunity and challenge in the utilization of marine natural products for food, pharmaceutical, and cosmetics industry purposes [1-7]. Approximately, 555 species of seaweed in Indonesia and most of the products have been exported as seaweed dried seaweed and processed [8-10]. In Indonesia, many studies conducted on red seaweed mainly *Eucheuma* sp. Seaweed grouped by chemical compounds they contain, so well known the producer of carrageenan seaweed (karagenofit), agar (agarofit), and alginate (alginofit). Based on these groupings, the red seaweed (*Rhodophyceae*) such as *Eucheuma cottonii*/*Kappaphycus alvarezii* and *Eucheuma spinosum* classified as a producer of carrageenan seaweed because it has high levels of carrageenan, approximately 62-68% dry weight [6,11-13].

Currently, Sindangkerta Village, District Cipatujah, Tasikmalaya, one of the villages on the south coast of the island of Java has a huge potential in the development of marine utilization of material products, particularly seaweed having high-selling power. Without processing seaweed into a better product, one of which carrageenan, of course, the sale value of raw or dried seaweed is not too high. When it becomes flour, carrageenan has a sale value higher than the selling seaweed before it is processed (raw) [14-16]. Here are some seaweed species most widely used in the village Sindangkerta, namely, lalamakan (*Dictyota dichotoma*), ades (*Gelidium* sp.), agar (*Gracilaria* sp.), and saribuhu (*Sargassum* sp.). With the aim to produce a product, that is, more commercial value is higher, we conducted a study of seaweed to be carrageenan, quality food grade. This study was done considering there has been no research on the specification of the carrageenan Sindangkerta product.

MATERIALS AND METHODS

Instrument

The instrument used was infrared (IR) spectrophotometer and Shimadzu Fourier transform infrared (FTIR)-8400.

Plant material

Plant material used in this study was simplisia seaweed lalamakan (*D. dichotoma*), ades (*Gelidium* sp.), Agar (*gracilaria* sp.), and saribuhu (*Sargassum* sp.) obtained from the Sindangkerta beach – Tasikmalaya. Type of seaweed was guided by LIPI book [17].

Methods

Seaweed samples were obtained randomly from Sindangkerta beach, at different growing distances and depths without regard to sample age.

Plant determination

The seaweed material was determined in the Taxonomy Laboratory, Department of Biology, Faculty of Science, University of Padjadjaran.

Seaweed was washed with clean water, clean of impurities such as sand and salt, then cut to small + 1 cm, then dried in an oven at 600°C to constant weight.

Extraction of carrageenan with distilled water

Extraction and its parameters based on the Departemen Kesehatan RI [18-20].

Seaweed was dried and weighed 2.5 g and then soaked in distilled water for 15 minutes and filtered with a cloth.

The extraction was done in the Erlenmeyer which was heated in a water bath shaker. First, the solvent preheated, after reaching a temperature of 90°C, seaweed inserted, and extraction time started counting. Comparison of seaweed and solvent is 1:30 (g/mL). The volume of solvent was kept constant by adding hot distilled water each time. After a certain time, the extraction was stopped by means of the filtrate separated from the dregs of seaweed. The filtrate was then collected into a beaker containing 96% ethanol with a ratio of 3 times the volume of the filtrate while stirring to form fibers hydrocolloids (carrageenan fibers). After allowed to stand about 30 minutes, the fiber was filtered and washed with distilled water until the washing water has a neutral pH. Carrageenan wet dried in an oven at 60°C until constant weighed to obtain the dry carrageenan (carrageenan paper). All of the above steps were repeated for each type of seaweed.

Extraction of carrageenan with 0.1 N KOH

Extraction with 0.1 N KOH was carried out similar to water extraction, but the seaweed was used two times more weigher.

Analysis characteristics of carrageenan

The yield

The yield of carrageenan obtained by comparing the weight of carrageenan obtained with seaweed weighed heavy. The yield was calculated by

% Yield=(weight carrageenan)/(Weight seaweed)×100%

Solubility

Testing was carried out by trying to dissolve the carrageenan to distilled water and 96% ethanol.

Assay test sulfate

Nearly 0.5 g (W_1) of sample hydrolyzed with 50 ml of HCl 0.1 N for 15 minutes at boiling temperature. Some 10 mL $BaCl_2$ 0.25M added little and simmered for 5 minutes. After cooling for 5 hrs, the precipitate is filtered using Whatman filter paper (no. 42 ashless) and subsequently burned in a furnace at 700°C for 1 hr. The remaining annealing in the form of heavy white ash was barium sulphate (W2).

Levels are calculated by

% Sulfate=(W_1/W_2)×100×0.4116

Identification of formation gel

Add a few grams of sample to a few ml of hot water (80°C) constant shake until dissolved and then cooled the solution at room temperature.

FTIR

About 1-2 mg of sample with a carefully blended with 300-400 mg KBr and pelleted by means of a rotary vacuum pump. Transparent pellets were formed and then inserted into the instrument. Samples and standard measured and automatically recorded in the form of the spectrum.

RESULTS AND DISCUSSION

Seaweed collection results

Seaweed is taken directly from the beach Sindangkerta, Tasikmalaya, between the months of October to December. Samples are taken at the distance grows and grows different depths and also with the different ages.

Plants determination results

Determination plants do in the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology. The purpose of this determination was to determine the species and families of plants used in the study. The result of determination showed that seaweed was a seaweed species lalamakan *D. dichotoma* (Hudson) J.V. Lamouroux of the family Phaeophyceae, species *Gracilaria* sp. (Greville, 1830) of the family Gracilariaceae, species *Gellidium* sp. of families Gelidiaceae, and species saribuhu *Sargassum* sp. of families Sargassaceae.

Extraction results

From extraction, using a distilled water to yield obtained *Gellidium* sp. amounting to 22.22%, *Gracilaria* sp. amounting to 21.35%, *D. dichotoma* amounting to 21.49%, and *Sargassum* sp. amounting to 26.66%.

As for the results of the yield of the extraction with 0.1N KOH solvent that was *Gellidium* sp. 10.83%, *Gracilaria* sp. amounting to 10.20%, *D. dichotoma* by 10.72%, and *Sargassum* sp. amounting to 10.16%.

Compared with distilled water, the low yield of solvent extraction using KOH due to the breakdown of the polymer by alkaline solutions. Hence, that the fibers were obtained during the deposition process becomes a bit. Unlike, the fibers obtained from the precipitation filtrate extraction with distilled water.

Total yield indicated how well the treatment was carried out on samples of seaweed. If the yield obtained great results, indicating that the processing was carried out has been quite good. If the yield of the resulting slightly, indicating the possibility of processing that is done is not good enough.

Manuhara *et al.* [21] studied K-carrageenan (RC) extracted from red algae originated from Karimun Jawa Island. They indicated that higher KCl concentration in extraction resulted in the increase of the carrageenan yield. Actually, the FAO Corporate Document Repository (available at http://www.fao.org/docrep/006/y4765e/y4765e0a.htm) has given a guide to the seaweed industry. Webber *et al.* [22] proposed an alternative method of extraction of carrageenan without previous alkaline treatment and ethanol precipitation using response surface methodology. They claimed that the carrageenan extract properties determined by the polynomial model were 31.17%. Mustapha *et al.* [23] stated in their alkaline extraction of red seaweed, *Eucheuma cottonii*, to yield semi-refined carrageenan (SRC) of kappa type. Temperatures 60-80°C was suitable for extraction of SRC. At 80°C using 1.0 M KOH produced SRC contained the highest purity of 3,6-anhydrogalactose, a lower heavy metal concentration gave highest rupture force.

Characteristics analysis of fiber

The results of organoleptic test (color)

The test results of fiber colors are extracted with a solvent distilled to *Gelidium* sp. brown, *Gracilaria* sp. amber, amber *D. dichotoma*, and *Sargassum* sp. brown.

The test results of fiber colors are extracted with 0.1N KOH solvent for *Gelidium* sp. brown, *Gracilaria* sp. amber, *D. dichotoma* brown color, and *Sargassum* sp. brown.

The results of organoleptic test (odor)

For the results of the smell test on fibers obtained from solvent extraction with distilled water that each fiber has a distinctive odor. The odor was different from the extraction results with 0.1 N KOH.

Test results solubility and gel formation

The test results solubility and gel forming fibers distilled solvent extraction (with ethanol 96%) for the four types of the same seaweed that was not soluble and does not form a gel.

The test results solubility and gel forming fibers 0.1N KOH solvent extraction (with ethanol 96%) for the four types of the same seaweed that was not soluble and did not form a gel.

The test results solubility and gelling of the hot distilled water (80°C) fiber extraction with distilled solvent to the four types of the same seaweed that was not dissolved, but it turned into a kind of gel fibers.

Solubility test results against hot distilled water (80°C) fiber 0.1N KOH solvent extraction for the four types of the same seaweed that was not dissolved, but it turned into a kind of gel fibers.

From the results, gel formation can be seen that the fibers resulting from the extraction were done included in the kappa carrageenan or iota carrageenan. As for the lambda carrageenan did not belong to it, because the results obtained should not form a gel for lambda carrageenan, according to the nature of the carrageenan listed in USP, that kappa carrageenan and iota carrageenan yielding gel whereas for lambda carrageenan does not form a gel.

For fiber from Sigma comparative carrageenan, the resulting gel texture is easily broken, then the fiber *Gellidium* sp. produces a gel which easily broken, as well as fibers produced from *Gracillaria* sp., *D. dichotoma*, and *Sargassum* sp., namely, textured gel easily broken.

Sulfate assay test results

Results of the analysis of the content of sulfate showed fiber obtained from solvent extraction with 0.1N KOH, respectively, were *Gellidium* sp. 4.474%, *Gracilaria* sp. 4.929%, 2.065% *D. dichotoma*, and *Sargassum* sp. 1.070%. Results of the analysis showed the content of sulfate fiber obtained from solvent extraction with distilled water, respectively, are *Gellidium* sp. 2.124%, *Gracilaria* sp. 4.540%, 7.043% *D. dichotoma*, and *Sargassum* sp. 1.015%. The results can be seen in Tables 1 and 2.

The concentration of the solvent used KOH significant effect on levels of sulfate of fiber carrageenan. Large concentrations of alkaline solution used at the time of extraction can make the sulfate content of carrageenan smaller. The addition of an alkaline solution of carrageenan in the manufacturing process could eliminate or reduce the levels of sulfate ester at C6 of a chain of 1-6-D-galactose. Sulfate esters which reacted with alkali to form sulfate salts thus more easily separated during the screening process. The loss of sulfate ester would form a ring which had a 3.6-anhydrogalactose straight chain, resulting in the formation of gel will easily occur. This result aligned with results of research conducted by Distantina *et al.* [24] which showed the influence of carrageenan sulfate content generated from solvent extraction with distilled water and solvent bases. Which also states that the use of 0.1N KOH solvent extraction with an increasingly long time, the levels of sulfates in carrageenan also decrease.

The results of the analysis of sulfate content of fiber extraction solvent seaweed well with distilled water and 0.1N KOH food grade not meet the standards under the Food Chemical Codex (FCC), the European Economic Community (EEC), and the Food Agriculture Organization (FAO) were 15-40%. Yasita and Rachmawati [25] claimed that they were able to have a food grade carrageenan from red algae (*E. cottonii*) using NaOH instead of KOH and also using H_2O_2. Mishra *et al*. [26], however, in their study, on yield and quality of carrageenan from *K. alvarezii* subjected to different physical and chemical treatments, showed that treatment with KOH gave better yield and quality gel.

Table 1: Results of sulfate test on fiber extraction with distilled water

Fiber sample	Result (%)
Gellidium sp.	2.124
Gracilaria sp.	4.540
Dictyota dichotoma	7.043
Sargassum sp.	1.015

Table 2: Results of sulfate test on fiber extraction with 0.1 N KOH

Fiber sample	Result (%)
Gellidium sp.	4.474
Gracilaria sp.	4.929
Dictyota dichotoma	2.065
Sargassum sp.	1.070

Infrared spectroscopy

For the results of the analysis by infrared spectroscopy, as can be seen in Table 2, almost all fibers showed absorption at wavenumbers that are characteristic of carrageenan, either for sulfate ester (1220-1260/cm), 3,6- anhydrogalactose (928-933/cm), galactose 4-sulfate (840-850/cm), or 3,6-anhiydrogalactose-2-sulfate (800-805/cm). These result in accordance with another researcher [27] and also refer to the reference of JECFA [14] on the FAO JECFA Monograph 4 to carrageenan. According to Pancomulyo *et al.* [28], carrageenan hydrocolloid was a compound which was a composition of long-chain polysaccharide compounds extracted from seaweed. Furthermore, according to Kordi and Ghufran [29], carrageenan was a linear polysaccharide and was a molecule galactans with units mainly in the form of glucose. Volery *et al*. (2004) [30] reported characterization of commercial carrageenans by FTIR spectroscopy using single-reflection attenuated total reflection and mentioned that the total preparation and analysis time was <5 minutes per sample.

The fibers obtained in this study have absorption at wavenumbers between 1000-1100/cm which are a common trait of the polysaccharides that indicate when their absorption at the wavenumbers length is a polysaccharide compound.

Then, Kordi and Ghufran also added, polysaccharide be composed of galactose units with a bond α (1,3) D-galactose and β (1,4) 3,6-anhidrogalaktosa alternately, both contain ester sulfate or without sulfate. The results shown by the infrared spectrum of each fiber obtained from the extraction with distilled solvent or solvent 0.1N KOH can be concluded that the fiber was carrageenan, as each fiber showed absorption at wavenumber that was characteristic of carrageenan by JECFA [14] on the FAO JECFA Monograph 4 (Tables 3-10).

CONCLUSIONS

Based on the extraction results obtained using the solvent extraction method with distilled water and 0.1N KOH solvent, it concluded that the extraction method could be used to obtain carrageenan from seaweed,

Table 3: IR spectrum of carrageenan standard (Sigma)

Wave number (cm^{-1})	Spectrum	Functional group
928-933	927.78	3,6-anhydrogalactose
840-850	846.76	Galactose-4-sulfate

IR: Infrared

Table 4: IR spectrum of *Gellidium* sp. extracted by distilled water

Wave number (cm^{-1})	Spectrum	Functional group
928-933	932.60	3,6-anhydrogalactose

IR: Infrared

Table 5: IR spectrum *Gracilaria* sp. extracted by distilled water

Wave number (cm^{-1})	Spectrum	Functional group
1220-1260	1257.61	Sulfate ester
928-933	931.63	3,6-anhydrogalactose
840-850	846.76	Galactose-4-sulfate
800-805	802.40	3,6-anhydrogalactose-2-sulfate

IR: Infrared

Table 6: IR spectrum *Sargassum* sp. extracted by distilled water

Wave number (cm^{-1})	Spectrum	Functional group
1220-1260	1232.53	Sulfate ester
800-805		

IR: Infrared

Table 7: IR spectrum *Gellidium* sp. extracted by KOH 0.1 N

Wave number (cm^{-1})	Spectrum	Functional group
1220-1260	1239.29	Sulfate ester
928-933	932.60	3,6-anhydrogalactose
840-850	848.69	Galactose-4-sulfate

IR: Infrared

Table 8: IR spectrum *Gracilaria* sp. extracted by KOH 0.1 N

Wave number (cm^{-1})	Spectrum	Functional group
928-933	928.78	3,6-anhydrogalactose
840-850	846.76	Galactose-4-sulfate

IR: Infrared

Table 9: IR spectrum *Dictyota dichotoma* extracted by KOH 0.1 N

Wavenumber (cm^{-1})	Spectrum	Functional group
928-933	930.67	3,6-anhydrogalactose

IR: Infrared

Table 10: IR spectrum *Sargassum* sp. extracted by 0.1 N

Wave number (cm^{-1})	Spectrum	Functional group
810-820	818.80	Galactose-6-sulfate

IR: Infrared

with the yield obtained ranged from 21-27% and 10-11% with distilled water and 0.1N KOH, respectively.

It concluded as well as that the fibers had some character carrageenans, except for the sulfate content of the fiber was not yet eligible carrageenan food grade standards established by the FAO, FCC, and EEC.

Based on this result, it suggests further for optimization of the method used to obtain results that can qualify the standards set by the FAO, FCC, and EEC.

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