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Optimization of Extraction and Quality Assessment Based on Physicochemical Properties of Carrageenan from Red Algae (*Kappaphycus alvarezii*) Origin of South Sulawesi Indonesia: A Recent Study

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ABSTRACT

One of the compounds that can be developed as raw material for drugs or excipients is carrageenan which can be obtained from the red algae *Kappaphycus alvarezii*. This species of red algae was chosen as the object of research because in addition to its high carrageenan content, this species is easily found in the waters of South Sulawesi Indonesia. This research was started with collecting, washing and processing the red algae, then carrageenan extraction was carried out using the modified extraction method. The calculation results repeatability and reproducibility show that the %RSD of both does not exceed 3%, therefore the carrageenan extraction method using technical ethanol precipitator and pro-analytical ethanol meets the requirements repeatability and reproducibility precision because it meets the Horwitz standard, which is a maximum RSD of 3%. The extracted products were separated, purified and dried to obtain carrageenan raw materials. Then, the products were analyzed physically and chemically, and also using spectrophotometry and chromatography to ensure that carrageenan obtained meets the quality standards as excipient raw materials in pharmaceutical preparations. The results obtained were that *Kappaphycus alvarezii* red algae raw material fulfil the quality of the Indonesian National Standard (SNI) and carrageenan produced meet the quality standards of carrageenan according to *Food Agriculture Organization* (FAO), Food Chemicals Codex (FCC) and European Economic Community (EEC).

Keywords: Kappaphycus alvarezii; carrageenan; raw materials drugs; excipients

1. INTRODUCTION

Red algae is a source of state income and a source of revenue for coastal communities. Apart from being used as food, beverages and medicines, some of the products processed from red algae such as gelatine, alginate and carrageenan are important compounds in the industry. Some types of red algae are found in Indonesia and have high economic value are gelatin-producing red algae (*agarophyte*), namely Gracilaria, Gelidium, Gelidiopsis, and Hypnea; algin-producing red algae, namely Sargasum and Turbinaria; carrageenan-producing red algae (*carrageenophyte*), namely *Eucheuma cottoni (Kappaphycus alvarezii)*, *Eucheuma spinosum* and *Eucheuma striatum* [1].

Most of the red algae in Indonesia are exported in dried form [2]. If viewed from an economic perspective, the price of processed red algae such as carrageenan is much higher than dried red algae. Therefore, to increase the added value of red algae and reduce imports of processed products, the processing of red algae into carrageenan in the country needs to be developed [1]. If

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Kappaphycus alvarezii are processed into carrageenan, they can provide a profit increase of up to 20 to 30 times [3].

Carrageenophytes are red algae which contain the main ingredients of carrageenan linear polysaccharides which are composed of D-galactose and L-galactose 3,6 anhydrogalactose units which are linked alternately by alpha-1,3 and beta-1,4 glycosidic bonds. There are three types of carrageenan, namely iota carrageenan or known as spinosum type, kappa carrageenan or known as cottoni type and lamda carrageenan. All three are distinguished by the nature of the jelly formed. Iota carrageenan produces soft and flexible jelly. Kappa carrageenan produces stiff, brittle, and hard jelly. Mean while, lambda carrageenan cannot produce jelly, but is viscous liquid in nature [4,5].

Carrageenan is a hydrocolloid compound which is a long chain polysaccharide compound extracted from red algae of the types of carrageenophytes, such as *Eucheuma sp*, *Chondrus sp*, *Hypnea sp*, and *Gigartina sp*. The polysaccharides were arranged from a number of galactose units with alternating α(1,3) D-galactose and β(1,4) 3,6-anhydrogalactose bonds, both containing sulfate esters or without sulfates [6].

Based on the stereotype of the molecular structure and the position of the sulfate ion, carrageenan is divided into three types, namely iota-carrageenan, kappa-carrageenan, and lambda-carrageenan. All three differ in the nature of gel reaction with proteins. Kappa-carrageenan produces strong gel, while iota-carrageenan produces soft gel and is easily formed. In addition, each carrageenan is also produced by different types of red algae. The solubility of carrageenan in water is influenced by several factors, namely temperature, the presence of other organic compounds, salts that are soluble in water, and the type of carrageenan itself [6].

Carrageenan isolation procedures from various red algae have been developed. Generally, this procedure consists of three working stages, namely; extraction, filtering, and precipitation. In the extraction stage, the velocity and solubility of carrageenan in water are influenced by the temperature and time of the process of joining all carrageenan fractions from red algae with water fractions used as solvent media [7].

The degree of carrageenan viscosity is influenced by concentration, temperature, and other molecules dissolved in the mixture. The viscosity of carrageenan solution will decrease rapidly, with increasing temperature. The types of carrageenan in forming a gel are divided into two different types: hard and soft types. The texture depends on the type of carrageenan, concentration, the presence of other ions, the presence of other solutions, and hydrocolloid compounds that do not form gels. If in the solution there are potassium ions, the kappa carrageenan gel tends to be more brittle than the iota carrageenan. The iota carrageenan gel elasticity is caused by the presence of 2 sulfate ions in the polymer [6].

Basically, the process of carrageenan production consists of the process of preparing raw materials, carrageenan extraction using extracting materials, purification, drying and milling. The process of obtaining raw materials for red algae consists of removing sand, mineral salts, and foreign objects that are still attached to the seaweed. Carrageenan extraction is carried out using hot water or hot alkaline solution [8].

The extraction process using bases is the most economical one. After being extracted, the dissolved carrageenan will be dried and milled into flour form with various gel grade. Carrageenan is a compound with a high molecular weight and is a polydispersed material. Carrageenan extract has a molecular weight ranging from 400-560 kDa, while Processed Euchema Seaweed (PES) has a molecular weight of 615 kDa.

Based on data from the International Medical Research Institute that the world's carrageenan needs are currently around 50 thousand tons and increase on average 3% per year. If the carrageenan needs can increase rapidly to 25% per year, the world's carrageenan needs in 2014 will reach 100 thousand tons. Or, the world's need for raw materials for carrageenan-producing red algae reaches

400 thousand dried tons (4 million wet tons). Data in 2009 said that the new red algae industry could absorb 14,300 tons of red algae raw material or about 57,200 tons of wet sample [9]. These data indicate that carrageenan has a very potential prospect to be developed as excipient raw materials in addition to medicinal raw materials in accordance with the ministry of health priority program.

The success of the carrageenan isolation process from red algae is highly dependent on several factors such as the environmental conditions in which seaweed grows, the purity of the organic solvents used, and the extraction method chosen. Separation of carrageenan from extracting material is carried out by filtration and precipitation. Filtering carrageenan extract generally still uses conventional filtering, namely filter cloth and filter press, in hot conditions intended to avoid gel formation [10]. Precipitation of carrageenan can be carried out, among others, by the method of gel press, KCl press, or precipitation with alcohol [11]. Wet carrageenan drying can be done by oven or air drying [12]. Drying using the oven is carried out at temperature of 60 ^{o}C [13]. Dried carrageenan is then mixed, sifted, standardized and mixed, then packaged in tightly closed containers [14].

Alkaline condition can be obtained by adding alkaline solutions such as NaOH, $Ca(OH)_2$, or KOH solutions so that the pH of the solution reaches 8 - 10. The volume of water used in extraction is 30-40 times the weight of seaweed. Extraction usually approaches the boiling temperature of around 90 – 95^{o}C for one to several hours. The use of alkaline has two functions, namely to help extracting the polysaccharides more completely and accelerate the elimination of 6-sulfate from the monomer unit to 3,6-anhydro-D-galactose so that it can increase gel strength and product reactivity to proteins [7,15,16]. Carrageenan extraction using (KOH) affects the increase in yield and quality of carrageenan produced [17].

There have been many procedures that have been carried out to obtain carrageenan, but the problems that are often encountered are the low carrageenan yield produced at the time of extraction and the low quality of carrageenan obtained after the deposition of carrageenan, making it difficult to obtain large amounts of carrageenan according to the desired quality standards to meet the needs of pharmaceutical industry. The approach chosen in solving the above problems is to optimize the extraction process in order to obtain higher carrageenan yield and optimization of purification techniques in order to obtain carrageenan that meets pharmaceutical quality standards. The purpose of this study is the production of carrageenan from *Kappaphycus alvarezii* red algae from South Sulawesi waters that meet pharmaceutical quality standards after going through the application of extraction techniques in optimal conditions so that it can be used as a reference for the development of industrial scale carrageenan.

2. EXPERIMENTAL

2.1 Sample Collection and Processing of *Kappaphycus alvarezii* Red Algae

Red algae samples from *Kappaphycus alvarezii* were taken from several red algae cultivation sites in South Sulawesi, namely Bantaeng, Takalar, Maros and Bone Districts. To ensure that the samples *Kappaphycus alvarezii* red algae were obtained correctly, the determination was made at the Faculty of Marine and Fisheries Laboratory, Hasanuddin University. Fresh red algae samples from *Kappaphycus alvarezii* collected from red algae cultivation in South Sulawesi waters were selected and washed with water until clean. Next, the samples are weighed and soaked in rice washing water with a ratio of 1:20 for 12 hours then lifted and washed with water then followed by soaking with water for 12 hours. After that, the samples were removed and cleaned with water then cut into small pieces (size 2-3 cm) to facilitate drying. Samples that had been cut into small pieces are dried in direct sunlight for 2-3 days.

2.2 Standardization of Red Algae *Kappaphycus alvarezii* Raw Materials

The standardization of dried red algae *Kappaphycus alvarezii* was carried out by measuring several quality parameters based on the latest Indonesian National Standard (SNI) provisions, namely analysis of moisture content, impurities rough, organoleptic test and characteristic test.

2.2.1 Water content analysis

Determination of water content was based on differences in sample weight before and after drying. The porcelain cup to be used, is dried first about 1 hour at 105°C, then cooled in a desiccator for 30 minutes and weighed until the weight is fixed (A). The sample weighed about 2 g (B) in the cup, dried in an oven at 105°C for several hours (5 hours) until the weight is fixed. The cup containing the sample was cooled in the desicator for 30 minutes then weighed until the weight remained (C). Water content is calculated by the formula:

$$Water\ content\ (\%) = \frac{(A + B) - Cx\ 100\ \%}{(B)}$$

2.2.2 Impurities rough analysis

Analysis of impurities rough in samples of dried red algae includes examination of the presence of salt, sand, coral, wood and other types. Weigh 250 grams of the sample in the Cup and record its weight (Wo). Separate and collect dirt (other types of seaweed, plastk, clams, corals and other foreign bodies) from seaweed. For the dirt stuck (sand and salt) is separated by being freed. Weigh the debris that has been collected and record the weight (Wd). Impurities rough content was calculated using the formula:

Impurities rough (%) = Wd/Wo x 100%

Note: Wo is a sample weight used for analysis (g); Wd is heavy dirt and other foreign objects (g)

2.2.3 Organoleptic analysis

Organoleptic tests were carried out on dried red algae samples. Dried red algae were tested for the quality of the appearance (shape, color, and appearance), odor and texture. Appearance: The criteria used in the quality test for the appearance of dried red algae were clean, fibrous lengths of 10-40 cm and whitish red in color evenly. Odor: The range of quality testing for the smell of dried red algae is the specific odor of red algae. Texture: The range of testing the quality of the texture of dried red algae is dry, clayey, easily broken between the stem and branches (thallus).

2.2.4 Characteristic test

Characteristic test of dried red algae samples included several tests, namely determination of water content, ash content, acid insoluble ash content, protein content, fat content, carbohydrate content, sulfate content and heavy metal content.

2.3 Optimization of Carrageenan Extraction

At this stage carrageenan extraction process was carried out with a variety of parameters, namely using alkaline solution at a concentration of 5-9%, heating temperature 70-100^{o}C and extraction time of 2-4 hours. Observations were made on one of the parameters which became an indicator of the quality of carrageenan, namely yield. Determination of the best conditions for extracting carrageenan was chosen based on the results of temperature treatment, alkali concentration and the best extraction time obtained from this study.

Optimization of the carrageenan extraction process was carried out with four treatments, namely the temperature of red algae heating temperature, alkali concentration and extraction duration and determination of the type of alcohol used as precipitating agent.

2.3.1 Determination of heating temperature of red algae

The dried red algae were weighed 500 g each and put in a container, then 10 liters of distilled water were added followed by KOH 9% (pH 9). Each of sample was boiled for 3 hours with the temperature

variation 70-100^{o}C. After that, filtration was done using gauze. The filtrate obtained was neutralized with hydrochloric acid and precipitated by the alcohol method (using ethanol) until carrageenan fibers were formed, then filtered again with gauze. After that, the sample was sundried for 2-4 days. Dried carrageenan was then milled to form powdered carrageenan.

2.3.2 Determination of alkaline concentration

Dried red algae *Kappaphycus alvarezii* was each weighed 500 g, then cooked in a pan using distilled water with a volume of 10 liters. Extraction of a red algae *Kappaphycus alvarezii* was conducted by cooking in alkaline conditions using a variation of the concentration of 5% KOH, 7% KOH and 9% KOH until the heating temperature reaches 90^{o}C. After the extraction process was finished, the samples were filtered using gauze in hot conditions, to separate between the filtrate in the form of soles (thick liquid) and solid residue (pulp). The filtrate obtained was neutralized with hydrochloric acid and precipitated by the alcohol method (using ethanol) until carrageenan fibers were formed, then filtered again with gauze. After that, the sample was sundried for 2-4 days. Dried carrageenan was then milled to form powdered carrageenan.

2.3.3 Determination of extraction time

The dried red algae were weighed 500 g each and put in a container then 10 liters of distilled water added with 9% KOH (pH 9). Each sample was boiled for 2 hours, 3 hours, and 4 hours with the temperature of 90^{o}C. After that, filtration was done using gauze. The filtrate obtained was neutralized with hydrochloric acid and precipitated by the alcohol method (using ethanol) until carrageenan fibers were formed, then filtered again with gauze. After that, sample was sundried for 2-4 days. Dried carrageenan was then milled to form powdered carrageenan.

2.3.4 Determination of the type of alcohol used as precipitating agent

Dried red algae were weighed 500 g then put in a pan containing 10 liters of distilled water. The pH of the solution was adjusted to pH 9 by using 9% KOH. Boiling was carried out for 3 hours at a temperature of 90°C. After the boiling process, the red algae extract was filtered with a gauze filter in hot conditions to facilitate the screening process. The filtrate obtained was neutralized with hydrochloric acid then precipitated by pouring alcohol (ethanol, methanol and isopropyl alcohol) into each carrageenan filtrate under constant stirring, so that carrageenan fibers were formed. The carrageenan fiber obtained was washed with fresh alcohol, then filtered and dried in direct sunlight for 2-4 days, then dried carrageenan fibers were milled to obtain carrageenan powder.

2.3.5 Determining the best conditions

Determining the best conditions to obtain carrageenan selected based on the quality parameters, namely yield, and other quality parameters.

2.4 Verification of Carrageenan Yield Determination Method

Verification of the method for determining the yield of carrageenan from red algae *Kappaphycus alvarezii* was carried out using 2 types of solvents, namely technical ethanol and ethanol pro-analytical. Each carrageenan extraction was repeated 6 times. Verification parameters tested were repeatability and reproducibility. The acceptance criteria refer to the Horwitz standard with the stipulation that the repeatability and reproducibility test of the Relative Standard Deviation (RSD) is a maximum of 3% [18].

Dried red algae were weighed 500 g then put in a pan containing 10 liters of distilled water. The pH of the solution was adjusted to pH 9 by using 9% KOH. Boiling was carried out for 3 hours at a temperature of 90°C. After the boiling process, the red algae extract was filtered with a gauze filter in hot conditions to facilitate the screening process. The filtrate obtained was neutralized with hydrochloric acid then precipitated by pouring alcohol (technical ethanol and ethanol pro-analysis),

into each carrageenan filtrate under constant stirring, so that carrageenan fibers were formed. The carrageenan fiber obtained was washed with fresh alcohol, then filtered and dried in direct sunlight for 2-4 days, then dried carrageenan fibers were milled to obtain carrageenan powder. Calculate the percent yield of the carrageenan and Relatif Standard Deviation (RSD).

2.5 Carrageenan Production in Optimum Conditions

The dried red algae samples were put into production container which contains water added with 9% KOH solution (pH 9), then warmed up to 90^{o}C for 3 hours under occasional stirring. After that, the samples were filtered in hot conditions using gauze. The filtrate obtained was neutralized with hydrochloric acid and carrageenan precipitation was carried out by pouring ethanol into each carrageenan filtrate, forming carrageenan fibers. The carrageenan fibers obtained were then washed with fresh ethanol, then filtered and dried in direct sunlight for 2-4 days, milled to obtain carrageenan powder. Carrageenan extraction was carried out repeatedly according to this procedure until the required carrageenan was obtained.

2.6 Physicochemical Analysis of Carrageenan

The characteristic of carrageenan obtained was analyzed including yield, viscosity, water content, ash content, content of acid insoluble ash, protein, fat, carbohydrates, whiteness, levels of sulfates, heavy metals, gel strength, whiteness, and melting points general.

Yield [19]: Carrageenan yield as extraction results was calculated based on the ratio between the weight of carrageenan produced by the weight of dry red algae used.

$$Yield\ (\%)\ = \frac{Dried\ carrageenan\ weight}{Dried\ red\ algae\ weight} x\ 100\ \%$$

Viscosity [19]: Viscosity is the retention measured from liquid to flow. The scientific unit of viscosity is poise (1 poise = 100 cP). The higher the viscosity indicates the greater the resistance of the liquid in question. Carrageenan solution with a concentration of 1.5% was heated in a boiling water bath under constant stirring until the temperature reached 75^{o}C. The viscosity was measured using Brookfield Viscometer. The spindle was first heated at 75^{o}C and then mounted to the instrument. The position of the spindle in the hot solution was adjusted, the viscometer was turned on and the temperature of the solution is measured. When the temperature of the solution reaches 75^{o}C and the viscosity value was observed by reading the viscometer on a scale of 1 to 100. Reading was carried out after 2 times one-minute full rotation for spindle no 1.

Water Content [20]: Determination of water content is based on differences in the weight of the sample before and after drying. The porcelain cup to be used, was dried first about 1 hour at 105°C, then cooled in a desiccator for 30 minutes and weighed until the weight was fixed (A). Samples were weighed approximately 2 g (B) in the cup, dried in an oven at a temperature of 100-105°C for 5 hours or until the weight fixed. The cup containing the sample was cooled in the desiccator for 30 minutes then weighed until the weight fixed (C). Water content was calculated by the formula:

$$Water\ content\ (\%)\ = \frac{(A+B)-C}{(B)} \text{ x } 100$$

Ash content [20]: Ash content is calculated based on the ash weight formed during combustion in the kiln at 550 °C until the form of ash is white. Porcelain dishes were dried in the oven for one hour at temperature 105°C, then cooled for 30 minutes in the desiccator and weighed until fixed weight (A) was obtained. 2 g of sample was weighed (B), put into a porcelain dish and heated on a Bunsen burner until it no longer smoked. After that, it was inserted into the furnace with a temperature of 550 °C for ± 12 hours. Next, the cup was cooled for 30 minutes in the desiccator, then weighed until a fixed weight (C) was obtained. Ash content was calculated using the formula:

$$Ash\ content\ (\%)\ = \frac{(A\ +\ B) - C}{(B)} x\ 100\ \%$$

Acid insoluble ash content [19]: Ashed carrageenan was boiled with 25 ml of 10% HCl for 5 minutes. Non-soluble ingredients were filtered using ash-free filter paper. Filter paper was burned in the same way as above, then cooled in a desiccator to be weighed. The levels of acid insoluble ash are calculated by the formula:

$$Acid\ insoluble\ ash\ content\ (\%)\ = \frac{Ash\ weight}{Sample\ weight} x\ 100\%$$

Protein content [20]: Determination of protein content was carried out using the micro Kjeldahl micro method. 0.75 g of sample was put into the Kjeldahl flask, then 6.25 g of K_2SO_4 and 0.6225 g of $CuSO_4$ were added as catalysts. A total of 15 ml of concentrated H_2SO_4 and 3 ml of $H_{\ 2}O_2$ were slowly added to the flask and allowed to stand for 10 minutes in the acid chamber. The next stage was destruction process at 410°C for 2 hours or until a clear solution obtained, the sample was left to reach room temperature and 50-75 ml of distilled water was added. An Erlenmeyer flask was prepared with 25 ml of 4% H_3BO_3 solution containing an indicator (0.1% bromocresol green and 0.1% (2: 1) red methyl as a distillate container. Kjeldahl flask was installed on a steam distillation circuit. 50 ml of $Na_2(SO_4)_3$ (alkaline). Distillation was carried out and the distillate was stored in the Erlenmeyer until the distillate volume reached 150 ml (the result of green colored distillate). The distillate was titrated with 0.2 N HCl, until the color changes to natural gray. The blank was carried out using the same steps. The sample test was done in duplicate. The protein content was determined by the formula:

$$Protein\ content\ (\%)\ = \frac{(A\ -\ B)\ x\ N\ HCl\ x\ 14{,}007\ x\ 6{,}2}{W\ (g)} x\ 100\ \%$$

Note : A = ml HCl sample titration; B = ml HCl blank titration

Lipid content [21]: Lipid flask that had been dried in the oven (105°C) was weighed until fixed weight (A) was obtained. A total of 2 g of sample (C) was wrapped in fat-free filter paper and then put into a fat sleeve. The sleeve was inserted into the Soxhlet tube. A total of 150 ml of chloroform was put into the flask. The sample was refluxed for 8 hours, after the solvent had turned clear, indicating that all the fat had been extracted. Then, the solvent in the fat flask was evaporated to separate the solvent and fat, then the fat flask was dried in an oven of 105°C for 30 minutes. After that, the sample was weighed until fixed weight (B) was obtained. Fat content was calculated by the formula:

$$Lipid\ content\ (\%)\ = \frac{(B\ -\ A)}{C} x\ 100\ \%$$

Carbohydrate content: Performed by calculating the remainders (by difference). Carbohydrate content (%) = 100% - (water + protein + lipid + ash content)

Whiteness [8]: The instrument used was the whiteness meter. 3 g of sample was placed in specialized container. Before the instrument was prepared and turned on, the standard instructions must be in zero position. There are three types of filters that can be used, namely blue, green and red with a wavelength of 425 nm, 550 nm and 520 nm respectively. The test was repeated several time until appropriate average value was obtained

Sulfate content [19]: The principle used was that the sulfate group that has been weighed and hydrolyzed, to be deposited as $BaSO_4$. The sample was weighed as much as 1 g and put into an Erlenmeyer flask which was added with 50 ml of 0.2 N HCl then refluxed until boiled for 6 hours for the solution to become clear. This solution was transferred into a Beaker and boiled. Next, 10 ml of $BaCl_2$ solution was added to the water bath for 2 hours. The precipitate formed was filtered with ash-

free filter paper and washed with boiling distilled water until free of chloride. The filter paper was dried into a drying oven, then blanched at 1000^{o}C until white ash was obtained. The ash was cooled in the desiccator was then weighed. The sulfate content calculation is as follow:

$$Sulfate\ content\ (\%) = \frac{P\ x\ 0{,}4116}{Sample\ weight} x\ 100\ \%$$

Note: 0,4116 = SO_4 atom relative mass divided by $BaSO_4$ atom relative mass
P = weight of $BaSO_4$ precipitate (g)

Heavy metals [21]: The principle used is removal of organic materials with dry ignition, then the residues are dissolved in dilute acid. The solution was dispersed in the flame inside the Atomic Absorption Spectrophotometer so that the absorption or emission of metals could be analyzed and measured at maximum wavelengths. The heavy metal content to be analyzed was Pb, Zn, Cu and As using Atomic Absorption Spectrophotometer. The procedure was as much as 5-6 ml of 6 N HCl was added to a dish containing ashes, then heated on an electric heater (hotplate) with low heating until dried. After that, 15 ml of 3 N HCl was added, then the dish was heated over the electric heater until it began to boil. After being cooled and filtered, the filtrate was put into the appropriate measuring flask. The solids were left as much as possible in the dish and diluted with water to the set mark. The blank was prepared using the same reagent. The AAS instrument was set according to the manual instruction. Metal standard, blank and sample solutions were then analyzed. During the determination of the sample, an inspection was carried out to ensure the standard value remained constant. Then a standard curve for each metal was produced (absorption value vs. metal concentration in µg/ml).

Gel strength [19]: A solution of 1.6% carrageenan and 0.16% KCl was heated in a boiling water bath under constant stirring until the temperature of 80^{o}C. The volume of the solution was made up to 50 ml. The hot solution was put into a mold about 4 cm in diameter and left at 10^{o}C for 2 hours. The gel in the mold was inserted into a measuring instrument (*curd tension meter*) so that the plunger that would come in contact with the gel was in the middle. The plunger was activated and observed. The reading was done when the spring returned. The calculation of gel strength was as follows:

Gel strength (dyne/cm^2) = (F/S) *x* 980 dyne/cm^2

Note: *F* = curve height; S = sensing rod surface area (cm^2)

Melting point [22]: Carrageenan solution with a concentration of 6.67% (w/w) was prepared with distilled water. Samples were incubated at 10^{o}C for ± 2 hours. Measurement of melting point was carried out by heating the carrageenan gel in the water bath. A steel ball was placed on top of the carrageenan gel, and when the steel ball fell into the base of the carrageenan gel, the temperature was expressed as the melting point of carrageenan.

Gelling point [23]: Carrageenan solution with a concentration of 6.67% (w/w) was prepared with distilled water in a 15 ml volume measuring cylinder. The temperature of the sample was lowered slowly by placing it in a container with ice. The gelling point was measured when the carrageenan solution begins to form a gel using Hanna digital thermometer.

3. RESULTS AND DISCUSSION

3.1 Preparation and Processing of Red Algae

Kappaphycus alvarezii red algae used in this study were collected by seaweed farmers on several cultivation locations in South Sulawesi, namely Bantaeng, Takalar, Maros and Bone Districts. To ensure that the correct type of red algae was used, sample determination was conducted in the Faculty of Maritime and Fishery Science Laboratory, Hasanuddin University.

This research was carried out with the stages of preparing raw materials for red algae, boiling, drying and milling of carrageenan. Preparation of raw materials began with washing the red algae *Kappaphycus alvarezii* to remove sand, mineral salts and other foreign objects that were still attached to red algae and then dried under direct sunlight to obtain dried red algae. The drying step was carried out with the aim of reducing the water content in wet red algae.

Standardization of Red Algae Raw Materials. According to the latest SNI, the *Kappaphycus alvarezii* type red algae quality standards are a maximum moisture content of 32%, a maximum foreign matter of 5% and specific odor of seaweed. The results of the analysis of water content in the red algae samples can be seen in Table 1.

Table 1. Moisture content of *Kappaphycus alvarezii* red algae samples

Moisture content of red algae raw materials (%)					
Sample origin	**1**	**2**	**3**	**Total**	**Average**
Bantaeng	14.68	14.43	14.85	43.96	14.65
Takalar	21.57	21.81	20.96	64.34	21.45
Maros	13.72	13.73	13.77	41.22	13.74
Bone	16.98	15.95	16.43	49.36	16.45

Water content is an important component because it is related to the quality of red algae. The water content of red algae in this study ranged from 13.74 - 21.45%. The highest water content was obtained from red algae obtained from Takalar (21.45%) and the lowest in red algae obtained from Maros (13.74%). Dried red algae that have a high moisture content will be more easily damaged when compared with low-moisture red algae. In addition, red algae are hygroscopic so that storage in a humid place will cause damage to occur more quickly. SNI sets the water content of dried red algae *Kappaphycus alvarezii* for maximum 32 %, thus the water content of the sample used in this study still meets the quality standards of red algae raw materials. The results of analysis of the levels of impurities rough in the red algae raw materials can be seen in Table 2.

Table 2. Impurities rough content in the red algae *Kappaphycus alvarezii*

Impurities rough content of red algae raw materials (%)					
Sample origin	**1**	**2**	**3**	**Total**	**Average**
Bantaeng	0.04	0.02	0.06	0.12	0.04
Takalar	0.06	0.08	0.04	0.18	0.06
Maros	0.02	0.02	0.04	0.08	0.03
Bone	0.06	0.04	0.04	0.14	0.05

Based on these data, obtained impurities rough content in dried red algae used in this study ranged from 0.03 - 0.06 %. The highest impurities rough content was obtained from red algae obtained from Takalar (0.06%) and the lowest in red algae obtained from Maros (0.03%). This raw material meets the requirements of SNI, namely the content of impurities rough for red algae raw material must not exceed 5 %.

Identification of dried red algae *Kappaphycus alvarezii* was carried out by organoleptic tests which included visual examination (shape, color and appearance), odor and texture. The appearance of dried red algae used is clean, fibrous, length of 10-40 cm and in uniform whitish red color; with the specific odor of red algae and the texture is dry, clayey, easily broken between the stem and branches.

Quality parameters of dried red algae *Kappaphycus alvarezii* other than stated in Indonesian National Standard (SNI) is the proximate analysis includes examinations of moisture, ash, protein, lipid, carbohydrate, acid insoluble ash and sulfate content. The proximate analysis of the whole red algae raw materials can be seen in Table 3.

Based on the results of proximate analysis of dried red algae, it is shown that the component with the largest percentage in dried red algae is carbohydrate ranged from 42.50 to 52.77%, then the ash content ranged from 29.37 to 32.10% and the water content ranging between 13.74 - 21.45%.

Meanwhile, protein and fat are the components with the smallest percentage, each of which ranged between 2.68 - 3.94% and 0.12 - 0.25%, respectively.

Table 3. Proximate analysis results of *Kappaphycus alvarezii* dried red algae

Sample origin	Water content (%)	Ash content (%)	Protein level (%)	Fat level (%)	Carbohydrate (difference) (%)	Acid insoluble ash content (%)	Sulfate content (%)
Bantaeng	14.65	29.37	3.94	0.21	51.82	1.50	6.09
Takalar	21.45	32.10	3.83	0.12	42.50	1.36	6.40
Maros	13.74	30.56	2.68	0.25	52.77	1.65	5.78
Bone	16.45	30.78	3.13	0.17	49.46	1.66	5.79

The results of analysis of ash content ranged from 29.37 - 32.10%. The highest ash content was obtained from red algae obtained from Takalar (32.10%) and the lowest in red algae obtained from Maros (29.37%). Ash content is related to the mineral content of the sample. Enough mineral content is needed for osmosis balance in maintaining its biological system [24]. The content of ashes in red algae consists mainly of sodium salt derived from sea water which attaches to the red algae thallus. According to Hirao [25], the ash content in red algae ranges from 15-40%.

The results of the analysis of protein content in this study ranged from 2.68 - 3.94%. The highest protein content was obtained from red algae obtained from Bantaeng (3.94%) and the lowest in red algae obtained from Maros (2.68%). Eidman [26] stated that in the period of algae exponential growth more protein is synthesized so that the formation of cell walls and food reserves is less, in these conditions a small supply of nitrogen and part of the protein synthesis process from photosynthetic activities will be converted into carbohydrate synthesis.

The results of the analysis of fat content obtained in this study ranged from 0.12 - 0.25%. The highest fat content was obtained from red algae obtained from Maros (0.25%) and the lowest in red algae obtained from Takalar (0.12%). This raw material meets SNI requirements, namely low-fat content.

The results of the analysis of carbohydrate levels in this study ranged from 42.50 - 52.77%. The highest carbohydrate content was obtained from red algae obtained from Maros (52.77%) and the lowest in red algae obtained from Takalar (42.50%). Increased carbohydrates are caused by an increase in fluoridean starch as a result of photosynthesis. Floridean starch is a galactose and glycerol compound that binds via glycosidic bonds [27]. Carbohydrates in *Kappaphycus alvarezii* are linear polysaccharide compounds consisting of D-galactose and L-galactose 3,6 anhydrogalactose units with either sulfate or without sulfate bound by (1,3) and (1,4) glycosidic bonds.

Table 4. Results of analysis of heavy metals in red algae raw materials *Kappaphycus alvarezii* from South Sulawesi

Heavy metal test parameters	Sample origin			
	Bantaeng	Takalar	Maros	Bone
- Lead (Pb) (ppm)	0.25	0.17	0.20	0.24
- Zinc (Zn) (ppm)	0.01	0.01	0.03	0.02
- Copper (Cu) (ppm)	0.44	0.87	3.18	3.66
- Arsenic (As) (ppm)	0.0069	0.00	0.00	0.0006

The values of *Kappaphycus alvarezii* red algae sulphate content were found ranging from 5.78 - 6.40 %. The highest sulphate content was obtained from red algae obtained from Takalar (6.40%) and the lowest in red algae obtained from Maros (5.78%). According to Guiseley et al. [14], High sulfate content causes more repulsive resisting forces between negatively charged sulfate groups, so that polymer chains are stiff and tightly attracted, so that viscosity will increase. The results of the analysis of heavy metal content in red algae raw materials can be seen in Table 4.

Results of analysis of heavy metals in red algae *Kappaphycus alvarezii* from South Sulawesi indicates that the raw materials contain heavy metals of lead ranged from 0.17 to 0.25 ppm; zinc ranged from 0.01 - 0.03 ppm; copper ranged between 0.44 to 3.66 ppm and arsenic ranged from 0.00 to 0.0069 ppm. The presence of Pb content indicates pollution in the waters, because red algae can absorb heavy metals from the waters through the absorption process. The presence of Zn content in red algae is probably caused by Zn accumulation through absorption or ion exchange process in red algae. Zn is an element or mineral needed by the algae. This process occurs through the red algae cell wall, which is then compounded with proteins and polysaccharides. The red algae of *Kappaphycus alvarezii* in this study contained relatively small amounts of As. The content of heavy metals in red algae raw material is relatively small so that it meets the standards set by EEC for maximum Pb content of 10 ppm, maximum Cu content of 50 ppm, maximum Zn content of 25 ppm and maximum As content of 3 ppm.

3.2 Optimization of Carrageenan Extraction

Carrageenan is a red algae gum from the type of *Kappaphycus alvarezii* which is extracted with hot alkaline solution. Red algae of *Kappaphycus alvarezii* type which are used as raw material for the production of carrageenan are from South Sulawesi waters. Extracting solution used in this study is KOH with a concentration of 5, 7 and 9%, and the extraction duration of 2 and 4 hours and temperature of 70-100°C. Research at this stage aimed to determine the best conditions for the carrageenan extraction process. Determination of the best conditions is selected based on the yield parameters.

Optimization of the carrageenan extraction process was carried out using four treatments, namely heating temperature, alkali concentration, extraction time and determining the type of alcohol used as precipitating agent.

3.2.1 Determination of heating temperature

The yield of carrageenan obtained following extraction at varied heating temperature ranged from 29.19 to 53.30 %. These results indicate that the most optimal heating temperature is 90^{o} C to obtain the highest carrageenan yield of 53.30%.

Table 5. Results of carrageenan yield obtained after extraction at varied heating temperatures

Temperature (^{o}C)	Red algae weight (g)	Carrageenan weight (g)	Yield (%)
70	499.9	145.9	29.19
80	499.6	209.1	41.85
90	499.8	266.4	53.30
100	499.9	227.6	45.53

3.2.2 Determination of alkali concentration

The yield of carrageenan obtained following extraction using varied concentration of alkali solution ranged between 36.12 to 48.04 %. These results indicate that the most optimal alkali (KOH) concentration is 9% to obtain the highest carrageenan yield of 48.04%.

Table 6. Results of carrageenan yield obtained after extraction using varied concentration of alkali solution

Alkali concentration (%)	Red algae weight (g)	Carrageenan weight (g)	Yield (%)
5	499.7	1805	36.12
7	499.9	200.3	40.07
9	499.8	240.1	48.04

3.2.3 Determination of extraction time

The yield of carrageenan obtained following extraction in varied duration ranged from 40.08 to 49.85%. These results indicate that the most optimal extraction time is 3 hours to obtain the highest carrageenan yield, which is 49.85%.

Table 7. Results of carrageenan yield obtained after extraction in varied duration

Duration (hour)	Red algae weight (g)	Carrageenan weight (g)	Yield (%)
2	499.7	200.3	40.08
3	499.9	249.2	49.85
4	499.8	240.1	48.04

3.2.4 Determination of the type of alcohol used as precipitating agent

The yield of carrageenan obtained in this study following separation process using several types of alcohol as precipitating agent ranged between 25.37 - 33.52%. These results indicate that the most optimal type of alcohol in separating carrageenan is ethanol to obtain the highest carrageenan yield of 33.52 %.

Table 8. Results of carrageenan yield obtained after separation using several types of alcohol

Types of alcohol	Red algae weight (g)	Carrageenan weight (g)	Yield (%)
Ethanol	499.7	167.5	33.52
Methanol	499.5	126.7	25.37
Isopropyl alcohol	499.5	137.5	27.53

The recovery is an important parameter in assessing the effectiveness of the process of producing carrageenan. Effective and efficient extraction process of raw materials in producing carrageenan can be seen from the yield value produced. The yield calculation was carried out to determine the percentage of carrageenan produced from dried red algae used based on temperature, KOH concentration and extraction time. The yields produced in this study still meet the minimum standard requirements for carrageenan yield determined by the Department of Commerce (1989), which is 25%. The results of the analysis show that temperature, KOH concentration and extraction time give a real influence on the yield of carrageenan produced. Treatment of 9% KOH concentration and extraction time 3 hours and a temperature of 90^{o}C also gives a different effect on the yield of carrageenan produced.

The KOH concentration affects the yield produced. This is presumably due to the higher concentration of KOH during the extraction causing the pH to increase and consequently increase the ability for extraction. The alkali treatment helps to extract the polysaccharide completely, also accelerates the formation of 3,6 anhydrogalactose during the extraction process. This is consistent with the statement of Glicksman [12] which states that kappa carrageenan is sensitive to potassium ions and calcium ions.

The carrageenan yield is also affected by the duration and temperature of extraction. The longer the extraction process and the higher the extraction temperature will increase the carrageenan yield. This is because the longer the red algae contact with heat and with the extracting solution, the more carrageenan is released from the cell wall and causes higher carrageenan yield.

3.3 Verification of Carrageenan Yield Determination Method

3.3.1 Repeatability precision test

Repeatability precision test of the method for determining the yield of carrageenan was carried out for 6 replications. The complete results can be seen in Table 9.

Table 9. Results of carrageenan yield obtained after separation using several types of alcohol

Replication	Carrageenan Yield (%)	
	Technical Ethanol	**Ethanol Pro-Analytical**
1	43.5	45.8
2	44.4	45.4
3	44.3	44.8
4	43.6	44.6
5	44.6	45.0
6	43.8	46.2
Average	44.03	45.30
SD	0.46	0.62
%RSD	1.0	1.4

The test results in the table above show that the highest yield of carrageenan was obtained in the extraction using pro-analytical ethanol precipitant, which was 45.30%. Based on the verification parameter tested, namely repeatability, each % RSD was obtained, namely 1.0% for technical ethanol and 1.4% for pro-analytical ethanol yield. The calculation results show that the RSD percentage of both does not exceed 3%, therefore the carrageenan extraction method using technical ethanol precipitator and pro-analytical ethanol meets the requirements of good repeatability precision because it meets the Horwitz standard, which is a maximum RSD of 3% [18].

3.3.2 Reproducibility precision test

In the reproducibility precision test, the yield of red algae *Kappaphycus alvarezii* was determined by two different analysts, each with 6 replications. The complete results can be seen in Table 10.

Table 10. Reproducibility Test of Carrageenan Yield from Red Algae *Kappaphycus alvarezii*

Replication	Carrageenan Yield (%)			
	Technical Ethanol		**Ethanol Pro-Analytical**	
	Analyst 1	**Analyst 2**	**Analyst 1**	**Analyst 2**
1	48.2	48.2	50.2	50.4
2	47.8	47.2	49.6	49.2
3	48.4	46.4	48.6	48.3
4	48.6	48.0	49.4	49.4
5	47.6	48.4	49.6	48.6
6	46.8	46.8	50.4	49.8
Average	47.9	47.5	49.6	49.3
SD	0.65	0.82	0.64	0.77
%RSD	1.4	1.7	1.3	1.6

Based on the data in the Table 10, the average % yield of carrageenan for analyst one and analyst two was 47.9% and 47.5% for the use of technical ethanol precipitant, respectively, and 49.6% and

49.3% for the use of pro-analytical ethanol precipitant. The results of this test showed that the highest yield of carrageenan was obtained in the extraction using pro-analytical ethanol precipitant. Based on the verification parameters tested, namely reproducibility, it was obtained that the % RSD was 1.4% and 1.7%, respectively, from analyst one and analyst two for technical ethanol precipitant and 1.3% and 1.6% for pro-analytical ethanol precipitant. Since both % RSD do not exceed 3%, the carrageenan extraction method using technical ethanol precipitator and pro-analytical ethanol meets the requirements of good reproducibility precision because it meets the Horwitz standard, which is a maximum RSD of 3% [18].

3.4 Production and Characterization of Carrageenan

The production of carrageenan from red algae *Kappaphycus alvarezii* was carried out under optimum conditions, namely using alkali (KOH) 9% at 90^{o}C for 3 hours and separating carrageenan by precipitation using ethanol solvent. This process produces carrageenan in powder form. Characteristics of carrageenan produced can be observed based on chemical properties (moisture content, ash content, acid insoluble ash content and sulfate content) and physical properties (gel strength, viscosity, white degree, melting point and gel point or the point of the point).

The samples were then boiled using distilled water by comparison (10: 1) of the weight of dried red algae in temperature close to boiling temperature (90^{o}C) for 3 hours. Naylor [23] reported that boiling carried out for several hours can accelerate the process of carrageenan extraction [28].

The extraction process was performed using alkaline solution (KOH). This was done by referring to the results of Sheng Yao's [23,29] study, that extraction carried out with alkali has the potential of gel formation 3 to 5 times stronger when compared to using water only. Gel formation is a phenomenon of combining or crossing polymer chains so that a three-dimensional mesh is formed, then the mesh will capture water in it to form a strong, rigid and elastic structure. Besides that, the addition of an alkaline solution aims to catalyze the 6-sulfate group from its monomer unit by forming 3,6-anhydrogalactose to increase its gelling strength. The addition of alkali was carried out until pH 9 was obtained, where the higher the pH used, the better gel formation and viscosity. According to past reference [30], it is stated that the potential for gel formation and viscosity of the carrageenan solution will decrease with decreasing pH [31].

Separation of carrageenan can be done by filtration and precipitation. Filtering the results of decoction in hot conditions, is intended to avoid the formation of gel when it is cold which can interfere with the separation process between carrageenan filtrate and its residue [27]. The last stage is the drying of carrageenan with direct sunlight for 2-4 days and continued with the process to obtain powdered carrageenan. Results of organoleptic test for the carrageenan obtained from *Kappaphycus alvarezii* can be seen in Table 11.

Table 11. Results of organoleptic test for the carrageenan obtained from *Kappaphycus alvarezii*

Sample origin	Results obtained	Results according to references	Remark
Bantaeng	The powder is yellowish white, slightly odor and tasteless	Brownish powder to white, slight odor and tasteless	+
Takalar	The powder is yellowish white, slightly odor and tasteless	Brownish powder to white, slight odor and tasteless	+
Maros	The powder is yellowish white, slightly odor and tasteless	Brownish powder to white, slight odor and tasteless	+
Bone	The powder is yellowish white, slightly odor and tasteless	Brownish powder to white, slight odor and tasteless	+

Note : (+) = sample to contain the carrageenan; (-) = The sample does not contain carrageenan

Based on research data in Table 11, test organoleptic test results for the obtained carrageenan are in accordance to the standard carrageenan that provides the same observations with the references in the form of a fine powder brownish yellow, with red algae distinctive smell and tasteless. These results agree with Arthur, 2000 which reported that carrageenan when extracted from red algae, the powder will be brownish yellow to white, in the form of powder that is slight odor and tasteless [32].

3.4.1 Chemical properties of carrageenan

The chemical properties of carrageenan from *Kappaphycus alvarezii* analyzed were water content, ash content, acid insoluble ash content and sulfate content. The results of the analysis of the chemical properties of carrageenan in this study can be seen in Table 12.

Table 12. The chemical properties of carrageenan obtained Kappaphycus alvarezii

	Sample origin			
Parameter	**Bantaeng**	**Takalar**	**Maros**	**Bone**
Water content (%)	10.67	11.87	6.17	8.92
Ash content (%)	16.42	15.96	16.70	16.68
Acid insoluble ash content (%)	1.36	0.89	1.16	1.55
Protein content (%)	2.54	2.74	5.27	4.58
Lipid content (%)	1.16	0.90	1.11	1.22
Rough fiber (%)	3.49	3.53	4.71	3.45
Carbohydrates (%)	69.22	68.19	70.75	68.60
Sulfate content (%)	15.57	15.55	15.34	15.66
Heavy metal (ppm):				
– Lead (Pb)	0.24	0.59	0.11	0.0037
– Zinc (Zn)	0.01	0.05	0.03	0.02
– Copper (Cu)	0.29	0.33	0.30	0.50
– Arsenic (As)	0.0068	0.0015	0.0010	0.0006

Water content states the amount of water and volatile materials contained in carrageenan. The moisture content of a product is usually determined by the conditions of drying, packaging and storage methods. Poor storage and drying conditions cause high water content in the product so that the material is damaged more quickly. Likewise, the less tight packaging conditions will increase the water content of the product so that the quality of the products produced decreases. Testing the water content is intended to determine the water content in carrageenan. The carrageenan water content is very influential on its shelf life, because it is closely related to the microbiological activities that occur during the carrageenan storage. Syarief and Halid [33] stated that the role of water in food is one of the factors that influence metabolic activities such as enzyme activity, microbial activity, and chemical activity, namely the occurrence of rancidity and non-enzymatic reactions, giving rise to changes in organoleptic properties and nutritional value. The results of measurements of water content as in table 10 where obtained values for carrageenan ranging from 6.17 - 11.87 %. Water content in the carrageenan produced from *Kappaphycus alvarezii* still meet the standards of quality specifications for carrageenan water content set by FAO and FCC, which is a maximum of 12%.

The combustion process in measuring the ash level causes organic substances in the material to burn and leave ash. The remaining ash is inorganic substances, which are minerals. Marine algae are industrial materials that are rich in minerals, such as Na, K, Ca and Mg. The levels of carrageenan ash produced from *Kappaphycus alvarezii* red algae are 15.96 - 16, 70%, and still meet the standards of quality specifications for carrageenan ash content set by FAO at 15-40%, while the FCC sets a maximum of 35%.

Acid-insoluble ash is partly acid-insoluble chloride salts, some of which are heavy metal salts and silica. High content of acid insoluble ash indicates contamination of mineral or metal residues that are

insoluble in acid on a product, such as silica (Si) found in nature as quartz, stone and sand. Acid insoluble ash content in carrageenan ranged between 0.89 – 1.55% . The highest content of acid insoluble ash was obtained from carrageenan from *Kappaphycus alvarezii* obtained from Bone, which amounted to 1.55%. The high content of acid insoluble ash in carrageenan from *Kappaphycus alvarezii* is presumably because insoluble minerals or metals found in carrageenan solutions cannot be reduced optimally during processing. In addition, the drying of raw materials and filtering techniques was not perfect, allowing filter aid to pass into the filtrate to be analyzed as the acid insoluble content.

Analysis of ash content was carried out to know the mineral content found in carrageenan in general. The value of ash content in foods indicates the amount of minerals contained in these foods [21]. Sudarmadji et al. [34] stated that minerals contained in a material can be divided into two types of salts, namely organic salts and inorganic salts. In addition to these two salts, sometimes minerals form as complex compounds that are organic in nature. Ash content is related to minerals of a material. The materials that evaporate during the combustion process in the form of water and other volatile materials will experience oxidation by producing CO_2. Red algae is a type of food that contains high enough minerals such as Na, K, Cl, and Mg. The content of acid insoluble ash obtained in this study still meet the standards set by the EEC of a maximum of 2%. Sulfate content is a parameter used for various types of polysaccharides found in red algae [35].

Results of the usual red algae extract are differentiated based on the content of sulfates. The *Kappaphycus alvarezii* carrageenan sulfate content values obtained ranged from 15.34 - 15.66 %. According to Guiseley et al. [14], high sulfate content causes more repulsive resisting forces between negatively charged sulfate groups, so that polymer chains are stiff and tightly attracted, so that viscosity will increase. Similarly, if the sulfate content is low, viscosity decreases. Another thing that can affect the high sulfate content in carrageenan *Kappaphycus alvarezii* is a raw material and extraction method. The sulfate content produced from carrageenan *Kappaphycus alvarezii* still meets the standards of quality specifications for carrageenan sulfate levels determined by EEC and FAO, ranging from 15 - 40%, while the FCC sets 18 - 40%.

Heavy metals are a type of metal found in samples such as lead, zinc, mercury, cadmium and arsenic, and high molecular weight lead. Analysis of heavy metals in the raw material of carrageenan is very important, among others, to determine whether carrageenan is safe to use or consume for pharmaceutical products (medicines) and food products.

Results of heavy metal analysis of carrageenan from *Kappaphycus alvarezii* red algae from South Sulawesi show that the carrageenan produced in this study contains heavy metals lead ranged from 0.0037 – 0.59 ppm; zinc ranged from 0.01 - 0.05 ppm; copper ranged between 0.29 – 0.50 ppm and arsenic ranged between 0.0006 - 0.0068 ppm. The content of heavy metals in carrageenan is relatively small so that it meets the standards set by EEC for maximum Pb content of 10 ppm, maximum Cu content of 50 ppm, maximum Zn content of 25 ppm and maximum As content of 3 ppm.

Table 13. Physical properties of carrageenan from *Kappaphycus alvarezii*

	Sample origin			
Parameter	**Bantaeng**	**Takalar**	**Maros**	**Bone**
Gel strength (dyne/cm^2)	446.40	449.50	442.66	441.95
Viscosity (cP)	20	20	40	40
Melting point (^{o} C)	46.98	49.65	44.40	43.60
Gelling point (^{o} C)	33.25	34.48	32.68	32.80
Amendment (%)	48.45	46.75	30.07	44.85
White degrees	43.18	46.67	39.67	40.96

3.4.2 Physical properties of carrageenan

The quality indicators of carrageenan based on the physical properties analyzed are gel strength, viscosity, whiteness, melting point and gelling point. The results of the analysis of the physical properties of carrageenan can be seen in Table 13.

The strength of the carrageenan gel obtained from the results of this study ranged from 441.95 - 449.50 dyne/cm^2. The highest gel strength was obtained from red algae obtained from Takalar (449.50 dyne/cm^2) and the lowest in red algae obtained from Bone (441.95 dyne/cm^2). Gel strength is the main physical property of carrageenan, because the strength of the gel shows the ability of carrageenan in gel formation. Glicksman [36] stated that one of the important physical properties of carrageenan is the ability to form gel or defined as gel strength. The strength of the gel from carrageenan is strongly influenced by the concentration of KOH, pH, temperature and time of extraction [37]. The high gel strength is due to lower sulfate content. Suryaningrum, 1988, stated that the increase in gel strength was directly proportional to 3,6-anhydrogalactose and inversely proportional to its sulfate content. The smaller the sulfate content the smaller the viscosity but the consistency of the gel increases [38]. Gel strength is very important to determine the best treatment in the process of carrageenan extraction. One of the important properties of carrageenan is being able to convert liquids into solids or change the shape of sol into a gel that is reversible. This ability causes carrageenan to be very widely used, both in the food and pharmaceutical fields.

Based on the data above, it can be seen that in general the pattern of the strength of the carrageenan gel produced is constant and the pattern is intermittent with carrageenan viscosity. This shows that the viscosity value is inversely proportional to the value of gel strength, i.e. if the viscosity is high then the gel strength tends to be low, and vice versa if the value of the viscosity obtained is low then the gel strength will be high. This is in line with the statement of Friedlander and Zelokovitch [39], that the increase in gel strength is directly proportional to the amount of the content of 3,6-anhydrogalactose and inversely proportional to the sulfate content. Furthermore, according to Moirano [37], that 3,6-anhydrogalactose causes irregular properties in polymers which will increase the potential for double helical formation so that gel formation is more quickly achieved.

Viscosity is one of the important physical properties of carrageenan. Viscosity testing is carried out to determine the level of carrageenan thickness as a solution at a certain concentration and temperature. Carrageenan viscosity is usually measured at 75^{o}C with a concentration of 1.5% [40]. Viscosity is a quality factor that is important for liquid and semi-liquid or pure products, where it is a measure and control to determine the quality of the final product [41]. Carrageenan viscosity has an effect on gel properties especially the gelling and melting point, where high carrageenan viscosity results in higher melting and gel formation rates than carrageenan which have low viscosity. Carrageenan viscosity value obtained from *Kappaphycus alvarezii* red algae ranged from 20-40 cP. Moirano [37] suggested that the viscosity of carrageenan solutions is mainly due to the nature of carrageenan as polyelectrolytes. The repulsion force between negative charges along the polymer chain, which is the sulfate ester, resulting in the chain bending over immobilized water. The value of viscosity produced in the study still meets the standards of carrageenan viscosity quality specifications set by FAO at least 5 cP.

The decrease in viscosity is caused by a decrease in sulfate content. According to Guiseley et al. [14], the viscosity in carrageenan is caused by the repulsion between the negatively charged sulfate groups along the polymer chain, causing the polymer chain to stiffen and be tightly attracted. Because the hydrophilic nature causes the molecule to be surrounded by immovable water, this is what causes the carrageenan viscosity value to increase. Another thing that also affects the viscosity value is the presence of divalent Ca2 + ions, Mg2 + found in carrageenan. These ions are thought to accumulate by red algae from the aquatic environment, the accumulation of these ions through absorption or ion exchange that occurs in the cell wall of the algae which is then compounded with polysaccharides and proteins [42].

Gelling point is the temperature at which the carrageenan solution in a certain concentration begins to form a gel, while the melting point is the opposite of the gel point, which is the temperature of the carrageenan solution melting at a certain concentration. Carrageenan can form a gel reversibly, meaning it forms a gel when cooling and melts again if heated.

Carrageenan gelling point measurement results obtained from *Kappaphycus alvarezii* red algae ranged between 32.68 - 34.48°C. The value of the melting point of carrageenan derived from red algae ranged from 43.60 to 49.65°C. This is presumably because the higher the temperature of the gelling point, the higher the temperature of the melting point. Moirano (1977 referenced in Suryaningrum et al. 1991) states that the melting point temperature of kappa carrageenan is 10-15^{o}C above the temperature of the geling point [42,37]. The temperature of gelling and melting point of carrageenan from *Kappaphycus alvarezii* obtained in this study is lower than expected. This is caused by lower sulfate content. Friedlander and Zelokovitch [39] stated that the temperature of gelling and melting point is directly proportional to the content of 3,6-anhydrogalactose and inversely proportional to the sulfate content. Furthermore Reen [43] stated that the presence of sulfates tends to cause polymers to be in the form of soles, so that the temperature of the gel point is difficult to form [43].

Whiteness is a general description of the color of a material in general. The whiteness of carrageenan is expected to approach 100% because high-quality carrageenan is usually colorless, so the application is broader. The results of measurements of the whiteness of carrageenan produced from *Kappaphycus alvarezii* red algae ranged from 39.67 - 46.67 %. The value of the whiteness in carrageenan can be caused by the raw materials used, filtering and precipitation techniques. Another thing that affects the value of the whiteness is the concentration of the extracting material because during the process, the alkaline condition of the extracting material can oxidize pigments to other colorless compounds so that the resulting product is whiter.

Chemically, the bleaching process is the oxidation or reduction of double bonds in color-forming compounds. Filtration process in carrageenan processing aims to separate crude fiber from the filtrate from red algae, the separation of coarse brown fibers will lead to the brighter the color of the filtrate produced. Another thing that affects the whiteness is the carrageenan drying technique. It is assumed that by using a vacuum oven dryer, the whiteness produced is higher when compared to sun drying. The whiteness values obtained in this study were slightly lower because in the washing process deliberately no bleach was added to reduce the use of chemicals in the process of making carrageenan.

4. CONCLUSION

Based on the results, we conclude that raw materials of *Kappaphycus alvarezii* red algae obtained from several farms in South Sulawesi in particular Bantaeng, Takalar, Maros and Bone meet the requirements of the quality standards of Indonesian National Standard (SNI), which include moisture content of not more than 32%, impurity levels of not more than 5% and organoleptically specific odor of seaweed or algae-specific odors. This red algae raw material also meets the EEC quality requirements, namely sulfate content is not more than 40%, ash content less than 40% and acid insoluble ash content of less than 2%.

By extraction under the optimum conditions using the raw material from standardized *Kappaphycus alvarezii* red algae then, carrageenan with the best quality can be produced with the physical properties namely the gel strength ranging from 441.95 to 449.50 dyne/cm^{2}, the viscosity ranging between 20-40 cP , the gelling point ranging from 32.68 - 34.48^{o}C, the melting point ranging from 43.60 - 49.65^{o}C, the yield ranging from 30.07 - 48.45% and whiteness ranging from 39.67 – 46.67% and carrageenan chemical properties, namely water content ranging from 6.17 - 11.87%; ash content ranging from 15.96 - 16.70%; acid insoluble ash content ranging from 0.89 - 1.55%; protein content ranging from 2.54 - 5.27%; fat content ranging from 0.90 - 1.22 %, crude fiber content ranging from 3.45 - 4.71 %; carbohydrate content ranging from 68.19 - 70.75%; sulfate content ranging from 15.34 - 15.66% and heavy metals Pb, Zn, Cu and As ranging from 0,0006 - 0,50 ppm. Carrageenan powder

obtained from *Kappaphycus alvarezii* red algae 99% has passed the 60 mesh and meets the quality standards issued by FAO, FCC and EEC.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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