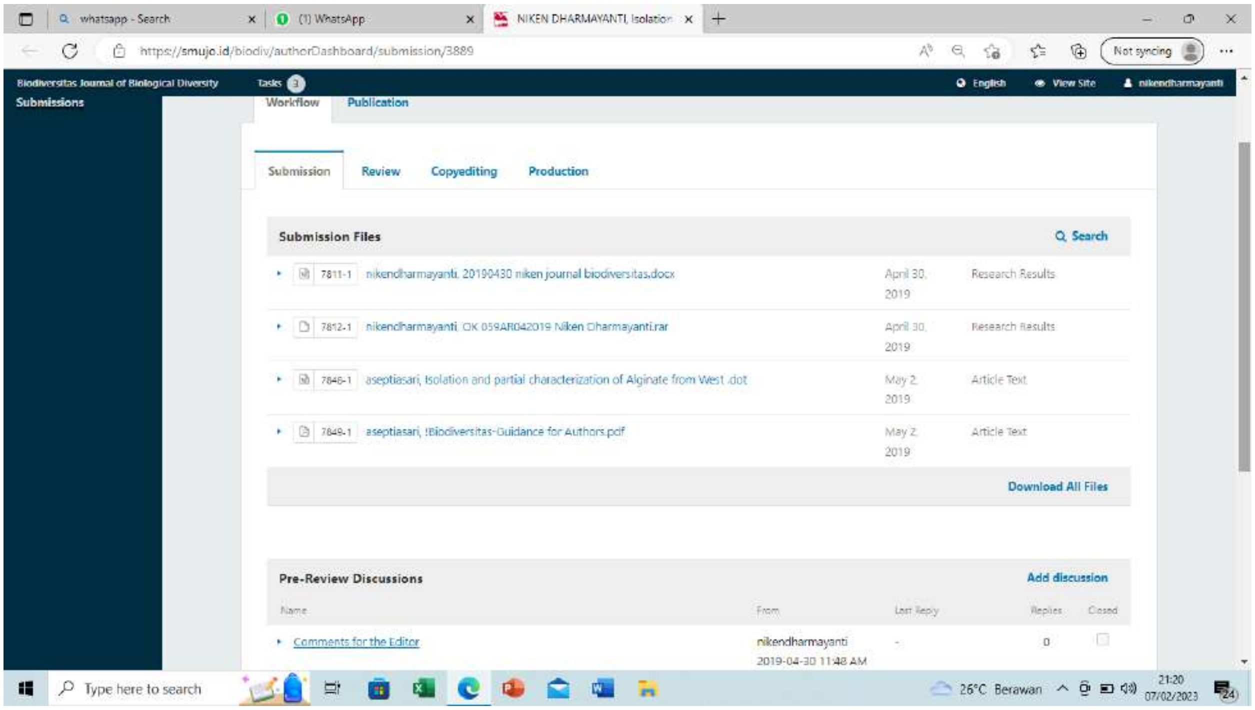
Bukti Submit Jurnal Biodiversitas "Isolation and partial characterization of alginate extracted from Sargassum polycystum collected from three habitats in Banten, Indonesia"



Isolation and partial characterization of Alginate from West of Java Indonesia Waters Seaweed *Sargassum* spp.

Abstract. Alginate is a polysaccharide organic linear polymer consisting of α-L guluronic acid monomers and β-D manuronic acid, or it can be a combination of these two monomers which may be useful for food and non-food purposes. Utilization of *Sargassum* spp seaweed as an alternative alginate source will reduce dependence on alginat imports, which is currently still 100% imported. Thus, the purpose of this study was to prepare and characterize alginates from *Sargassum* spp seaweed obtained from three locations with different ecological characteristics. Isolation alginate with partial hydrolysis separated guluronate acid and manuronic acid followed by freeze drying and measurements qualitatively and quantitatively using FTIR. The results showed that alginat from *Sargassum* spp. of Lima Island, Ujung Kulon and Binuangeun gave rendemen were 11,48 %**,** 18,62 % and 5,75 % respectively, a viscosity of 35 cps, 62,50 cps and 81,33cps respectively. The test result of partial hydrolysis of alginat showed that gulluronic block (GG) in the alginate polymer of Lima Island, Ujung Kulon and Binuangeun were 67,60 %, 59,00 % and 41,40% respectively. This relates to the nature of the gel formed. Alginat of Lima Island tend to be more rigid and less flexible than the alginate from Ujung Kulon and Binuangeun. The variations in the concentration of Manuronate and Guluronate from the three ecologies of *Sargassum* in Western Java are different variations.

Key words: alginat, characterization, guluronat , Java, manuronat, partial, *Sargassum* spp, west

Abbreviations : FTIR = Fourier Transform Infrared Spectroscopy

Running title: Alginat partial characterization of *Sargassum polycistum*

INTRODUCTION

Brown algae are better known as alginate sources. Alginate is needed in various industries, functions as a gelling agent (gelling agent), stabilizer (stabilizer), emulsifier (emulsifier), suspension (suspending agent), and dispersing a product. In the food industry, alginate compounds are added ingredients for butter, ice cream and milk. In the cosmetics industry it functions as a water binder so that it easily penetrates the skin tissue and is perfectly bound. In the textile industry it functions as a binder of water (thickener) in tasting batik (Suptijah, 2002; Sinurat and Murdinah, 2007).

Alginate consisting of a group of polysaccharides can be found in brown algae tissue. Although all brown algae contain alginate, only a few species of brown algae are used commercially in the subtropical blood today such as Macrocystis pyrifera, Ascophyllumnodosum, Laminaria hyperborea, Laminaria digitata, Ecklonia maxima, and Lessonia nigrescans. Whereas in Indonesia more use of Sargassum sp., Turbinaria sp., Hormophysa sp., And Padina sp., (Rasyid, 2003).

Sargassum spp. is an alginate-producing seaweed. The opportunity to increase alginate production will require data on Sargassum spp. Seaweed species. and its content in an effort to manage sustainable alginate resources. This study aims to obtain ecological, morphological, molecular and alginate structure of Sargassum spp. in western Java so it is known that there is a relationship between the Sargassum spp gene. against the alginate structure. The character of alginate structure is needed in the development of sustainable indigenous species. Sargassum spp. as wild seaweed can be known based on the place of growth has the potential as a source of specific alginate raw material.

Increasing demand for increasingly high industries, encourages to explore and develop potentially industrial species. Ekas Bay on Lombok Island presents brown macroalgae stocks that vary with season and species, for Padina biomas it reaches 97.85 ± 12.63 and 79.54 ± 2.53 tons in May to June and November. For Sargassaceae species it reached 669.70 ± 109.64 and 147.70 ± 77.97 tons in May to June and November. The best alginate results occur during the period May to June, Padina can produce 9.10 ± 0.06 tons of alginate dry weight. Interestingly, Sargassum extraction allows producing 207.61 ± 0.42 tons of alginate dry weight. This study shows that wild Sargassaceae is an attractive stock in terms of biomass, alginate character is influenced by the ratio of manuronate and guluronate. (Setyawidati et al., 2018).

According to King (1983) and Saraswathi et al. (2003), the alginate content of seaweed varies greatly depending on the type of brown seaweed extracted. According to Winarno (1990), alginate formed in Sargassum seaweed cell walls reaches 40% of the total dry weight and plays an important role in maintaining the talus tissue structure. Talus seaweed Sargassum spp. has a variety of shapes and sizes, from the talus in the form of rods that collect in a bundle to the large talus which sometimes shows an outer shape like tall plants, the shape of the talus can affect the content of alginate.

Alginophite is a type of alginate-producing seaweed. The types of alginate-producing brown seaweed are Sargasssum spp., Turbinaria spp., Laminaria spp., Ascophyllum spp., And Macrocystis spp. Sargassum spp. and Turbinaria spp. mostly found in Indonesian marine waters, while Laminaria spp., Ascophyllum spp. and Macrocystis spp. often found in sub-tropical waters.

Potential type

Sargassum spp. not yet cultivated in Indonesia, Sargassum spp. still very limited. In the world of Sargassum spp. there are about 400 species, while in Indonesia there are 12 types, namely: Sargassum duplicatum, S. hitrix, S. echinocarpum, S. gracillinum, S. obtuspfolium, S. binderi, S. polycystum, S. microphylum, S. crassifolium, S aquafolium, S. vulgare, and S. polyceratium.

alginate structure

Alginofit is a family of brown seaweed. The difference in the main chemical compounds contained in seaweed shows the characteristics of the seaweed. The characteristic of these chemical compounds shows the extraction process. Similarly, in a variety of benefits and the use of each of these chemical compounds, there are many different from one another. Specific differences in characteristics are caused by differences in the types of seaweed, different locations of growing seaweed and its growing season.

Alginate is a long chain polymer consisting of α- (1→4) -linked L-guluronic acid (G) and β- (1→4) -linked D-mannuronic acid (M). The content ratio (G) and (M) is referred to as the G / M ratio. The ideal structure of the alginate formula is shown in Figure 1.

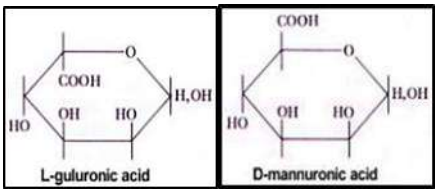


Figure 1. The ideal structure of the alginate formula

The G / M ratio will greatly affect the quality of alginate, the higher the G content the alginate thickness will be the higher the opposite if the M ratio is more the level of viscosity is lower. Alginophytes that grow in waters consist of 3 genera, namely: Sargassum with 7 species, Turbinaria 2 species, and Hormophisa. The structure of the G / M ratio can be seen in Figure 2.

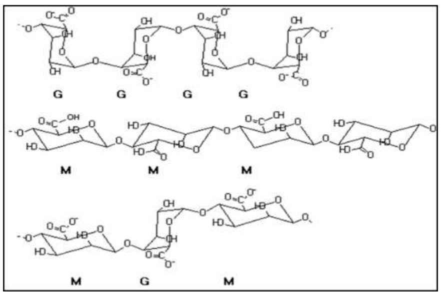


Figure 2. G / M alginate ratio

Based on this description, a study of the alginate content of Sargassum spp. Seaweed was carried out. which is found in the waters of Western Java to find out the types of Sargassum spp. which has the potential to produce alginate for the development of indigenous species producing sustainable alginate. Isolation study and partial characterization of alginate from Sargassum spp. from the west Java waters of Indonesia, the purpose is to identify the structure of sodium alginate based on the chemical composition of manuronate and guluronate on alginate products from Sargassum spp. which has ecological variations and morphological variations in western Java.

MATERIALS AND METHODS

A. Time and Place

The study was carried out in February 2018 until April 2019. Ecological observations were carried out in three locations in western Indonesia, namely in the waters of Pulau Lima, Ujung Kulon and Binuangeun including the values of diversity index, uniformity and dominance. Ecological support includes, depth, current speed, brightness, pH, temperature, salinity, DO and for wave height using data from BMKG (Meteorology and Geophysics Agency). Morphological observations carried out on descriptive explanations following Mattio L 2009 consisted of holdfast parameters, leaf shape, leaf margin, main thalus shape and vesicle form. Observations of Sargassum spp seaweed morphometrics were carried out at the Biology Laboratory, Fisheries High School including measurements of holdfast diameter, main stem diameter, leaf length, leaf width, vesicle diameter and total thalus length.

B. Location for Sampling and Handling

The geographical conditions of western Indonesia are surrounded by three major waters, namely the Java Sea in the north, the Sunda Strait in the west, and the Indonesian Ocean in the south. The sampling location consisted of the waters of Pulau Lima, Ujung Kulon and Binuangeun. The location of S. Polycystum sampling is presented in Figure 3.

Figure 3. Locations of Pulau Lima, Ujung Kulon and Binuangeun show locations for sampling *Sargassum polycystum*: point 1 ($6^0$3'39 "S, $106^0$09'20" E), point 2 ($6^0$48'15 "S, $105^0$29'5" E), and point 3 ($6^0$49'16 "S, $105^0$56'14" E).

Collection and identification of samples of Sargassum spp. at the lowest tide at each study location. Samples were collected by the transect method along the coast, and each was photographed. then taken to the Jakarta Fisheries College Biology Laboratory, for further analysis, while very low amounts collected were photographed and identified. Seaweed is stored in a plastic bag, cleaned, sorted according to genus, weighed in fresh condition, dried with wind, and then ready to examine morphological and morphometric characteristics.

C. Research Methods

1) Materials and Research Tools

The material used in the study included the types of seaweed in the three locations found on the western coast of Java, precisely in three locations, namely Pulau Lima, Ujung Kulon and Binuangeun.

The research tools used were plastic sheeting, square paralon with a size of 0.5 x 0.5 m, large and small plastic bags, thermometers, hand refractometers, pH meters, Water Quality Checkers, digital scales, underwater cameras, meters roll, label, ruler, permanent marker, bucket, dropper pipette, dark bottle, tissue, 4 liter plastic jerry can, and scissors. Map of western Java map. For orientation in the field, multi-parameter devices are used to measure temperature, salinity, DO, pH, and flouting drodge to measure current speed, secchi disk for measuring brightness, wave pole for measuring wave height, polyethylene bottles for water samples, GPS for get a geographical position, a digital camera for shooting the latest conditions and stationery.

2) Ecological Data Collection

The method used in the study is a survey method with sampling techniques using the transect method along the lines 50 - 100 m. The research was divided into 1-3 stations, with quadratic squares of 50 x 50 cm2, on the left or right side of the transect line following the direction of the arrival of the sea water flow. Each seaweed sampling location has 1-3 stations with 3 transects or repetitions. Monitoring to see abiotic components (dead coral, sand and others) and biotic components (other types of seaweed, seagrass and other living biota). The main parameters observed in each station were the density of diversity, uniformity and dominance of seaweed using Microsoft Excel software. While the supporting parameters are measurements of physical factors namely temperature, depth, salinity, current velocity, water clarity and measurement of chemical factors namely pH, nitrate, phosphate and Dissolved Oxigen. Seaweed sampling is carried out at low tide. All types of seaweed that have been taken and put into plastic that has been labeled, then separated according to type. After that the wet weight was weighed and photographed for documentation.

The type of seaweed was identified by using the identification book Trono (1997); Atmadja (1996); Anggadiredja et al., (2006). The data obtained were then analyzed using the Shannon – Wienner diversity index to determine its diversity, Simpson's dominance index to determine its dominance, Pielou's evenness index to determine its evenness and Sorensen's similarity index to find out the similarity of its species (Odum 1971).

Seawater extraction for water quality is carried out during sampling in intertidal seaweed areas. Measurement of temperature, pH and salinity of seawater, according to Alaerts and Santika (1987) was carried out by using a multi tester to measure the temperature into the waters which will be observed for 10 minutes. Temperature measurements were repeated 3 times in the seaweed sampling area. Depth measurements were carried out at low tide, by using a ruler dipped in the waters in the seaweed sampling area. Salinity measurements using a hand refractometer, salinity measurements were repeated 3 times, namely in the seaweed collection area. Calibrated pH measurements to measure pH into seawater using the correction factor of distilled water added with salt according to salinity at the sampling location. PH measurements were repeated 3 times. Flow velocity measurements are carried out using floating drodge. Floating drodge is released by its anchor or loads into the body of the water, allowing the buoy to move with the flow. Calculated how many seconds the floating drodge was released, all the strings were stretched to that distance, repeated 3 times and the results were evenly distributed.

The data used includes primary data and secondary data. Primary data is obtained by conducting surveys and direct observations in the field. Secondary data was obtained from the Meteorology, Climatology and Geophysics Agency and the Central Statistics Agency (BPS), secondary data covering the physical properties found in western Java waters throughout the year.

Characteristics of alginate test

1) Sample Preparation

The alginate content contained in Sargassum spp. influenced by species and location. The monomer composition in the structure of the alginate polymer is one of the factors that determine the quality of alginate. This study was carried out at 3 different locations of the Sargassum type which were dominant at that location on the alginate content and monomer composition in the structure of the alginate polymer.

2) Materials and Equipment

Brown seaweed consists of 3 dominant samples in each location, technical grade chemicals for extraction processes include Sodium carbonate, calcium chloride, Chloride acid, 70% alcohol, hydrogen peroxide and p.a grade chemicals for the analysis of alginate monomers. The equipment needed includes equipment for alginate extraction, while the test equipment is the Visfeter brand Brookfild, the Shimadzu Prestige Fourier Transform Infrared Spectroscopy (FTIR) and the Shimadzu brand Spectrophotometer.

3) Data Collection

Alginate Extraction

The extraction process of alginate is as follows, the raw material is Sargassum sp. cleaned then weighed 200 grams then added acetic acid solution according to the treatment. Ingredients that have been mixed in the blender to get the result in the form of seaweed pulp. Seaweed porridge that has been obtained is then heated at 800C and stirred for 10 minutes. After that it is filtered and squeezed to get the results in the form of coarse alginate liquid which is then dried at 650C for 24 hours, after drying it is then pressed to get coarse alginate flour. The flow chart for making alginate flour can be seen in Figure 3.

Na-Alginate Extraction Process

The main process of extracting brown alginofit seaweed into Na-alginate is divided into 4 stages namely immersion (pre extraction), extraction, bleaching and purification. Or the first immersion stage is carried out in an alkaline solution and an acid solution. The second (stage) extraction was carried out in an alkaline atmosphere by cooking using an extracting solution (Na_2CO_3, NaOH). Stage (third) bleaching using a solution of NaClO or H_2O_2. Stage (fourth) purification is divided into 3 phases, namely the formation of alginic acid, the formation of sodium alginate and the formation of pure sodium alginate.

Immersion

Soaking seaweed in $CaCl_2$ solution aims to dissolve laminarin, mannitol, dyes and salts. Besides that, this treatment also serves as an effort to dissolve the remaining impurities in seaweed. According to Tanikawa (1985), alginic acid precipitates under conditions of $pH < 3$, presumably in this condition the alginate component will be stable in the raw material of the immersion process. While immersion in alkaline solutions aims for deproteinization (Tseng, 1946).

Extraction

The brown seaweed extraction process is carried out in alkaline conditions. The goal is to separate the cellulose content from alginate. The extracting material that can be used is Na_2CO_3 or NaOH. Chou and Chiang (1976) state that high concentrations of Na_2CO_3 (3-5%) can cause a decrease in product yield and viscosity. This is because the alkaline solution can damage the alginic acid compound by shortening the polymer chain into oligosaccharides which in turn degrades to 4-deoxy-5-ketouronic acid. Extraction carried out by heating will also affect the alginate produced. This heating process not only makes extraction processes easier but can also extract the weight of higher alginate molecules so that they can increase product yield and viscosity (Chou and Chiang, 1976).

Bleaching

The bleaching process aims to dissolve the dyes namely phenolic compounds contained in the polymeric alginate bonds so that a clearer solution can be obtained. Pale material commonly used in the process of extracting seaweed is NaCIO or H2O2. The bleaching process with NaCIO does not cause foam and takes place relatively quickly (Yani, 1988). However Tseng (1946) suggested that the NaCIO used was no more than 1%, because it would cause the alginate to be oxidized and degraded.

The bleaching material of H2O2 used is intended to purify the extract and cellulose so that it can be separated by centrifuge or filtration. According to Wood et al., (1966), H2O2 has a strong power to free oxygen, and this material can be used for oxidation reactions at low temperatures. The reaction equation for the bleaching process using H2O2 is as follows:

Formation of Alginic Acid

To bind alginate contained in the bleached filtrate, HCI acid or H2SO4 sulfuric acid is added gradually. This process is not an easy polymer chain in alginic acid which is very susceptible to the addition of strong acids. This depends on how the acid can penetrate the alginate particles contained in the extracted filtrate. Binding of H + ions with alginate during the process of forming alginic acid runs relatively quickly (McHugh, 1987).

Deposition of Na-alginate

In the formation of sodium alginate, alginic acid that has been formed is added with alkaline solution containing Na + ions such as NaOH and Na2CO3. The purpose of the formation of sodium alginate is to get a more stable alginate compound. According to McHugh (1987), the exchange of H + ions with Na + ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid.

Withdrawal of Sodium Alginate

Withdrawal of Na-alginate compounds from sodium alginate solution can be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonim (1976), 1% sodium alginate starts to show the separation process in a solution of 10% isopropanol or in ethanol 20% as well as its boiling point. The melting point of isopropanol (secondary alcohol) is lower than ethanol (primary alcohol). In an effort to withdraw sodium alginate the use of isopropanol is more efficient than ethanol. Formation of pure sodium alginate is done by attracting the water content contained in the solution. This pure Na-alginate is then dried in an oven and after that it can be ground into Na-alginate flour.

After the water content contained in the anatomic alginate solution is pulled out, pure sodium alginate is formed. Sodium alginate is then dried in an oven and ground to form sodium alginate flour. The characteristics of alginic acid and sodium alginate were tested compared to Table 1. Characteristics of alginic acid and sodium alginate and Table 2. Quality specifications of alginic acid and sodium alginate. According to Glicksman (1983), alginic acid is described as a hydrophilic colloidal carbohydrate extracted with alkali salt from various types of brown seaweed. Chemically, alginate is a pure polymer of uronic acid arranged in a long linear chain. The chemical formula of alginate is (C6H8O6) n with the number n between 80 to 83 (Schoffel and Link, 1993). There are two types of monomers that make up alginic acid, namely β-D-mannopyranosil uronate or D-mannuronic acid and α-L-gulopyranosil uronate or L-guluronic acid. Of the two types of monomers, alginic acid can be a homopolymer consists of similar monomers namely D-mannuronic acid only or L-guluronic acid only (Winarno, 1996).

Homopolymers of D-mannuronic acid (polymannuronic acid) are formed by repeating D-mannuronic acid with β- (1,4) bonds and hydrogen bonds between hydroxyl groups on C3 atoms with oxygen atoms on adjacent hexose rings. The homopolymer form of L-Guluronic acid is more rigid than D-Mannuronic acid homopolymer (Atkins et al., 1971 in Anonim, 1976). Alginate with a high proportion of L-guluronic acid homopolymers tends to form stiff, regas and bersineresis gels. On the other hand, the higher proportion of D-mannuronic acid homopolymers tends to form a gel that is more elastic, does not regas and does not show high syneresis (Glicksman, 1983).

Data analysis of molecular structure and quality of alginate.

Variables observed in alginate include yield test, moisture content test, ash content test, viscosity test, pH test (Bahar, 2012), and structural tests with FTIR Alginate or algin is a compound contained in brown seaweed cell walls (Phaeopyceace) other than cellulose and pectin. Alginate compounds are alginic acid polysaccharides in the form of Sodium, Calcium, Potassium and Magnesium salts (Satari, 1996).

Alginate partial hydrolysis test

The composition of the polyguluronic, polimanuronate and mixed segments between manuronate and guluronate in alginate determined the quality of alginate. To isolate manuronic acid (M) and guluronate (G) on alginate molecules carried out by Partial Hydrolysis of Alginate by: 5.00 g Alginate in HCl 0.3 N at 100 ° C for 2 hours was obtained: The soluble fraction was identified as a block MG. Bonding The hydroxyl between M and G is easily hydrolyzed by insoluble-fraction acid more resistant to acid hydrolysis, again dissolved by adding alkali and fractionation by adjusting the pH at 2.85, so that the GG block settles and the MM block dissolves.

Partial analysis and characterization of alginate

The yield of alginate was measured for yield, which is as a percentage of the ratio between the final weight of alginate produced by the initial weight of Sargassum spp seaweed (in%).

Variables observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test (Bahar, 2012), and structural tests with FTIR. The data obtained were analyzed by multi-variant analysis, PCA, discriminant and clustering.
Analysis of alginate functional groups was carried out using a fourier transform infra red (FTIR) spectrophotometer (Perkin Elmer, spectrum one) based on the method of Barth (2000). Samples plus KBr (1: 100) then mashed until evenly mixed. Then presses with a vacuum pump for 15 minutes, and read the absorbance at wave numbers 400-4000 cm-1. From the resulting curve, the type of bond and its functional group are determined based on FTIR references.

MATERIALS AND METHODS
Materials and tools
The material used is Sargassum spp seaweed from three locations, Pulau Lima, Ujung Kulon and Binuangeun. Chemicals used for soaking (HCl) extracting materials (Na2CO3) forming ingredients of alginic acid (HCl) purifying materials Na-alginate (iso propyl alcohol and NaOH) all from Merck and other materials for physical and chemical analysis. Technical grade chemicals, for extraction processes include Sodium carbonate, calcium chloride, Cloria Acid, 70% alcohol, hydrogen peroxide and p.a grade chemicals for the analysis of alginate monomers.
The tool used for extracting Na alginate is a large capacity capacity of 20 L and a small dang of 10 L. Glas beaker with a capacity of 5 L (Pyrex) universal pH indicator paper, sitting scale (4 digit scale) mill machine, 7 ton power anchoring hydrolic filte press with 100 mesh cloth screen, filter (40 mesh) stainless steel thermometer, analyzer (viscometer) brook stable mycro filed TAXT2 texture analysis system)
The test equipment is the viscometer of the Brookfild brand, the Shimadzu Prestige Fourier Transform Infrared Spectroscopy (FTIR) brand and the Shimadzu brand Spectrophotometer.
Research methods
1. Na alginate extraction
The research method used is the experimental method through laboratory experiments, namely the process of making Na alginate with observations at the stage
extraction and filtration, replications were carried out three times. Observation of the chemical content (moisture content, clean unpure water, pH) and physical (yield, viscosity) of the Na alginate produced was carried out to see the characteristics of Na alginate from three locations.
The Na alginate extraction process was carried out using the Yunizal (2004) method modified with Sargassum spp seaweed weighed 1 kg, then washed with clean water 2 times as heavy the sample was then soaked with 1% HCl 2 times the sample weight for 60 minutes. Then rinsed with clean water and then extracted with a solution of 2% Na2CO3 in a volume of 60 liters with 2 stages of boiling which is the first stage of boiling with a temperature of 60oC and boiling time 60 minutes after it is crushed with a grinding machine until it is boiled again at 60oC and boiling time 60 minutes. Then filtering using the hydrolic filter press can easily add a filter aid, namely diatomaceous earth (celite) to facilitate filtration. Separation is carried out using a filter press consisting of fine filter cloth and filter aid. The screening method is continued by bleaching using 2% NaOCl. 450 ml NaOCl was added in 30 liters of alginate filtrate, stirred then left for 30 minutes, then added 10% HCl and left for 30 minutes to form alginic acid which was white or ivory yellow to pH 2-3. After the pH is reached, it is neutralized by adding dilute NaOH until it reaches pH 7-8 while stirring for homogenization of the solution of isoprophyl alcohol (IPA), adding that alginate has reached a neutral pH to obtain Na-alginate fiber and then the obtained fiber is dried in the form of Na-alginate.
Rendement
The Na-alginate yield obtained from the extraction process of seaweed Sargassum spp was calculated based on the weight of Na-alginate after drying on the dry weight of the raw material. Calculate the yield of alginate Na using the following formula:
Addendum (%) = (weight of Na - final alginate (g) / weight of initial seaweed (g)) x 100%
Viscosity
Viscosity analysis refers to JECFA (2007), observations were made at a 1-5% solid concentration to determine the relationship between concentration and solution viscosity. Na-alginate (sample) was weighed as much as 7.5 g in weigh paper. 492.5 g of distilled water are weighed in a 500 mL glass beaker so that the sample and distilled water when added reach total weight 500 g. Alginate is included in a 500 mL glass beaker containing distilled water and stirrer gradually. Akudes are heated and stirred once to reach 75oC, after a constant temperature the solution is heated for 25 minutes. Stirring I was carried out at minute 1 for 1 minute, stirring II at 25 minutes. Beaker glass is covered with aluminum foil to prevent water loss in the heating process due to evaporation, then the solution temperature is lowered to reach 75oC. The measurement of solution viscosity was measured using RVA (Rapid Visco Analyzer) spindle 2 at 30 rpm, waiting until the spindle needle was stable (up to 6 times rotation). Viscosity is expressed in centiPoise (cP).
1). PH value
A sample of 3 g was weighed and then put into a 300 mL glass beaker then added 197 g of distilled water until the total weight was 200 g. The sample is heated while stirring using a stirer until it dissolves at a temperature of 60-80 oC. Then the electrode is dipped into the sample solution which was previously calibrated. The pH value is obtained according to what is shown on the screen. Then the electrode is rinsed with distilled water.

2). Partial hydrolysis of alginate

The alginate content contained in each species is different. The monomer composition in the alginate polymer structure is one of the factors that determine the quality of alginate, therefore in this study carried out on Sargassum spp from three locations, namely from Pulau Lima, Ujung Kulon and Binuangeun. The composition of the polyguluronic, polimanuronate and mixed segments between manuronate and guluronate in alginate determined the quality of alginate. To isolate manuronic acid (M) and guluronate (G) on alginate molecules carried out by partial alginate hydrolysis (Subaryono 2018), by:

- 5.00 g of alginate in HCl 0.3 N at 100oC for 2 hours is obtained:
- The soluble fraction is identified as MG block. The hydroxyl bond between M and G is easily hydrolyzed by acid.
- The insoluble fraction is more resistant to acid hydrolysis, again dissolved by adding alkali and fractionation by adjusting the pH at 2.85, so that the GG block settles and the MM block dissolves.

3). FTIR Analysis

As much as 2 mg of alginate sample is put into a small bottle and 200 ml KBr is added, then stirred until homogeneous. The mixture is then placed on the die, pressed for several minutes until it is pelleted. The pellets are then put into the sample and their absorption is measured at 4000-400nm wavelength, alginate is at peak / peak wavelength 1030/1080 nm.

RESULTS AND DISCUSSION

1. Characteristics of seaweed

Raw materials of seaweed obtained from western Java in Indonesia consist of Pulau Lima, Ujung Kulon and Binuangeun. Images of Sargassumspp seaweed are presented in Figures 5, 6, 7.

Figure 5. Sargassum *spp* seaweed from Lima Island

Figure 6. *Sargassum* spp seaweed from Ujung Kulon

Figure 7. Sargassum *spp* seaweed from Binuangeun

Based on the analysis of the quality of Sargassum spp seaweed from Pulau Lima, Ujung Kulon and Binuangeun which includes yields, water content, CAW and impurities obtained as shown in Table 3.

Table 3. Chemical composition of Sargassum *spp* Seaweed from 3 West Java Locations in Indonesia

Location	Lima Island			Ujung Kulon			Binuangeun			Standard
Chemical compotition	1	2	mean	1	2	mean	1	2	mean	
Rendemen (%)	12,96	11,22	12,09	17,78	14,70	16,24	29,54	22,00	25,77	-
Water content (%)	09,00	09,50	09,25	12,75	11,00	11,88	13,00	12,50	12,75	< 15 %
CAW (%)	76,64	71,49	74,06	67,69	65,42	66,55	75,59	78,24	76,92	>50 %
Impurities (%)	13,80	09,32	11,56	33,88	3,50	3,69	25,27	26,15	25,71	< 3 %

Seaweed yields obtained from the results of wet seaweed drying, yield differences from the three locations, the highest yield of Sargassum spp. from Binuangeun 25.77%.

Sargassum water content has been met which is a maximum of 15%. According to Winarno (1996), the value of the water content of seaweed is influenced by the drying process. The highest water content of Ujung Kulon 14.50% still meets the requirements of the SNI standard.

CAW provides information on the cleanliness of seaweed from dirt, sand and rock attached. Based on Table 2, it can be seen that the CAW values of Pulau Lima, Ujung kulon and Binuangeun were obtained, which were 76.64%, 67.69% and 75.59% respectively. This value gives the meaning that Sargassum spp. which is used clean and free of dirt. These results are in accordance with the quality requirements of dried seaweed based on SNI No. 2690.1.2015 which is a minimum of 50%.

The highest impurity level of the three locations is from Binuangeun (25.71%) impurity consisting of sand, rock, coral and litter consisting of plastic impurities, waste leaves left by humans in Binuangeun because it is close to residential dwellings. Impurity Rate of Sargassum spp. from Pulau Lima (11.56%) consisting of sand and coral and the level of impurity of Sargassum spp. the lowest is from the location of Ujung Kulon (3.69%) containing sand and coral because of the location of Sargassum spp. far from the residential areas of Taman Jaya village, Sumur District, Pandeglang Regency, West Java Province, the results are the cleanest.

2. Alginate extraction

The results of the study based on the physical characteristics of the quality of Na-alginate Sargassum spp from three locations are presented in Table 4.

Table 4. The mean value of physical quality analysis of Na alginate *Sargassum* spp from three locations

Karakteristik	Lokasi			
	Pulau Lima	Ujung Kulon	Binuangeun	Standar
Rendemen (%)	11,48 ± 0,79	18,62 ± 0,84	05,75 ± 0,11	> 18,00*
Viskositas (cP)	35,00 ± 7,07	62,50± 3,53	81,33 ± 1,88	> 27,00**

Note : SNI* and ** Food Chemical Codex (2004)

Rendemen

Sargassum *spp*. from Ujung Kulon it has the highest Na alginate content with the highest yield value (18.62 + 0.84)% followed by the location of Pulau Lima with an average yield yield (11.48 + 0.79)% this is influenced by the cleanliness of the location the impurity consists only of sand and coral, for the Binuangeun location the lowest Na alginate yield (05.75 + 0.11)% this is influenced by the amount of sand, rock, coral and litter impurities because it is close to the population occupancy. The results of the alginate yield test in the extraction of *Sargassum* spp. presented in Figure 8.

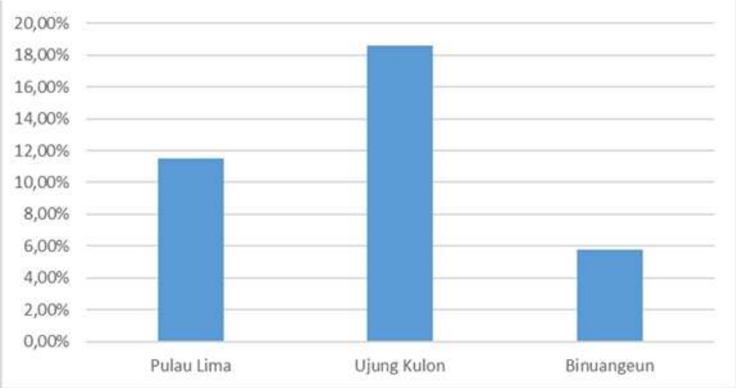


Figure 8. Results of alginate yield test in extraction of Sargassum spp. (%)

Alginate yield produced from seaweed is influenced by habitat (light intensity, wave size or current and aquatic nutrition), age of brown seaweed and brown seaweed handling techniques after harvest, before and the extraction process used (Basmal et al 2012) because other factors are carried out with the same treatment in three locations, so the size of the waves or currents affect the size of the yield of the three different locations. Binuangeun has a shallow depth of 40.00 cm with the shortest total thalus length of (31.82) cm where Sargassum is overgrown at the lowest ebb in the form of inundation affected by current velocity (0.24, 0.14 and 0.03) and based on the Meteorological Agency , Climatology and Geophysics (BMKG - maritime.bmkg.go.id) waves in the area of Pulau Lima in the western part of the Java Sea, the waters of the northern coast of Java are included in the Slight Sea / Small group with a wave size of 0.5 - 1.25 m, while Ujung Kulon and Binuangeun belong to the Moderate Sea / Moderate group, with a wave size of 1.25 - 2.50 m, this causes the thalus length in Binuangeun to be shorter than the total thalus length of Sargassum in Ujung Kulon and Pulau Lima.

Viscosity

The highest mean viscosity was Sargassum spp originating from Binuangeun (81.33 + 1.88) cP, Ujung Kulon (62.50 + 3.53) cP, and Pulau Lima (35.00 + 7.07) cP. According to Subaryono (2009) states that the high content of water insoluble material and low alginate viscosity is caused by the low purity of the alginate produced. Na-alginate thickness is divided into three levels, namely low viscosity (<60 cP), medium thickness (60-110 cP) and high thickness (110-800 cP). Based on this division, the viscosity of Na alginate on the island of Lima is included in low viscosity (Syafarini 2009; Subaryono and Apriani 2010). Na alginate viscosity varies greatly from 10-5000 cP (1% Na alginate solution in water), depending on the final destination of the product (Basmal et al.2012). Sodium alginate for food usually has a lower viscosity than sodium alginate for textiles. Seaweed from the tropics (warm water) generally produces alginates with low viscosity (McHugh 2008). Seaweed with a long thalus length will produce Na alginate with low viscosity, whereas if used with seaweed with a short thalus (20-40) cm it will produce high viscosity. Raw material for Sargassum spp. obtained from Binuangeun waters has a small thalus length (less than 20 - 40 cm) Sinurat E. Marliani R. 2017. The results of the alginate viscosity in the extraction of Sargassum spp. are presented in Figure 9.

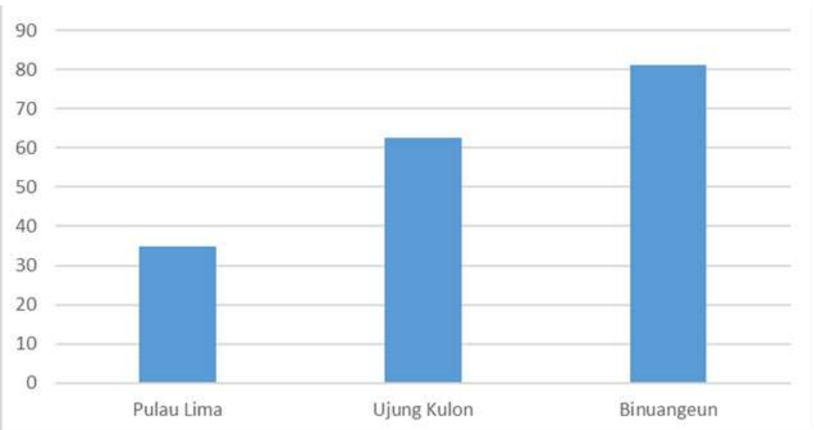


Figure 9. Test results for alginate viscosity in extraction of Sargassum spp. (Cps)

Alginate viscosity is influenced by several factors, including temperature, solution level and degree of polymerization. Na alginate viscosity value is highly dependent on the age of brown seaweed harvesting, extraction techniques (concentration, temperature, pH and the presence of polyvalent metal cations) and the weight of seaweed molecules extracted (Basmal el al 2012; McHugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80oC, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the thickness using RVA (rapid viscoanalyzer). Anggadireja et al (2008) stated that the higher the drying temperature the higher the viscosity value. It is assumed that the increase in drying temperature will increase the formation of the amount of sulfate esters so that viscosity increases.

Chemical Characteristics of Na-alginate Sargassum spp.

Chemical analysis of Sargassum spp. Na-alginate seaweed. includes the value of water content, ash content, and pH value presented in Table 5.

Table 5. Mean value of chemical quality analysis of Na alginate Sargasum spp from three locations

Characteristics	Locations			
	Lima Island	Ujung Kulon	Binuangeun	Standard
Water content (%)	12,42 ± 0,30	10,23 ± 1,68	13,31 ± 0,77	5-20**
Ash content (%)	26,68 ± 2,48	24,94 ± 4,41	26,69 ± 0,82	18,00-27,00*
pH	7,39 ± 0,03	7,03 ± 2,60	6,05 ± 0,57	3,5 - 10***

Description: (* Food Chemical Codex (2004), **) Winarno (1996)

Sargassum spp seaweed from Binuangeun has a Na alginate content with higher water content and ash content while the mean pH (6.05+ 0.57) is lower than Sargassum spp from Pulau Lima and Ujung Kulon.

Water content

Drying is a process of reducing a part of the water content of the material. Water content of the material is the amount of water contained in the material expressed in percent (%). The water content in Na alginate from the three locations in the study was in accordance with international quality standards provided that the drying losses were <15% (FCC 2004). The mean water content of Na alginate seaweed Sargassum spp from three locations is presented in Table 5. The results of the observations showed that the mean value of Na alginate water content from Binuangeun was the highest. The higher the purity of alginate results in the difficulty of the water coming out of the matrix during the drying process. Alginate is a polymer with the ability to hold water very well so that the higher the purity of the alginate, the better the ability to hold water (Suwarda, 2016).

Water content can affect the shelf life of a product. Products that have low water content usually have a longer shelf life than products that have high water content (Siswati 2002). Water is an important component in food ingredients because water can affect the appearance, texture and taste of food. The water content in food ingredients also determines acceptability. Diversity and durability of food ingredients (Winarno 2008). The water content allowed in Na alginate is between 5-20%, while the water content allowed by the FCC is <15%. When compared to research ith some standards, the resulting Na-alginate water content meets the standard (FCC 2004). JECFA also stated that the alginate water content as food additives must have a maximum water content of 15% (FAO 2009).

Ash

Ash content is important to know because it can determine the purity level of the product from unwanted components. Based on the analysis of the resulting ash content it can be seen that commercial alginate generally has a maximum ash content of 27% when compared to the three locations the ash content used still meets JECFA requirements standards (McHugh 2008).

pH

Na alginate with a mean pH of 6 from Binuangeun was lower than the pH of Ujung Kulon and Pulau Lima.

The results of the overall chemical and physical analysis that has been carried out by Na alginate produced from Binuangeun are more suitable for use in non-food products, this is related to the quality produced. Binuangeun's results have brighter wana can be formulated according to the needs and desired physicochemical properties, especially those related to gel formation, thickness, binding of water so that it can retain moisture, while Lima Island is more suitable for food products. Food alginate Na food products must have low water content, low ash content and neutral pH (Yunizal 2004). Alginate characteristics of Pulau Lima, Ujung Kulon and Binuangeun meet alginate standards as food grade, but when viewed from viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry.

3. Partial Hydrolysis of Alginate

The results of isolation of manuronic acid (M) and guluronate (G) on alginate molecules were carried out by partial hydrolysis of alginate, obtained by GG block deposits as presented in Table 6.

Table 6. Results of Alginate Partial Hydrolysis

Locations of Sargassum spp	Blok GG	Blok MM	Blok MG	Blok M	Blok G
Pulau Lima	67,60	59,00	41,40	27,00	35,00
Ujung Kulon	50,00	5,40	6,00	9,60	30
Binuangeun	55	70	63	37	45

The highest component G is alginate from Pulau Lima with the results of the viscosity test as listed in Figure 8 and according to the functional group analysis which is qualitatively proven on the FTIR curve as shown in Figure 10.

Analysis of functional groups of sodium alginate from extraction of Sargassum spp from three locations

Uptake of functional groups from Sargassum spp from these three locations is seen in the FTIR curve of Figure 10 and Table 7.

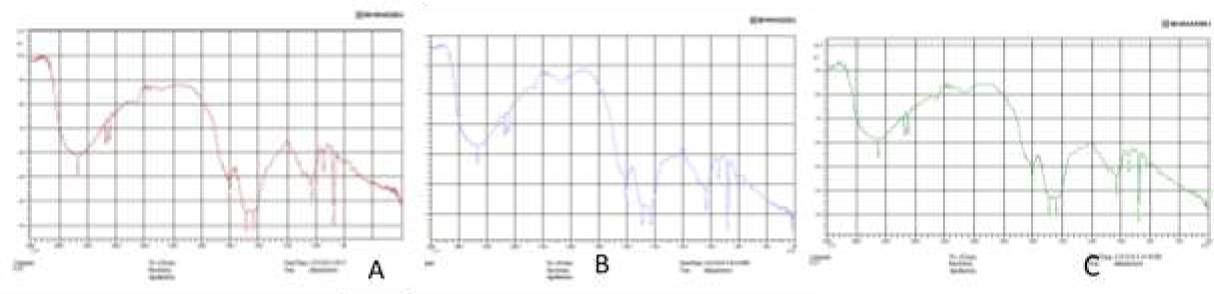


Figure 10. FTIR curve Alginate Island A. Lima, B. Ujung Kulon, C. Binuangeun

Tabel 7. Gugus fungsi pada kurva FTIR

Bilangan gelombang cm-1	% Transmitan (% T)			Gugus Fungsi
	Pulau Lima	Ujung Kulon	Binuangeun	
3427.51-3448.72	63.09	53.88	52.89	O-H streching i hidrogen antar mo
1608.63	50.98	41.15	50.29	C=O
1411.89	38.72	33.42	38.49	Ikatan -C-OH
1091-1093.64	53.62	41.02	48.50	COOH, C-O stre C-O-C streching
1170	64.41	48.21	56.67	C-0 streching C-C stretching C-C-C bending
1029.99 – 1033.85	48.94	37.16	45.59	C-O stretching C-O-C stretching
947.05	62.31	47.11	54.50	C-O stretching C-C-H stretching
817.82 – 875.68	59.11	30.96	35.04	C-C stretching C-C-H stretching C-O bending

The sodium alginate spectrum showed the presence of hydroxyl (-OH) groups, carbonyl groups (-COO-), –C-OH and –COOH bonds, C-O streching, C-C streching, and C-O bending as seen in FTIR Curves (figure 10). According to Jesus Ivan Murillo –Alvares et al absorption at wave numbers 1608.63 cm-1, 1411.89cm-1 and 1091 cm-1-1093.64 cm-1 (in the wave number area 1091 cm-1 -1093,64 cm-1 (in the area wave number 1608 cm-1 to wave number area 1091 cm-1), if higher than absorption at wave number 1029 cm-1-1033 cm-1, 947 cm-1 and 817,82cm-1 - 875,68 (at the area of wave number 1029 cm-1 to 817 cm-1) indicates that the alginate polymer consists of a higher proportion of guluronic monomers, whereas absorption at wave numbers 1315 cm-1, 1170 cm-1, 1029 cm-1-1033 cm-1, 947 cm-1 and 817.82-875.68cm-1 were higher indicating that the alginate polymer consisted of the proportion of manuronic monomers. In the results of the FTIR curve showed that the absorption at wave number 1608.63cm-1,1411.89cm-1, and 1091cm-1 -1093.64 cm-1 in Binuangeun alginate products provide higher absorption compared to Pulau Lima and Ujung Kulon alginates, but d The results of the GG block partial deposit test (Table 8.) showed the lowest guluronate group (41.40%). This is possible at wave number 1029.99 cm-1-1033.85 cm-1 giving quite high uptake of C-O stretching and C-O-C stretching uptake. Whereas the Pulau Lima and Ujun Kulon alginate products showed a higher proportion of guluronate monomers than manuronates according to the results of the quantitative test of GG block partial deposits namely Pulau Lima, Ujung kulon and Binuangeun respectively with 67.60%, 59.00% and 41.40%.

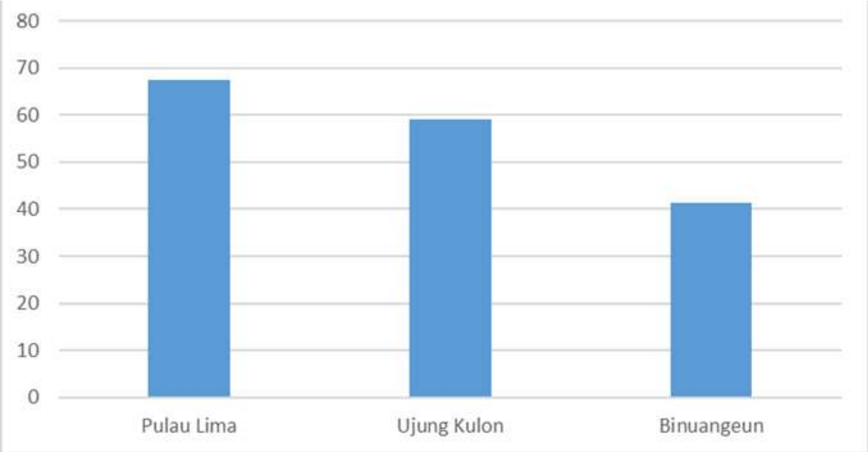


Figure 11. Results of GG block deposition test on alginate structure (%)

The combination of viscosity and gel strength of the two characteristics of Guluronat and Manuronat is the determinant of whether or not the hydrogel is produced, while the other characters are supporting information. Inconclusion of this study showed that alginate from Sargassum spp. from Lima Island, Ujung Kulon and Binuangeun, the yields were 11.48%, 18.62% and 5.75% respectively. Alginate from Binuangeun has the best physico-chemical characteristics compared to the others. With a high viscosity value, the viscosity is 35 cps, 62.50 cps and 81.33cps respectively. The characteristics of Na alginate produced from Binuangeun have relatively higher quality. Alginate characteristics of Lima Island, Ujung Kulon and Binuangeun has met alginate standards as food grade, but when viewed from viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry. The results of the partial alginate hydrolysis test showed that guluronic block (GG) in alginate polymers on the islands of Lima, Ujung Kulon and Binuangeun were 67.60%, 59.00% and 41.40%, respectively. This is related to the gel properties formed. Alginate from Pulau Lima tends to be stiffer and less flexible than alginate from Ujung Kulon and Binuangeun. Variations in the concentration of Manuronate and Guluronate from the three ecology of Sargassum in West Java have different variations.

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ISOLATION AND PARTIAL CHARACTERIZATION OF ALGINATE FROM WEST OF JAVA INDONESIA WATERS SEAWEED *Sargassum* spp.

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ISOLATION AND PARTIAL CHARACTERIZATION OF ALGINATE FROM WEST OF JAVA INDONESIA WATERS SEAWEED *Sargassum* spp.

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Abstract. Alginate is a polysaccharide organic linear polymer consisting of α-L guluronic acid monomers and β-D manuronic acid, or it can be a combination of these two monomers which may be useful for food and non-food purposes. Utilization of *Sargassum* spp seaweed as an alternative alginate source will reduce dependence on alginat imports, which is currently still 100% imported. Thus, the purpose of this study was to prepare and characterize alginates from *Sargassum* spp seaweed obtained from three locations with different ecological characteristics. Isolation alginate with partial hydrolysis separated guluronate acid and manuronic acid followed by freeze drying and measurements qualitatively and quantitatively using FTIR. The results showed that alginat from *Sargassum* spp. of Lima Island, Ujung Kulon and Binuangeun gave rendemen were 11,48 %**,** 18,62 % and 5,75 % respectively, a viscosity of 35 cps, 62,50 cps and 81,33cps respectively. The test result of partial hydrolysis of alginat showed that gulluronic block (GG) in the alginate polymer of Lima Island, Ujung Kulon and Binuangeun were 67,60 %, 59,00 % and 41,40% respectively. This relates to the nature of the gel formed. Alginat of Lima Island tend to be more rigid and less flexible than the alginate from Ujung Kulon and Binuangeun. The variations in the concentration of Manuronate and Guluronate from the three ecologies of *Sargassum* in Western Java are different variations.
Key words: alginat, characterization, gulurona t , Java, manuronat, partial, *Sargassum* spp, west

Abbreviations : FTIR = Fourier Transform Infrared Spectroscopy

Running title: Alginat partial characterization of *Sargassum polycistum*

INTRODUCTION (10 PT)

Brown algae are better known as alginate sources. Alginate is needed in various industries, functions as a gelling agent (gelling agent), stabilizer (stabilizer), emulsifier (emulsifier), suspension (suspending agent), and dispersing a product. In the food industry, alginate compounds are added ingredients for butter, ice cream and milk. In the cosmetics industry it functions as a water binder so that it easily penetrates the skin tissue and is perfectly bound. In the textile industry it functions as a binder of water (thickener) in tasting batik (Suptijah, 2002; Sinurat and Murdinah, 2007).

Alginate consisting of a group of polysaccharides can be found in brown algae tissue. Although all brown algae contain alginate, only a few species of brown algae are used commercially in the subtropical blood today such as Macrocystis pyrifera, Ascophyllumnodosum, Laminaria hyperborea, Laminaria digitata, Ecklonia maxima, and Lessonia nigrescans. Whereas in Indonesia more use of Sargassum sp., Turbinaria sp., Hormophysa sp., And Padina sp., (Rasyid, 2003).

Sargassum spp. is an alginate-producing seaweed. The opportunity to increase alginate production will require data on Sargassum spp. Seaweed species. and its content in an effort to manage sustainable alginate resources. This study aims to obtain ecological, morphological, molecular and alginate structure of Sargassum spp. in western Java so it is known that there is a relationship between the Sargassum spp gene. against the alginate structure. The character of alginate structure is needed in the development of sustainable indigenous species. Sargassum spp. as wild seaweed can be known based on the place of growth has the potential as a source of specific alginate raw material.

Increasing demand for increasingly high industries, encourages to explore and develop potentially industrial species. Ekas Bay on Lombok Island presents brown macroalgae stocks that vary with season and species, for Padina biomas it reaches 97.85 ± 12.63 and 79.54 ± 2.53 tons in May to June and November. For Sargassaceae species it reached 669.70 ± 109.64 and 147.70 ± 77.97 tons in May to June and November. The best alginate results occur during the period May to June, Padina can produce 9.10 ± 0.06 tons of alginate dry weight. Interestingly, Sargassum extraction allows producing 207.61 ± 0.42 tons of alginate dry weight. This study shows that wild Sargassaceae is an attractive stock in terms of biomass, alginate character is influenced by the ratio of manuronate and guluronate. (Setyawidati et al., 2018).

According to King (1983) and Saraswathi et al. (2003), the alginate content of seaweed varies greatly depending on the type of brown seaweed extracted. According to Winarno (1990), alginate formed in Sargassum seaweed cell walls reaches

40% of the total dry weight and plays an important role in maintaining the talus tissue structure. Talus seaweed Sargassum spp. has a variety of shapes and sizes, from the talus in the form of rods that collect in a bundle to the large talus which sometimes shows an outer shape like tall plants, the shape of the talus can affect the content of alginate.

Alginophite is a type of alginate-producing seaweed. The types of alginate-producing brown seaweed are Sargasssum spp., Turbinaria spp., Laminaria spp., Ascophyllum spp., And Macrocystis spp. Sargassum spp. and Turbinaria spp. mostly found in Indonesian marine waters, while Laminaria spp., Ascophyllum spp. and Macrocystis spp. often found in sub-tropical waters.

Potential type

Sargassum spp. not yet cultivated in Indonesia, Sargassum spp. still very limited. In the world of Sargassum spp. there are about 400 species, while in Indonesia there are 12 types, namely: Sargassum duplicatum, S. hitrix, S. echinocarpum, S. gracillinum, S. obtuspfolium, S. binderi, S. polycystum, S. microphylum, S. crassifolium, S aquafolium, S. vulgare, and S. polyceratium.

alginate structure

Alginofit is a family of brown seaweed. The difference in the main chemical compounds contained in seaweed shows the characteristics of the seaweed. The characteristic of these chemical compounds shows the extraction process. Similarly, in a variety of benefits and the use of each of these chemical compounds, there are many different from one another. Specific differences in characteristics are caused by differences in the types of seaweed, different locations of growing seaweed and its growing season.

Alginate is a long chain polymer consisting of α- (1→4) -linked L-guluronic acid (G) and β- (1→4) -linked D-mannuronic acid (M). The content ratio (G) and (M) is referred to as the G / M ratio. The ideal structure of the alginate formula is shown in Figure 1.

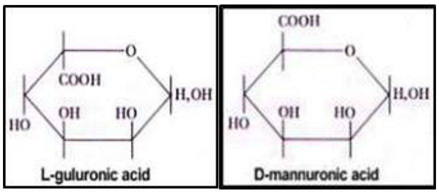

Figure 1. The ideal structure of the alginate formula

The G / M ratio will greatly affect the quality of alginate, the higher the G content the alginate thickness will be the higher the opposite if the M ratio is more the level of viscosity is lower. Alginophytes that grow in waters consist of 3 genera, namely: Sargassum with 7 species, Turbinaria 2 species, and Hormophisa. The structure of the G / M ratio can be seen in Figure 2.

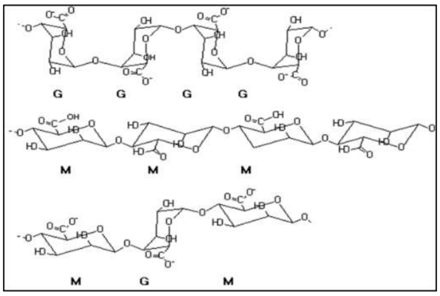

Figure 2. G / M alginate ratio

Based on this description, a study of the alginate content of Sargassum spp. Seaweed was carried out. which is found in the waters of Western Java to find out the types of Sargassum spp. which has the potential to produce alginate for the development of indigenous species producing sustainable alginate. Isolation study and partial characterization of alginate from Sargassum spp. from the west Java waters of Indonesia, the purpose is to identify the structure of sodium alginate based on the chemical composition of manuronate and guluronate on alginate products from Sargassum spp. which has ecological variations and morphological variations in western Java.

MATERIALS AND METHODS

A. Time and Place

The study was carried out in February 2018 until April 2019. Ecological observations were carried out in three locations in western Indonesia, namely in the waters of Pulau Lima, Ujung Kulon and Binuangeun including the values of diversity index, uniformity and dominance. Ecological support includes, depth, current speed, brightness, pH, temperature, salinity, DO and for wave height using data from BMKG (Meteorology and Geophysics Agency). Morphological observations carried out on descriptive explanations following Mattio L 2009 consisted of holdfast parameters, leaf shape, leaf margin, main thalus shape and vesicle form. Observations of Sargassum spp seaweed morphometrics were carried out at the Biology Laboratory, Fisheries High School including measurements of holdfast diameter, main stem diameter, leaf length, leaf width, vesicle diameter and total thalus length.

B. Location for Sampling and Handling

The geographical conditions of western Indonesia are surrounded by three major waters, namely the Java Sea in the north, the Sunda Strait in the west, and the Indonesian Ocean in the south. The sampling location consisted of the waters of Pulau Lima, Ujung Kulon and Binuangeun. The location of S. Polycystum sampling is presented in Figure 3.

Figure 3. Locations of Pulau Lima, Ujung Kulon and Binuangeun show locations for sampling *Sargassum polycystum*: point 1 ($6^0$3'39 "S, $106^0$09'20" E), point 2 ($6^0$48'15 "S, $105^0$29'5" E), and point 3 ($6^0$49'16 "S, $105^0$56'14" E).

Collection and identification of samples of Sargassum spp. at the lowest tide at each study location. Samples were collected by the transect method along the coast, and each was photographed. then taken to the Jakarta Fisheries College Biology Laboratory, for further analysis, while very low amounts collected were photographed and identified. Seaweed is stored in a plastic bag, cleaned, sorted according to genus, weighed in fresh condition, dried with wind, and then ready to examine morphological and morphometric characteristics.

C. Research Methods

1) Materials and Research Tools

The material used in the study included the types of seaweed in the three locations found on the western coast of Java, precisely in three locations, namely Pulau Lima, Ujung Kulon and Binuangeun.

The research tools used were plastic sheeting, square paralon with a size of 0.5 x 0.5 m, large and small plastic bags, thermometers, hand refractometers, pH meters, Water Quality Checkers, digital scales, underwater cameras, meters roll, label, ruler, permanent marker, bucket, dropper pipette, dark bottle, tissue, 4 liter plastic jerry can, and scissors. Map of western Java map. For orientation in the field, multi-parameter devices are used to measure temperature, salinity, DO, pH, and flouting drodge to measure current speed, secchi disk for measuring brightness, wave pole for measuring wave height, polyethylene bottles for water samples, GPS for get a geographical position, a digital camera for shooting the latest conditions and stationery.

2) Ecological Data Collection

The method used in the study is a survey method with sampling techniques using the transect method along the lines 50 - 100 m. The research was divided into 1-3 stations, with quadratic squares of 50 x 50 cm2, on the left or right side of the transect line following the direction of the arrival of the sea water flow. Each seaweed sampling location has 1-3 stations with 3 transects or repetitions. Monitoring to see abiotic components (dead coral, sand and others) and biotic components (other types of seaweed, seagrass and other living biota). The main parameters observed in each station were the density of diversity, uniformity and dominance of seaweed using Microsoft Excel software. While the supporting parameters are measurements of physical factors namely temperature, depth, salinity, current velocity, water clarity and measurement of chemical factors namely pH, nitrate, phosphate and Dissolved Oxigen. Seaweed sampling is carried out at low tide. All types of seaweed that have been taken and put into plastic that has been labeled, then separated according to type. After that the wet weight was weighed and photographed for documentation.

The type of seaweed was identified by using the identification book Trono (1997); Atmadja (1996); Anggadiredja et al., (2006). The data obtained were then analyzed using the Shannon – Wienner diversity index to determine its diversity, Simpson's dominance index to determine its dominance, Pielou's evenness index to determine its evenness and Sorensen's similarity index to find out the similarity of its species (Odum 1971).

Seawater extraction for water quality is carried out during sampling in intertidal seaweed areas. Measurement of temperature, pH and salinity of seawater, according to Alaerts and Santika (1987) was carried out by using a multi tester to measure the temperature into the waters which will be observed for 10 minutes. Temperature measurements were repeated 3 times in the seaweed sampling area. Depth measurements were carried out at low tide, by using a ruler dipped in the waters in the seaweed sampling area. Salinity measurements using a hand refractometer, salinity measurements were repeated 3 times, namely in the seaweed collection area. Calibrated pH measurements to measure pH into seawater using the correction factor of distilled water added with salt according to salinity at the sampling location. PH measurements were repeated 3 times. Flow velocity measurements are carried out using floating drodge. Floating drodge is released by its anchor or loads into the body of the water, allowing the buoy to move with the flow. Calculated how many seconds the floating drodge was released, all the strings were stretched to that distance, repeated 3 times and the results were evenly distributed.

The data used includes primary data and secondary data. Primary data is obtained by conducting surveys and direct observations in the field. Secondary data was obtained from the Meteorology, Climatology and Geophysics Agency and the Central Statistics Agency (BPS), secondary data covering the physical properties found in western Java waters throughout the year.

Characteristics of alginate test

1) Sample Preparation

The alginate content contained in Sargassum spp. influenced by species and location. The monomer composition in the structure of the alginate polymer is one of the factors that determine the quality of alginate. This study was carried out at 3 different locations of the Sargassum type which were dominant at that location on the alginate content and monomer composition in the structure of the alginate polymer.

2) Materials and Equipment

Brown seaweed consists of 3 dominant samples in each location, technical grade chemicals for extraction processes include Sodium carbonate, calcium chloride, Chloride acid, 70% alcohol, hydrogen peroxide and p.a grade chemicals for the analysis of alginate monomers. The equipment needed includes equipment for alginate extraction, while the test equipment is the Visfeter brand Brookfild, the Shimadzu Prestige Fourier Transform Infrared Spectroscopy (FTIR) and the Shimadzu brand Spectrophotometer.

3) Data Collection

Alginate Extraction

The extraction process of alginate is as follows, the raw material is Sargassum sp. cleaned then weighed 200 grams then added acetic acid solution according to the treatment. Ingredients that have been mixed in the blender to get the result in the form of seaweed pulp. Seaweed porridge that has been obtained is then heated at 800C and stirred for 10 minutes. After that it is filtered and squeezed to get the results in the form of coarse alginate liquid which is then dried at 650C for 24 hours, after drying it is then pressed to get coarse alginate flour. The flow chart for making alginate flour can be seen in Figure 3.

Na-Alginate Extraction Process

The main process of extracting brown alginofit seaweed into Na-alginate is divided into 4 stages namely immersion (pre extraction), extraction, bleaching and purification. Or the first immersion stage is carried out in an alkaline solution and an acid solution. The second (stage) extraction was carried out in an alkaline atmosphere by cooking using an extracting solution (Na_2CO_3, NaOH). Stage (third) bleaching using a solution of NaClO or H_2O_2. Stage (fourth) purification is divided into 3 phases, namely the formation of alginic acid, the formation of sodium alginate and the formation of pure sodium alginate.

Immersion

Soaking seaweed in $CaCl_2$ solution aims to dissolve laminarin, mannitol, dyes and salts. Besides that, this treatment also serves as an effort to dissolve the remaining impurities in seaweed. According to Tanikawa (1985), alginic acid precipitates under conditions of pH <3, presumably in this condition the alginate component will be stable in the raw material of the immersion process. While immersion in alkaline solutions aims for deproteinization (Tseng, 1946).

Extraction

The brown seaweed extraction process is carried out in alkaline conditions. The goal is to separate the cellulose content from alginate. The extracting material that can be used is Na_2CO_3 or NaOH. Chou and Chiang (1976) state that high concentrations of Na_2CO_3 (3-5%) can cause a decrease in product yield and viscosity. This is because the alkaline solution can damage the alginic acid compound by shortening the polymer chain into oligosaccharides which in turn degrades to 4-deoxy-5-ketouronic acid. Extraction carried out by heating will also affect the alginate produced. This heating process not only makes extraction processes easier but can also extract the weight of higher alginate molecules so that they can increase product yield and viscosity (Chou and Chiang, 1976).

Bleaching

The bleaching process aims to dissolve the dyes namely phenolic compounds contained in the polymeric alginate bonds so that a clearer solution can be obtained. Pale material commonly used in the process of extracting seaweed is NaCIO or H_2O_2. The bleaching process with NaCIO does not cause foam and takes place relatively quickly (Yani, 1988). However Tseng (1946) suggested that the NaCIO used was no more than 1%, because it would cause the alginate to be oxidized and degraded. The bleaching material of H_2O_2 used is intended to purify the extract and cellulose so that it can be separated by centrifuge or filtration. According to Wood et al., (1966), H_2O_2 has a strong power to free oxygen, and this material can be used for oxidation reactions at low temperatures. The reaction equation for the bleaching process using H_2O_2 is as follows:

Formation of Alginic Acid

To bind alginate contained in the bleached filtrate, HCI acid or H_2SO_4 sulfuric acid is added gradually. This process is not an easy polymer chain in alginic acid which is very susceptible to the addition of strong acids. This depends on how the acid can penetrate the alginate particles contained in the extracted filtrate. Binding of H + ions with alginate during the process of forming alginic acid runs relatively quickly (McHugh, 1987).

Deposition of Na-alginate

In the formation of sodium alginate, alginic acid that has been formed is added with alkaline solution containing Na + ions such as NaOH and Na_2CO_3. The purpose of the formation of sodium alginate is to get a more stable alginate compound. According to McHugh (1987), the exchange of H + ions with Na + ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid.

Withdrawal of Sodium Alginate

Withdrawal of Na-alginate compounds from sodium alginate solution can be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonim (1976), 1% sodium alginate starts to show the separation process in a solution of 10% isopropanol or in ethanol 20% as well as its boiling point. The melting point of isopropanol (secondary alcohol) is lower than ethanol (primary alcohol). In an effort to withdraw sodium alginate the use of isopropanol is more efficient than ethanol. Formation of pure sodium alginate is done by attracting the water content contained in the solution. This pure Na-alginate is then dried in an oven and after that it can be ground into Na-alginate flour.

After the water content contained in the anatomic alginate solution is pulled out, pure sodium alginate is formed. Sodium alginate is then dried in an oven and ground to form sodium alginate flour. The characteristics of alginic acid and sodium alginate were tested compared to Table 1. Characteristics of alginic acid and sodium alginate and Table 2. Quality specifications of alginic acid and sodium alginate. According to Glicksman (1983), alginic acid is described as a hydrophilic colloidal carbohydrate extracted with alkali salt from various types of brown seaweed. Chemically, alginate is a pure polymer of uronic acid arranged in a long linear chain. The chemical formula of alginate is $(C_6H_8O_6)$ n with the number n between 80 to 83 (Schoffel and Link, 1993). There are two types of monomers that make up alginic acid, namely β-D-mannopyranosil uronate or D-mannuronic acid and α-L-gulopyranosil uronate or L-guluronic acid. Of the two types of monomers, alginic acid can be a homopolymer consists of similar monomers namely D-mannuronic acid only or L-guluronic acid only (Winarno, 1996).

Homopolymers of D-mannuronic acid (polymannuronic acid) are formed by repeating D-mannuronic acid with β- (1,4) bonds and hydrogen bonds between hydroxyl groups on C3 atoms with oxygen atoms on adjacent hexose rings. The homopolymer form of L-Guluronic acid is more rigid than D-Mannuronic acid homopolymer (Atkins et al., 1971 in Anonim, 1976). Alginate with a high proportion of L-guluronic acid homopolymers tends to form stiff, regas and bersineresis gels. On the other hand, the higher proportion of D-mannuronic acid homopolymers tends to form a gel that is more elastic, does not regas and does not show high syneresis (Glicksman, 1983).

Data analysis of molecular structure and quality of alginate.

Variables observed in alginate include yield test, moisture content test, ash content test, viscosity test, pH test (Bahar, 2012), and structural tests with FTIR Alginate or algin is a compound contained in brown seaweed cell walls (Phaeopyceace) other than cellulose and pectin. Alginate compounds are alginic acid polysaccharides in the form of Sodium, Calcium, Potassium and Magnesium salts (Satari, 1996).

Alginate partial hydrolysis test

The composition of the polyguluronic, polimanuronate and mixed segments between manuronate and guluronate in alginate determined the quality of alginate. To isolate manuronic acid (M) and guluronate (G) on alginate molecules carried out by Partial Hydrolysis of Alginate by: 5.00 g Alginate in HCl 0.3 N at 100 ° C for 2 hours was obtained: The soluble fraction was identified as a block MG. Bonding The hydroxyl between M and G is easily hydrolyzed by insoluble-fraction acid more resistant to acid hydrolysis, again dissolved by adding alkali and fractionation by adjusting the pH at 2.85, so that the GG block settles and the MM block dissolves.

Partial analysis and characterization of alginate

The yield of alginate was measured for yield, which is as a percentage of the ratio between the final weight of alginate produced by the initial weight of Sargassum spp seaweed (in%).

Variables observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test (Bahar, 2012), and structural tests with FTIR. The data obtained were analyzed by multi-variant analysis, PCA, discriminant and clustering.

Analysis of alginate functional groups was carried out using a fourier transform infra red (FTIR) spectrophotometer (Perkin Elmer, spectrum one) based on the method of Barth (2000). Samples plus KBr (1: 100) then mashed until evenly mixed. Then presses with a vacuum pump for 15 minutes, and read the absorbance at wave numbers 400-4000 cm-1. From the resulting curve, the type of bond and its functional group are determined based on FTIR references.

MATERIALS AND METHODS

Materials and tools

The material used is Sargassum spp seaweed from three locations, Pulau Lima, Ujung Kulon and Binuangeun. Chemicals used for soaking (HCl) extracting materials (Na2CO3) forming ingredients of alginic acid (HCl) purifying materials Na-alginate (iso propyl alcohol and NaOH) all from Merck and other materials for physical and chemical analysis. Technical grade chemicals, for extraction processes include Sodium carbonate, calcium chloride, Cloria Acid, 70% alcohol, hydrogen peroxide and p.a grade chemicals for the analysis of alginate monomers.

The tool used for extracting Na alginate is a large capacity capacity of 20 L and a small dang of 10 L. Glas beaker with a capacity of 5 L (Pyrex) universal pH indicator paper, sitting scale (4 digit scale) mill machine, 7 ton power anchoring hydrolic filte press with 100 mesh cloth screen, filter (40 mesh) stainless steel thermometer, analyzer (viscometer) brook stable mycro filed TAXT2 texture analysis system)

The test equipment is the viscometer of the Brookfild brand, the Shimadzu Prestige Fourier Transform Infrared Spectroscopy (FTIR) brand and the Shimadzu brand Spectrophotometer.

Research methods

1. Na alginate extraction

The research method used is the experimental method through laboratory experiments, namely the process of making Na alginate with observations at the stage

extraction and filtration, replications were carried out three times. Observation of the chemical content (moisture content, clean unpure water, pH) and physical (yield, viscosity) of the Na alginate produced was carried out to see the characteristics of Na alginate from three locations.

The Na alginate extraction process was carried out using the Yunizal (2004) method modified with Sargassum spp seaweed weighed 1 kg, then washed with clean water 2 times as heavy the sample was then soaked with 1% HCl 2 times the sample weight for 60 minutes. Then rinsed with clean water and then extracted with a solution of 2% Na2CO3 in a volume of 60 liters with 2 stages of boiling which is the first stage of boiling with a temperature of 60oC and boiling time 60 minutes after it is crushed with a grinding machine until it is boiled again at 60oC and boiling time 60 minutes. Then filtering using the hydrolic filter press can easily add a filter aid, namely diatomaceous earth (celite) to facilitate filtration. Separation is carried out using a filter press consisting of fine filter cloth and filter aid. The screening method is continued by bleaching using 2% NaOCl. 450 ml NaOCl was added in 30 liters of alginate filtrate, stirred then left for 30 minutes, then added 10% HCl and left for 30 minutes to form alginic acid which was white or ivory yellow to pH 2-3. After the pH is reached, it is neutralized by adding dilute NaOH until it reaches pH 7-8 while stirring for homogenization of the solution of isoprophyl alcohol (IPA), adding that alginate has reached a neutral pH to obtain Na-alginate fiber and then the obtained fiber is dried in the form of Na-alginate.

Rendement

The Na-alginate yield obtained from the extraction process of seaweed Sargassum spp was calculated based on the weight of Na-alginate after drying on the dry weight of the raw material. Calculate the yield of alginate Na using the following formula:

Addendum (%) = (weight of Na - final alginate (g) / weight of initial seaweed (g)) x 100%

Viscosity

Viscosity analysis refers to JECFA (2007), observations were made at a 1-5% solid concentration to determine the relationship between concentration and solution viscosity. Na-alginate (sample) was weighed as much as 7.5 g in weigh paper. 492.5 g of distilled water are weighed in a 500 mL glass beaker so that the sample and distilled water when added reach total weight 500 g. Alginate is included in a 500 mL glass beaker containing distilled water and stirrer gradually. Akudes are heated and stirred once to reach 75oC, after a constant temperature the solution is heated for 25 minutes. Stirring I was carried out at minute 1 for 1 minute, stirring II at 25 minutes. Beaker glass is covered with aluminum foil to prevent water loss in the heating process due to evaporation, then the solution temperature is lowered to reach 75oC. The measurement of solution viscosity was measured using RVA (Rapid Visco Analyzer) spindle 2 at 30 rpm, waiting until the spindle needle was stable (up to 6 times rotation). Viscosity is expressed in centiPoise (cP).

1). PH value

A sample of 3 g was weighed and then put into a 300 mL glass beaker then added 197 g of distilled water until the total weight was 200 g. The sample is heated while stirring using a stirer until it dissolves at a temperature of 60-80 oC. Then the electrode is dipped into the sample solution which was previously calibrated. The pH value is obtained according to what is shown on the screen. Then the electrode is rinsed with distilled water.

2). Partial hydrolysis of alginate

The alginate content contained in each species is different. The monomer composition in the alginate polymer structure is one of the factors that determine the quality of alginate, therefore in this study carried out on Sargassum spp from three locations, namely from Pulau Lima, Ujung Kulon and Binuangeun. The composition of the polyguluronic, polimanuronate and mixed segments between manuronate and guluronate in alginate determined the quality of alginate. To isolate manuronic acid (M) and guluronate (G) on alginate molecules carried out by partial alginate hydrolysis (Subaryono 2018), by:

- 5.00 g of alginate in HCl 0.3 N at 100oC for 2 hours is obtained:
- The soluble fraction is identified as MG block. The hydroxyl bond between M and G is easily hydrolyzed by acid.
- The insoluble fraction is more resistant to acid hydrolysis, again dissolved by adding alkali and fractionation by adjusting the pH at 2.85, so that the GG block settles and the MM block dissolves.

3). FTIR Analysis

As much as 2 mg of alginate sample is put into a small bottle and 200 ml KBr is added, then stirred until homogeneous. The mixture is then placed on the die, pressed for several minutes until it is pelleted. The pellets are then put into the sample and their absorption is measured at 4000-400nm wavelength, alginate is at peak / peak wavelength 1030/1080 nm.

RESULTS AND DISCUSSION

1. Characteristics of seaweed

Raw materials of seaweed obtained from western Java in Indonesia consist of Pulau Lima, Ujung Kulon and Binuangeun. Images of Sargassumspp seaweed are presented in Figures 5, 6, 7.

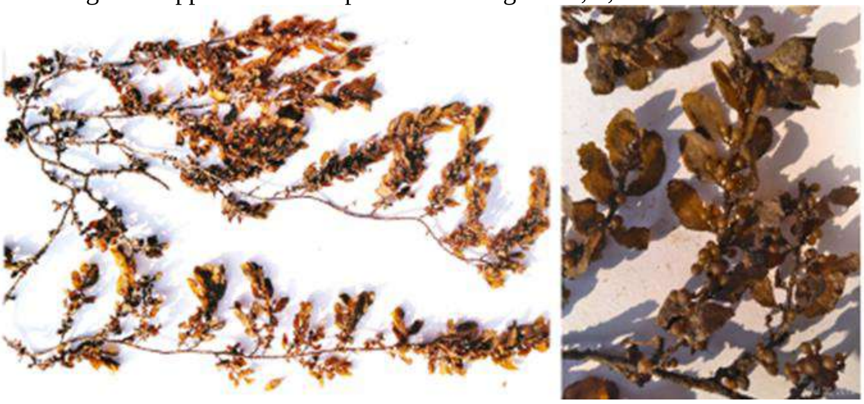

Figure 5. Sargassum *spp* seaweed from Lima Island

Figure 6. *Sargassum* spp seaweed from Ujung Kulon

Figure 7. Sargassum *spp* seaweed from Binuangeun

Based on the analysis of the quality of Sargassum spp seaweed from Pulau Lima, Ujung Kulon and Binuangeun which includes yields, water content, CAW and impurities obtained as shown in Table 3.

Table 3. Chemical composition of Sargassum *spp* Seaweed from 3 West Java Locations in Indonesia

Location	Lima Island			Ujung Kulon			Binuangeun			Standard
Chemical compotition	1	2	mean	1	2	mean	1	2	mean	
Rendemen (%)	12,96	11,22	12,09	17,78	14,70	16,24	29,54	22,00	25,77	-

Water content (%)	09,00	09,50	09,25	12,75	11,00	11,88	13,00	12,50	12,75	< 15 %
CAW (%)	76,64	71,49	74,06	67,69	65,42	66,55	75,59	78,24	76,92	>50 %
Impurities (%)	13,80	09,32	11,56	33,88	3,50	3,69	25,27	26,15	25,71	< 3 %

Seaweed yields obtained from the results of wet seaweed drying, yield differences from the three locations, the highest yield of Sargassum spp. from Binuangeun 25.77%.

Sargassum water content has been met which is a maximum of 15%. According to Winarno (1996), the value of the water content of seaweed is influenced by the drying process. The highest water content of Ujung Kulon 14.50% still meets the requirements of the SNI standard.

CAW provides information on the cleanliness of seaweed from dirt, sand and rock attached. Based on Table 2, it can be seen that the CAW values of Pulau Lima, Ujung kulon and Binuangeun were obtained, which were 76.64%, 67.69% and 75.59% respectively. This value gives the meaning that Sargassum spp. which is used clean and free of dirt. These results are in accordance with the quality requirements of dried seaweed based on SNI No. 2690.1.2015 which is a minimum of 50%.

The highest impurity level of the three locations is from Binuangeun (25.71%) impurity consisting of sand, rock, coral and litter consisting of plastic impurities, waste leaves left by humans in Binuangeun because it is close to residential dwellings. Impurity Rate of Sargassum spp. from Pulau Lima (11.56%) consisting of sand and coral and the level of impurity of Sargassum spp. the lowest is from the location of Ujung Kulon (3.69%) containing sand and coral because of the location of Sargassum spp. far from the residential areas of Taman Jaya village, Sumur District, Pandeglang Regency, West Java Province, the results are the cleanest.

2. Alginate extraction

The results of the study based on the physical characteristics of the quality of Na-alginate Sargassum spp from three locations are presented in Table 4.

Table 4. The mean value of physical quality analysis of Na alginate *Sargassum* spp from three locations

Karakteristik	Lokasi			
	Pulau Lima	Ujung Kulon	Binuangeun	Standar
Rendemen (%)	11,48 ± 0,79	18,62 ± 0,84	05,75 ± 0,11	> 18,00*
Viskositas (cP)	35,00 ± 7,07	62,50± 3,53	81,33 ± 1,88	> 27,00**

Note : SNI* and ** Food Chemical Codex (2004)

Rendemen

Sargassum *spp*. from Ujung Kulon it has the highest Na alginate content with the highest yield value (18.62 + 0.84)% followed by the location of Pulau Lima with an average yield yield (11.48 + 0.79)% this is influenced by the cleanliness of the location the impurity consists only of sand and coral, for the Binuangeun location the lowest Na alginate yield (05.75 + 0.11)% this is influenced by the amount of sand, rock, coral and litter impurities because it is close to the population occupancy. The results of the alginate yield test in the extraction of *Sargassum* spp. presented in Figure 8.

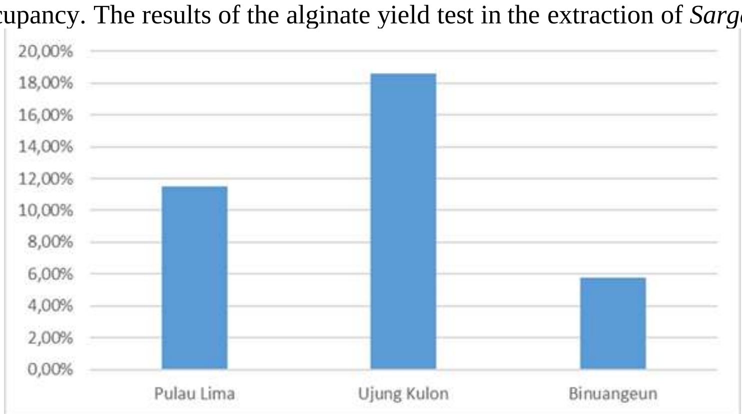

Figure 8. Results of alginate yield test in extraction of Sargassum spp. (%)

Alginate yield produced from seaweed is influenced by habitat (light intensity, wave size or current and aquatic nutrition), age of brown seaweed and brown seaweed handling techniques after harvest, before and the extraction process used (Basmal et al 2012) because other factors are carried out with the same treatment in three locations, so the size of the waves or currents affect the size of the yield of the three different locations. Binuangeun has a shallow depth of 40.00 cm with the shortest total thalus length of (31.82) cm where Sargassum is overgrown at the lowest ebb in the form of inundation affected by current velocity (0.24, 0.14 and 0.03) and based on the Meteorological Agency , Climatology and Geophysics (BMKG - maritime.bmkg.go.id) waves in the area of Pulau Lima in the western part of the Java Sea, the waters of the northern coast of Java are included in the Slight Sea / Small group with a wave size of 0.5 - 1.25 m, while Ujung Kulon and Binuangeun belong to the Moderate Sea / Moderate group, with a wave size of 1.25 - 2.50 m, this causes the thalus length in Binuangeun to be shorter than the total thalus length of Sargassum in Ujung Kulon and Pulau Lima. Viscosity

The highest mean viscosity was Sargassum spp originating from Binuangeun (81.33 + 1.88) cP, Ujung Kulon (62.50 + 3.53) cP, and Pulau Lima (35.00 + 7.07) cP. According to Subaryono (2009) states that the high content of water insoluble material and low alginate viscosity is caused by the low purity of the alginate produced. Na-alginate thickness is divided into three levels, namely low viscosity (<60 cP), medium thickness (60-110 cP) and high thickness (110-800 cP). Based on this division, the viscosity of Na alginate on the island of Lima is included in low viscosity (Syafarini 2009; Subaryono and Apriani 2010). Na alginate viscosity varies greatly from 10-5000 cP (1% Na alginate solution in water), depending on the final destination of the product (Basmal et al.2012). Sodium alginate for food usually has a lower viscosity than sodium alginate for textiles. Seaweed from the tropics (warm water) generally produces alginates with low viscosity (McHugh 2008). Seaweed with a long thalus length will produce Na alginate with low viscosity, whereas if used with seaweed with a short thalus (20-40) cm it will produce high viscosity. Raw material for Sargassum spp. obtained from Binuangeun waters has a small thalus length (less than 20 - 40 cm) Sinurat E. Marliani R. 2017. The results of the alginate viscosity in the extraction of Sargassum spp. are presented in Figure 9.

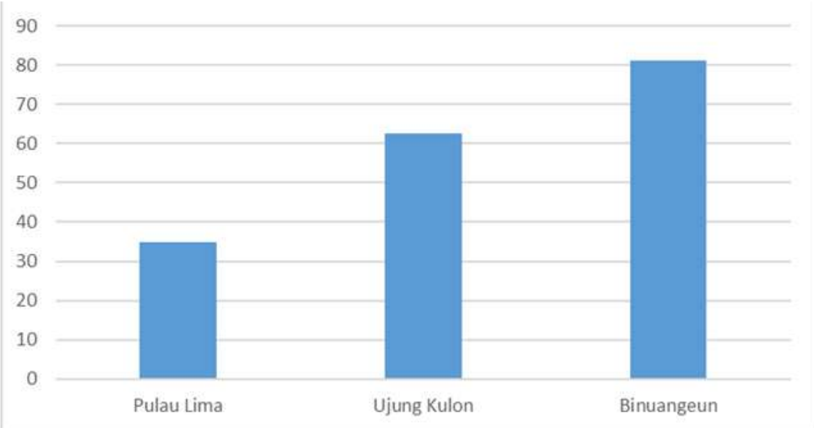

Figure 9. Test results for alginate viscosity in extraction of Sargassum spp. (Cps)

Alginate viscosity is influenced by several factors, including temperature, solution level and degree of polymerization. Na alginate viscosity value is highly dependent on the age of brown seaweed harvesting, extraction techniques (concentration, temperature, pH and the presence of polyvalent metal cations) and the weight of seaweed molecules extracted (Basmal el al 2012; McHugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80oC, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the thickness using RVA (rapid viscoanalyzer). Anggadireja et al (2008) stated that the higher the drying temperature the higher the viscosity value. It is assumed that the increase in drying temperature will increase the formation of the amount of sulfate esters so that viscosity increases.

Chemical Characteristics of Na-alginate Sargassum spp.

Chemical analysis of Sargassum spp. Na-alginate seaweed. includes the value of water content, ash content, and pH value presented in Table 5.

Table 5. Mean value of chemical quality analysis of Na alginate Sargasum spp from three locations

Characteristics	Locations			
	Lima Island	Ujung Kulon	Binuangeun	Standard
Water content (%)	12,42 ± 0,30	10,23 ± 1,68	13,31 ± 0,77	5-20**
Ash content (%)	26,68 ± 2,48	24,94 ± 4,41	26,69 ± 0,82	18,00-27,00*
pH	7,39 ± 0,03	7,03 ± 2,60	6,05 ± 0,57	3,5 - 10***

Description: (* Food Chemical Codex (2004), **) Winarno (1996)

Sargassum spp seaweed from Binuangeun has a Na alginate content with higher water content and ash content while the mean pH (6.05+ 0.57) is lower than Sargassum spp from Pulau Lima and Ujung Kulon.

Water content

Drying is a process of reducing a part of the water content of the material. Water content of the material is the amount of water contained in the material expressed in percent (%). The water content in Na alginate from the three locations in the study was in accordance with international quality standards provided that the drying losses were <15% (FCC 2004). The mean water content of Na alginate seaweed Sargassum spp from three locations is presented in Table 5. The results of the observations showed that the mean value of Na alginate water content from Binuangeun was the highest. The higher the purity of alginate results in the difficulty of the water coming out of the matrix during the drying process. Alginate is a polymer with the ability to hold water very well so that the higher the purity of the alginate, the better the ability to hold water (Suwarda, 2016).

Water content can affect the shelf life of a product. Products that have low water content usually have a longer shelf life than products that have high water content (Siswati 2002). Water is an important component in food ingredients because water can affect the appearance, texture and taste of food. The water content in food ingredients also determines acceptability. Diversity and durability of food ingredients (Winarno 2008). The water content allowed in Na alginate is between 5-20%, while the water content allowed by the FCC is <15%. When compared to research ith some standards, the resulting Na-alginate water content meets the standard (FCC 2004). JECFA also stated that the alginate water content as food additives must have a maximum water content of 15% (FAO 2009).

Ash

Ash content is important to know because it can determine the purity level of the product from unwanted components. Based on the analysis of the resulting ash content it can be seen that commercial alginate generally has a maximum ash content of 27% when compared to the three locations the ash content used still meets JECFA requirements standards (McHugh 2008).

pH

Na alginate with a mean pH of 6 from Binuangeun was lower than the pH of Ujung Kulon and Pulau Lima.

The results of the overall chemical and physical analysis that has been carried out by Na alginate produced from Binuangeun are more suitable for use in non-food products, this is related to the quality produced. Binuangeun's results have brighter wana can be formulated according to the needs and desired physicochemical properties, especially those related to gel formation, thickness, binding of water so that it can retain moisture, while Lima Island is more suitable for food products. Food alginate Na food products must have low water content, low ash content and neutral pH (Yunizal 2004). Alginate characteristics of Pulau Lima, Ujung Kulon and Binuangeun meet alginate standards as food grade, but when viewed from viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry.

3. Partial Hydrolysis of Alginate

The results of isolation of manuronic acid (M) and guluronate (G) on alginate molecules were carried out by partial hydrolysis of alginate, obtained by GG block deposits as presented in Table 6.

Table 6. Results of Alginate Partial Hydrolysis

Locations of Sargassum spp	Blok GG	Blok MM	Blok MG	Blok M	Blok G
Pulau Lima	67,60	59,00	41,40	27,00	35,00
Ujung Kulon	50,00	5,40	6,00	9,60	30
Binuangeun	55	70	63	37	45

The highest component G is alginate from Pulau Lima with the results of the viscosity test as listed in Figure 8 and according to the functional group analysis which is qualitatively proven on the FTIR curve as shown in Figure 10.

Analysis of functional groups of sodium alginate from extraction of Sargassum spp from three locations

Uptake of functional groups from Sargassum spp from these three locations is seen in the FTIR curve of Figure 10 and Table 7.

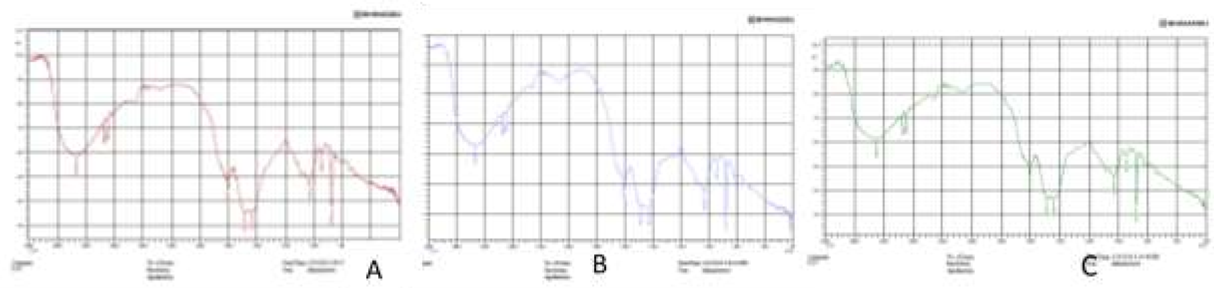

Figure 10. FTIR curve Alginate Island A. Lima, B. Ujung Kulon, C. Binuangeun

Tabel 7. Gugus fungsi pada kurva FTIR

Bilangan gelombang cm-1	% Transmitan (% T)			Gugus Fungsi
	Pulau Lima	Ujung Kulon	Binuangeun	
3427.51-3448.72	63.09	53.88	52.89	O-H streching i hidrogen antar mo
1608.63	50.98	41.15	50.29	C=O
1411.89	38.72	33.42	38.49	Ikatan -C-OH
1091-1093.64	53.62	41.02	48.50	COOH, C-O stre C-O-C streching
1170	64.41	48.21	56.67	C-0 streching C-C stretching C-C-C bending
1029.99 – 1033.85	48.94	37.16	45.59	C-O stretching C-O-C stretching
947.05	62.31	47.11	54.50	C-O stretching C-C-H stretching
817.82 – 875.68	59.11	30.96	35.04	C-C stretching C-C-H stretching C-O bending

The sodium alginate spectrum showed the presence of hydroxyl (-OH) groups, carbonyl groups (-COO-), –C-OH and –COOH bonds, C-O streching, C-C streching, and C-O bending as seen in FTIR Curves (figure 10). According to Jesus Ivan Murillo –Alvares et al absorption at wave numbers 1608.63 cm-1, 1411.89cm-1 and 1091 cm-1-1093.64 cm-1 (in the wave number area 1091 cm-1 -1093,64 cm-1 (in the area wave number 1608 cm-1 to wave number area 1091 cm-1), if higher than absorption at wave number 1029 cm-1-1033 cm-1, 947 cm-1 and 817,82cm-1 - 875,68 (at the area of wave number 1029 cm-1 to 817 cm-1) indicates that the alginate polymer consists of a higher proportion of guluronic monomers, whereas absorption at wave numbers 1315 cm-1, 1170 cm-1, 1029 cm-1-1033 cm-1, 947 cm-1 and 817.82-875.68cm-1 were higher indicating that the alginate polymer consisted of the proportion of manuronic monomers. In the results of the FTIR curve showed that the absorption at wave number 1608.63cm-1,1411.89cm-1, and 1091cm-1 -1093.64 cm-1 in Binuangeun alginate products provide higher absorption compared to Pulau Lima and Ujung Kulon alginates, but d The results of the GG block partial deposit test (Table 8.) showed the lowest guluronate group (41.40%). This is possible at wave number 1029.99 cm-1-1033.85 cm-1 giving quite high uptake of C-O stretching and C-O-C stretching uptake. Whereas the Pulau Lima and Ujun Kulon alginate products showed a higher proportion of guluronate monomers than manuronates according to the results of the quantitative test of GG block partial deposits namely Pulau Lima, Ujung kulon and Binuangeun respectively with 67.60%, 59.00% and 41.40%.

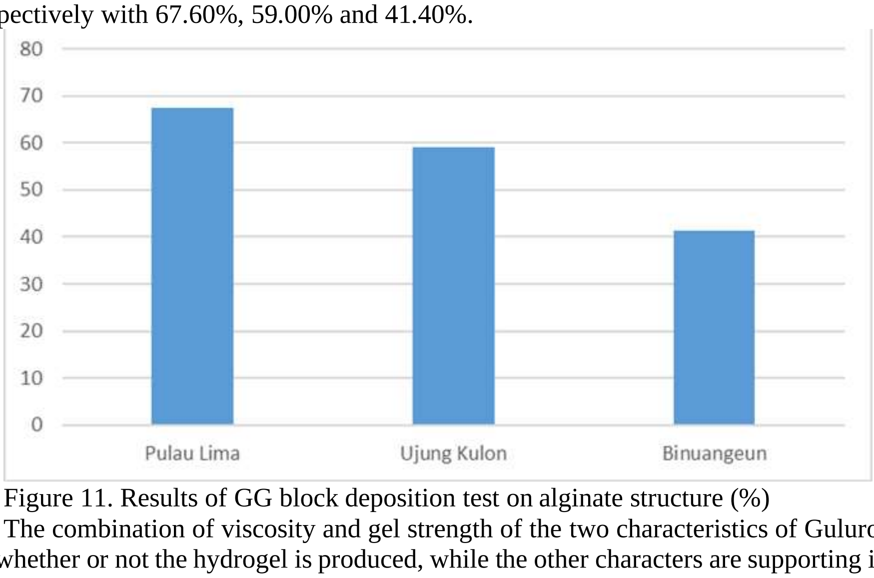

Figure 11. Results of GG block deposition test on alginate structure (%)

The combination of viscosity and gel strength of the two characteristics of Guluronat and Manuronat is the determinant of whether or not the hydrogel is produced, while the other characters are supporting information. Inconclusion of this study showed that alginate from Sargassum spp. from Lima Island, Ujung Kulon and Binuangeun, the yields were 11.48%, 18.62% and 5.75% respectively. Alginate from Binuangeun has the best physico-chemical characteristics compared to the others. With a high viscosity value, the viscosity is 35 cps, 62.50 cps and 81.33cps respectively. The characteristics of Na alginate produced from Binuangeun have relatively higher quality. Alginate characteristics of Lima Island, Ujung Kulon and Binuangeun has met alginate standards as food grade, but when viewed from viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry. The results of the partial alginate hydrolysis test showed that guluronic block (GG) in alginate polymers on the islands of Lima, Ujung Kulon and Binuangeun were 67.60%, 59.00% and 41.40%, respectively. This is related to the gel properties formed. Alginate from Pulau Lima tends to be stiffer and less flexible than alginate from Ujung Kulon and Binuangeun. Variations in the concentration of Manuronate and Guluronate from the three ecology of Sargassum in West Java have different variations.

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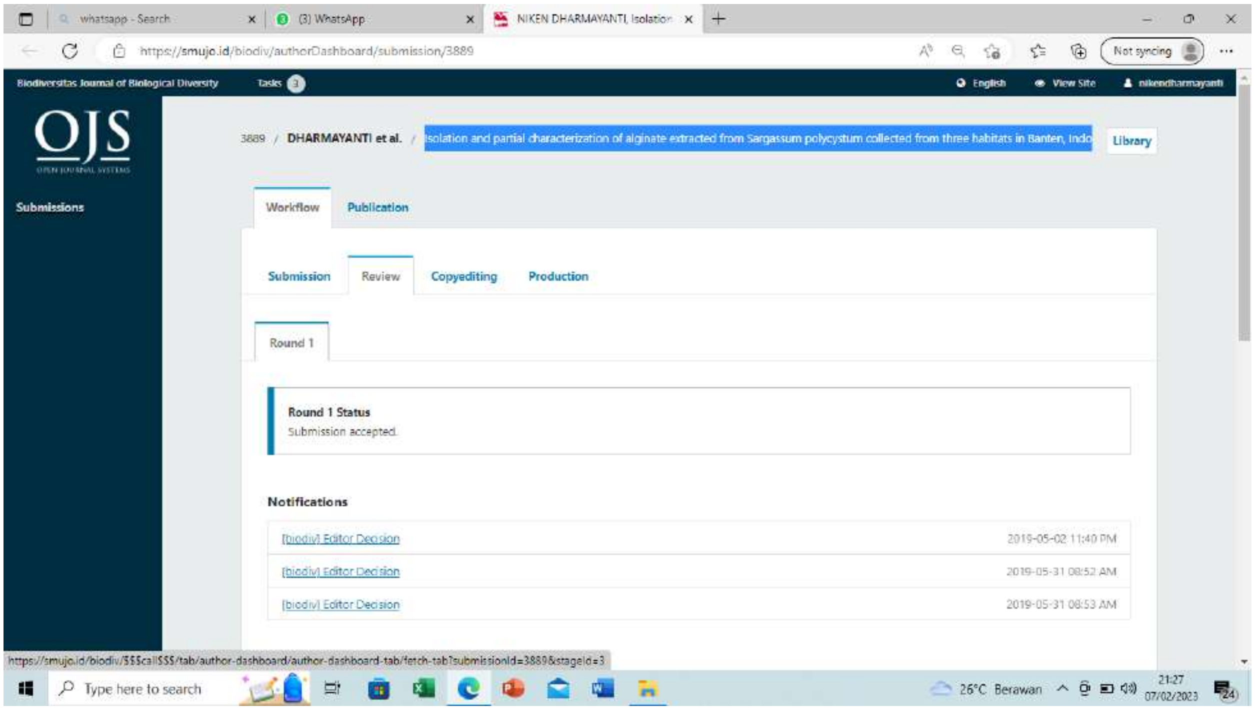
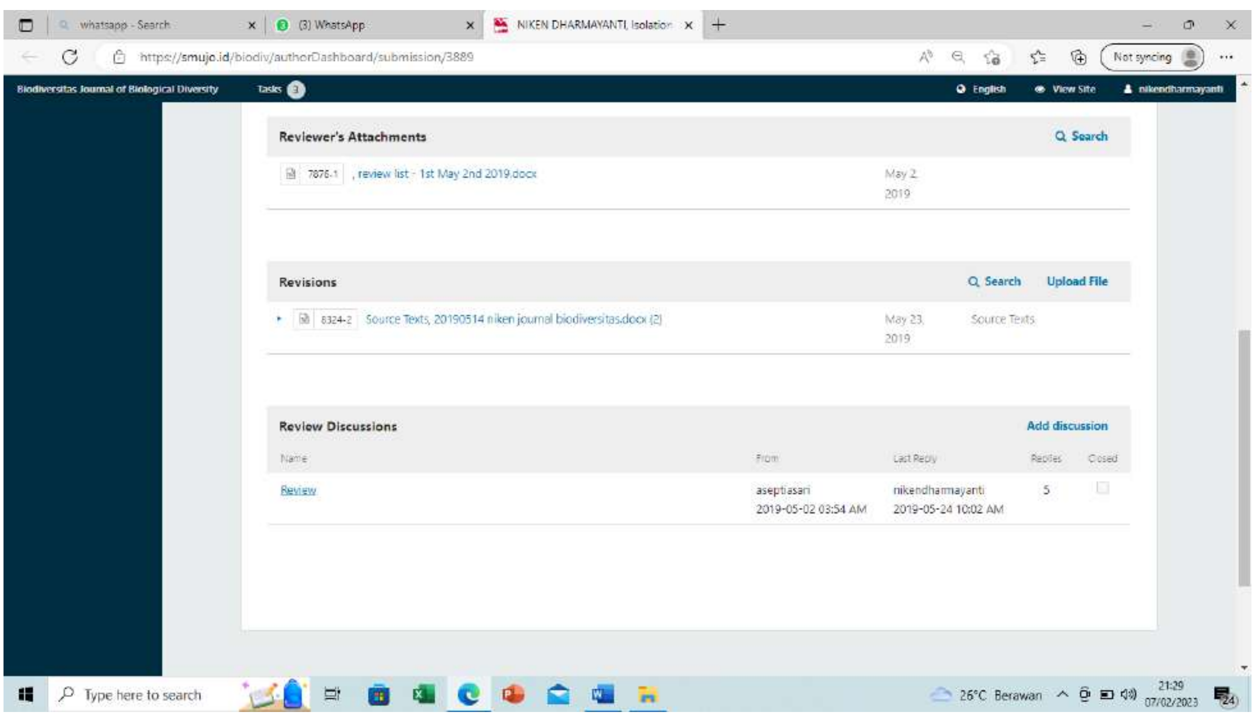
Bukti Review Jurnal Biodiversitas “Isolation and partial characterization of alginate extracted from Sargassum polycystum collected from three habitats in Banten, Indonesia”





Review list :

Number of Line in the manuscript		Should be / Suggestion
Abstract	:	Still more than 200 words (222 words), should be reduce
1,2	:	Isolation and partial characterization of Alginate from *Sargassum* spp. of West Java Indonesia
12	:	this study was to prepare and characterize alginates which extracted from *Sargassum* spp seaweed...
13-14	:	Isolation alginate with partial hydrolysis separated guluronate acid and manuronic acid followed by freeze drying, then measured by qualitatively and quantitatively parameters using FTIR.
15	:	…. provided yield were ….. respectively, and also the viscosity were … and 81,33 cps
16	:	…. showed that guluronic block (GG) …
18-19	:	There are differences in the concentration of the components of manuronate and guluronate in 3 locations grown of Sargassum spp. in West Java
20	:	alginat, characterization, guluronat, manuronat, partial hydrolisis, *Sargassum* spp, West Java
24-25	:	Brown algae are better known as alginate sources. Alginate is needed in various industries, functions as a gelling agent (gelling agent), stabilizer (stabilizer), emulsifier (emulsifier), suspension (suspending agent), and dispersing a product agent.
27	:	functions as a water binder
28	:	as a binder of water water binder (thickener) in batik dyeing tasting batik
30	:	only a few species of brown algae are used commercially and can be found in subtropical climate suc as…..in the subtropical blood today such
32	:	Whereas in Indonesia more use of Sargassum sp., Turbinaria sp., Hormophysa sp., And Padina sp., Whereas in Indonesia, the more common types are …
33-34	:	The opportunity to increase alginate production will need an information of Sargassum spp. species will require data on Sargassum spp. Seaweed species. and its content in an effort to manage sustainable alginate resources.
35-36	:	…. so it is known that there is a relationship between the Sargassum spp gene. against the alginate structure. …. so that the relationship between genes from sargassum and its structure will be known.
38	:	….place of growth has the potential as a source of specific alginate raw material and can be a potential source as a raw material for alginate.
39	:	Increasing demand for increasingly high industries, encourages to explore and develop potentially industrial species. Increasing demand requires an increase in industrial quality and encourages exploration of potential species.
40	:	….. species, it should be written by .
40-41	:	…for Padina biomas it reaches 97.85 ± 12.63 and 79.54 ± 2.53 tons in May to

		June and November.
44	:	… This study shows showed that wild …
47		…. alginate formed in Sargassum seaweed cell walls …
48		…. and plays played an important …
48-49		The Thallus of Sargassum Talus seaweed Sargassum spp. has a variety of shapes and sizes
50		The types of alginate-producing **from** brown seaweed
72-73		…the higher of the G content, the alginate thickness will be the higher **than** the opposite if the M ratio is **higher** more the level of viscosity is lower
74		… namely: **i.e** Sargassum …
79-81		… content of Sargassum spp. **which is found in the Western Java sea** Seaweed was **is needed to carried out and to develop the potential indigenous species that could produce alginat**. which is found in the waters of Western Java to find out the types of Sargassum spp. which has the potential to produce alginate for the development of indigenous species producing sustainable alginate.
82		… the purpose is **aims** to …
88 and 96		Namely **i.e**
426		are more suitable for use in non-food products **more suitable for non-food products**
429		Food alginate Na food products **alginate for food** must have low water content, low ash content and neutral pH
430-431		but when viewed from **its** viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry.
442		The highest component G is alginate **The highest component of G in alginate is obtained** from Pulau Lima
468-469		The combination of viscosity and gel strength of the two characteristics of Guluronat and Manuronat is the determinant **factors to produced hydrogel that other characteristics** of whether or not the hydrogel is produced, while the other characters are supporting information.
469-470		. …Inconclusion of **The result of** this study showed that **the yields of** alginate from Sargassum spp. from Lima Island, Ujung Kulon and Binuangeun, the yields were …
478-479		**There was** Variations in the concentration of Manuronate and Guluronate from the three ecology of Sargassum in West Java have different variations.

Words/paragraph in yellow line : **preferably deleted**
The bold word : is the correct one

Isolation and partial characterization of alginate extracted from *Sargassum polycystum* in West Java, Indonesia

The utilization of *Sargassum polycystum* as an alternative alginate source may reduce the dependence on alginate import as currently alginate demands in Indonesia are 100% supplied from overseas. The purpose of this study was to characterize alginate extracted from *Sargassum polycystum* obtained from three locations with different ecological characteristics. Isolation of alginate was conducted through partial hydrolysis to separate gulurunate acid and mannurunate acid followed by freeze drying and then the parameters were measured qualitatively and quantitatively using FTIR. The results showed that alginate extracted from *Sargassum polycystum* collected from Lima Island, Ujung Kulon and Binuangeun were 11,48 %**,** 18,62 % and 5,75 %, respectively with the viscosity were 35 cP, 62,50 cP and 81,33 cP, respectively. The test result of partial hydrolysis of alginate showed that guluronic block (GG) in the alginate polymer of Lima Island, Ujung Kulon and Binuangeun were 67,60 %, 59,00 % and 41,40%, respectively. These relate to the nature of the gel formed. The alginate from Lima Island tend to be more rigid and less flexible than that from Ujung Kulon and Binuangeun. The findings of this study suggest that there are differences in the concentration of the components of mannurunate and gulurunate of *Sargassum polycystum* across different locations in West Java.

Keywords: Alginate, characteristics, partial hydrolysis, *Sargassum polycystum*

Abbreviations : FTIR = Fourier Transform Infrared Spectroscopy

Running title: Alginate partial characterization of *Sargassum polycistum*

INTRODUCTION

Brown algae is known as alginate sources. Alginate is needed in various industries for various purposes including as gelling agent, stabilizer, emulsifier, suspending agent and dispersing agent. In food industry, alginate compounds are added as ingredient in butter, ice cream and milk. In cosmetics industry it function as water binder so that the cosmetic components are perfectly bound and easily penetrate skin tissue. In textile industry it serves as water binder (thickener) in batik dyeing (Suptijah 2002; Sinurat and Murdinah 2007).

Alginophyte is a family of brown seaweeds. The difference in the main chemical compounds contained in seaweed shows the characteristics of the seaweed, suggesting different extraction process among seaweeds. Similarly, the benefits and uses of these chemical compounds also vary one another. These differences are caused by different species of seaweed, different location and growing season.

Alginate consists of group of polysaccharides and can be found in brown algae tissue. Alginate is a long chain polymer consisting of α- (1$\rightarrow$4) -linked L-guluronic acid (G) and β- (1$\rightarrow$4) -linked D-mannuronic acid (M). The content ratio (G) and (M) is referred to as the G / M ratio. The G / M ratio will greatly affect the quality of alginate in which the higher is the G content, the higher is the alginate thickness, and vice versa.

Although all brown algae contain alginate, only few species of brown algae are used commercially and can be found in subtropical climate such as *Macrocystis pyrifera*, *Ascophyllumn odosum*, *Laminaria hyperborea*, *Laminaria digitata*, *Ecklonia maxima*, and *Lessonia nigrescans*. Whereas in Indonesia, the common species are *Sargassum* sp., *Turbinaria* sp., *Hormophysa* sp., and *Padina* sp. (Rasyid 2007). Alginophyte that grow in water consists of three genera, i.e *Sargassum* with seven species, *Turbinaria* with two species, and *Hormophisa*.

Increasing demands on alginate requires the enhancement of the quality of industry in which one of the aspect is encouraging the exploration of potential species as alginate sources. According to King (1983) and Saraswathi et al. (2003), the alginate content of seaweed varies greatly depending on the species of brown seaweed extracted. For example, a study showed that brown macroalgae stocks found at Ekas Bay in Lombok Island vary across seasons and species. The biomass sourced from *Padina* reached 97.85 ± 12.63 and 79.54 ± 2.53 tons in May to June and November, respectively while *Sargassaceae* species produced 669.70 ± 109.64 and 147.70 ± 77.97 tons in May to June and November, respectively. The highest alginate yields were produced during the period May to June in which *Padina* can produce 9.10

± 0.06 tons of dry alginate while *Sargassum* can produce 207.61 ± 0.42 tons of dry alginate. This study demontrates that wild Sargassaceae is a potential source of alginate which influenced by the ratio of manuronate and guluronate (Setyawidati et al. 2018). According to Winarno (1990), alginate formed in *Sargassum* cell walls reached 40% of the total dry weight and played an important role in maintaining the tissue structure of thallus. The thallus of *Sargassum* has a variety of shapes and sizes, ranging from in the form of rods and unite in a bundle to in the form of large thallus with outer shape like tall plants. The shape of the thallus can affect alginate content.

There is opportunity to increase alginate production in Indonesia but this will need information of *Sargassum* spp. and its contents to manage alginate resources sustainably. The genus of *Sargassum* consists of 400 species while in Indonesia there are 12 species namely *Sargassum duplicatum, S. hitrix, S. echinocarpum, S. gracillinum, S. obtuspfolium, S. binderi, S. polycystum, S. microphylum, S. crassifolium, S aquafolium, S. vulgare,* and *S. polyceratium.*

Sargassum polycystum is an alginate-producing seaweed. So far, *Sargassum polycystum* grow wild and have not been cultivated in Indonesia. This study was aimed to obtain the ecological, morphological, molecular and structure of alginate extracted from *Sargassum polycystum* in western Java so that the relationship between genes from *Sargassum* and its structure can be revealed. This aim will be achieved through isolation and partial characterization of alginate extracted from *Sargassum polycystum* collected from West Java waters to identify the structure of sodium alginate based on the chemical composition of manuronate and guluronate.

MATERIALS AND METHODS

Study period and location

The study was carried out in February 2018 until April 2019. There were three sampling locations, i.e Lima Island, Ujung Kulon and Binuangeun. The location of S. *polycystum* sampling is presented in Figure 1. The geographical conditions of western Java are surrounded by three major waters, i.e the Java Sea in the north, the Sunda Strait in the west, and the Indonesian Ocean in the south.

Figure 1. Three locations for sampling of *Sargassum polycystum* in: (1) Lima Island ($6^0$3'39 "S, $106^0$09'20" E); (2) Ujung Kulon ($6^0$48'15 "S, $105^0$29'5" E); and (3) Binuangeun ($6^0$49'16 "S, $105^0$56'14" E).

Sampling procedure

Collection and identification of samples of *Sargassum polycystum* was conducted during the lowest tide at each study location. Samples were collected using transect method along the coast. Each sample was photographed and then taken to the Jakarta Fisheries University for identification and further analysis. Seaweed was stored in a plastic bag, cleaned, sorted according to genus, weighed in fresh condition, wind dried, and then ready for alginate extraction and partial hydrolysis conducted in Chemistry Laboratory, Fish Processing Technology Department, Jakarta Fisheries University. Analysis of functional group using FTIR was undertaken in Chemistry Laboratory, Department of Chemistry, University of Indonesia.

Laboratory analysis

Isolation and partial characterization of alginate from *Sargassum polycystum* included chemical composition of *Sargassum polycystum*, alginate extraction, characterization of alginate (i.e rendement viscosity, water content, ash content, pH), partial hydrolysis of alginate (i.e isolation of mannuronic acid and guluronate acid, analysis of functional group analysis which is qualitatively proven on the FTIR curve).

Materials and equipments

Three samples of *Sargassum polycystum* from each location were prepared for extraction processes with materials included natrium carbonate, calcium chloride, chloride acid, alcohol 70%, peroxide hydrogen, aquades, Ca_2Cl_2 4%, HCl 2%, Na_2CO 34%, $Ca_2Cl_2$10%, Ca_2Cl_2 5%, HCl 5%, dan Alkohol 95%. Partial hydrolysis used HCl 37% and NaOH 5 mol and p.a grade chemicals for the analysis of alginate monomers. The equipment needed included equipment for alginate extraction, while the test equipment is the brookfield brand viscometer, the Shimadzu Prestige Fourier Transform Infrared Spectroscopy (FTIR) and the Shimadzu Spectrophotometer.

Data collection

Alginate Extraction

The extraction of alginate as follows: the raw material of *Sargassum polycystum* was cleaned, weighed at 200 grams then added with acetic acid solution according to the treatment. The mixed material was blended into seaweed pulp, heated at 80^{o}C and stirred for 10 minutes. After that it was filtered and squeezed into the form of coarse alginate liquid which was then dried at 65^{o}C for 24 hours. After being dried it was then pressed to get coarse alginate flour.

Na-Alginate Extraction Process

The main process of extracting Na-alginate was divided into four stages namely immersion (pre extraction), extraction, bleaching and purification. Immersion stage was carried out in an alkaline solution and anacid solution. Extraction was carried out in an alkaline atmosphere by cooking using extracting solutions (Na2CO3, NaOH). Bleaching used solution of NaOCl or H2O2. Purification was divided into three phases, i.e the formation of alginic acid, the formation of sodium alginate and the formation of pure sodium alginate.

Immersion

Soaking seaweed in CaCl2 solution was aimed to dissolve laminarin, mannitol, dyes and salts. This treatment also served to dissolve the remaining impurities in seaweed. According to Tanikawa (1985), alginic acid precipitated under the conditions of pH <3 in which in this condition the alginate component will be stable in the raw material during the immersion process. While immersion in alkaline solutions was aimed for deproteinization (Tseng and Sweeney 1946).

Extraction

The brown seaweed extraction process is carried out in alkaline conditions. The goal is to separate the cellulose content from alginate. The extracting material that can be used is Na2CO3 or NaOH. Chou and Chiang (1976) state that high concentrations of Na2CO3 (3-5%) can cause a decrease in product yield and viscosity. This is because the alkaline solution can damage the alginic acid compound by shortening the polymer chain into oligosaccharides which in turn degrades to 4-deoxy-5-ketouronic acid. Extraction carried out by heating will also affect the alginate produced. This heating process not only makes extraction processes easier but can also extract the weight of higher alginate molecules so that they can increase product yield and viscosity (Chou and Chiang 1976).

Deposition of Na-alginate

In the formation of sodium alginate, alginic acid that has been formed is added with alkaline solution containing Na + ions such as NaOH and Na2CO3. The purpose of the formation of sodium alginate is to get a more stable alginate compound. According to Mc Hugh (1987), the exchange of H + ions with Na + ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid.

Withdrawal of Sodium Alginate

Withdrawal of Na-alginate compounds from sodium alginate solution can be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonym (1976), 1% sodium alginate starts to show the separation process in a solution of 10% isopropanol or in ethanol 20% as well as its boiling point. The melting point of isopropanol (secondary alcohol) is lower than ethanol (primary alcohol). In an effort to withdraw sodium alginate the use of isopropanol is more efficient than ethanol. Formation of pure sodium alginate is done by attracting the water content contained in the solution. This pure Na-alginate is then dried in an oven and after that it can be ground into Na-alginate flour.

After the water content contained in the anatomic alginate solution is pulled out, pure sodium alginate is formed. Sodium alginate is then dried in an oven and ground to form sodium alginate flour. The characteristics of alginic acid and sodium alginate were tested compared to Table 1. Characteristics of alginic acid and sodium alginate and Table 2. Quality specifications of alginic acid and sodium alginate. According to Glicksman (1983), alginic acid is described as a hydrophilic colloidal carbohydrate extracted with alkali salt from various types of brown seaweed. Chemically, alginate is a pure polymer of uronic acid arranged in a long linear chain. The chemical formula of alginate is (C6H8O6) n with the number n between 80 to 83 (Schoeffel and Link 1993). There are two types of monomers that make up alginic acid, namely β-D-mannopyranosil uronate or D-mannuronic acid and α-L-gulopyranosil uronate or L-guluronic acid. Of the two types of monomers, alginic acid can be a homopolymer consists of similar monomers namely D-mannuronic acid only or L-guluronic acid only (Winarno 1996).

Homopolymers of D-mannuronic acid (polymannuronic acid) are formed by repeating D-mannuronic acid with β- (1,4) bonds and hydrogen bonds between hydroxyl groups on C3 atoms with oxygen atoms on adjacent hexose rings. The homopolymer form of L-Guluronic acid is more rigid than D-Mannuronic acid homopolymer (Atkins et al. 1971 in Anonym 1976). Alginate with a high proportion of L-guluronic acid homopolymers tends to form stiff, rigid and syneresis gels. On the other hand, the higher proportion of D-mannuronic acid homopolymers tends to form a gel that is more elastic, does not rigid and does not show high syneresis (Glicksman 1983).

Data analysis of molecular structure and quality of alginate.

Variables observed in alginate include yield test, moisture content test, ash content test, viscosity test, pH test (Bahar and Arif 2012), and structural tests with FTIR Alginate or alginate is a compound contained in brown seaweed cell walls (Phaeophyceae) other than cellulose and pectin. Alginate compounds are alginic acid polysaccharides in the form of Sodium, Calcium, Potassium and Magnesium salts (Satari 1996).

Rendement

The Na-alginate yield obtained from the extraction process of seaweed *Sargassum polycystum* was calculated based on the weight of Na-alginate after drying on the dry weight of the raw material. Calculate the yield of alginate Na using the following formula:

Addendum (%) = (weight of Na - final alginate (g) / weight of initial seaweed (g)) x 100%

Viscosity

Viscosity analysis refers to JECFA (2007), observations were made at a 1-5% solid concentration to determine the relationship between concentration and solution viscosity. Na-alginate (sample) was weighed as much as 7.5 g in weigh paper. 492.5 g of distilled water are weighed in a 500 mL glass beaker so that the sample and distilled water when added reach total weight 500 g. Alginate is included in a 500 mL glass beaker containing distilled water and stirrer gradually. Aquades are heated and stirred once to reach 75ºC, after a constant temperature the solution is heated for 25 minutes. Stirring I was carried out at minute 1 for 1 minute, stirring II at 25 minutes. Beaker glass is covered with aluminum foil to prevent water loss in the heating process due to evaporation, then the solution temperature is lowered to reach 75ºC. The measurement of solution viscosity was measured using RVA (Rapid Viscos Analyzer) spindle 2 at 30 rpm, waiting until the spindle needle was stable (up to 6 times rotation). Viscosity is expressed in centipoise (cP).

Water content

A sample of 2 g was weighed and then put into a porcelain cup. The sample is heated by oven at temperature 105ºC for 24 hours. Then keep the sample in the desiccator for 5 minutes. Finally, the sample weighed till the value stable. Calculate the water content using the following formula:

Water content (%) = (weight of final sample (g) / weight of initial sample (g)) x 100%

Ash content

The final sample from water content continued heated by ignition furnace at temperature 600ºC for 24 hours. Then keep the sample in the desiccator for 5 minutes. Finally, the sample weighed till the value stable. Calculate the ash content using the following formula:

Ash content (%) = (weight of final ash sample (g) / weight of initial sample (g)) x 100%

PH value

A sample of 3 g was weighed and then put into a 300 mL glass beaker then added 197 g of distilled water until the total weight was 200 g. The sample is heated while stirring using a stirrer until it dissolves at a temperature of 60-80ºC. Then the electrode is dipped into the sample solution which was previously calibrated. The pH value is obtained according to what is shown on the screen. Then the electrode is rinsed with distilled water.

Alginate partial hydrolysis test

The composition of the polyguluronic, polimanuronate and mixed segments between manuronate and guluronate in alginate determined the quality of alginate. To isolate manuronic acid (M) and guluronate (G) on alginate molecules carried out by Partial Hydrolysis of Alginate by: 5.00 g Alginate in HCl 0.3 N at 100 ° C for 2 hours was obtained: The soluble fraction was identified as a block MG. Bonding The hydroxyl between M and G is easily hydrolyzed by insoluble-fraction acid more resistant to acid hydrolysis, again dissolved by adding alkali and fractionation by adjusting the pH at 2.85, so that the GG block settles and the MM block dissolves.

Analysis of alginate functional groups was carried out using a Fourier Transform Infrared (FTIR) spectrophotometer (Perkin Elmer, spectrum one) based on the method of Barth (2000). Samples plus KBr (1: 100) then mashed until evenly mixed. Then presses with a vacuum pump for 15 minutes, and read the absorbance at wave lengths 400-4000 cm-1. From the resulting curve, the type of bond and its functional group are determined based on FTIR references.

FTIR Analysis

As much as 2 mg of alginate sample is put into a small bottle and 200 ml KBr is added, then stirred until homogeneous. The mixture is then placed on the die, pressed for several minutes until it is pelleted. The pellets are then put into the sample and their absorption is measured at 4000-400nm wavelength; alginate is at peak / peak wavelength 1030/1080 nm.

RESULTS AND DISCUSSION

Characteristics of seaweed

Raw materials of seaweed obtained from western Java in Indonesia consist of Lima island, Ujung Kulon and Binuangeun. Images of *Sargassum polycystum* seaweed are presented in Figures 5, 6, 7.

Based on the analysis of the quality of *Sargassum polycystum* seaweed from Lima island, Ujung Kulon and Binuangeun which includes yields, water content, CAW and impurities obtained as shown in Table 3.

Seaweed yields obtained from the results of wet seaweed drying, yield differences from the three locations, the highest yield of *Sargassum polycystum* from Binuangeun 25.77%.

Sargassum water content has been met which is a maximum of 15%. According to Winarno (1996), the value of the water content of seaweed is influenced by the drying process. The highest water content of Ujung Kulon 14.50% still meets the requirements of the SNI standard.

CAW provides information on the cleanliness of seaweed from dirt, sand and rock attached. Based on Table 2, it can be seen that the CAW values of Lima island, Ujung kulon and Binuangeun were obtained, which were 76.64%, 67.69% and 75.59% respectively. This value gives the meaning that *Sargassum polycystum* which is used clean and free of dirt. These results are in accordance with the quality requirements of dried seaweed based on SNI No. 2690.1.2015 which is a minimum of 50%.

Figure 5. *Sargassum polycystum* seaweed from Lima Island

Figure 6. *Sargassum polycystum* seaweed from Ujung Kulon

Figure 7. *Sargassum polycystum* seaweed from Binuangeun

Table 3. Chemical composition of *Sargassum polycystum* Seaweed from 3 West Java Locations in Indonesia

Location	Lima Island			Ujung Kulon			Binuangeun			Standard
Chemical compotition	1	2	mean	1	2	Mean	1	2	mean	
Rendemen (%)	12,96	11,22	12,09	17,78	14,70	16,24	29,54	22,00	25,77	-
Water content (%)	09,00	09,50	09,25	12,75	11,00	11,88	13,00	12,50	12,75	< 15 %
CAW (%)	76,64	71,49	74,06	67,69	65,42	66,55	75,59	78,24	76,92	>50 %
Impurities (%)	13,80	09,32	11,56	33,88	3,50	3,69	25,27	26,15	25,71	20-30%

Table 4. The mean value of physical quality analysis of Na alginate *Sargassum polycystum* from three locations

Karakteristik	Lokasi			
	Lima island	Ujung Kulon	Binuangeun	Standar
Rendemen (%)	11,48 ± 0,79	18,62 ± 0,84	05,75 ± 0,11	> 18,00*
Viskositas (cP)	35,00 ± 7,07	62,50± 3,53	81,33 ± 1,88	> 27,00**

Note : SNI* and ** Food Chemical Codex (2004)

The highest impurity level of the three locations is from Binuangeun (25.71%) impurity consisting of sand, rock, coral and litter consisting of plastic impurities, waste leaves left by humans in Binuangeun because it is close to residential dwellings. Impurity Rate of *Sargassum polycystum* from Lima island (11.56%) consisting of sand and coral and the level of impurity of *Sargassum polycystum* the lowest is from the location of Ujung Kulon (3.69%) containing sand and coral because of the location of *Sargassum polycystum* far from the residential areas of Taman Jaya village, Sumur District, Pandeglang Regency, West Java Province, the results are the cleanest.

Alginate extraction

The results of the study based on the physical characteristics of the quality of Na-alginate *Sargassum polycystum* from three locations are presented in Table 4.

Rendemen

Sargassum polycystum from Ujung Kulon it has the highest Na alginate content with the highest yield value (18.62 + 0.84)% followed by the location of Lima island with an average yield (11.48 + 0.79)% this is influenced by the cleanliness of the location the impurity consists only of sand and coral, for the Binuangeun location the lowest Na alginate yield (05.75 + 0.11)% this is influenced by the amount of sand, rock, coral and litter impurities because it is close to the population occupancy. The results of the alginate yield test in the extraction of *Sargassum polycystum* presented in Figure 8.

Alginate yield produced from seaweed is influenced by habitat (light intensity, wave size or current and aquatic nutrition), age of brown seaweed and brown seaweed handling techniques after harvest, before and the extraction process used (Basmal et al. 2012) because other factors are carried out with the same treatment in three locations, so the size of the waves or currents affect the size of the yield of the three different locations. Binuangeun has a shallow depth of 40.00 cm with the shortest total thallus length of (31.82) cm where Sargassum is overgrown at the lowest ebb in the form of inundation affected by current velocity (0.24, 0.14 and 0.03) and based on the Meteorological Agency , Climatology and Geophysics (BMKG - maritime.bmkg.go.id) waves in the area of Lima island in the western part of the Java Sea, the waters of the northern coast of Java are included in the Slight Sea / Small group with a wave size of 0.5 - 1.25 m, while Ujung Kulon and Binuangeun belong to the Moderate Sea / Moderate group, with a wave size of 1.25 - 2.50 m, this causes the thallus length in Binuangeun to be shorter than the total thallus length of *Sargassum polycystum* in Ujung Kulon and Lima island.

Viscosity

The highest mean viscosity was *Sargassum polycystum* originating from Binuangeun (81.33 + 1.88) cP, Ujung Kulon (62.50 + 3.53) cP, and Lima island (35.00 + 7.07) cP. According to Subaryono (2009) states that the high content of water insoluble material and low alginate viscosity is caused by the low purity of the alginate produced. Na-alginate thickness is divided into three levels, namely low viscosity (<60 cP), medium thickness (60-110 cP) and high thickness (110-800 cP). Based on this division, the viscosity of Na alginate on the island of Lima is included in low viscosity (Syafarini 2009; Subaryono and Apriani 2010). Na alginate viscosity varies greatly from 10-5000 cP (1% Na alginate solution in water), depending on the final destination of the product (Basmal et al. 2013). Sodium alginate for food usually has a lower viscosity than sodium alginate for textiles. Seaweed from the tropics (warm water) generally produces alginates with low viscosity (Mc Hugh 2008). Seaweed with a long thallus length will produce Na alginate with low viscosity, whereas if used with seaweed with a short thallus (20-40) cm it will produce high viscosity. Raw material for *Sargassum polycystum* obtained from Binuangeun waters has a small thallus length (less than 20 - 40 cm) (Sinurat and Marliani 2017). The results of the alginate viscosity in the extraction of *Sargassum polycystum* are presented in Figure 9.

Alginate viscosity is influenced by several factors, including temperature, solution level and degree of polymerization. Na alginate viscosity value is highly dependent on the age of brown seaweed harvesting, extraction techniques (concentration, temperature, pH and the presence of polyvalent metal cations) and the weight of seaweed molecules extracted (Basmal el al 2013; Mc Hugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80oC, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the thickness using RVA (rapid viscoanalyzer). Anggadiredja et al. (2011) stated that the higher the drying temperature the higher the viscosity value. It is assumed that the increase in drying temperature will increase the formation of the amount of sulfate esters so that viscosity increases.

Chemical Characteristics of Na-alginate Sargassum polycystum

Chemical analysis of Na-alginate *Sargassum polycystum* includes the value of water content, ash content, and pH value presented in Table 5.

Sargassum polycystum seaweed from Binuangeun has a Na alginate content with higher water content and ash content while the mean pH (6.05+ 0.57) is lower than *Sargassum polycystum* from Lima island and Ujung Kulon.

Water content

Drying is a process of reducing a part of the water content of the material. Water content of the material is the amount of water contained in the material expressed in percent (%). The water content in Na alginate from the three locations in the study was in accordance with international quality standards provided that the drying losses were <15% (FCC 2004). The mean water content of Na alginate seaweed *Sargassum polycystum* from three locations is presented in Table 5. The results of the observations showed that the mean value of Na alginate water content from Binuangeun was the highest. The higher the purity of alginate results in the difficulty of the water coming out of the matrix during the drying process. Alginate is a polymer with the ability to hold water very well so that the higher the purity of the alginate, the better the ability to hold water (Suwarda 2016).

Water content can affect the shelf life of a product. Products that have low water content usually have a longer shelf life than products that have high water content (Siswati dan Diyanah 2002). Water is an important component in food ingredients because water can affect the appearance, texture and taste of food. The water content in food ingredients also determines acceptability. Diversity and durability of food ingredients (Winarno 2008). The water content allowed in Na alginate is between 5-20%, while the water content allowed by the FCC is <15%. When compared to some research standards, water content of Na-alginate meets the standard (FCC 2004). JECFA also stated that the water content of food additives alginate maximum 15% (FAO 2009).

Ash Content

Ash content is important to know because it can determine the purity level of the product from unwanted components. Based on the analysis of the resulting ash content it can be seen that commercial alginate generally has a maximum ash content of 27% when compared to the three locations the ash content used still meets JECFA requirements standards (Mc Hugh 1987).

pH

Na alginate with a mean pH of 6 from Binuangeun was lower than the pH of Ujung Kulon and Lima island.

The results of the overall chemical and physical analysis that has been carried out by Na alginate produced from Binuangeun more suitable for non-food products, this is related to the quality produced. Binuangeun's results have brighter can be formulated according to the needs and desired physicochemical properties, especially those related to gel formation, thickness, binding of water so that it can retain moisture, while Lima Island is more suitable for food products. Alginate for food must have low water content, low ash content and neutral pH (Yunizal 2004). Alginate characteristics of Lima island, Ujung Kulon and Binuangeun meet alginate standards as food grade, but from its viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry.

Partial Hydrolysis of Alginate

The results of isolation of manuronic acid (M) and guluronate (G) on alginate molecules were carried out by partial hydrolysis of alginate, obtained by GG block deposits as presented in Table 6.

Figure 8. Results of alginate yield test in extraction of *Sargassum polycystum* (%)

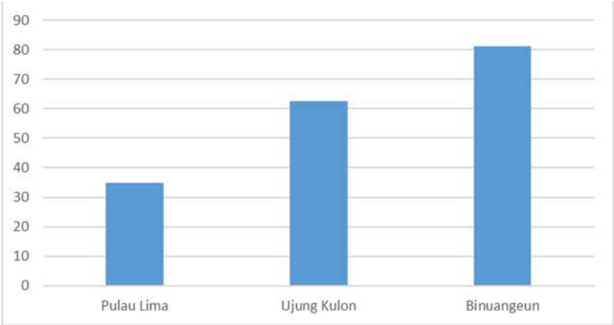


Figure 9. Test results for alginate viscosity in extraction of *Sargassum polycystum* (cP)

Table 5. Mean value of chemical quality analysis of Na alginate *Sargassum polycystum* from three locations

Characteristics	Locations			
	Lima Island	**Ujung Kulon**	**Binuangeun**	**Standard**
Water content (%)	12,42 ± 0,30	10,23 ± 1,68	13,31 ± 0,77	5-20**
Ash content (%)	26,68 ± 2,48	24,94 ± 4,41	26,69 ± 0,82	18,00-27,00*
pH	7,39 ± 0,03	7,03 ± 2,60	6,05 ± 0,57	3,5 - 10***

Description: (* Food Chemical Codex (2004), **) Winarno (1996)

Table 6. Results of Alginate Partial Hydrolysis

Locations of *Sargassum polycystum*	Blok GG	Blok MM	Blok MG	Blok M	Blok G
Lima island	67,60	59,00	41,40	27,00	35,00
Ujung Kulon	50,00	5,40	6,00	9,60	30
Binuangeun	55	70	63	37	45

Table 7. Functional groups on the FTIR curve

Wavelength	% Transmittance (% T)			Functional groups
cm-1	Lima island	Ujung Kulon	Binuangeun	
3427.51-3448.72	63.09	53.88	52.89	O-H stretching hydrogen bonds between molecules
1608.63	50.98	41.15	50.29	C=O
1411.89	38.72	33.42	38.49	Bond -C-OH
1091-1093.64	53.62	41.02	48.50	COOH, C-O stretching C-O-C stretching
1170	64.41	48.21	56.67	C-0 stretching
				C-C stretching
				C-C-C bending

The highest component of G in alginate is obtained from Lima island with the results of the viscosity test as listed in Figure 8 and according to the functional group analysis which is qualitatively proven on the FTIR curve as shown in Figure 10.

Analysis of functional groups of sodium alginate from extraction of *Sargassum polycystum* from three locations

Uptake of functional groups from *Sargassum polycystum* from these three locations is seen in the FTIR curve of Figure 10 and Table 7.

The sodium alginate spectrum showed the presence of hydroxyl (-OH) groups, carbonyl groups (-COO-), –C-OH and –COOH bonds, C-O stretching, C-C stretching, and C-O bending as seen in FTIR Curves (figure 10). According to Alvares et al. 2018, absorption at wave lengths 1608.63 cm-1, 1411.89cm-1 and 1091 cm-1-1093.64 cm-1 (in the wave length area 1091 cm-1 -1093,64 cm-1 (in the area wave length 1608 cm-1 to wave length area 1091 cm-1), if higher than absorption at wave length 1029 cm-1-1033 cm-1, 947 cm-1 and 817,82cm-1 - 875,68 (at the area of wave length 1029 cm-1 to 817 cm-1) indicates that the alginate polymer consists of a higher proportion of guluronic monomers, whereas absorption at wave lengths 1315 cm-1, 1170 cm-1, 1029 cm-1-1033 cm-1, 947 cm-1 and 817.82-875.68cm-1 were higher indicating that the alginate polymer consisted of the proportion of manuronic monomers. In the results of the FTIR curve showed that the absorption at wave length 1608.63cm-1,1411.89cm-1, and 1091cm-1 -1093.64 cm-1 in Binuangeun alginate products provide higher absorption compared to Lima island and Ujung Kulon alginates, but d The results of the GG block partial deposit test (Table 8.) showed the lowest guluronate group (41.40%). This is possible at wave length 1029.99 cm-1-1033.85 cm-1 giving quite high uptake of C-O stretching and C-O-C stretching uptake. Whereas the Lima island and Ujung Kulon alginate products showed a higher proportion of guluronate monomers than manuronates according to the results of the quantitative test of GG block partial deposits namely Lima island, Ujung kulon and Binuangeun respectively with 67.60%, 59.00% and 41.40%.

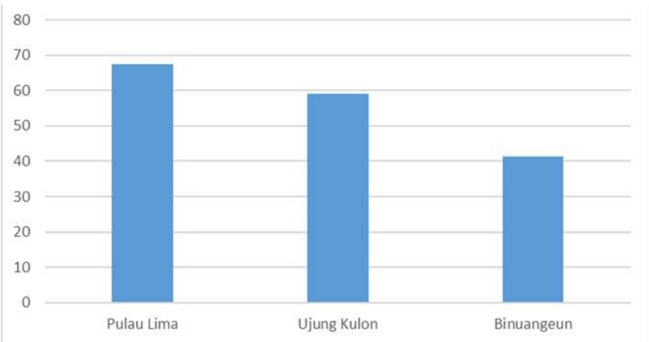


Figure 11. Results of GG block deposition test on alginate structure (%)

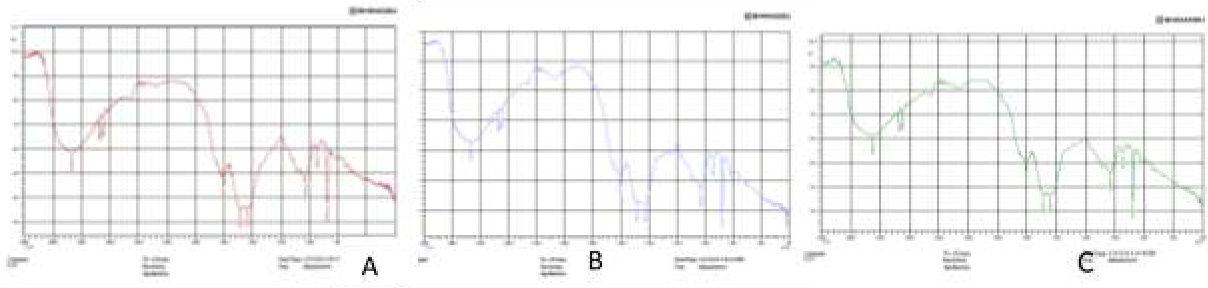


Figure 10. FTIR curve Alginate Island A. Lima, B. Ujung Kulon, C. Binuangeun

The combination of viscosity and gel strength of the two characteristics of Guluronate and Manuronate is the determinant factors to produced hydrogel that other characteristics, while the other characters are supporting information. The result of this study showed that the yields of alginate from *Sargassum polycystum* from Lima Island, Ujung Kulon and Binuangeun, were 11.48%, 18.62% and 5.75% respectively. Alginate from Binuangeun has the best physico-chemical characteristics compared to the others. With a high viscosity value, the viscosity is 35 cP, 62.50 cP and 81.33cP respectively. The characteristics of Na alginate produced from Binuangeun have relatively higher quality. Alginate characteristics of Lima Island, Ujung Kulon and Binuangeun has met alginate standards as food grade, but when viewed from viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry. The results of the partial alginate hydrolysis test showed that guluronic block (GG) in alginate polymers on the Lima Island, Ujung Kulon and Binuangeun were 67.60%, 59.00% and 41.40%, respectively. This is related to the gel properties formed. Alginate from Lima island tends to be stiffer and less flexible than alginate from Ujung Kulon and Binuangeun. There were variations in the concentration of Manuronate and Guluronate from the three ecology of Sargassum in West Java.

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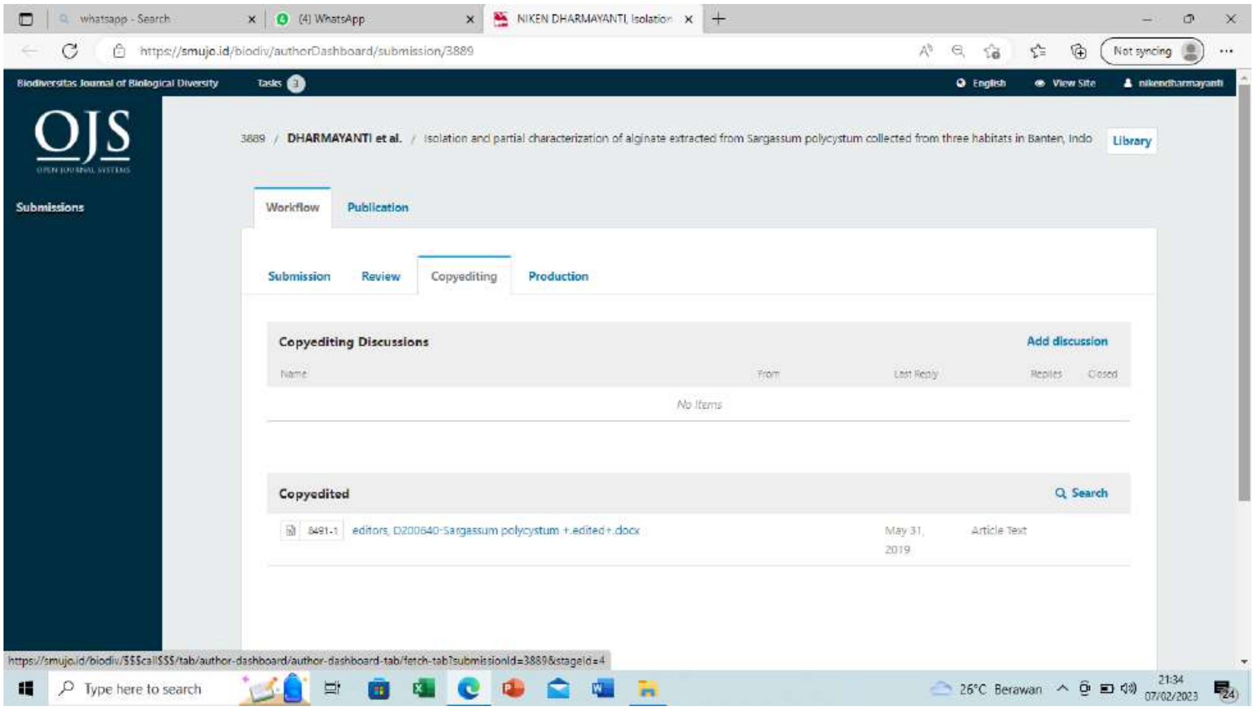

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Isolation and partial characterization of alginate extracted from *Sargassum polycystum* collected from three habitats in Banten, Indonesia

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Abstract. *Dharmayanti N, Supriatna J, Abinawanto, Yasman. 2019. Isolation and partial characterization of Alginate from* Sargassum polycystum *of Banten, Indonesia. Biodiversitas 20: 1776-1785.* The utilization of *Sargassum polycystum* as an alternative alginate source may reduce the dependence on alginate import as currently alginate demands in Indonesia are 100% supplied from overseas. The purpose of this study was to characterize alginate extracted from *Sargassum polycystum* obtained from three locations with different ecological characteristics. Isolation of alginate was conducted through partial hydrolysis to separate guluronic acid and mannuronic acid followed by freeze-drying and then the parameters were measured qualitatively and quantitatively using FTIR. The results showed that alginate extracted from *Sargassum polycystum* collected from Lima Island, Ujung Kulon and Binuangeun were 11.48**%,** 18.62%, and 5.75%, respectively with the viscosity were 35 cP, 62.50 cP, and 81.33 cP, respectively. The test result of partial hydrolysis of alginate showed that guluronic block (GG) in the alginate polymer of Lima Island, Ujung Kulon and Binuangeun were 67.60%, 59.00%, and 41.40%, respectively. These relate to the nature of the gel formed. The alginate from Lima Island tends to be more rigid and less flexible than that from Ujung Kulon and Binuangeun. The findings of this study suggest that there are differences in the concentration of the components of mannuronate and guluronate of *Sargassum polycystum* across different locations in Banten, Indonesia.

Keywords: Alginate, characteristics, partial hydrolysis, *Sargassum polycystum*

INTRODUCTION

Brown algae are known as alginate sources. Alginate, an anionic heteropolysaccharide extracted from natural brown algae, has useful properties for the food, chemical, medical, and agricultural industries (Inoe 2018). Alginate is needed in various industries for various purposes including as gelling agent, stabilizer, emulsifier, suspending agent and dispersing agent. In food industry, alginate compounds are added as ingredient in butter, ice cream, and milk. In cosmetics industry, it functions as water binder so that the cosmetic components are perfectly bound and easily penetrate skin tissue.

Alginophyte is a family of brown seaweeds. The difference in the main chemical compounds contained in seaweed shows the characteristics of the seaweed, suggesting different extraction process among seaweeds. Similarly, the benefits and uses of these chemical compounds also vary one another. These differences are caused by different species of seaweed, different location and growing season (Pereira 2018).

Alginate consists of group of polysaccharides and can be found in brown algae tissue. Alginate is a long chain polymer consisting of α- (1$\rightarrow$4)-linked L-guluronic acid (G) and β- (1$\rightarrow$4)-linked D-mannuronic acid (M). The content ratio (G) and (M) is referred to as the G / M ratio. The G / M ratio will greatly affect the quality of alginate in which the higher is the G content, the higher is the alginate thickness, and vice versa.

Although all brown algae contain alginate, only few species of brown algae are used commercially and can be found in subtropical climates such as *Macrocystis pyrifera*, *Ascophyllum odosum*, *Laminaria hyperborea*, *Laminaria digitata*, *Ecklonia maxima*, and *Lessonia nigrescans*. Whereas in Indonesia, the common species are *Sargassum* sp., *Turbinaria* sp., *Hormophysa* sp., and *Padina* sp. (Rasyid 2007). Alginophyte that grow in water consists of three genera, i.e., *Sargassum* with seven species, *Turbinaria* with two species, and *Hormophisa*.

Increasing demands on alginate require the enhancement of the quality of industry in which one of the aspects is encouraging the exploration of potential species as alginate sources. The alginate content of seaweed varies greatly depending on the species of brown seaweed extracted. For example, a study showed that brown macroalgae stocks found at Ekas Bay in Lombok Island vary across seasons and species. The biomass sourced from *Padina* reached 97.85 ± 12.63 and 79.54 ± 2.53 tons in May to June and November, respectively while *Sargassaceae* species produced 669.70 ± 109.64 and 147.70 ± 77.97 tons in May to June and November, respectively. The highest alginate yields were produced during the period May to June in which *Padina* can produce 9.10 ± 0.06 tons of dry alginate while *Sargassum* can produce 207.61 ± 0.42 tons of dry alginate. This study demonstrates that wild Sargassaceae is a potential source of alginate which is influenced by the ratio of mannuronate and guluronate (Setyawidati et al. 2018). Alginate formed in *Sargassum* cell walls reached 40% of the total dry weight and played an important role in maintaining the tissue structure of thallus. The thallus of *Sargassum* has a variety of shapes and sizes, ranging from in the form of rods and unite in a bundle to in the form of large thallus with outer shape like tall plants. The shape of the thallus can affect alginate content (Widyartini et al. 2017).

There is opportunity to increase alginate production in Indonesia to manage alginate resources sustainably but this will need information of *Sargassum* spp. and its contents. The genus of *Sargassum* consists of 400 species while in Indonesia there are 12 species namely *Sargassum duplicatum*, *S. hitrix*, *S. echinocarpum*, *S. gracillinum*, *S. obtuspfolium*, *S. binderi*, *S. polycystum*, *S. microphylum*, *S. crassifolium*, *S aquafolium*, *S. vulgare*, and *S. polyceratium*.

Sargassum polycystum is an alginate-producing seaweed. So far, *Sargassum polycystum* grow wild and have not been cultivated in Indonesia. This study was aimed to obtain the ecological, morphological, molecular and structure of alginate extracted from *Sargassum polycystum* in western Java so that the relationship between genes from *Sargassum* and its structure can be revealed. This aim will be achieved through isolation and partial characterization of alginate extracted from *Sargassum polycystum* collected from Banten waters to identify the structure of sodium alginate based on the chemical composition of mannuronate and guluronate.

MATERIALS AND METHODS

Study period and location

The study was carried out in February 2018 until April 2019 in Banten Province (western Java), Indonesia. There were three sampling locations, i.e., Lima Island (6° 00’05” S, 106°09'18" E), Ujung Kulon (6°48'15" S, 105°29'5" E), and Binuangeun (6°49'16" S, 105°56'14" E). The location of S. *polycystum* sampling is presented in Figure 1. The geographical conditions of western Java are surrounded by three major waters, i.e., the Java Sea in the north, the Sunda Strait in the west, and the Indian Ocean in the south.

Sampling procedure

Collection and identification of samples of *Sargassum polycystum* were conducted during the lowest tide at each study location. Samples were collected using transect method along the coast. Each sample was photographed and then taken to the Jakarta Fisheries University for identification and further analysis. Seaweed was stored in a plastic bag, cleaned, sorted according to genus, weighed in fresh condition, wind-dried, and then ready for alginate extraction and partial hydrolysis conducted in Chemistry Laboratory, Department of Fish Processing Technology, Jakarta Fisheries University, Jakarta, Indonesia. Analysis of functional group using FTIR (Fourier Transform Infrared Spectroscopy) was undertaken in Chemistry Laboratory, Department of Chemistry, University of Indonesia, Depok, Indonesia.

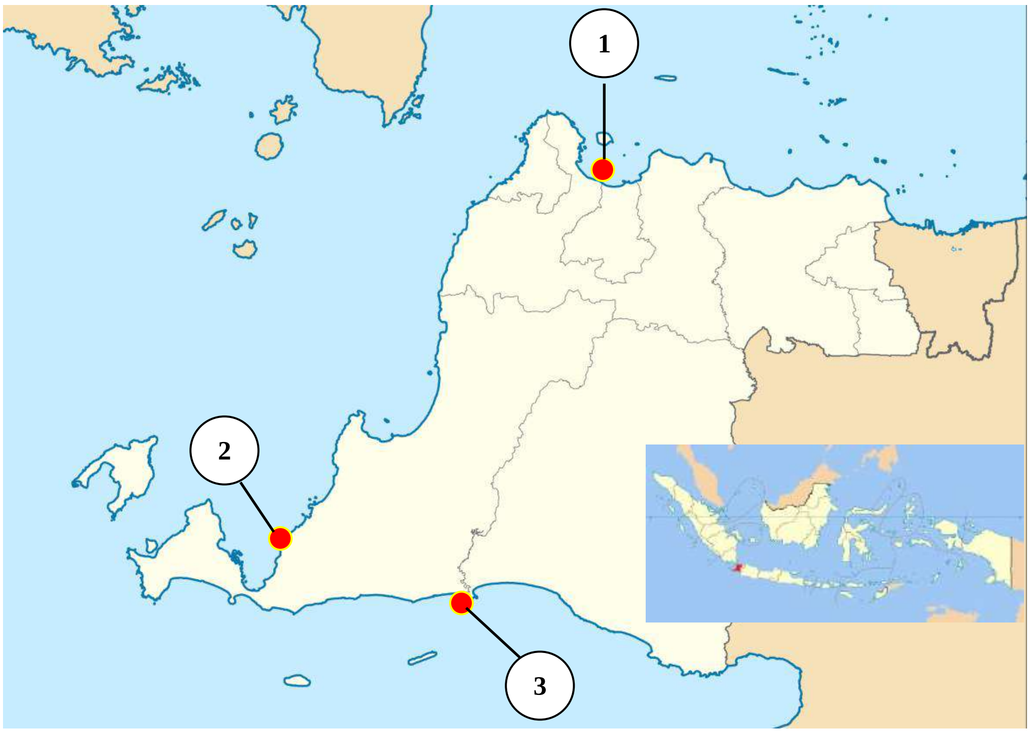


Figure 1. Three locations for sampling of *Sargassum polycystum* in Banten Province, Indonesia: 1. Lima Island; 2. Ujung Kulon; and 3. Binuangeun

Laboratory analysis

Isolation and partial characterization of alginate from *Sargassum polycystum* included chemical composition of *Sargassum polycystum*, alginate extraction, characterization of alginate (i.e., rendement viscosity, water content, ash content, pH), partial hydrolysis of alginate (i.e., isolation of mannuronic acid and guluronate acid, analysis of functional group analysis which is qualitatively proven on the FTIR curve).

Materials and equipment

Three samples of *Sargassum polycystum* from each location were prepared for extraction processes with materials included natrium carbonate, calcium chloride, chloride acid, alcohol 70%, peroxide hydrogen, aquadest, Ca_2Cl_2 4%, HCl 2%, Na_2CO 34%, $Ca_2Cl_2$10%, Ca_2Cl_2 5%, HCl 5%, dan Alkohol 95%. Partial hydrolysis used HCl 37% and NaOH 5 mol and p.a grade chemicals for the analysis of alginate monomers. The equipment needed included equipment for alginate extraction, while the test equipment is the Brookfield brand viscometer, the Shimadzu Prestige Fourier Transform Infrared Spectroscopy (FTIR) and the Shimadzu Spectrophotometer.

Data collection

Alginate extraction

The extraction of alginate as follows: the raw material of *Sargassum polycystum* was cleaned, weighed at 200 grams then added with acetic acid solution according to the treatment. The mixed material was blended into seaweed pulp, heated at 80ºC and stirred for 10 minutes. After that, it was filtered and squeezed into the form of coarse alginate liquid which was then dried at 65ºC for 24 hours. After being dried it was then pressed to get coarse alginate flour.

Na-alginate extraction process

The main process of extracting Na-alginate was divided into four stages namely immersion (pre-extraction), extraction, bleaching, and purification. Immersion stage was carried out in an alkaline solution and an acid solution. Extraction was carried out in an alkaline atmosphere by cooking using extracting solutions (Na_2CO_3, NaOH). Bleaching used solution of NaOCl or H_2O_2. Purification was divided into three phases, i.e., the formation of alginic acid, the formation of sodium alginate and the formation of pure sodium alginate.

Immersion

Soaking seaweed in $CaCl_2$ solution was aimed to dissolve laminarin, mannitol, dyes, and salts. This treatment also served to dissolve the remaining impurities in seaweed. According to Silva et al. (2015), alginic acid precipitated under the conditions of pH <3 in which in this condition the alginate component will be stable in the raw material during the immersion process. While immersion in alkaline solutions was aimed for deproteinization (Kamaruddin et al. 2015).

Extraction

The brown seaweed extraction process was carried out in alkaline conditions. The goal was to separate the cellulose content from alginate. The extracting material that can be used is Na_2CO_3 or NaOH. Lee and Mooney (2012) state that high concentrations of Na_2CO_3 (3-5%) can cause a decrease in product yield and viscosity. This is because the alkaline solution can damage the alginic acid compound by shortening the polymer chain into oligosaccharides which in turn degrades to 4-deoxy-5-ketouronic acid. Extraction carried out by heating will also affect the alginate produced. This heating process not only makes extraction processes easier but can also extract the weight of higher alginate molecules so that they can increase product yield and viscosity.

Deposition of Na-alginate

In the formation of sodium alginate, alginic acid that had been formed was added with alkaline solution containing Na+ions such as NaOH and Na_2CO_3. The purpose of the formation of sodium alginate is to get a more stable alginate compound. According to Mc Hugh (1987), the exchange of H+ions with Na+ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid.

Withdrawal of sodium alginate

Withdrawal of Na-alginate compounds from sodium alginate solution can be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonym (1976), 1% sodium alginate starts to show the separation process in a solution of 10% isopropanol or in ethanol 20% as well as its boiling point. The melting point of isopropanol (secondary alcohol) is lower than ethanol (primary alcohol). To withdraw sodium alginate, the use of isopropanol is more efficient than ethanol. Formation of pure sodium alginate was done by attracting the water content contained in the solution. This pure Na-alginate was then dried in an oven and after that, it can be ground into Na-alginate flour.

After the water content contained in the anatomic alginate solution was pulled out, pure sodium alginate was formed. Sodium alginate was then dried in an oven and ground to form sodium alginate flour. The characteristics of alginic acid and sodium alginate were tested compared to Table 1. Characteristics of alginic acid and sodium alginate and Table 2. Quality specifications of alginic acid and sodium alginate. According to Glicksman (1983), alginic acid is described as a hydrophilic colloidal carbohydrate extracted with alkali salt from various types of brown seaweed. Chemically, alginate is a pure polymer of uronic acid arranged in a long linear chain. The chemical formula of alginate is $(C_6H_8O_6)$ n with the number n between 80 to 83 (Schoeffel and Link 1993). There are two types of monomers that make up alginic acid, namely β-D-mannopyranosyl uronate or D-mannuronic acid and α-L-glucopyranosyl uronate or L-guluronic acid. Of the two types of monomers, alginic acid can be a homopolymer consists of similar monomers namely D-mannuronic acid only or L-guluronic acid only. Homopolymers of D-mannuronic acid (poly mannuronic acid) are formed by repeating D-mannuronic acid with β-(1,4) bonds and hydrogen bonds between hydroxyl groups on C3 atoms with oxygen atoms on adjacent hexose rings. The homopolymer form of L-guluronic acid is more rigid than D-mannuronic acid homopolymer (Rajendran et al. 2016) Alginate with a high proportion of L-guluronic acid homopolymers tends to form stiff, rigid and syneresis gels. On the other hand, the higher proportion of D-mannuronic acid homopolymers tends to form a gel that is more elastic, does not rigid and does not show high syneresis (Glicksman 1983).

Data analysis of molecular structure and quality of alginate

Variables observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test and structural tests with FTIR. Alginate is a compound contained in brown seaweed cell walls (Phaeophyceae) other than cellulose and pectin.

Rendement

The Na-alginate yield obtained from the extraction process of seaweed *Sargassum polycystum* was calculated based on the weight of Na-alginate after drying on the dry weight of the raw material. The yield of Na-alginate was calculated using the following formula:

Addendum (%) = (weight of Na-final alginate (g) / weight of initial seaweed (g)) x 100%

Viscosity

Viscosity analysis referred to JECFA (2007). Observations were made at a 1-5% solid concentration to determine the relationship between concentration and solution viscosity. Na-alginate (sample) was weighed as much as 7.5 g in weigh paper. As much as 492.5 g of distilled water was weighed in a 500 mL glass beaker so that the sample and distilled water had total weight of 500 g. Alginate was included in a 500 mL glass beaker containing distilled water and stirred gradually. Aquades was heated and stirred once to reach 75ºC, after a constant temperature the solution was heated for 25 minutes. Stirring I was carried out at minute 1 for 1 minute, stirring II at 25 minutes. Beaker glass was covered with aluminum foil to prevent water loss in the heating process due to evaporation, then the solution temperature was lowered to reach 75ºC. The measurement of solution viscosity was measured using RVA (Rapid Visco Analyzer) spindle 2 at 30 rpm, waited until the spindle needle was stable (up to 6 times rotation). Viscosity is expressed in centipoise (cP).

Water content

A sample of 2 g was weighed and then put into a porcelain cup. The sample was heated by oven at temperature 105ºC for 24 hours and keep the sample in the desiccator for 5 minutes. Finally, the sample was weighed until the value was stable. Water content was calculated using the following formula:

Water content (%) = (weight of final sample (g) / weight of initial sample (g)) x 100%

Ash content

The final sample from water content continued to be heated using ignition furnace at temperature 600ºC for 24 hours. Then the sample was kept in the desiccator for 5 minutes. Finally, the sample was weighed until the value was stable. Ash content was calculated using the following formula:

Ash content (%) = (weight of final ash sample (g) / weight of initial sample (g)) x 100%

pH value

A sample of 3 g was weighed and then put into a 300 mL glass beaker then added 197 g of distilled water until the total weight was 200 g. The sample was heated while being stirred using a stirrer until it dissolved at a temperature of 60-80ºC. Then the electrode was dipped into the sample solution which was previously calibrated. The pH value was obtained according to what shown on the screen. Then the electrode was rinsed with distilled water.

Alginate partial hydrolysis test

The composition of poly guluronic, poly mannuronate and mixed segments between mannuronate and guluronate in alginate determine the quality of alginate (Gomez 2018). To isolate mannuronic acid (M) and guluronate (G) on alginate molecules, we carried out Partial Hydrolysis of Alginate by 5.00 g alginate in HCl 0.3 N at 100° C for 2 hours. The soluble fraction was identified as a block MG. Bonding the hydroxyl between M and G was easily hydrolyzed by insoluble-fraction acid more resistant to acid hydrolysis, again dissolved by adding alkali and fractionation by adjusting the pH at 2.85, so that the GG block settled and the MM block dissolved.

Analysis of alginate functional groups was carried out using a Fourier Transform Infrared (FTIR) spectrophotometer (Perkin Elmer, spectrum one) based on the method of van Rossum (2000). Samples plus KBr (1: 100) was then mashed until evenly mixed. Then it was pressed with a vacuum pump for 15 minutes, and read the absorbance at wavelengths of 400-4000 cm^{-1}. From the resulted curve, the type of bond and its functional group were determined based on FTIR references.

FTIR Analysis

As much as 2 mg of alginate sample was put into a small bottle and 200 ml KBr was added, then stirred until homogeneous. The mixture was then placed on the die, pressed for several minutes until it formed pellet. The pellets were then put into the sample and their absorption was measured at 4000-400nm wavelength. Alginate was at peak at wavelength 1030/1080 nm.

RESULTS AND DISCUSSION

Characteristics of seaweed

Raw materials of *Sargassum polycystum* obtained from Lima island, Ujung Kulon and Binuangeun of from western Java are presented in Figures 5, 6, 7. Analysis of the quality of *Sargassum polycystum* seaweed from those three locations which includes yields, water content, CAW, and impurities is shown in Table 3.

The yields of dried seaweed differ across the three locations with the highest yield of *Sargassum polycystum* was from Binuangeun with 25.77%. Overall, the water content of all samples is below 15%. According to Winarno (1996), the water content of seaweed is influenced by the drying process. The highest water content was found in the sample from Ujung Kulon with 14.50% but this still meets the requirements by the SNI standard.

Figure 5. *Sargassum polycystum* seaweed from Lima Island, Banten Province, Indonesia

Figure 6. *Sargassum polycystum* seaweed from Ujung Kulon, Banten Province, Indonesia

Figure 7. *Sargassum polycystum* seaweed from Binuangeun, Banten Province, Indonesia

Table 3. Chemical composition of *Sargassum polycystum* seaweed from three locations in Banten, Indonesia

Chemical composition	Lima Island			Ujung Kulon			Binuangeun			Standard
	1	2	Mean	1	2	Mean	1	2	Mean	
Rendement (%)	12.96	11.22	12.09	17.78	14.70	16.24	29.54	22.00	25.77	-
Water content (%)	09.00	09.50	09.25	12.75	11.00	11.88	13.00	12.50	12.75	< 15%
CAW (%)	76.64	71.49	74.06	67.69	65.42	66.55	75.59	78.24	76.92	>50%
Impurities (%)	13.80	09.32	11.56	33.88	3.50	3.69	25.27	26.15	25.71	20-30%

Table 4. The mean value of physical quality analysis of Na alginate extracted from *Sargassum polycystum* collected from three locations in Banten, Indonesia

Characteristics	Site			
	Lima Island	Ujung Kulon	Binuangeun	Standard
Rendement (%)	11.48+0.79	18.62+0.84	05.75+0.11	> 18.00*
Viscosity (cP)	35.00+7.07	62.50±3.53	81.33+1.88	> 27.00**

Note: BSN (2015)* and ** Food Chemical Codex (2004)

CAW provides information on the cleanliness of seaweed from dirt, sand, and rock attached. Based on Table 2, it can be seen that the CAW values of the samples from Lima Island, Ujung Kulon and Binuangeun were 76.64%, 67.69%, and 75.59%, respectively. These values mean that the samples of *Sargassum polycystum* were clean and free of dirt. These results are in accordance with the quality requirements of dried seaweed based on SNI No. 2690.1.2015 which suggest minimum value of 50% (BSN 2015).

The highest impurity level of the three locations was from Binuangeun (25.71%) in which the samples contain sand, rock, coral and the wastes produced by humans including plastic as Binuangeun is close to residential dwellings. Impurity rate of *Sargassum polycystum* from Lima Island was 11.56%, consisting of sand and coral. The lowest impurity level was from Ujung Kulon (3.69%), indicating the cleanest samples which contain sand and coral as the sampling location was far from the residential areas of Taman Jaya village, Sumur Sub-district, Pandeglang District, Banten Province.

Alginate extraction

The physical characteristics of the quality of Na-alginate *Sargassum polycystum* from three locations are presented in Table 4.

Rendement

Sargassum polycystum from Ujung Kulon has the highest Na alginate content (18.62%+0.84%) followed by that from Lima Island with an average 11.48%+0.79% which is likely influenced by the cleanliness of the location which consists only of sand and coral. In contrast, samples from Binuangeun has the lowest Na alginate yield (5.75%+0.11%) which might be influenced by the amount of sand, rock, coral and litter contained because it is close to human settlement. The results of the alginate yield test in the extraction of *Sargassum polycystum* are presented in Figure 8.

Alginate yield produced by seaweed is influenced by habitat (i.e. light intensity, sea currents, and aquatic nutrition), age of brown seaweed, the handling techniques of brown seaweed during collection, and the extraction process used (Basmal et al. 2012). Because this study used the same treatment across three locations, so habitat and sea currents are likely the influencing factors on the yield of alginate. Binuangeun has shallow water with depth of 40.00 cm and the shortest total thallus length was 31.82 cm where *Sargassum* overgrew at the lowest ebb in the form of inundation affected by current velocity (0.24, 0.14, and 0.03). Based on the Meteorological, Climatology and Geophysics Agency (BMKG-maritime.bmkg.go.id) waves in the area of Lima Island are classified as Slight Sea/Small group with wave size of 0.5-1.25 m, while in Ujung Kulon and Binuangeun are belong to Moderate Sea/Moderate group with wave size of 1.25-2.50. This condition causes the thallus length of *Sargassum polycystum* in Binuangeun to be shorter than that in Ujung Kulon and Lima Island.

Viscosity

The highest mean viscosity was *Sargassum polycystum* originating from Binuangeun (81.33+1.88) cP, followed by that from Ujung Kulon (62.50+3.53) cP, and Lima Island (35.00+7.07) cP. The high content of water-insoluble material and low alginate viscosity is caused by the low purity of the alginate produced. Na-alginate thickness is divided into three levels, namely low viscosity (<60 cP), medium viscosity (60-110 cP) and high viscosity (110-800 cP). Based on this division, the viscosity of Na alginate from Lima Island is categorized as low viscosity (Manev et al. 2015). Sodium alginate for food usually has a lower viscosity than sodium alginate for textiles. Seaweed from the tropics (warm water) generally produces alginates with low viscosity (Mc Hugh 2008). Seaweed with a long thallus length will produce Na alginate with low viscosity, whereas if used with seaweed with a short thallus (20-40) cm it will produce high viscosity. Possible differences in the location where it grows is one of the causes of the difference in the value of the resulting viscosity (Hamrun 2018). The results of the alginate viscosity extracted from *Sargassum polycystum* are presented in Figure 9.

Alginate viscosity is influenced by several factors, including temperature, solution level and degree of polymerization. Na alginate viscosity value is highly dependent on the age of brown seaweed when harvested, extraction techniques (concentration, temperature, pH and the presence of polyvalent metal cations) and the weight of seaweed molecules extracted (Mc Hugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80ºC, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the thickness using RVA (Rapid Visco Analyzer). Anggadiredja 2011 stated that the higher is the drying temperature, the higher is the viscosity value. It is assumed that the increase in drying temperature will increase the formation of the amount of sulfate esters so that viscosity increases.

Chemical characteristics of Na-alginate Sargassum polycystum

Chemical analysis of Na-alginate extracted from *Sargassum polycystum* including the value of water content, ash content, and pH value are presented in Table 5. *Sargassum polycystum* seaweed from Binuangeun has Na alginate with higher water content and ash content than that from Lima Island and Ujung Kulon, while the mean pH (6.05+0.57) is lower.

Water content

Drying is a process of reducing a part of the water content of the material. Water content of the material is the amount of water contained in the material expressed in percent (%). The water content in Na alginate from the three study locations was in accordance with international quality standards in which the drying losses were <15% (FCC 2004). The mean water content of Na alginate seaweed *Sargassum polycystum* from the three study locations is presented in Table 5. The results of the observations showed that the mean value of Na alginate water content from Binuangeun was the highest. The higher the purity of alginate results in the difficulty of the water coming out of the matrix during the drying process. Alginate is a polymer with the ability to hold water very well so that the higher the purity of the alginate, the better the ability to hold water (Lee and Mooney 2011).

Water is an important component in food ingredients because water can affect the appearance, texture, and taste of food. The water content in food ingredients also determines acceptability and diversity as well as durability of food ingredients. The water content allowed in Na alginate is between 5-20%, while the water content allowed by the FCC is <15%. When compared to some research standards, water content of Na-alginate meets the standard (FCC 2004). JECFA also stated that the water content of food additives of alginate is maximum at 15% (FAO 2009).

Ash content

Ash content is important to know because it can determine the purity level of the product from unwanted components (Chee et al. 2011) . Based on the analysis it can be seen that commercial alginate generally has a maximum ash content of 27%. This means that alginate extracted from the three locations still meets JECFA requirements standards in term of the ash content contained (Mc Hugh 1987).

pH

Na alginate extracted from Binuangeun with a mean pH of 6 was lower than the pH of Ujung Kulon and Lima Island. The results of the overall chemical and physical analysis suggest that Na alginate produced from Binuangeun is more suitable for non-food products as this is related to the quality produced. The results from Binuangeun have brighter color which can be formulated according to the needs and desired physicochemical properties, especially those related to gel formation, thickness, binding of water so that it can retain moisture. On the other hand, Na alginate produced from Lima Island is more suitable for food products. Alginate for food must have low water content, low ash content and neutral pH (Puspita 2017). Alginate characteristics extracted from *Sargassum polycystum* from Lima Island, Ujung Kulon and Binuangeun meet alginate standards as food grade, but the alginate from Binuangeun has high level of viscosity according to the needs of the non-food industry.

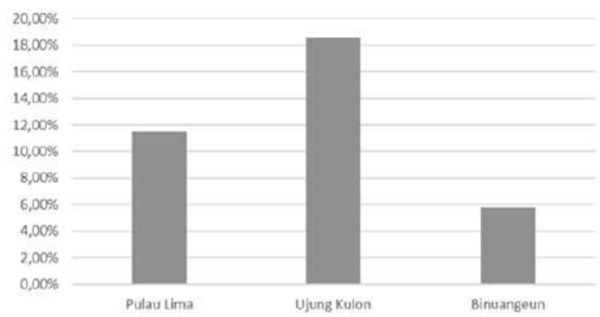


Figure 8. Results of alginate yield test in extraction of *Sargassum polycystum* (%)

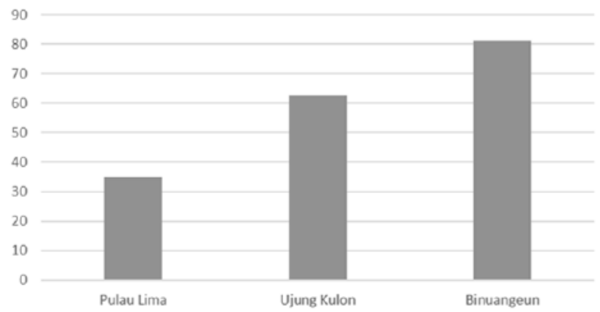


Figure 9. Test results for alginate viscosity in extraction of *Sargassum polycystum* (cP)

Table 5. Mean value of chemical quality analysis of Na alginate *Sargassum polycystum* from three locations in Banten, Indonesia

Characteristics	Locations			
	Lima Island	**Ujung Kulon**	**Binuangeun**	**Standard***
Water content (%)	12.42+0.30	10.23+1.68	13.31+0.77	5-20
Ash content (%)	26.68+2.48	24.94+4.41	26.69+0.82	18.00-27.00
pH	7.39+0.03	7.03+ 2.60	6.05+0.57	3.5-10

Note: * Food Chemical Codex (2004)

Table 6. Results of alginate partial hydrolysis

Locations of *Sargassum* spp.	Blok GG	Blok MM	Blok MG	Blok M	Blok G
Pulau Lima	67.60	27.00	5.40	30	70
Ujung Kulon	59.00	35.00	6.00	37	63
Binuangeun	41.40	50.00	9.60	55	45

Table 7. Functional groups on the FTIR curve

Wavelength (cm^{-1})	% Transmitan (%T)			Functional groups
	Pulau Lima	**Ujung Kulon**	**Binuangeun**	
3427.51-3448.72	63.29	53.88	62,80	O-H stretching (hydrogen bonds between molecules)
1608.63	50.98	41.15	50.29	C=O stretching
1411.89	38.72	33.42	38.49	-C-OH stretching
1091-1093.64	53.62	41.02	48.50	COOH, C-O stretching, C-O-C stretching
1170	64.41	48.21	56.67	C-O stretching, C-C stretching, C-C-C bending
1029.99-1033.85	48.94	37.16	45.59	C-O stretching, C-O-C stretching
947.05	62.31	47.11	54.50	C-O stretching, C-C-H stretching
817.82-875.68	39.92	30.96	35.04	C-C stretching, C-C-H stretching, C-O bending

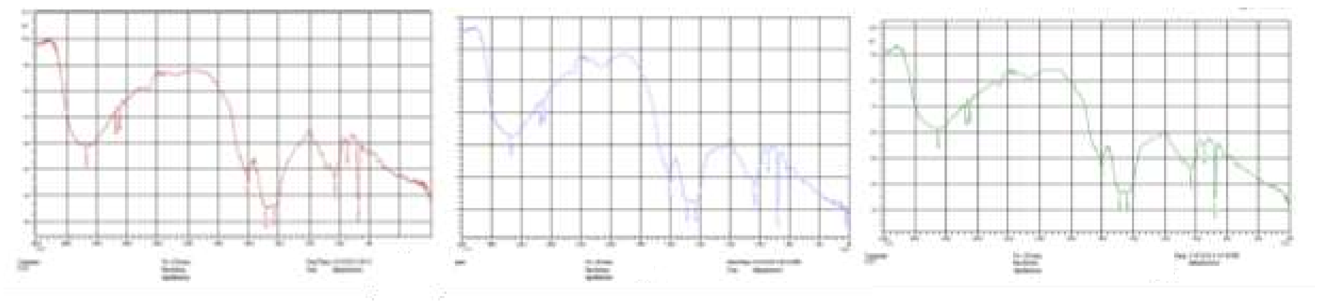
Partial hydrolysis of alginate

The results of isolation of mannuronic acid (M) and guluronate (G) on alginate molecules were carried out by partial hydrolysis of alginate, obtained by GG block deposits as presented in Table 6. The highest component of G in alginate is obtained from Lima Island with the results of viscosity test as listed in Figure 8 and according to the functional group analysis which is qualitatively proven on the FTIR curve as shown in Figure 10.

Analysis of functional groups of sodium alginate from extraction of *Sargassum polycystum* from three locations

Uptake of functional groups from *S. polycystum* from three locations can be seen in the FTIR curve presented in Figure 10 and Table 7. The sodium alginate spectrum showed the presence of hydroxyl (-OH) groups, carbonyl groups (-COO-),-C-OH and-COOH bonds, C-O stretching, C-C stretching, and C-O bending as seen in FTIR curves (Figure 10). According to Alvares et al. (2018), absorption at wavelengths of 1608.63 cm^{-1}, 1411.89 cm^{-1} and 1091 cm^{-1} - 1093.64 cm^{-1} (in the wavelength area 1091 cm^{-1} - 1093,64 cm^{-1}, if higher than absorption at wavelength 1029 cm^{-1} - 1033 cm^{-1}, 947 cm^{-1} and 817,82 cm^{-1} - 875,68 (at the wavelength area of 1029 cm^{-1} to 817 cm^{-1}) indicates that the alginate polymer consists of a higher proportion of guluronic monomers, whereas absorption at wavelengths of 1315 cm^{-1}, 1170 cm^{-1}, 1029 cm^{-1} - 1033 cm^{-1}, 947 cm^{-1} and 817.82-875.68 cm^{-1} were higher indicating that the alginate polymer consisted of the proportion of mannuronic monomers.

In the results of the FTIR curve showed that the absorption at wavelength 1608.63 cm^{-1}, 1411.89 cm^{-1}, and 1091 cm^{-1} - 1093.64 cm^{-1} in Binuangeun alginate products provide higher absorption compared to Lima Island and Ujung Kulon alginates. However, the results of the GG block partial deposit test (Table 8) showed that alginate extracted from Binuangeun had the lowest guluronate group (41.40%). This is possible at wavelength of 1029.99 cm^{-1} -1033.85 cm^{-1} giving quite high uptake of C-O stretching and C-O-C stretching. Whereas, Lima Island and Ujung Kulon alginate products showed a higher proportion of guluronate monomers than mannuronate. According to the quantitative test of GG block, partial deposits for Lima Island, Ujung Kulon and Binuangeun resulted in 67.60%, 59.00%, and 41.40%, respectively.



A **B** **C**

Figure 10. FTIR curve of alginate: A. Lima Island, B. Ujung Kulon, C. Binuangeun

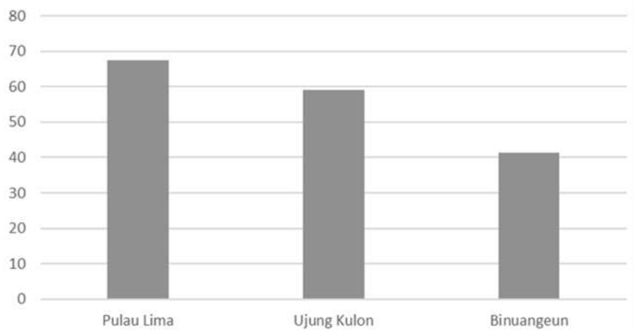


Figure 11. Results of GG block deposition test on alginate structure (%)

In conclusion, the combination of viscosity and gel strength of guluronate and mannuronate is the determinant factors to produce hydrogel than other characteristics, while the other characters are supporting ones. The results of this study showed that the yields of alginate from *Sargassum polycystum* from Lima Island, Ujung Kulon and Binuangeun were 11.48%, 18.62%, and 5.75%, respectively. Alginate from Binuangeun has the best physicochemical characteristics compared to the others. The viscosity of alginate from Lima Island, Ujung Kulon and Binuangeun were 35 cP, 62.50 cP, and 81.33cP, respectively. The characteristics of Na alginate extracted from Binuangeun have relatively higher quality. Alginate characteristics extracted from Lima Island, Ujung Kulon and Binuangeun have met alginate standards as food grade, but when viewed from viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry. The results of the partial alginate hydrolysis test showed that guluronic block (GG) in alginate polymers on the Lima Island, Ujung Kulon and Binuangeun were 67.60%, 59.00%, and 41.40%, respectively. This is related to the gel properties formed. Alginate from Lima Island tends to be stiffer and less flexible than alginate from Ujung Kulon and Binuangeun. Our study showed that there were variations in the concentration of mannuronate and guluronate from the three habitats of *Sargassum* in Banten.

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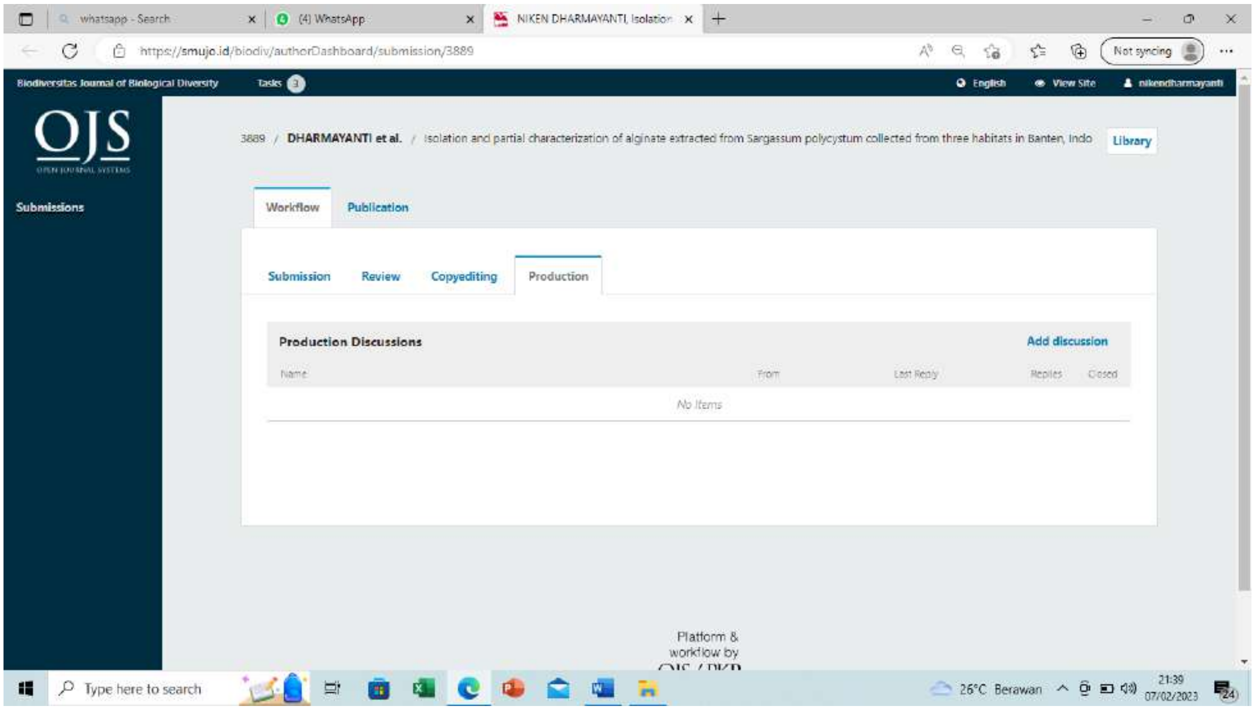

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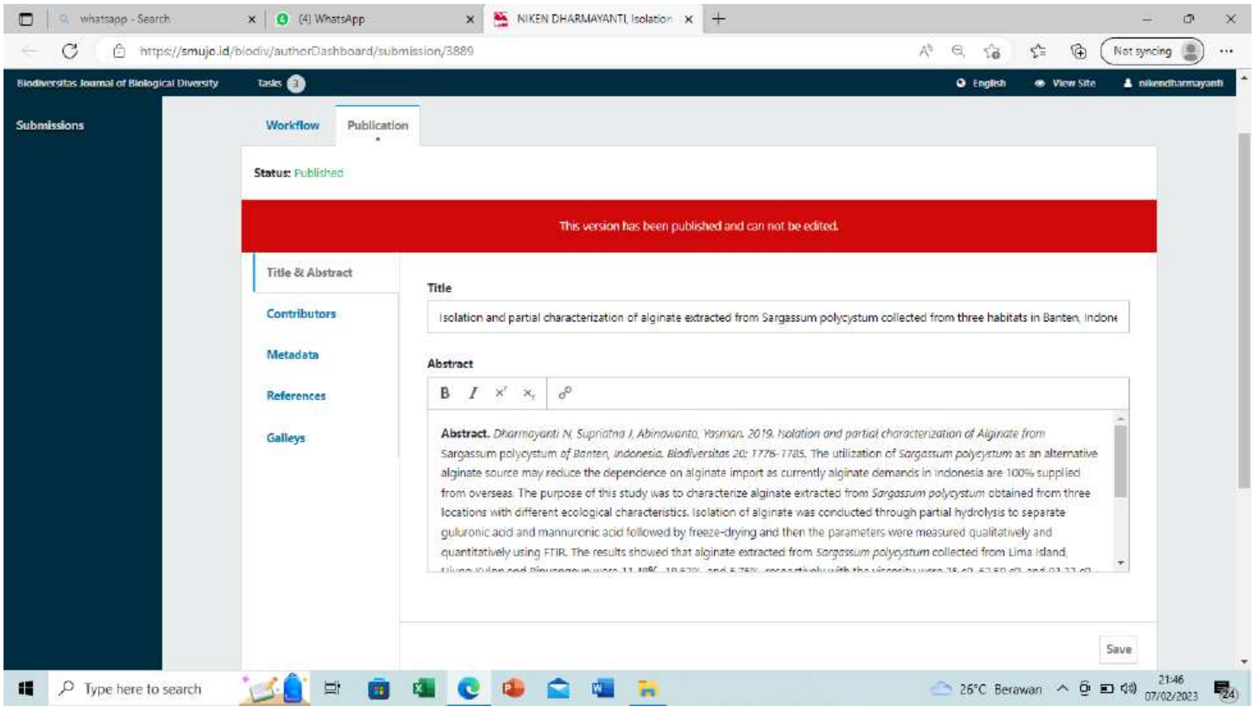

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Isolation and partial characterization of alginate extracted from *Sargassum polycystum* collected from three habitats in Banten, Indonesia

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Abstract. *Dharmayanti N, Supriatna J, Abinawanto, Yasman. 2019. Isolation and partial characterization of Alginate from* Sargassum polycystum *of Banten, Indonesia. Biodiversitas 20: 1776-1785.* The utilization of *Sargassum polycystum* as an alternative alginate source may reduce the dependence on alginate import as currently alginate demands in Indonesia are 100% supplied from overseas. The purpose of this study was to characterize alginate extracted from *Sargassum polycystum* obtained from three locations with different ecological characteristics. Isolation of alginate was conducted through partial hydrolysis to separate guluronic acid and mannuronic acid followed by freeze-drying and then the parameters were measured qualitatively and quantitatively using FTIR. The results showed that alginate extracted from *Sargassum polycystum* collected from Lima Island, Ujung Kulon and Binuangeun were 11.48**%,** 18.62%, and 5.75%, respectively with the viscosity were 35 cP, 62.50 cP, and 81.33 cP, respectively. The test result of partial hydrolysis of alginate showed that guluronic block (GG) in the alginate polymer of Lima Island, Ujung Kulon and Binuangeun were 67.60%, 59.00%, and 41.40%, respectively. These relate to the nature of the gel formed. The alginate from Lima Island tends to be more rigid and less flexible than that from Ujung Kulon and Binuangeun. The findings of this study suggest that there are differences in the concentration of the components of mannuronate and guluronate of *Sargassum polycystum* across different locations in Banten, Indonesia.

Keywords: Alginate, characteristics, partial hydrolysis, *Sargassum polycystum*

INTRODUCTION

Brown algae are known as alginate sources. Alginate, an anionic heteropolysaccharide extracted from natural brown algae, has useful properties for the food, chemical, medical, and agricultural industries (Inoe 2018). Alginate is needed in various industries for various purposes including as gelling agent, stabilizer, emulsifier, suspending agent and dispersing agent. In food industry, alginate compounds are added as ingredient in butter, ice cream, and milk. In cosmetics industry, it functions as water binder so that the cosmetic components are perfectly bound and easily penetrate skin tissue.

Alginophyte is a family of brown seaweeds. The difference in the main chemical compounds contained in seaweed shows the characteristics of the seaweed, suggesting different extraction process among seaweeds. Similarly, the benefits and uses of these chemical compounds also vary one another. These differences are caused by different species of seaweed, different location and growing season (Pereira 2018).

Alginate consists of group of polysaccharides and can be found in brown algae tissue. Alginate is a long chain polymer consisting of α- (1→4)-linked L-guluronic acid (G) and β- (1→4)-linked D-mannuronic acid (M). The content ratio (G) and (M) is referred to as the G / M ratio. The G / M ratio will greatly affect the quality of alginate in which the higher is the G content, the higher is the alginate thickness, and vice versa.

Although all brown algae contain alginate, only few species of brown algae are used commercially and can be found in subtropical climates such as *Macrocystis pyrifera*, *Ascophyllum odosum*, *Laminaria hyperborea*, *Laminaria digitata*, *Ecklonia maxima*, and *Lessonia nigrescans*. Whereas in Indonesia, the common species are *Sargassum* sp., *Turbinaria* sp., *Hormophysa* sp., and *Padina* sp. (Rasyid 2007). Alginophyte that grow in water consists of three genera, i.e., *Sargassum* with seven species, *Turbinaria* with two species, and *Hormophisa*.

Increasing demands on alginate require the enhancement of the quality of industry in which one of the aspects is encouraging the exploration of potential species as alginate sources. The alginate content of seaweed varies greatly depending on the species of brown seaweed extracted. For example, a study showed that brown macroalgae stocks found at Ekas Bay in Lombok Island vary across seasons and species. The biomass sourced from *Padina* reached 97.85 ± 12.63 and 79.54 ± 2.53 tons in May to June and November, respectively while *Sargassaceae* species produced 669.70 ± 109.64 and 147.70 ± 77.97 tons in May to June and November, respectively. The highest alginate yields were produced during the period May to June in which *Padina* can produce 9.10 ± 0.06 tons of dry alginate while *Sargassum* can produce 207.61 ± 0.42 tons of dry alginate. This study demonstrates that wild Sargassaceae is a potential source of alginate which is influenced by the ratio of mannuronate and guluronate (Setyawidati et al. 2018). Alginate formed in *Sargassum* cell walls reached 40% of the total dry weight and played an important role in maintaining the tissue structure of thallus. The thallus of *Sargassum* has a variety of shapes and sizes, ranging from in the form of rods and unite in a bundle to in the form of large thallus with outer shape like tall plants. The shape of the thallus can affect alginate content (Widyartini et al. 2017).

There is opportunity to increase alginate production in Indonesia to manage alginate resources sustainably but this will need information of *Sargassum* spp. and its contents. The genus of *Sargassum* consists of 400 species while in Indonesia there are 12 species namely *Sargassum duplicatum*, *S. hitrix, S. echinocarpum, S. gracillinum, S. obtuspfolium*, *S. binderi*, *S. polycystum*, *S. microphylum*, *S. crassifolium*, *S aquafolium*, *S. vulgare*, and *S. polyceratium*.

Sargassum polycystum is an alginate-producing seaweed. So far, *Sargassum polycystum* grow wild and have not been cultivated in Indonesia. This study was aimed to obtain the ecological, morphological, molecular and structure of alginate extracted from *Sargassum polycystum* in western Java so that the relationship between genes from *Sargassum* and its structure can be revealed. This aim will be achieved through isolation and partial characterization of alginate extracted from *Sargassum polycystum* collected from Banten waters to identify the structure of sodium alginate based on the chemical composition of mannuronate and guluronate.

MATERIALS AND METHODS

Study period and location

The study was carried out in February 2018 until April 2019 in Banten Province (western Java), Indonesia. There were three sampling locations, i.e., Lima Island (6° 00’05” S, 106°09'18" E), Ujung Kulon (6°48'15" S, 105°29'5" E), and Binuangeun (6°49'16" S, 105°56'14" E). The location of S. *polycystum* sampling is presented in Figure 1. The geographical conditions of western Java are surrounded by three major waters, i.e., the Java Sea in the north, the Sunda Strait in the west, and the Indian Ocean in the south.

Sampling procedure

Collection and identification of samples of *Sargassum polycystum* were conducted during the lowest tide at each study location. Samples were collected using transect method along the coast. Each sample was photographed and then taken to the Jakarta Fisheries University for identification and further analysis. Seaweed was stored in a plastic bag, cleaned, sorted according to genus, weighed in fresh condition, wind-dried, and then ready for alginate extraction and partial hydrolysis conducted in Chemistry Laboratory, Department of Fish Processing Technology, Jakarta Fisheries University, Jakarta, Indonesia. Analysis of functional group using FTIR (Fourier Transform Infrared Spectroscopy) was undertaken in Chemistry Laboratory, Department of Chemistry, University of Indonesia, Depok, Indonesia.

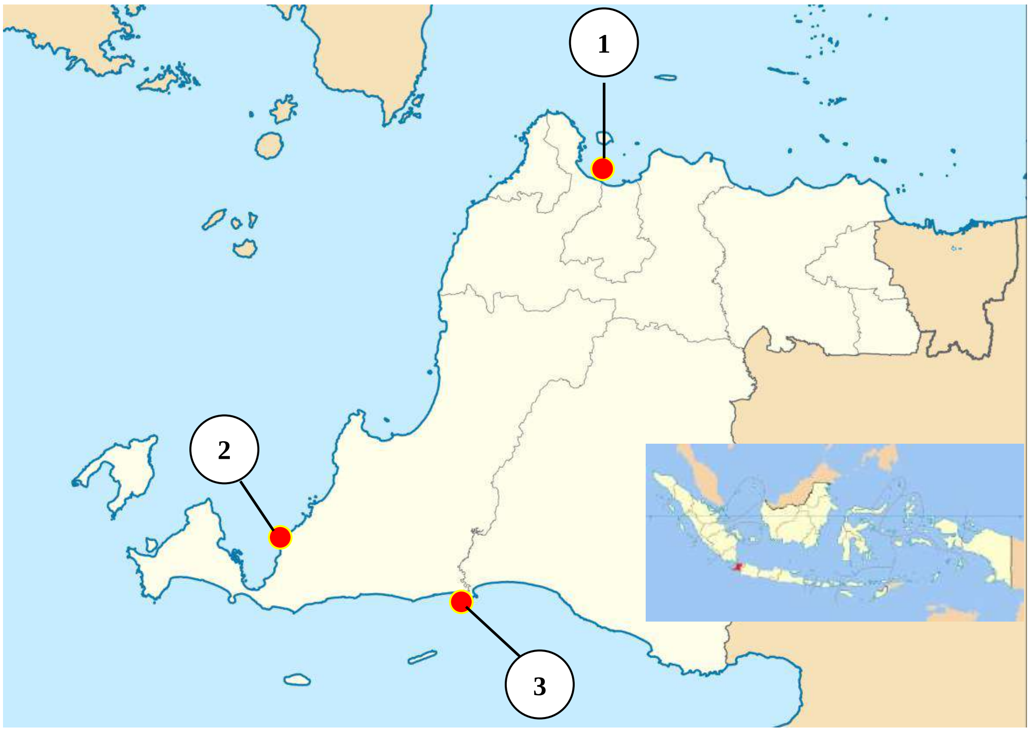


Figure 1. Three locations for sampling of *Sargassum polycystum* in Banten Province, Indonesia: 1. Lima Island; 2. Ujung Kulon; and 3. Binuangeun

Laboratory analysis

Isolation and partial characterization of alginate from *Sargassum polycystum* included chemical composition of *Sargassum polycystum*, alginate extraction, characterization of alginate (i.e., rendement viscosity, water content, ash content, pH), partial hydrolysis of alginate (i.e., isolation of mannuronic acid and guluronate acid, analysis of functional group analysis which is qualitatively proven on the FTIR curve).

Materials and equipment

Three samples of *Sargassum polycystum* from each location were prepared for extraction processes with materials included natrium carbonate, calcium chloride, chloride acid, alcohol 70%, peroxide hydrogen, aquadest, Ca_2Cl_2 4%, HCl 2%, Na_2CO 34%, $Ca_2Cl_2$10%, Ca_2Cl_2 5%, HCl 5%, dan Alkohol 95%. Partial hydrolysis used HCl 37% and NaOH 5 mol and p.a grade chemicals for the analysis of alginate monomers. The equipment needed included equipment for alginate extraction, while the test equipment is the Brookfield brand viscometer, the Shimadzu Prestige Fourier Transform Infrared Spectroscopy (FTIR) and the Shimadzu Spectrophotometer.

Data collection

Alginate extraction

The extraction of alginate as follows: the raw material of *Sargassum polycystum* was cleaned, weighed at 200 grams then added with acetic acid solution according to the treatment. The mixed material was blended into seaweed pulp, heated at 80ºC and stirred for 10 minutes. After that, it was filtered and squeezed into the form of coarse alginate liquid which was then dried at 65ºC for 24 hours. After being dried it was then pressed to get coarse alginate flour.

Na-alginate extraction process

The main process of extracting Na-alginate was divided into four stages namely immersion (pre-extraction), extraction, bleaching, and purification. Immersion stage was carried out in an alkaline solution and an acid solution. Extraction was carried out in an alkaline atmosphere by cooking using extracting solutions (Na_2CO_3, NaOH). Bleaching used solution of NaOCl or H_2O_2. Purification was divided into three phases, i.e., the formation of alginic acid, the formation of sodium alginate and the formation of pure sodium alginate.

Immersion

Soaking seaweed in $CaCl_2$ solution was aimed to dissolve laminarin, mannitol, dyes, and salts. This treatment also served to dissolve the remaining impurities in seaweed. According to Silva et al. (2015), alginic acid precipitated under the conditions of pH <3 in which in this condition the alginate component will be stable in the raw material during the immersion process. While immersion in alkaline solutions was aimed for deproteinization (Kamaruddin et al. 2015).

Extraction

The brown seaweed extraction process was carried out in alkaline conditions. The goal was to separate the cellulose content from alginate. The extracting material that can be used is Na_2CO_3 or NaOH. Lee and Mooney (2012) state that high concentrations of Na_2CO_3 (3-5%) can cause a decrease in product yield and viscosity. This is because the alkaline solution can damage the alginic acid compound by shortening the polymer chain into oligosaccharides which in turn degrades to 4-deoxy-5-ketouronic acid. Extraction carried out by heating will also affect the alginate produced. This heating process not only makes extraction processes easier but can also extract the weight of higher alginate molecules so that they can increase product yield and viscosity.

Deposition of Na-alginate

In the formation of sodium alginate, alginic acid that had been formed was added with alkaline solution containing Na+ions such as NaOH and Na_2CO_3. The purpose of the formation of sodium alginate is to get a more stable alginate compound. According to Mc Hugh (1987), the exchange of H+ions with Na+ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid.

Withdrawal of sodium alginate

Withdrawal of Na-alginate compounds from sodium alginate solution can be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonym (1976), 1% sodium alginate starts to show the separation process in a solution of 10% isopropanol or in ethanol 20% as well as its boiling point. The melting point of isopropanol (secondary alcohol) is lower than ethanol (primary alcohol). To withdraw sodium alginate, the use of isopropanol is more efficient than ethanol. Formation of pure sodium alginate was done by attracting the water content contained in the solution. This pure Na-alginate was then dried in an oven and after that, it can be ground into Na-alginate flour.

After the water content contained in the anatomic alginate solution was pulled out, pure sodium alginate was formed. Sodium alginate was then dried in an oven and ground to form sodium alginate flour. The characteristics of alginic acid and sodium alginate were tested compared to Table 1. Characteristics of alginic acid and sodium alginate and Table 2. Quality specifications of alginic acid and sodium alginate. According to Glicksman (1983), alginic acid is described as a hydrophilic colloidal carbohydrate extracted with alkali salt from various types of brown seaweed. Chemically, alginate is a pure polymer of uronic acid arranged in a long linear chain. The chemical formula of alginate is $(C_6H_8O_6)$ n with the number n between 80 to 83 (Schoeffel and Link 1993). There are two types of monomers that make up alginic acid, namely β-D-mannopyranosyl uronate or D-mannuronic acid and α-L-glucopyranosyl uronate or L-guluronic acid. Of the two types of monomers, alginic acid can be a homopolymer consists of similar monomers namely D-mannuronic acid only or L-guluronic acid only. Homopolymers of D-mannuronic acid (poly mannuronic acid) are formed by repeating D-mannuronic acid with β-(1,4) bonds and hydrogen bonds between hydroxyl groups on C3 atoms with oxygen atoms on adjacent hexose rings. The homopolymer form of L-guluronic acid is more rigid than D-mannuronic acid homopolymer (Rajendran et al. 2016) Alginate with a high proportion of L-guluronic acid homopolymers tends to form stiff, rigid and syneresis gels. On the other hand, the higher proportion of D-mannuronic acid homopolymers tends to form a gel that is more elastic, does not rigid and does not show high syneresis (Glicksman 1983).

Data analysis of molecular structure and quality of alginate

Variables observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test and structural tests with FTIR. Alginate is a compound contained in brown seaweed cell walls (Phaeophyceae) other than cellulose and pectin.

Rendement

The Na-alginate yield obtained from the extraction process of seaweed *Sargassum polycystum* was calculated based on the weight of Na-alginate after drying on the dry weight of the raw material. The yield of Na-alginate was calculated using the following formula:

Addendum (%) = (weight of Na-final alginate (g) / weight of initial seaweed (g)) x 100%

Viscosity

Viscosity analysis referred to JECFA (2007). Observations were made at a 1-5% solid concentration to determine the relationship between concentration and solution viscosity. Na-alginate (sample) was weighed as much as 7.5 g in weigh paper. As much as 492.5 g of distilled water was weighed in a 500 mL glass beaker so that the sample and distilled water had total weight of 500 g. Alginate was included in a 500 mL glass beaker containing distilled water and stirred gradually. Aquades was heated and stirred once to reach 75ºC, after a constant temperature the solution was heated for 25 minutes. Stirring I was carried out at minute 1 for 1 minute, stirring II at 25 minutes. Beaker glass was covered with aluminum foil to prevent water loss in the heating process due to evaporation, then the solution temperature was lowered to reach 75ºC. The measurement of solution viscosity was measured using RVA (Rapid Visco Analyzer) spindle 2 at 30 rpm, waited until the spindle needle was stable (up to 6 times rotation). Viscosity is expressed in centipoise (cP).

Water content

A sample of 2 g was weighed and then put into a porcelain cup. The sample was heated by oven at temperature 105ºC for 24 hours and keep the sample in the desiccator for 5 minutes. Finally, the sample was weighed until the value was stable. Water content was calculated using the following formula:

Water content (%) = (weight of final sample (g) / weight of initial sample (g)) x 100%

Ash content

The final sample from water content continued to be heated using ignition furnace at temperature 600ºC for 24 hours. Then the sample was kept in the desiccator for 5 minutes. Finally, the sample was weighed until the value was stable. Ash content was calculated using the following formula:

Ash content (%) = (weight of final ash sample (g) / weight of initial sample (g)) x 100%

pH value

A sample of 3 g was weighed and then put into a 300 mL glass beaker then added 197 g of distilled water until the total weight was 200 g. The sample was heated while being stirred using a stirrer until it dissolved at a temperature of 60-80ºC. Then the electrode was dipped into the sample solution which was previously calibrated. The pH value was obtained according to what shown on the screen. Then the electrode was rinsed with distilled water.

Alginate partial hydrolysis test

The composition of poly guluronic, poly mannuronate and mixed segments between mannuronate and guluronate in alginate determine the quality of alginate (Gomez 2018). To isolate mannuronic acid (M) and guluronate (G) on alginate molecules, we carried out Partial Hydrolysis of Alginate by 5.00 g alginate in HCl 0.3 N at 100° C for 2 hours. The soluble fraction was identified as a block MG. Bonding the hydroxyl between M and G was easily hydrolyzed by insoluble-fraction acid more resistant to acid hydrolysis, again dissolved by adding alkali and fractionation by adjusting the pH at 2.85, so that the GG block settled and the MM block dissolved.

Analysis of alginate functional groups was carried out using a Fourier Transform Infrared (FTIR) spectro-photometer (Perkin Elmer, spectrum one) based on the method of van Rossum (2000). Samples plus KBr (1: 100) was then mashed until evenly mixed. Then it was pressed with a vacuum pump for 15 minutes, and read the absorbance at wavelengths of 400-4000 cm^{-1}. From the resulted curve, the type of bond and its functional group were determined based on FTIR references.

FTIR Analysis

As much as 2 mg of alginate sample was put into a small bottle and 200 ml KBr was added, then stirred until homogeneous. The mixture was then placed on the die, pressed for several minutes until it formed pellet. The pellets were then put into the sample and their absorption was measured at 4000-400nm wavelength. Alginate was at peak at wavelength 1030/1080 nm.

RESULTS AND DISCUSSION

Characteristics of seaweed

Raw materials of *Sargassum polycystum* obtained from Lima island, Ujung Kulon and Binuangeun of from western Java are presented in Figures 5, 6, 7. Analysis of the quality of *Sargassum polycystum* seaweed from those three locations which includes yields, water content, CAW, and impurities is shown in Table 3.

The yields of dried seaweed differ across the three locations with the highest yield of *Sargassum polycystum* was from Binuangeun with 25.77%. Overall, the water content of all samples is below 15%. According to Winarno (1996), the water content of seaweed is influenced by the drying process. The highest water content was found in the sample from Ujung Kulon with 14.50% but this still meets the requirements by the SNI standard.

Figure 5. *Sargassum polycystum* seaweed from Lima Island, Banten Province, Indonesia

Figure 6. *Sargassum polycystum* seaweed from Ujung Kulon, Banten Province, Indonesia

Figure 7. *Sargassum polycystum* seaweed from Binuangeun, Banten Province, Indonesia

Table 3. Chemical composition of *Sargassum polycystum* seaweed from three locations in Banten, Indonesia

Chemical composition	Lima Island			Ujung Kulon			Binuangeun			Standard
	1	2	Mean	1	2	Mean	1	2	Mean	
Rendement (%)	12.96	11.22	12.09	17.78	14.70	16.24	29.54	22.00	25.77	-
Water content (%)	09.00	09.50	09.25	12.75	11.00	11.88	13.00	12.50	12.75	< 15%
CAW (%)	76.64	71.49	74.06	67.69	65.42	66.55	75.59	78.24	76.92	>50%
Impurities (%)	13.80	09.32	11.56	33.88	3.50	3.69	25.27	26.15	25.71	20-30%

Table 4. The mean value of physical quality analysis of Na alginate extracted from *Sargassum polycystum* collected from three locations in Banten, Indonesia

Characteristics	Site			
	Lima Island	Ujung Kulon	Binuangeun	Standard
Rendement (%)	11.48+0.79	18.62+0.84	05.75+0.11	> 18.00*
Viscosity (cP)	35.00+7.07	62.50±3.53	81.33+1.88	> 27.00**

Note: BSN (2015)* and ** Food Chemical Codex (2004)

CAW provides information on the cleanliness of seaweed from dirt, sand, and rock attached. Based on Table 2, it can be seen that the CAW values of the samples from Lima Island, Ujung Kulon and Binuangeun were 76.64%, 67.69%, and 75.59%, respectively. These values mean that the samples of *Sargassum polycystum* were clean and free of dirt. These results are in accordance with the quality requirements of dried seaweed based on SNI No. 2690.1.2015 which suggest minimum value of 50% (BSN 2015).

The highest impurity level of the three locations was from Binuangeun (25.71%) in which the samples contain sand, rock, coral and the wastes produced by humans including plastic as Binuangeun is close to residential dwellings. Impurity rate of *Sargassum polycystum* from Lima Island was 11.56%, consisting of sand and coral. The lowest impurity level was from Ujung Kulon (3.69%), indicating the cleanest samples which contain sand and coral as the sampling location was far from the residential areas of Taman Jaya village, Sumur Sub-district, Pandeglang District, Banten Province.

Alginate extraction

The physical characteristics of the quality of Na-alginate *Sargassum polycystum* from three locations are presented in Table 4.

Rendement

Sargassum polycystum from Ujung Kulon has the highest Na alginate content (18.62%+0.84%) followed by that from Lima Island with an average 11.48%+0.79% which is likely influenced by the cleanliness of the location which consists only of sand and coral. In contrast, samples from Binuangeun has the lowest Na alginate yield (5.75%+0.11%) which might be influenced by the amount of sand, rock, coral and litter contained because it is close to human settlement. The results of the alginate yield test in the extraction of *Sargassum polycystum* are presented in Figure 8.

Alginate yield produced by seaweed is influenced by habitat (i.e. light intensity, sea currents, and aquatic nutrition), age of brown seaweed, the handling techniques of brown seaweed during collection, and the extraction process used (Basmal et al. 2012). Because this study used the same treatment across three locations, so habitat and sea currents are likely the influencing factors on the yield of alginate. Binuangeun has shallow water with depth of 40.00 cm and the shortest total thallus length was 31.82 cm where *Sargassum* overgrew at the lowest ebb in the form of inundation affected by current velocity (0.24, 0.14, and 0.03). Based on the Meteorological, Climatology and Geophysics Agency (BMKG-maritime.bmkg.go.id) waves in the area of Lima Island are classified as Slight Sea/Small group with wave size of 0.5-1.25 m, while in Ujung Kulon and Binuangeun are belong to Moderate Sea/Moderate group with wave size of 1.25-2.50. This condition causes the thallus length of *Sargassum polycystum* in Binuangeun to be shorter than that in Ujung Kulon and Lima Island.

Viscosity

The highest mean viscosity was *Sargassum polycystum* originating from Binuangeun (81.33+1.88) cP, followed by that from Ujung Kulon (62.50+3.53) cP, and Lima Island (35.00+7.07) cP. The high content of water-insoluble material and low alginate viscosity is caused by the low purity of the alginate produced. Na-alginate thickness is divided into three levels, namely low viscosity (<60 cP), medium viscosity (60-110 cP) and high viscosity (110-800 cP). Based on this division, the viscosity of Na alginate from Lima Island is categorized as low viscosity (Manev et al. 2015). Sodium alginate for food usually has a lower viscosity than sodium alginate for textiles. Seaweed from the tropics (warm water) generally produces alginates with low viscosity (Mc Hugh 2008). Seaweed with a long thallus length will produce Na alginate with low viscosity, whereas if used with seaweed with a short thallus (20-40) cm it will produce high viscosity. Possible differences in the location where it grows is one of the causes of the difference in the value of the resulting viscosity (Hamrun 2018). The results of the alginate viscosity extracted from *Sargassum polycystum* are presented in Figure 9.

Alginate viscosity is influenced by several factors, including temperature, solution level and degree of polymerization. Na alginate viscosity value is highly dependent on the age of brown seaweed when harvested, extraction techniques (concentration, temperature, pH and the presence of polyvalent metal cations) and the weight of seaweed molecules extracted (Mc Hugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80ºC, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the thickness using RVA (Rapid Visco Analyzer). Anggadiredja 2011 stated that the higher is the drying temperature, the higher is the viscosity value. It is assumed that the increase in drying temperature will increase the formation of the amount of sulfate esters so that viscosity increases.

Chemical characteristics of Na-alginate Sargassum polycystum

Chemical analysis of Na-alginate extracted from *Sargassum polycystum* including the value of water content, ash content, and pH value are presented in Table 5. *Sargassum polycystum* seaweed from Binuangeun has Na alginate with higher water content and ash content than that from Lima Island and Ujung Kulon, while the mean pH (6.05+0.57) is lower.

Water content

Drying is a process of reducing a part of the water content of the material. Water content of the material is the amount of water contained in the material expressed in percent (%). The water content in Na alginate from the three study locations was in accordance with international quality standards in which the drying losses were <15% (FCC 2004). The mean water content of Na alginate seaweed *Sargassum polycystum* from the three study locations is presented in Table 5. The results of the observations showed that the mean value of Na alginate water content from Binuangeun was the highest. The higher the purity of alginate results in the difficulty of the water coming out of the matrix during the drying process. Alginate is a polymer with the ability to hold water very well so that the higher the purity of the alginate, the better the ability to hold water (Lee and Mooney 2011).

Water is an important component in food ingredients because water can affect the appearance, texture, and taste of food. The water content in food ingredients also determines acceptability and diversity as well as durability of food ingredients. The water content allowed in Na alginate is between 5-20%, while the water content allowed by the FCC is <15%. When compared to some research standards, water content of Na-alginate meets the standard (FCC 2004). JECFA also stated that the water content of food additives of alginate is maximum at 15% (FAO 2009).

Ash content

Ash content is important to know because it can determine the purity level of the product from unwanted components (Chee et al. 2011) . Based on the analysis it can be seen that commercial alginate generally has a maximum ash content of 27%. This means that alginate extracted from the three locations still meets JECFA requirements standards in term of the ash content contained (Mc Hugh 1987).

pH

Na alginate extracted from Binuangeun with a mean pH of 6 was lower than the pH of Ujung Kulon and Lima Island. The results of the overall chemical and physical analysis suggest that Na alginate produced from Binuangeun is more suitable for non-food products as this is related to the quality produced. The results from Binuangeun have brighter color which can be formulated according to the needs and desired physicochemical properties, especially those related to gel formation, thickness, binding of water so that it can retain moisture. On the other hand, Na alginate produced from Lima Island is more suitable for food products. Alginate for food must have low water content, low ash content and neutral pH (Puspita 2017). Alginate characteristics extracted from *Sargassum polycystum* from Lima Island, Ujung Kulon and Binuangeun meet alginate standards as food grade, but the alginate from Binuangeun has high level of viscosity according to the needs of the non-food industry.

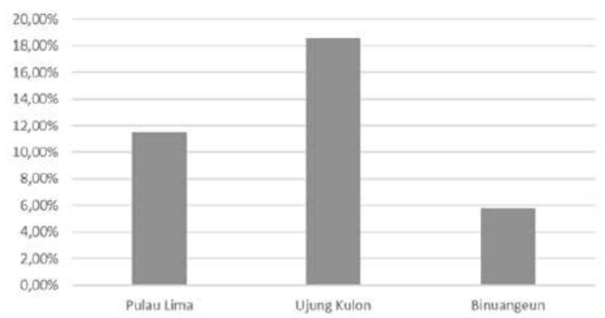


Figure 8. Results of alginate yield test in extraction of *Sargassum polycystum* (%)

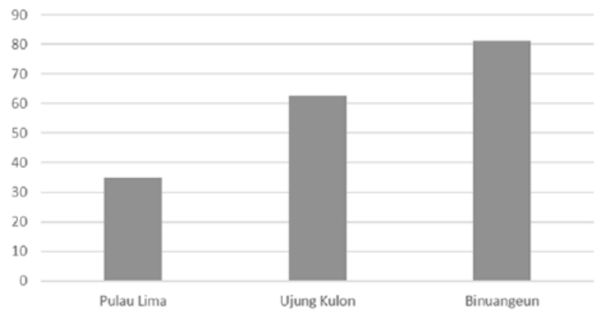


Figure 9. Test results for alginate viscosity in extraction of *Sargassum polycystum* (cP)

Table 5. Mean value of chemical quality analysis of Na alginate *Sargassum polycystum* from three locations in Banten, Indonesia

Characteristics	Locations			
	Lima Island	Ujung Kulon	Binuangeun	Standard*
Water content (%)	12.42+0.30	10.23+1.68	13.31+0.77	5-20
Ash content (%)	26.68+2.48	24.94+4.41	26.69+0.82	18.00-27.00
pH	7.39+0.03	7.03+ 2.60	6.05+0.57	3.5-10

Note: * Food Chemical Codex (2004)

Table 6. Results of alginate partial hydrolysis

Locations of *Sargassum* spp.	Blok GG	Blok MM	Blok MG	Blok M	Blok G
Pulau Lima	67.60	27.00	5.40	30	70
Ujung Kulon	59.00	35.00	6.00	37	63
Binuangeun	41.40	50.00	9.60	55	45

Table 7. Functional groups on the FTIR curve

Wavelength (cm^{-1})	% Transmitan (%T)			Functional groups
	Pulau Lima	Ujung Kulon	Binuangeun	
3427.51-3448.72	63.29	53.88	62,80	O-H stretching (hydrogen bonds between molecules)
1608.63	50.98	41.15	50.29	C=O stretching
1411.89	38.72	33.42	38.49	-C-OH stretching
1091-1093.64	53.62	41.02	48.50	COOH, C-O stretching, C-O-C stretching
1170	64.41	48.21	56.67	C-O stretching, C-C stretching, C-C-C bending
1029.99-1033.85	48.94	37.16	45.59	C-O stretching, C-O-C stretching
947.05	62.31	47.11	54.50	C-O stretching, C-C-H stretching
817.82-875.68	39.92	30.96	35.04	C-C stretching, C-C-H stretching, C-O bending

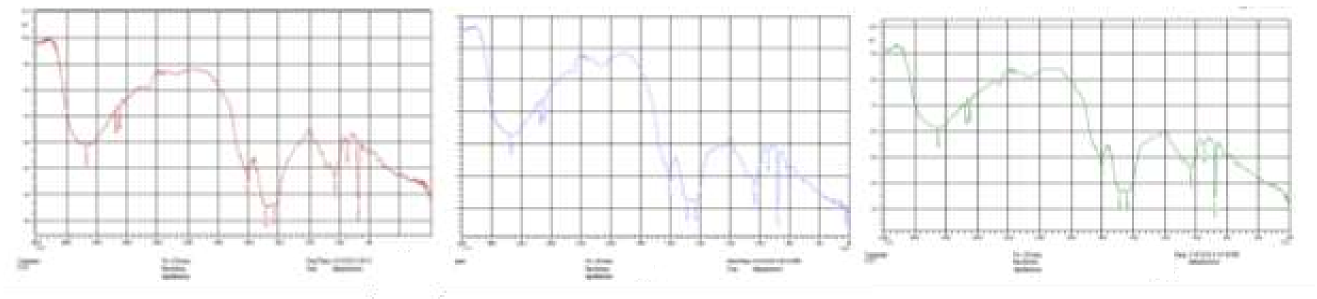
Partial hydrolysis of alginate

The results of isolation of mannuronic acid (M) and guluronate (G) on alginate molecules were carried out by partial hydrolysis of alginate, obtained by GG block deposits as presented in Table 6. The highest component of G in alginate is obtained from Lima Island with the results of viscosity test as listed in Figure 8 and according to the functional group analysis which is qualitatively proven on the FTIR curve as shown in Figure 10.

Analysis of functional groups of sodium alginate from extraction of *Sargassum polycystum* from three locations

Uptake of functional groups from *S. polycystum* from three locations can be seen in the FTIR curve presented in Figure 10 and Table 7. The sodium alginate spectrum showed the presence of hydroxyl (-OH) groups, carbonyl groups (-COO-),-C-OH and-COOH bonds, C-O stretching, C-C stretching, and C-O bending as seen in FTIR curves (Figure 10). According to Alvares et al. (2018), absorption at wavelengths of 1608.63 cm^{-1}, 1411.89 cm^{-1} and 1091 cm^{-1} - 1093.64 cm^{-1} (in the wavelength area 1091 cm^{-1} - 1093,64 cm^{-1}, if higher than absorption at wavelength 1029 cm^{-1} - 1033 cm^{-1}, 947 cm^{-1} and 817,82 cm^{-1} - 875,68 (at the wavelength area of 1029 cm^{-1} to 817 cm^{-1}) indicates that the alginate polymer consists of a higher proportion of guluronic monomers, whereas absorption at wavelengths of 1315 cm^{-1}, 1170 cm^{-1}, 1029 cm^{-1} - 1033 cm^{-1}, 947 cm^{-1} and 817.82-875.68 cm^{-1} were higher indicating that the alginate polymer consisted of the proportion of mannuronic monomers.

In the results of the FTIR curve showed that the absorption at wavelength 1608.63 cm^{-1}, 1411.89 cm^{-1}, and 1091 cm^{-1} - 1093.64 cm^{-1} in Binuangeun alginate products provide higher absorption compared to Lima Island and Ujung Kulon alginates. However, the results of the GG block partial deposit test (Table 8) showed that alginate extracted from Binuangeun had the lowest guluronate group (41.40%). This is possible at wavelength of 1029.99 cm^{-1} -1033.85 cm^{-1} giving quite high uptake of C-O stretching and C-O-C stretching. Whereas, Lima Island and Ujung Kulon alginate products showed a higher proportion of guluronate monomers than mannuronate. According to the quantitative test of GG block, partial deposits for Lima Island, Ujung Kulon and Binuangeun resulted in 67.60%, 59.00%, and 41.40%, respectively.



A **B** **C**

Figure 10. FTIR curve of alginate: A. Lima Island, B. Ujung Kulon, C. Binuangeun

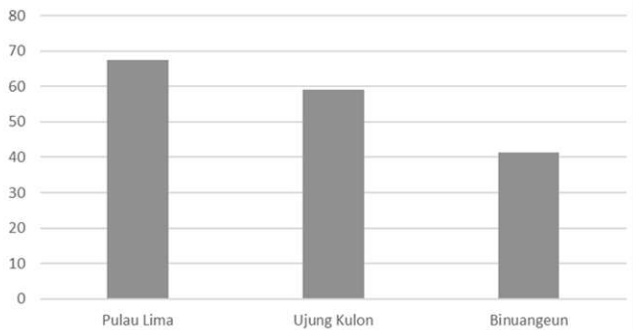


Figure 11. Results of GG block deposition test on alginate structure (%)

In conclusion, the combination of viscosity and gel strength of guluronate and mannuronate is the determinant factors to produce hydrogel than other characteristics, while the other characters are supporting ones. The results of this study showed that the yields of alginate from *Sargassum polycystum* from Lima Island, Ujung Kulon and Binuangeun were 11.48%, 18.62%, and 5.75%, respectively. Alginate from Binuangeun has the best physicochemical characteristics compared to the others. The viscosity of alginate from Lima Island, Ujung Kulon and Binuangeun were 35 cP, 62.50 cP, and 81.33cP, respectively. The characteristics of Na alginate extracted from Binuangeun have relatively higher quality. Alginate characteristics extracted from Lima Island, Ujung Kulon and Binuangeun have met alginate standards as food grade, but when viewed from viscosity, Binuangeun has the highest viscosity according to the needs of the non-food industry. The results of the partial alginate hydrolysis test showed that guluronic block (GG) in alginate polymers on the Lima Island, Ujung Kulon and Binuangeun were 67.60%, 59.00%, and 41.40%, respectively. This is related to the gel properties formed. Alginate from Lima Island tends to be stiffer and less flexible than alginate from Ujung Kulon and Binuangeun. Our study showed that there were variations in the concentration of mannuronate and guluronate from the three habitats of *Sargassum* in Banten.

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