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Characteristics of alginate content on *Sargassum polycystum* C.A. Agardh from western Java, Indonesia

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Abstract. Utilization of *Sargassum polycystum* seaweed as an alternative alginate source will reduce dependence on alginate imports, which is currently still 100% imported. Thus, the purpose of this study was to characterize alginates from *S. polycystum* seaweed obtained from three locations with different ecological characteristics. Alginate isolation by partial hydrolysis separated guluronic acid (G) and manuronic acid (M) followed by freeze-dried and measured qualitatively and quantitatively using FTIR. A standard curve was made to calibrate the concentration of alginate in each location. The results showed that alginate rendement from *S. polycystum* of Lima Island, Ujung Kulon, and Binuangeun were 11.48, 18.62, and 5.75%, respectively. The linear regression equation of alginate polymer composition of M/G from Lima Island, Ujung Kulon, and Binuangeun standard curve were $y = -14.171x + 68.13$ $R^2 = 0.9242$, $y = -6.6279x + 33.776$ $R^2 = 0.9811$ and $y = -9.6763x + 59.558$ $R^2 = 0.9042$ respectively. The concentrations of alginate polymers on Lima Island, Ujung Kulon and Binuangeun M/G % were 1.35%, 1.44%, and 2.33%, respectively. It can be concluded that the variations in the concentration of manuronic and guluronic from the three ecologies of *S. polycystum* in western of Java were different variations.

Keywords: alginate, ecological characteristics, *S. polycystum*

1. Introduction

According to BPS (2018), Indonesia imported about 1,650 tons of alginate every year. As much as 50% of the imported alginate was used for textile industry, 30% for food, 6% for paper production, 5% for welding rods production, and the other 5% for pharmaceutical purposes (Kusumawati 2018). There is an opportunity to increase alginate production in Indonesia to manage alginate resources sustainability but this will need information about *S. polycystum* and its contents. The genus of *Sargassum* consists of 400 species while in Indonesia there are 12 species named *S. duplicatum*, *S. hitrix*, *S. echinocarpum*, *S. gracillinum*, *S. obtusifolium*, *S. binderi*, *S. polycystum*, *S. microphyllum*, *S. crassifolium*, *S. aquafolium*, *S. vulgare*, and *S. polyceratium* (Kadi 2005). *S. polycystum* is an alginate-producing seaweed. So far, *S. polycystum* grow wild and have not been cultivated in Indonesia. This study was aimed to obtain the alginate's content characteristics qualitatively and quantitatively from *S. polycystum* in western of Java so that the relationship between alginate contents of *Sargassum* and its



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Locations can be revealed. This aim was achieved through isolation and partial characterization of alginate extracted from *S. polycystum* collected from Lima Island, Ujung Kulon, and Binuangeun waters to identify the contents of sodium alginate based on the chemical composition of mannuronate and guluronate by linear regression analysis equation.

2. Materials and methods

The study was carried out in February 2018 until June 2019 in western of Java, Indonesia. There were three sampling locations, i.e., Lima Island ($6^{\circ}00'05''$ S, $106^{\circ}09'18''$ E), Ujung Kulon ($6^{\circ}48'15''$ S, $105^{\circ}29'5''$ E), and Binuangeun ($6^{\circ}49'16''$ S, $105^{\circ}56'14''$ E). The location of *S. polycystum* sampling is presented in figure 1. The geographical conditions of western Java are surrounded by three major water, i.e., the Java Sea in the north, the Sunda Strait in the west, and the Indian Ocean in the south.



Figure 1. Three sampling locations, i.e., (1) Lima Island ($6^{\circ}00'05''$ S, $106^{\circ}09'18''$ E), (2) Ujung Kulon ($6^{\circ}48'15''$ S, $105^{\circ}29'5''$ E), and (3) Binuangeun ($6^{\circ}49'16''$ S, $105^{\circ}56'14''$ E).

2.1. Materials

Three samples of *S. polycystum* from each location were prepared for the extraction process. The extraction process used sodium carbonate, calcium chloride, hydrochloric acid, alcohol 70%, hydrogen peroxide, distilled water, Ca_2Cl_2 4%, HCl 2%, Na_2CO_3 34%, Ca_2Cl_2 10%, Ca_2Cl_2 5%, HCl 5%, and alcohol 95% while partial hydrolyzation used HCl 37% and NaOH 5 mol and p.a grade chemicals for the analysis of alginate monomers. The measurement equipment needed were viscometer (Brookfield), FTIR (Shimadzu) and spectrophotometer (Shimadzu).

2.2. Sampling preparation

Sample collection and identification of *S. polycystum* were conducted during the lowest tide at each studied location. Samples were collected using the 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 was undertaken in Chemistry Laboratory, Department of Chemistry, University of Indonesia, Depok, Indonesia.

2.3. Laboratory analysis

Quality analysis of *S. polycystum* was rendement, water content, and impurities. Character analysis of alginate from *S. polycystum* was rendement, viscosity, water content, ash content, color, and pH. FTIR analysis was done to isolation of mannuronic acid and guluronate acid.

2.4. Alginate extraction and characterization

Prior to alginate extraction, the seaweed was soaked in CaCl_2 solution to dissolve laminarin, mannitol, dyes, and salts. The alginate was extracted under alkaline condition using Na_2CO_3 and NaOH. The Na-alginate produced was precipitated using isopropanol and was subsequently dried in an oven and grounded to form sodium alginate flour. The characteristic of sodium alginate was observed using FTIR.

Partial hydrolysis of alginate was carried out 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. The insoluble-fraction was further dissolved by adding alkali solution and fractionated by adjusting the pH at 2.85. The result of partial hydrolysis was dried by freeze drier. Analysis of alginate functional groups was carried out using FTIR spectrophotometer (Perkin Elmer, spectrum one). Samples plus KBr (1:100) was mashed until evenly mixed. Then it was pressed with a vacuum pump for 15 minutes, and read the absorbance at wavelengths of $400\text{-}4,000\text{ cm}^{-1}$. From the resulted curve, the type of bond and its functional group were determined based on FTIR references. Alginate has a peak at wavelength 1,030/1,080 nm.

2.5. Standard curve

M/G concentration of 1 g alginate were varied from 0, 25, 50, 75, and 100% then the transmittances were measured. By plotting alginate concentration as x axis and transmittance as y axis, regression equations were obtained. Determination of M/G concentrations from alginate samples was done based on optimum conditions for previous observation.

3. Results and discussion

3.1. Viscosity

The highest alginate viscosity obtained from *S. 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 low alginate viscosity was caused by the low purity of the alginate produced. Na-alginate viscosity is divided into three levels, namely low viscosity (<60 cP), medium viscosity (60-110 cP) and high viscosity (110-800) cP. Based on this definition, the viscosity of Na-alginate from Lima Island was categorized as low viscosity. Sodium alginate for alginate extraction. *S. polycystum* from Ujung Kulon had the highest Na-alginate content ($18.62\% \pm 0.84\%$) followed by that from Lima Island with an average $11.48\% \pm 0.79\%$ which was likely influenced by the cleanliness of the location which consists only of sand and coral. In contrast, samples from Binuangeun had the lowest Na-alginate yield ($5.75\% \pm 0.11\%$) which might be influenced by the amount of sand, rock, coral and litter contained because it is close to human settlement. 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* 2013). Because this study used the same treatment across three locations, habitat and sea currents were likely the influencing factors on the yield of alginate.

Binuangeun has shallow water with a depth of 40.00 cm so the shortest total thallus length was 31.82 cm. The habitat where *Sargassum* has grown was 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 *S. polycystum* in Binuangeun is shorter than in Ujung Kulon and Lima Island. 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 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. Differences locations of *S. polycystum* grown might be one of the causes of the difference in the value of the resulting viscosity (Hamrun 2018). 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 (McHugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80°C, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the viscosity using rapid visco analyzer (RVA). Anggadiredja (2008) stated that the higher drying temperature, the higher viscosity value. It is assumed that increasing drying temperature will increase more sulfate esters so that viscosity will increase.

3.2. 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 (Yamamoto *et al* 2011). GG block deposits were obtained as listed in table 1.

Table 1. Alginate partial hydrolysis results.

Locations of <i>S. polycystum</i>	Blok MM	Blok GG	Blok MG	Blok M	Blok G
Lima Island	22.00	77.00	1.00	23	77
Ujung Kulon	28.70	62.00	9.30	30	70
Binuangeun	47.00	45.00	8.00	47	53

The highest G component was alginate from Lima Island which had a relatively low viscosity and stiff, compared to Ujung Kulon and Binuangeun. Thus this results matched with the results of viscosity measurements as shown in figure 2 and they matched with the results of functional group analysis which were qualitatively proven by the FTIR curve as presented in figure 3 and table 2.

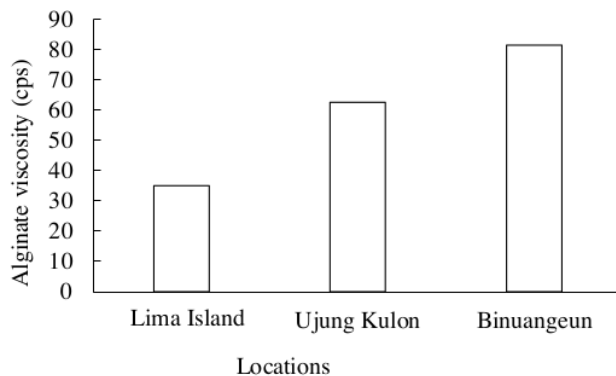


Figure 2. Test results for alginate viscosity at the extraction of *S. polycystum* (cps).

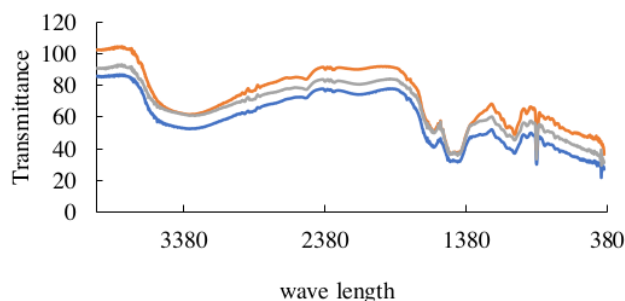


Figure 3. FTIR Curve of Alginate from Lima Island, Ujung Kulon, Binuangeun, — Ujung Kulon, — Pulau Lima, — Binuangen.

Table 2. The functional groups on th FTIR.

Wavelength cm ⁻¹	% Transmitan (% T)			Functional group
	Pulau Lima	Ujung Kulon	Binuangeun	
3427.51-3448.72	63.29	62.80	53.88	O-H stretching
1608.63	50.98	50.29	41.15	C=O
1411.89	38.72	38.49	33.42	ending -C-OH
1091-1093.64	53.62	48.50	41.02	COOH, C-O stretching
1170	64.41	56.67	48.21	O-C stretching
				C-O stretching
				C-C stretching
1029.99-1033.85	48.94	45.59	37.16	C-C bending
				C-O stretching
				O-C stretching
947.05	62.31	54.50	47.11	C-O stretching
817.82 -875.68	39.92	35.04	30.96	C-H stretching
				C-C stretching
				C-C-H stretching
				C-O bending

A calibration curve was made to see the linearity between concentration of analytes in samples with regions measure given. Linearity was evaluated from graph, namely by plotting absorbance as a function of analyte concentration, which is normally called a calibration curve (figures 4-6).

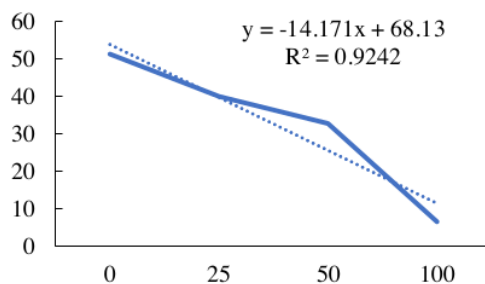


Figure 4. Alginate's Lima Island M/G calibration curve (before pre-concentration).

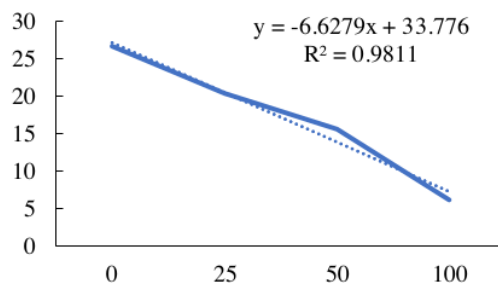


Figure 5. Alginat's Ujung Kulon M/G calibration curve (before pre-concentration).

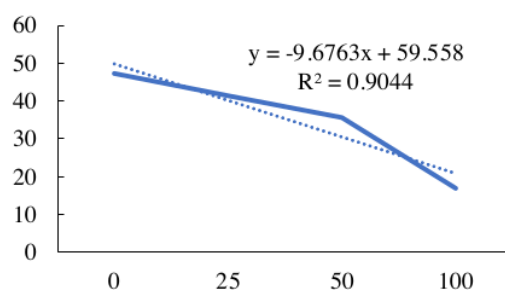


Figure 6. Alginat's Binuangeun M/G calibration curve (before pre-concentration).

The results of the partial alginate hydrolysis test showed that alginate polymers on Lima Island, Ujung Kulon and Binuangeun M/G % were 1.35, 1.44, and 2.33%, respectively. Our study showed that there were variations in the concentration of mannuronate and guluronate from the three habitats of *Sargassum* in western Java. It can be concluded that the variations in the concentration of manuronic and guluronic from the three ecologies of *Sargassum polycystum* in Western of Java were different variations.

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