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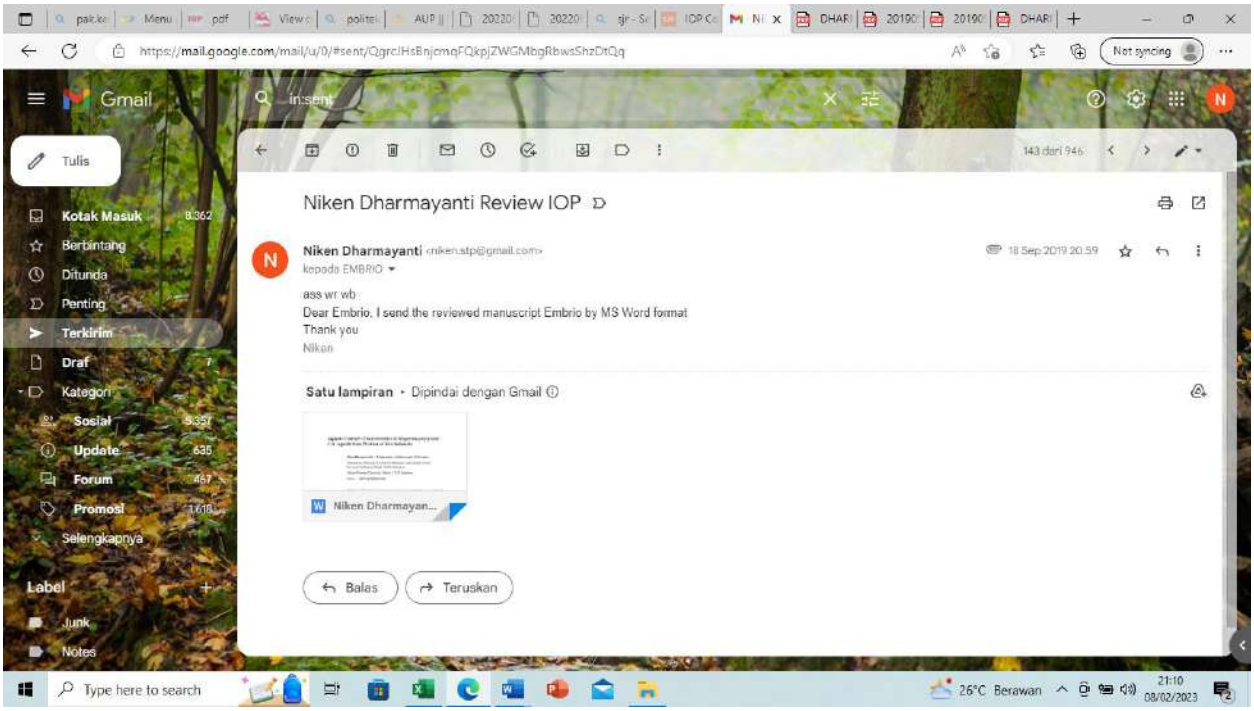


Alginate Content's Characteristics of *Sargassum polycystum* C.A. Agardh from Western of Java Indonesia

Niken Dharmayanti[1,2] , J.Supriatna[1], A.Abinawanto[1], Y.Yasman[1]

[1]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Indonesia, Depok, 16424, Indonesia

[2]Jakarta Fisheries University, Jakarta, 12520, Indonesia

Email : niken.stp@gmail.com

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 gulurunic acid (G) and manurunic acid (M) followed by freeze dried and measured qualitatively and quantitatively using FTIR. Standard curve was made to calibrate the concentration of Alginate in each location. The results showed that alginat 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 the 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: alginat, characteristics, ecologies, *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 opportunity to increase alginate production in Indonesia to manage alginate resources sustainability but this will need information of *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. obtuspfolium, S. binderi, S. polycystum, S. microphylum, 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 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 Juny 2019 in western of 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.

Figure 1. 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).

2.1 Materials

Three samples of *Sargassum polycystum* from each location were prepared for extraction process. The extraction process used natrium carbonate, calcium chloride, chloride acid, alcohol 70%, peroxide hydrogen, aquadest, Ca2Cl2 4%, HCl 2%, Na2CO 34%, Ca2Cl210%, Ca2Cl2 5%, HCl 5%, dan Alkohol 95% while partial hydrolization 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 Prestige Fourier Transform Infrared Spectroscopy) 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 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.

2.2.2. Laboratory analysis

Quality analysis of *S. polycystum* were rendement, water content, CAW and impurities. Character analysis of alginate from *S. polycystum* were rendement, viscosity, water content, ash

content,colour and pH. FTIR analysis was done to isolation of mannuronic acid and guluronate acid.

2.3. Data collection

Seaweed was soaking 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 Silva et al. (2015), alginic acid precipitated under the conditions of pH <3 in which 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). The brown seaweed extraction process was carried out in alkaline conditions. The goal was to separate the cellulose content from alginate. The extracting materials were Na2CO3 and NaOH. Lee et.al (2005) stated 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 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. In the formation of sodium alginate, alginic acid that had been formed was added with alkaline solution containing Na+ions such as NaOH and Na2CO3. The purpose of the formation of sodium alginate was to get a more stable alginate compound. According to Mc Hugh (2008), the exchange of H+ions with Na+ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid. Withdrawal of Na-alginate compounds from sodium alginate solution was be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonym (1976), 1% sodium alginate starts to separate in a solution of 10% isopropanol or in ethanol 20%. 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 was 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. Measurements observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test and functional groups analysis with FTIR. Alginate is a compound contained in brown seaweed cell walls (*Phaeophyceae*) other than cellulose and pectin. 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. 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. Hydroxyl bond between M and G was easily hydrolyzed by acid. Insoluble-fraction was more resistant to acid hydrolysis, so it was dissolved by adding alkali and fractionated by adjusting the pH at 2.85, so that the GG block settled and the MM block dissolved. The result of partial hydrolysis were dried by freeze drier. Analysis of alginate functional groups was carried out using a Fourier Transform Infrared (FTIR) spectro-photometer (Perkin Elmer, spectrum one). 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. Two 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 transmittance was measured at 4000-400nm wavelength. Alginate was at peak at wavelength 1030/1080 nm.

2.2.4. Standard Curve

M/G concentration of 1 g alginate were varied from 0%; 25%; 50%; 75%; 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 as the procedure previous. The transmittance obtained from samples were plotted to regression equations and got M/G concentrations.

3. RESULTS AND DISCUSSION

3.1 Viscosity

The highest alginate's viscosity obtained from *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 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%±0.84%) followed by that from Lima Island with an average 11.48%±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%±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, so habitat and sea currents were likely the influencing factors on the yield of alginate. Binuangeun has shallow water with depth of 40.00 cm so the shortest total thallus length was 31.82 cm. The habitat where *Sargassum* 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 *Sargassum 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 (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. Differences locations of *Sargassum 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 (Mc Hugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80^{o}C, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the viscosity using RVA (Rapid Visco Analyzer). 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.

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 *S. polycystum*	**Blok MM**	**Blok GG**	**Blok MG**	**Blok M**	**Blok G**
Pulau Lima	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.

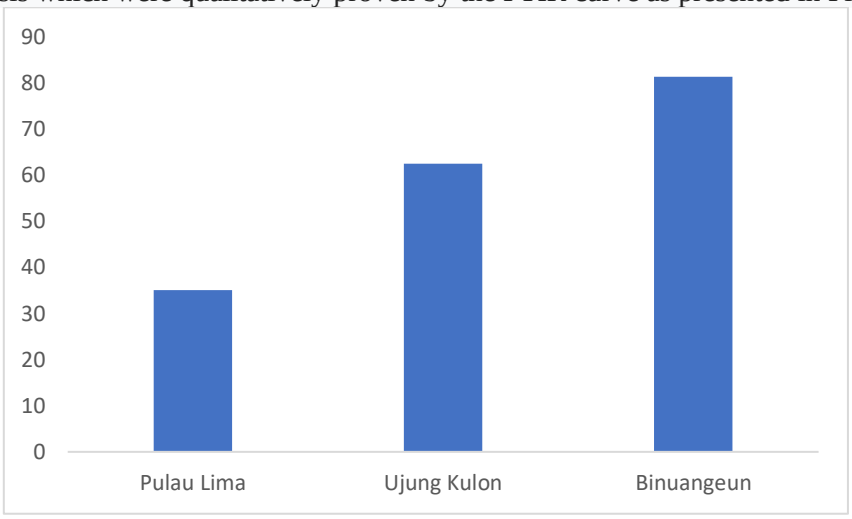


Figure 2. Test results for alginate viscosity at extraction of *S. polycystum* (cps)
Qualitative test *Sargassum* by FTIR

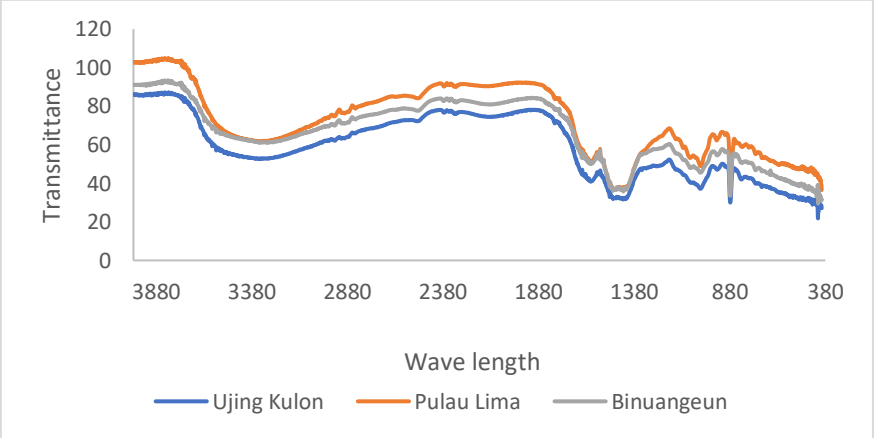


Figure 3. FTIR Curve of Alginat from Lima Island, Ujung Kulon, Binuangeun

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 streching
1608.63	50.98	50.29	41.15	C=O
1411.89	38.72	38.49	33.42	bending -C-OH
1091-1093.64	53.62	48.50	41.02	COOH, C-O streching C-O-C streching
1170	64.41	56.67	48.21	C-O streching C-C stretching C-C-C bending
1029.99 – 1033.85	48.94	45.59	37.16	C-O stretching C-O-C stretching
947.05	62.31	54.50	47.11	C-O stretching

Wavelength cm-1	% Transmitan (% T)			Functional group
	Pulau Lima	Ujung Kulon	Binuangeun	
817.82 – 875.68	39.92	35.04	30.96	C-C-H stretching C-C stretching C-C-H stretching C-O bending

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 normal called a calibration curve.

Alginate's Lima Island M/G calibration

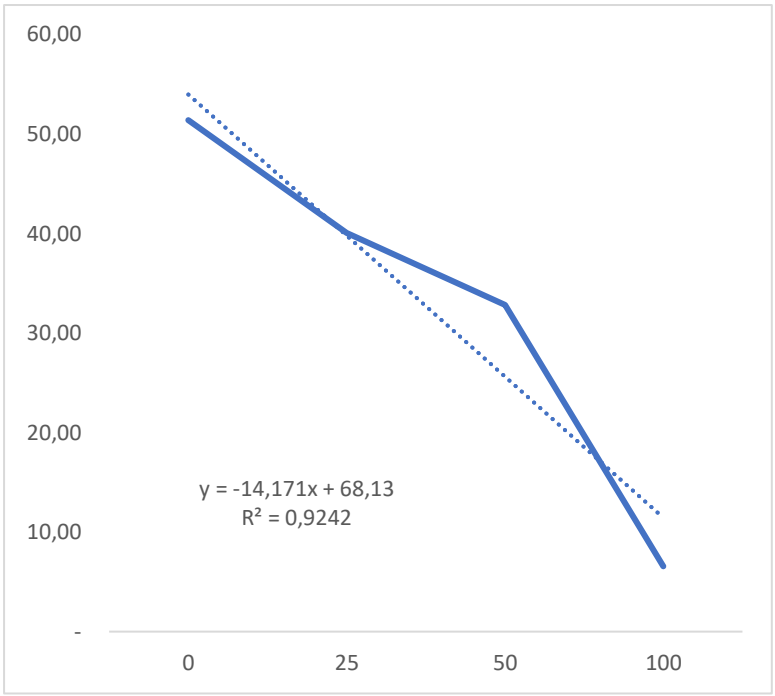


Figure 4. Alginate's Lima Island M/G calibration Curve (Before Pre-concentration)

The regression equation of Alginate's Lima Island was y = -14,171x + 68,13 with a correlation coefficient (R) = 0.9242.

Alginate's Ujung Kulon calibration

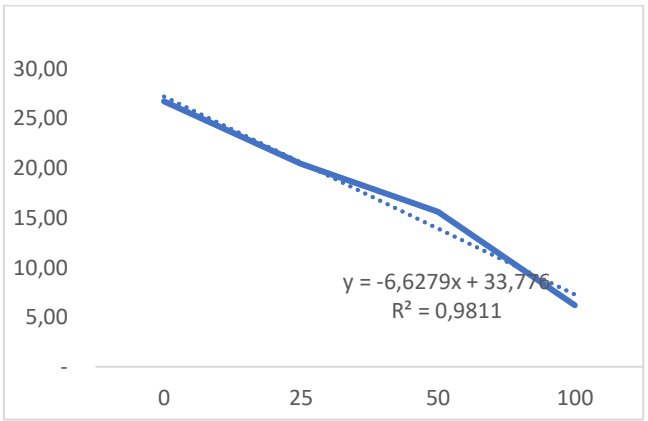


Figure 5. Alginate's Ujung Kulon M/G calibration Curve (Before Pre-concentration)

The regression equation of Alginate's Ujung Kulon was y = -6,6279x + 33,776 with a correlation coefficient (R) = 0.9811.
Alginate's Binuangeun

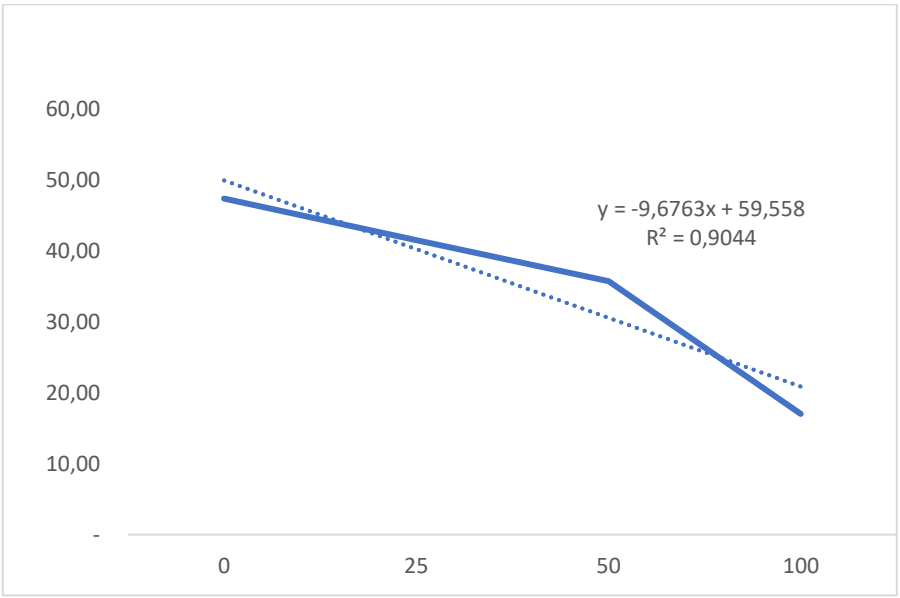


Figure 6. Alginate's Binuangeun M/G calibration Curve (Before Pre-concentration)

The regression equation of Alginate's Binuangeun was y = -9,6763x + 59,558 with a correlation coefficient (R) = 0.9044.
The results of the partial alginate hydrolysis test showed that alginate polymers on the 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 of 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.

ACKNOWLEDGEMENTS
We thank Ms. Petra Spliethoff and the NICHE Project CDI Team (Center for Development Innovation), Wageningen University & Research, Netherlands who have provided doctoral scholarship assistance at the University of Indonesia, Depok. We also thank Research Center for Oceanography, LIPI, Jakarta, Research Center for Biology, LIPI, Cibinong, Bogor, Research Center for Fisheries Biotechnology and Processing, KKP, Jakarta, KKP Loka Ujung Kulon, TPI Binuangeun, BAPPL Karangantu, Biology, Environment, Chemistry and Biotechnology Laboratory of STP, Jakarta, Molecular-Biology and Chemistry Laboratory of FMIPA UI and ILRC UI Jakarta which have helped carry out this research

REFERENCES
Anggadiredja J, Zatnika A, Purwoto H. Istini S 2008, Rumput Laut. Jakarta, Penebar Swadaya.
Anonym, 1976. Keto Algin Hydrophilic Derivation of Alginic Acid for Scientific Water Control 2nd ed New York. Kelco Division of Merck and Co.
Basmal J., Utomo B. S. B., Tazwir, Murdiyanah, Wikanta T., Marraskuranto E., Kusunawati R., 2013 [Producing alginate from seaweed *Sargassum*]. Penebar Swadaya, 91 pp. [in Indonesian]

BPS [Badan Pusat Statistik], 2018. Statistik Indonesia 2018. Badan Pusat Statistik, Jakarta.

Hamrun N, Thalib B, Tahir D, Kasim S, Nugraha AS. 2018. Physical characteristics test (water content and viscosity) of extraction sodium alginate brown algae (phaeophyta) species padina sp. as basic material for production dental impression material. J Dentomaxillofacial Science 3:84 - 87.

Kadi, A. 2005. Beberapa Catatan Kehadiran Marga Sargassum di Perairan Indonesia. Jakarta: Bidang Sumberdaya Laut, Puslitbang Oseanologi-LIPI

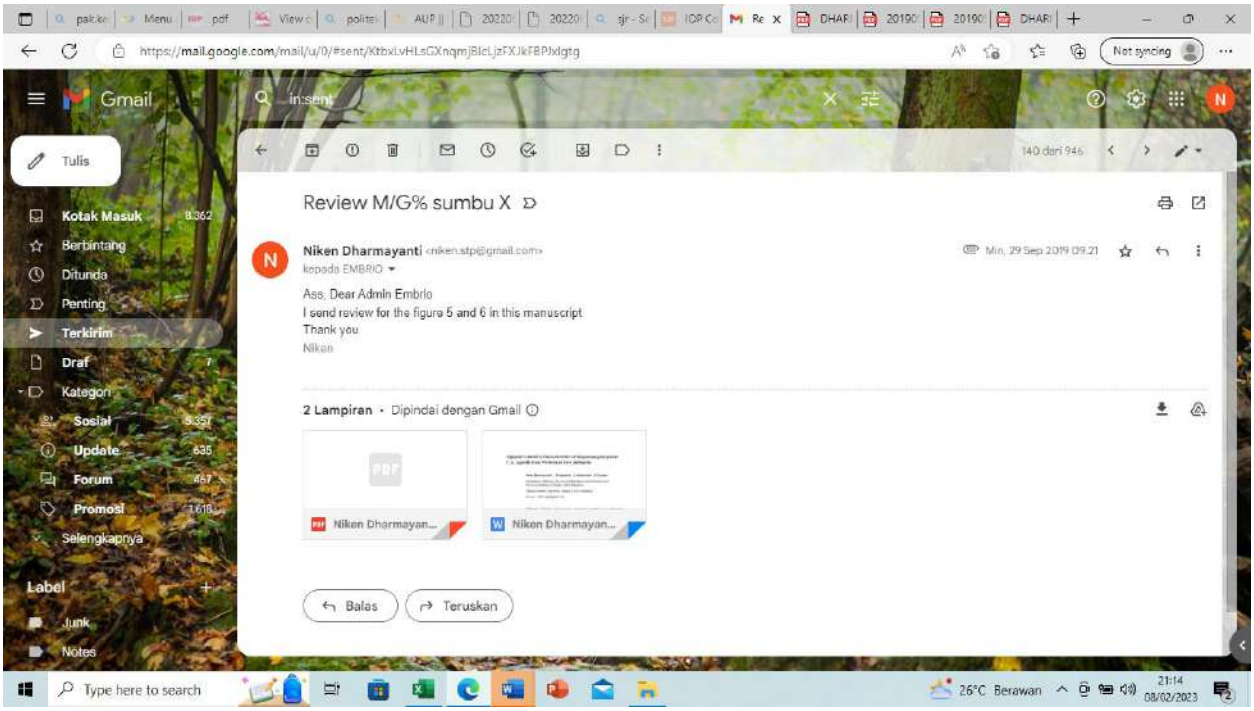
Kusumawati R., Basmal J., Utomo B.B. (2018). Physicochemical Characteristics Of Sodium Alginate Extracted From Turbinaria Sp. And Sargassum sp. Squalen Bull. of Mar. and Fish. Postharvest and Biotech. 13 (2) 2018, 79-84

Lee I-Min, Eric J Shiroma, Felipe Lobelo, Pekka Puska, Steven N Blair, Peter T Katzmarzyk, 2012. Eff ect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet 2012; 380: 219–29

Mc Hugh, 2008. Production, properties and uses of alginates New South Wales Univ., Campbell (Australia). Dept. of Chemistry)

Yamamoto, K., Ishikawa, C., Katano, H., Yasumoto, T., and Mori, N. (2011). Fucoxanthin and Its Deacetylated Product, Fucoxanthinol, Induce Apoptosis of Primary Effusion Lymphomas. Cancer Letters, 300, 225-234

Perbaikan Review 29/09/2019



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3. RESULTS AND DISCUSSION

3.1 Viscosity

The highest alginate's viscosity obtained from *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 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, so habitat and sea currents were likely the influencing factors on the yield of alginate. Binuangeun has shallow water with depth of 40.00 cm so the shortest total thallus length was 31.82 cm. The habitat where *Sargassum* 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 *Sargassum 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 (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. Differences locations of *Sargassum 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 (Mc Hugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80^{o}C, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the viscosity using RVA (Rapid Visco Analyzer). 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.

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 *S. polycystum*	**Blok MM**	**Blok GG**	**Blok MG**	**Blok M**	**Blok G**
Pulau Lima	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.

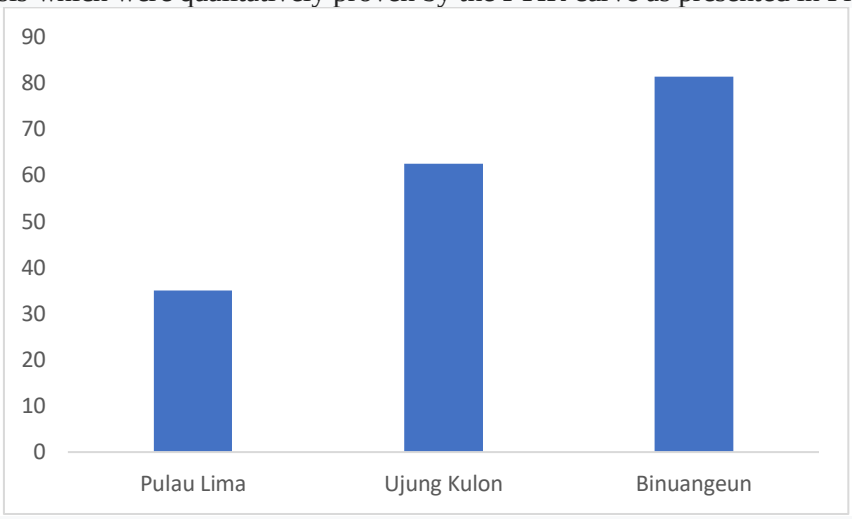

Figure 2. Test results for alginate viscosity at extraction of *S. polycystum* (cps)
Qualitative test *Sargassum* by FTIR

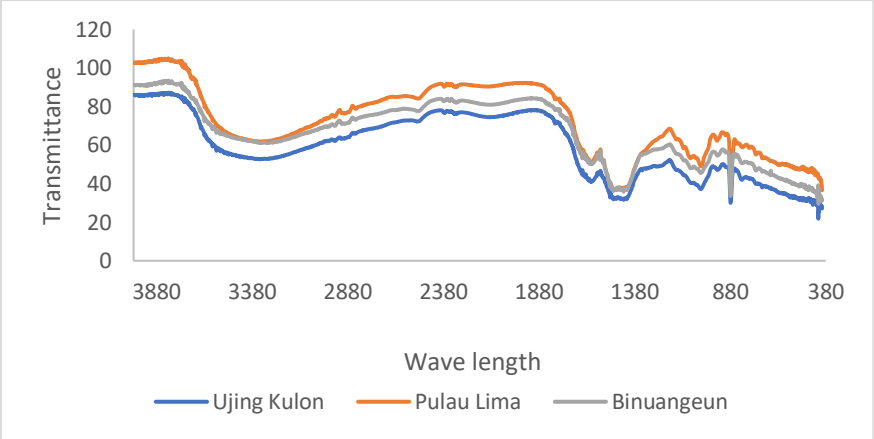

Figure 3. FTIR Curve of Alginat from Lima Island, Ujung Kulon, Binuangeun

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 streching
1608.63	50.98	50.29	41.15	C=O
1411.89	38.72	38.49	33.42	bending -C-OH
1091-1093.64	53.62	48.50	41.02	COOH, C-O streching C-O-C streching
1170	64.41	56.67	48.21	C-O streching C-C stretching C-C-C bending
1029.99 – 1033.85	48.94	45.59	37.16	C-O stretching C-O-C stretching
947.05	62.31	54.50	47.11	C-O stretching

Wavelength cm-1	% Transmitan (% T)			Functional group
	Pulau Lima	Ujung Kulon	Binuangeun	
817.82 – 875.68	39.92	35.04	30.96	C-C-H stretching C-C stretching C-C-H stretching C-O bending

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 normal called a calibration curve.

Alginate's Lima Island M/G calibration

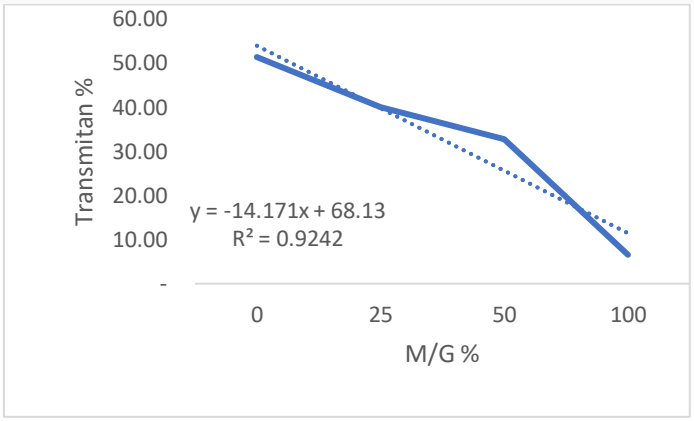

Figure 4. Alginate's Lima Island M/G calibration Curve

The regression equation of Alginate's Lima Island was y = -14,171x + 68,13 with a correlation coefficient (R) = 0.9242.

Alginate's Ujung Kulon calibration

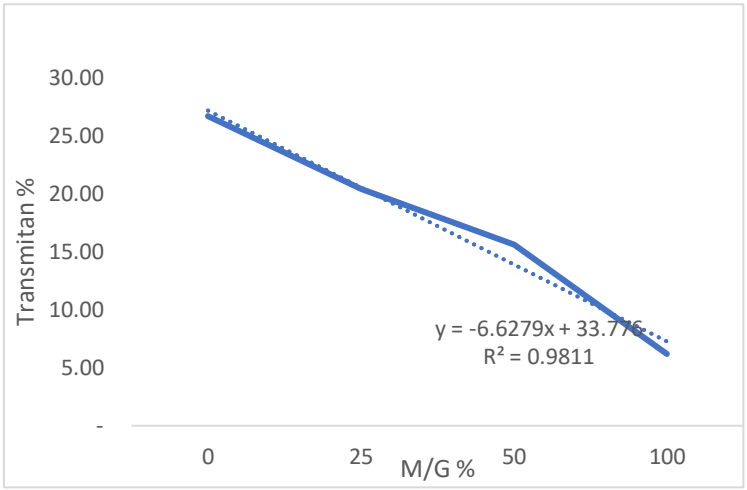

Figure 5. Alginate's Ujung Kulon M/G calibration Curve

The regression equation of Alginate's Ujung Kulon was y = -6,6279x + 33,776 with a correlation coefficient (R) = 0.9811.

content,colour and pH. FTIR analysis was done to isolation of mannuronic acid and guluronate acid.

2.3. Data collection

Seaweed was soaking 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 Silva et al. (2015), alginic acid precipitated under the conditions of pH <3 in which 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). The brown seaweed extraction process was carried out in alkaline conditions. The goal was to separate the cellulose content from alginate. The extracting materials were Na2CO3 and NaOH. Lee et.al (2005) stated 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 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. In the formation of sodium alginate, alginic acid that had been formed was added with alkaline solution containing Na+ions such as NaOH and Na2CO3. The purpose of the formation of sodium alginate was to get a more stable alginate compound. According to Mc Hugh (2008), the exchange of H+ions with Na+ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid. Withdrawal of Na-alginate compounds from sodium alginate solution was be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonym (1976), 1% sodium alginate starts to separate in a solution of 10% isopropanol or in ethanol 20%. 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 was 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. Measurements observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test and functional groups analysis with FTIR. Alginate is a compound contained in brown seaweed cell walls (*Phaeophyceae*) other than cellulose and pectin. 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. 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. Hydroxyl bond between M and G was easily hydrolyzed by acid. Insoluble-fraction was more resistant to acid hydrolysis, so it was dissolved by adding alkali and fractionated by adjusting the pH at 2.85, so that the GG block settled and the MM block dissolved. The result of partial hydrolysis were dried by freeze drier. Analysis of alginate functional groups was carried out using a Fourier Transform Infrared (FTIR) spectro-photometer (Perkin Elmer, spectrum one). 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. Two 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 transmittance was measured at 4000-400nm wavelength. Alginate was at peak at wavelength 1030/1080 nm.

2.2.4. Standard Curve

M/G concentration of 1 g alginate were varied from 0%; 25%; 50%; 75%; 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

Alginate's Binuangeun

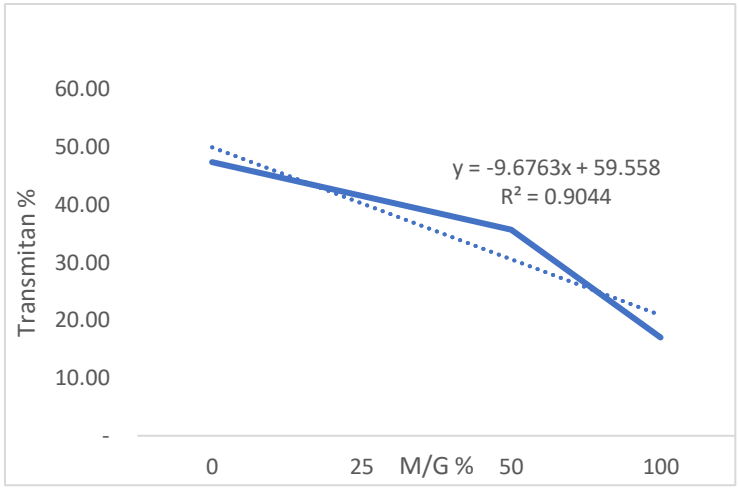

Figure 6. Alginate's Binuangeun M/G calibration Curve (Before Pre-concentration)

The regression equation of Alginate's Binuangeun was y = -9,6763x + 59,558 with a correlation coefficient (R) = 0.9044.

The results of the partial alginate hydrolysis test showed that alginate polymers on the 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 of 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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REFERENCES

Anggadiredja J, Zatnika A, Purwoto H. Istini S 2008, Rumput Laut. Jakarta, Penebar Swadaya.

Anonym, 1976. Keto Algin Hydrophilic Derivation of Alginic Acid for Scientific Water Control 2nd ed New York. Kelco Division of Merck and Co.

Basmal J., Utomo B. S. B., Tazwir, Murdiyanah, Wikanta T., Marraskuranto E., Kusunawati R., 2013 [Producing alginate from seaweed *Sargassum*]. Penebar Swadaya, 91 pp. [in Indonesian]

BPS [Badan Pusat Statistik], 2018. Statistik Indonesia 2018. Badan Pusat Statistik, Jakarta.

Hamrun N, Thalib B, Tahir D, Kasim S, Nugraha AS. 2018. Physical characteristics test (water content and viscosity) of extraction sodium alginate brown algae (phaeophyta) species padina sp.

as basic material for production dental impression material. J Dentomaxillofacial Science 3:84 - 87.
Kadi, A. 2005. Beberapa Catatan Kehadiran Marga Sargassum di Perairan Indonesia. Jakarta: Bidang Sumberdaya Laut, Puslitbang Oseanologi-LIPI
Kusumawati R., Basmal J., Utomo B.B. (2018). Physicochemical Characteristics Of Sodium Alginate Extracted From Turbinaria Sp. And Sargassum sp. Squalen Bull. of Mar. and Fish. Postharvest and Biotech. 13 (2) 2018, 79-84
Lee I-Min, Eric J Shiroma, Felipe Lobelo, Pekka Puska, Steven N Blair, Peter T Katzmarzyk, 2012. Eff ect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet 2012; 380: 219–29
Mc Hugh, 2008. Production, properties and uses of alginates New South Wales Univ., Campbell (Australia). Dept. of Chemistry)
Yamamoto, K., Ishikawa, C., Katano, H., Yasumoto, T., and Mori, N. (2011). Fucoxanthin and Its Deacetylated Product, Fucoxanthinol, Induce Apoptosis of Primary Effusion Lymphomas. Cancer Letters, 300, 225-234

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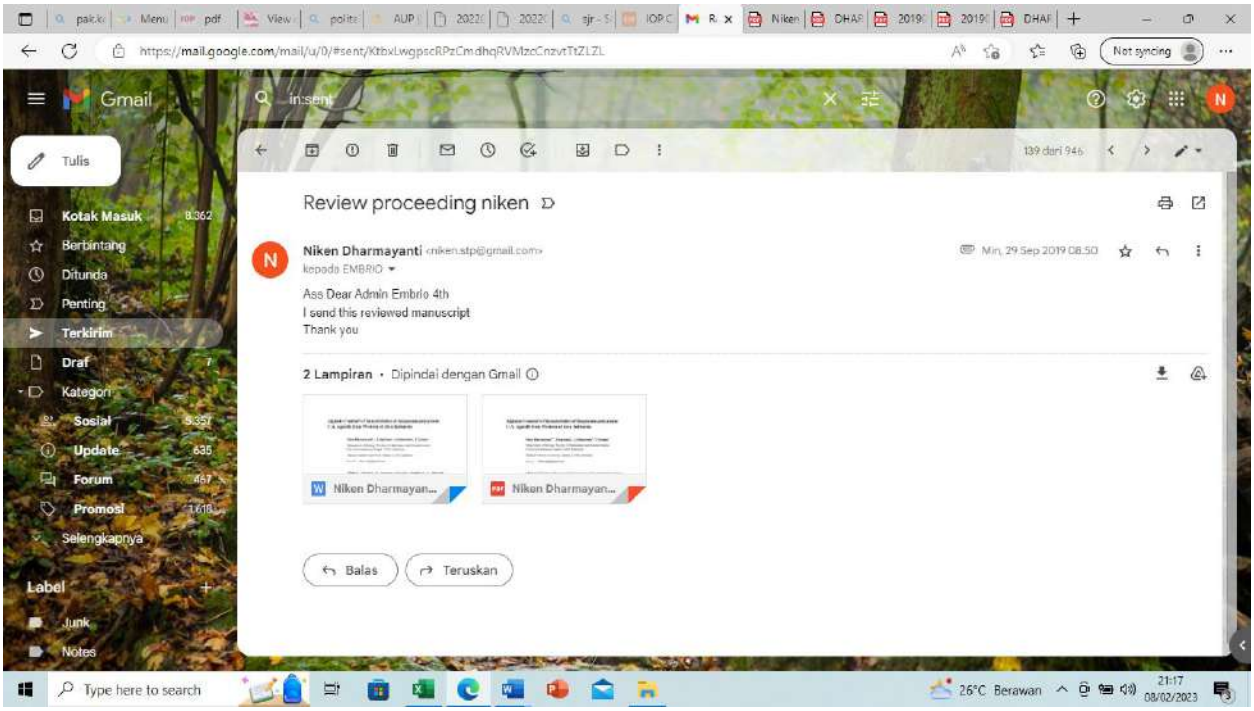

Alginate Content's Characteristics of *Sargassum polycystum* C.A. Agardh from Western of Java Indonesia

Niken Dharmayanti[1,2] , J.Supriatna[1], A.Abinawanto[1], Y.Yasman[1]

[1]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Indonesia, Depok, 16424, Indonesia

[2]Jakarta Fisheries University, Jakarta, 12520, Indonesia

Email : niken.stp@gmail.com

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 gulurunic acid (G) and manurunic acid (M) followed by freeze dried and measured qualitatively and quantitatively using FTIR. Standard curve was made to calibrate the concentration of Alginate in each location. The results showed that alginat 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 the 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: alginat, characteristics, ecologies, *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 opportunity to increase alginate production in Indonesia to manage alginate resources sustainability but this will need information of *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. obtuspfolium, S. binderi, S. polycystum, S. microphylum, 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 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 Juny 2019 in western of 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.

Figure 1. 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).

2.1 Materials

Three samples of *Sargassum polycystum* from each location were prepared for extraction process. The extraction process used natrium carbonate, calcium chloride, chloride acid, alcohol 70%, peroxide hydrogen, aquadest, Ca2Cl2 4%, HCl 2%, Na2CO 34%, Ca2Cl210%, Ca2Cl2 5%, HCl 5%, dan Alkohol 95% while partial hydrolization 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 Prestige Fourier Transform Infrared Spectroscopy) 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 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.

2.2.2. Laboratory analysis

Quality analysis of *S. polycystum* were rendement, water content, CAW and impurities. Character analysis of alginate from *S. polycystum* were rendement, viscosity, water content, ash

content,colour and pH. FTIR analysis was done to isolation of mannuronic acid and guluronate acid.

2.3. Data collection

Seaweed was soaking 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 Silva et al. (2015), alginic acid precipitated under the conditions of pH <3 in which 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). The brown seaweed extraction process was carried out in alkaline conditions. The goal was to separate the cellulose content from alginate. The extracting materials were Na2CO3 and NaOH. Lee et.al (2005) stated 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 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. In the formation of sodium alginate, alginic acid that had been formed was added with alkaline solution containing Na+ions such as NaOH and Na2CO3. The purpose of the formation of sodium alginate was to get a more stable alginate compound. According to Mc Hugh (2008), the exchange of H+ions with Na+ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid. Withdrawal of Na-alginate compounds from sodium alginate solution was be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonym (1976), 1% sodium alginate starts to separate in a solution of 10% isopropanol or in ethanol 20%. 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 was 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. Measurements observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test and functional groups analysis with FTIR. Alginate is a compound contained in brown seaweed cell walls (*Phaeophyceae*) other than cellulose and pectin. 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. 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. Hydroxyl bond between M and G was easily hydrolyzed by acid. Insoluble-fraction was more resistant to acid hydrolysis, so it was dissolved by adding alkali and fractionated by adjusting the pH at 2.85, so that the GG block settled and the MM block dissolved. The result of partial hydrolysis were dried by freeze drier. Analysis of alginate functional groups was carried out using a Fourier Transform Infrared (FTIR) spectro-photometer (Perkin Elmer, spectrum one). 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. Two 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 transmittance was measured at 4000-400nm wavelength. Alginate was at peak at wavelength 1030/1080 nm.

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M/G concentration of 1 g alginate were varied from 0%; 25%; 50%; 75%; 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 as the procedure previous. The transmittance obtained from samples were plotted to regression equations and got M/G concentrations.

3. RESULTS AND DISCUSSION

3.1 Viscosity

The highest alginate's viscosity obtained from *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 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%±0.84%) followed by that from Lima Island with an average 11.48%±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%±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, so habitat and sea currents were likely the influencing factors on the yield of alginate. Binuangeun has shallow water with depth of 40.00 cm so the shortest total thallus length was 31.82 cm. The habitat where *Sargassum* 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 *Sargassum 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 (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. Differences locations of *Sargassum 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 (Mc Hugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80^{o}C, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the viscosity using RVA (Rapid Visco Analyzer). 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.

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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

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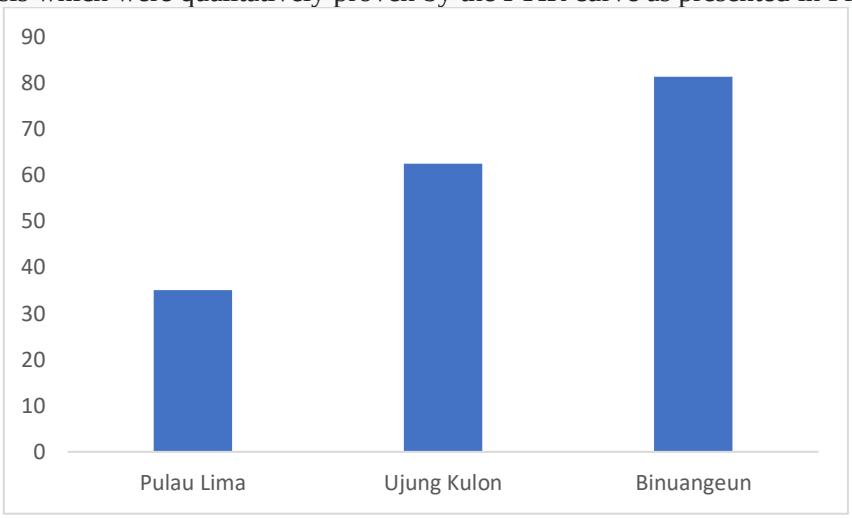

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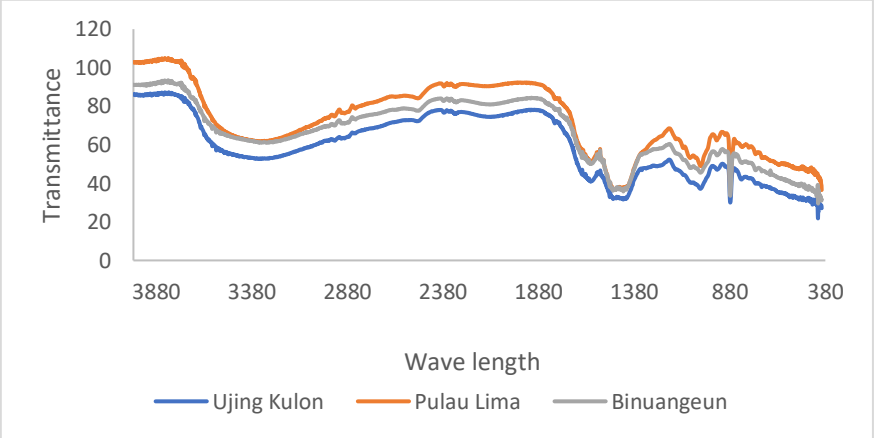

Figure 3. FTIR Curve of Alginat from Lima Island, Ujung Kulon, Binuangeun

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Wavelength cm-1	**% Transmitan (% T)**			**Functional group**
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3427.51-3448.72	63.29	62,80	53.88	O-H streching
1608.63	50.98	50.29	41.15	C=O
1411.89	38.72	38.49	33.42	bending -C-OH
1091-1093.64	53.62	48.50	41.02	COOH, C-O streching C-O-C streching
1170	64.41	56.67	48.21	C-O streching C-C stretching C-C-C bending
1029.99 – 1033.85	48.94	45.59	37.16	C-O stretching C-O-C stretching
947.05	62.31	54.50	47.11	C-O stretching

Wavelength cm-1	% Transmitan (% T)			Functional group
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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 normal called a calibration curve.

Alginate's Lima Island M/G calibration

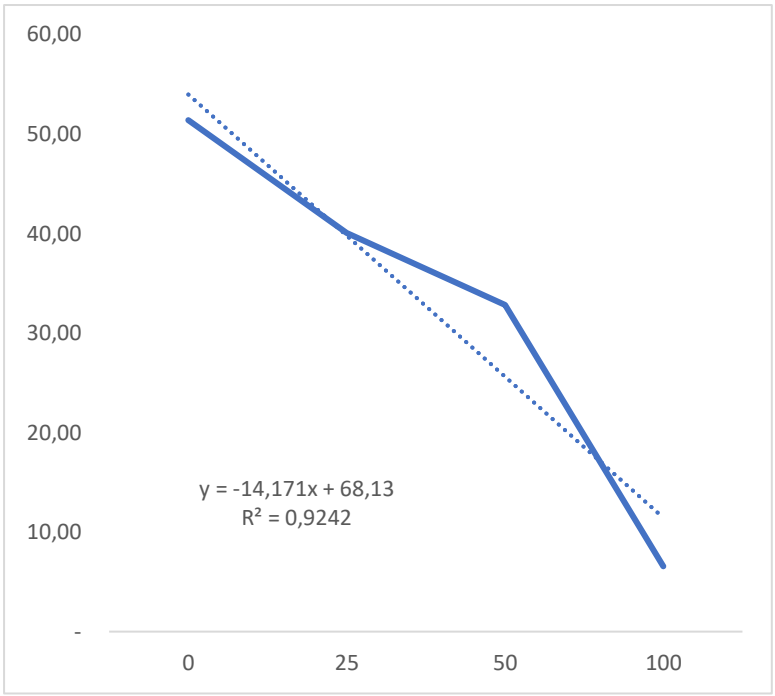

Figure 4. Alginate's Lima Island M/G calibration Curve (Before Pre-concentration)

The regression equation of Alginate's Lima Island was y = -14,171x + 68,13 with a correlation coefficient (R) = 0.9242.

Alginate's Ujung Kulon calibration

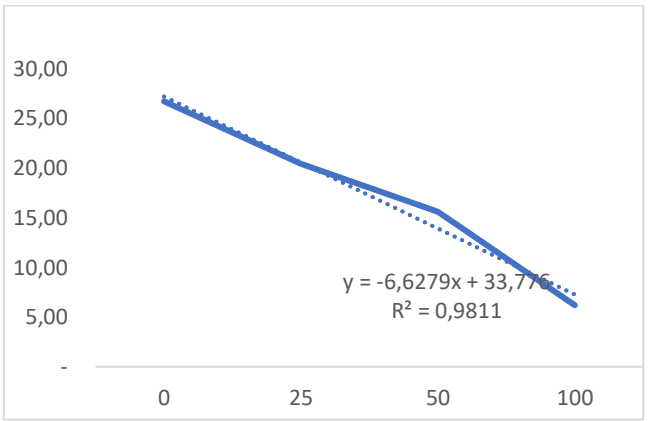

Figure 5. Alginate's Ujung Kulon M/G calibration Curve (Before Pre-concentration)

The regression equation of Alginate's Ujung Kulon was y = -6,6279x + 33,776 with a correlation coefficient (R) = 0.9811.
Alginate's Binuangeun

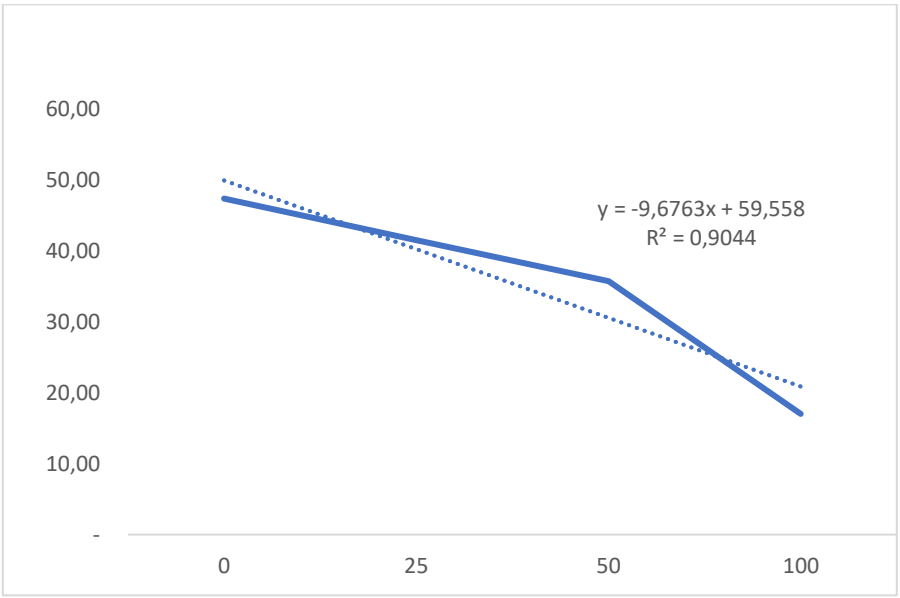

Figure 6. Alginate's Binuangeun M/G calibration Curve (Before Pre-concentration)

The regression equation of Alginate's Binuangeun was y = -9,6763x + 59,558 with a correlation coefficient (R) = 0.9044.
The results of the partial alginate hydrolysis test showed that alginate polymers on the 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 of 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.

ACKNOWLEDGEMENTS
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REFERENCES
Anggadiredja J, Zatnika A, Purwoto H. Istini S 2008, Rumput Laut. Jakarta, Penebar Swadaya.
Anonym, 1976. Keto Algin Hydrophilic Derivation of Alginic Acid for Scientific Water Control 2nd ed New York. Kelco Division of Merck and Co.
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BPS [Badan Pusat Statistik], 2018. Statistik Indonesia 2018. Badan Pusat Statistik, Jakarta.

Hamrun N, Thalib B, Tahir D, Kasim S, Nugraha AS. 2018. Physical characteristics test (water content and viscosity) of extraction sodium alginate brown algae (phaeophyta) species padina sp. as basic material for production dental impression material. J Dentomaxillofacial Science 3:84 - 87.

Kadi, A. 2005. Beberapa Catatan Kehadiran Marga Sargassum di Perairan Indonesia. Jakarta: Bidang Sumberdaya Laut, Puslitbang Oseanologi-LIPI

Kusumawati R., Basmal J., Utomo B.B. (2018). Physicochemical Characteristics Of Sodium Alginate Extracted From Turbinaria Sp. And Sargassum sp. Squalen Bull. of Mar. and Fish. Postharvest and Biotech. 13 (2) 2018, 79-84

Lee I-Min, Eric J Shiroma, Felipe Lobelo, Pekka Puska, Steven N Blair, Peter T Katzmarzyk, 2012. Eff ect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet 2012; 380: 219–29

Mc Hugh, 2008. Production, properties and uses of alginates New South Wales Univ., Campbell (Australia). Dept. of Chemistry)

Yamamoto, K., Ishikawa, C., Katano, H., Yasumoto, T., and Mori, N. (2011). Fucoxanthin and Its Deacetylated Product, Fucoxanthinol, Induce Apoptosis of Primary Effusion Lymphomas. Cancer Letters, 300, 225-234

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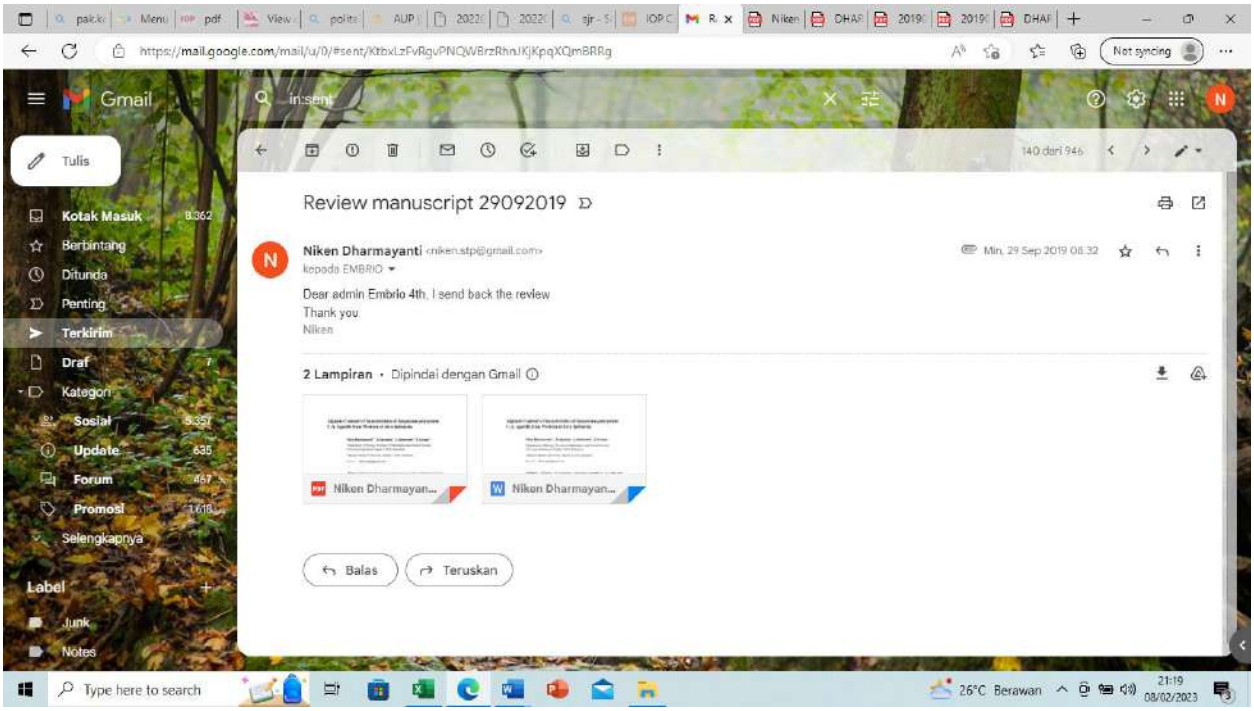

Alginate Content's Characteristics of *Sargassum polycystum* C.A. Agardh from Western of Java Indonesia

Niken Dharmayanti[1,2] , J.Supriatna[1], A.Abinawanto[1], Y.Yasman[1]

[1]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Indonesia, Depok, 16424, Indonesia

[2]Jakarta Fisheries University, Jakarta, 12520, Indonesia

Email : niken.stp@gmail.com

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 gulurunic acid (G) and manurunic acid (M) followed by freeze dried and measured qualitatively and quantitatively using FTIR. Standard curve was made to calibrate the concentration of Alginate in each location. The results showed that alginat 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 the 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: alginat, characteristics, ecologies, *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 opportunity to increase alginate production in Indonesia to manage alginate resources sustainability but this will need information of *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. obtuspfolium, S. binderi, S. polycystum, S. microphylum, 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 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 Juny 2019 in western of 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.

Figure 1. 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).

2.1 Materials

Three samples of *Sargassum polycystum* from each location were prepared for extraction process. The extraction process used natrium carbonate, calcium chloride, chloride acid, alcohol 70%, peroxide hydrogen, aquadest, Ca2Cl2 4%, HCl 2%, Na2CO 34%, Ca2Cl210%, Ca2Cl2 5%, HCl 5%, dan Alkohol 95% while partial hydrolization 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 Prestige Fourier Transform Infrared Spectroscopy) 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 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.

2.2.2. Laboratory analysis

Quality analysis of *S. polycystum* were rendement, water content, CAW and impurities. Character analysis of alginate from *S. polycystum* were rendement, viscosity, water content, ash

content,colour and pH. FTIR analysis was done to isolation of mannuronic acid and guluronate acid.

2.3. Data collection

Seaweed was soaking 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 Silva et al. (2015), alginic acid precipitated under the conditions of pH <3 in which 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). The brown seaweed extraction process was carried out in alkaline conditions. The goal was to separate the cellulose content from alginate. The extracting materials were Na2CO3 and NaOH. Lee et.al (2005) stated 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 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. In the formation of sodium alginate, alginic acid that had been formed was added with alkaline solution containing Na+ions such as NaOH and Na2CO3. The purpose of the formation of sodium alginate was to get a more stable alginate compound. According to Mc Hugh (2008), the exchange of H+ions with Na+ions runs slowly depending on the alkali speed penetrating into the particles of alginic acid. Withdrawal of Na-alginate compounds from sodium alginate solution was be done using alcohol. Alcohol commonly used is methanol (methyl alcohol) or isopropanol (isopropyl alcohol). According to Anonym (1976), 1% sodium alginate starts to separate in a solution of 10% isopropanol or in ethanol 20%. 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 was 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. Measurements observed in alginate included yield test, moisture content test, ash content test, viscosity test, pH test and functional groups analysis with FTIR. Alginate is a compound contained in brown seaweed cell walls (*Phaeophyceae*) other than cellulose and pectin. 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. 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. Hydroxyl bond between M and G was easily hydrolyzed by acid. Insoluble-fraction was more resistant to acid hydrolysis, so it was dissolved by adding alkali and fractionated by adjusting the pH at 2.85, so that the GG block settled and the MM block dissolved. The result of partial hydrolysis were dried by freeze drier. Analysis of alginate functional groups was carried out using a Fourier Transform Infrared (FTIR) spectro-photometer (Perkin Elmer, spectrum one). 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. Two 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 transmittance was measured at 4000-400nm wavelength. Alginate was at peak at wavelength 1030/1080 nm.

2.2.4. Standard Curve

M/G concentration of 1 g alginate were varied from 0%; 25%; 50%; 75%; 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 as the procedure previous. The transmittance obtained from samples were plotted to regression equations and got M/G concentrations.

3. RESULTS AND DISCUSSION

3.1 Viscosity

The highest alginate's viscosity obtained from *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 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%±0.84%) followed by that from Lima Island with an average 11.48%±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%±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, so habitat and sea currents were likely the influencing factors on the yield of alginate. Binuangeun has shallow water with depth of 40.00 cm so the shortest total thallus length was 31.82 cm. The habitat where *Sargassum* 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 *Sargassum 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 (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. Differences locations of *Sargassum 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 (Mc Hugh 2008). The temperature at the time of making the solution for the analysis of viscosity Na-alginate should not exceed 80^{o}C, if it exceeds this temperature the solution will be degraded so that it is difficult to analyze the viscosity using RVA (Rapid Visco Analyzer). 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.

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 *S. polycystum*	**Blok MM**	**Blok GG**	**Blok MG**	**Blok M**	**Blok G**
Pulau Lima	22,00	77,00	1,00	23	77
Ujung Kulon	28,70	62,00	9,30	30	70
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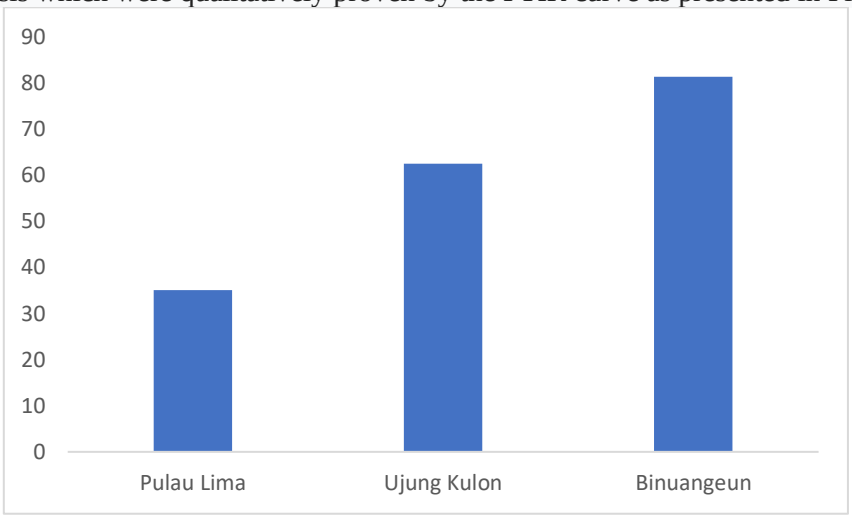

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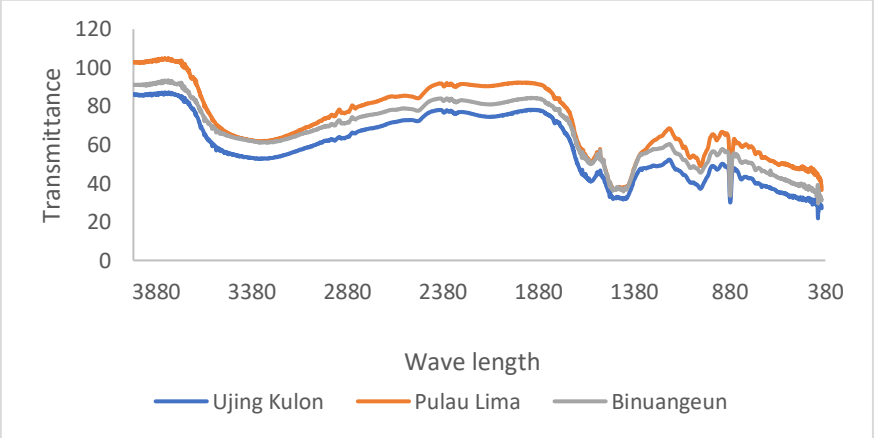

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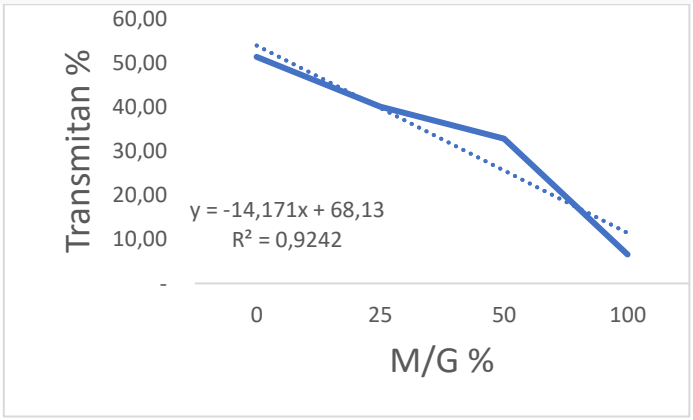

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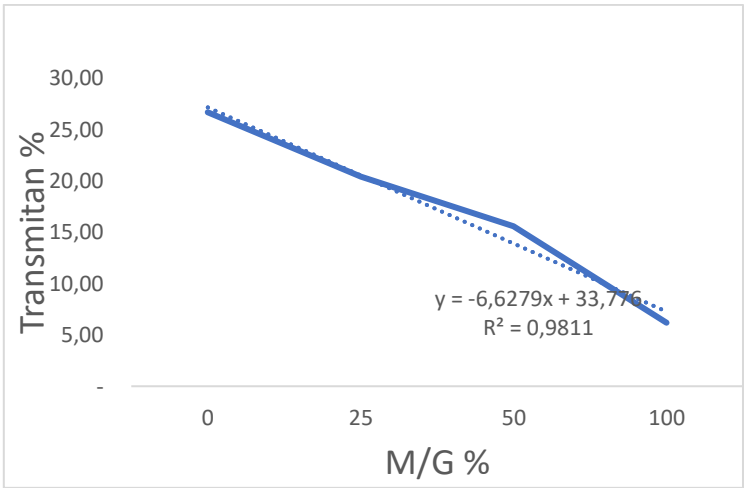

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Alginate's Binuangeun

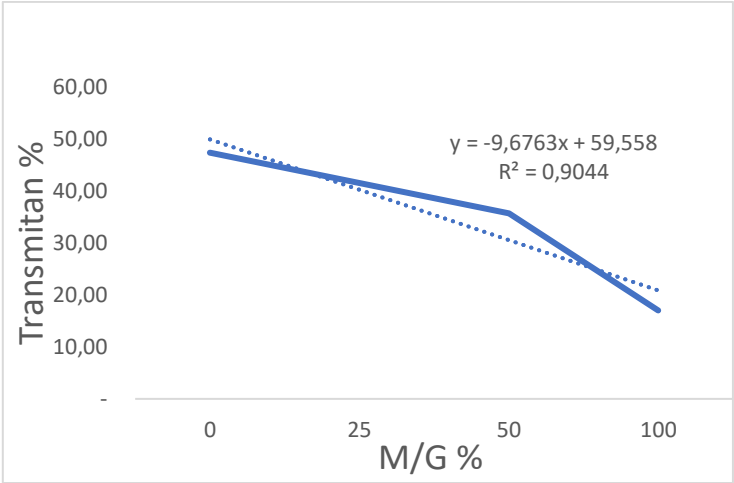

Figure 6. Alginate's Binuangeun M/G calibration Curve

The regression equation of Alginate's Binuangeun was y = -9,6763x + 59,558 with a correlation coefficient (R) = 0.9044.

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REFERENCES

Anggadiredja J, Zatnika A, Purwoto H. Istini S 2008, Rumput Laut. Jakarta, Penebar Swadaya.

Anonym, 1976. Keto Algin Hydrophilic Derivation of Alginic Acid for Scientific Water Control 2nd ed New York. Kelco Division of Merck and Co.

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as basic material for production dental impression material. J Dentomaxillofacial Science 3:84 - 87.
Kadi, A. 2005. Beberapa Catatan Kehadiran Marga Sargassum di Perairan Indonesia. Jakarta: Bidang Sumberdaya Laut, Puslitbang Oseanologi-LIPI
Kusumawati R., Basmal J., Utomo B.B. (2018). Physicochemical Characteristics Of Sodium Alginate Extracted From Turbinaria Sp. And Sargassum sp. Squalen Bull. of Mar. and Fish. Postharvest and Biotech. 13 (2) 2018, 79-84
Lee I-Min, Eric J Shiroma, Felipe Lobelo, Pekka Puska, Steven N Blair, Peter T Katzmarzyk, 2012. Eff ect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet 2012; 380: 219–29
Mc Hugh, 2008. Production, properties and uses of alginates New South Wales Univ., Campbell (Australia). Dept. of Chemistry)
Yamamoto, K., Ishikawa, C., Katano, H., Yasumoto, T., and Mori, N. (2011). Fucoxanthin and Its Deacetylated Product, Fucoxanthinol, Induce Apoptosis of Primary Effusion Lymphomas. Cancer Letters, 300, 225-234

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

N Dharmayanti[1,2*], J Supriatna[1], A Abinawanto[1] and Y Yasman[1]

[1]Department of Biology, Faculty of Mathematics and Natural Science, University of Indonesia, Depok, 16424, Indonesia
[2]Jakarta Fisheries University, Jakarta, 12520, Indonesia

*E-mail: niken.stp@gmail.com

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 gulurunic acid (G) and manurunic 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. obtuspfolium, S. binderi, S. polycystum, S. microphylum, 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

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°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 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°00'05" S, 106°09'18" E), (2) Ujung Kulon (6°48'15" S, 105°29'5" E), and (3) Binuangeun (6°49'16" S, 105°56'14" E).

2.1. Materials

Three samples of *S. polycystum* from each location were prepared for the extraction process. The extraction process used natrium carbonate, calcium chloride, chloride acid, alcohol 70%, peroxide hydrogen, distilled water, Ca_2Cl_2 4%, HCl 2%, Na_2CO 34%, Ca_2Cl_2 10%, Ca_2Cl_2 5%, HCl 5%, dan alkohol 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 400-4,000 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%±0.84%) followed by that from Lima Island with an average 11.48%±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%±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^{o}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 *S. polycystum*	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.

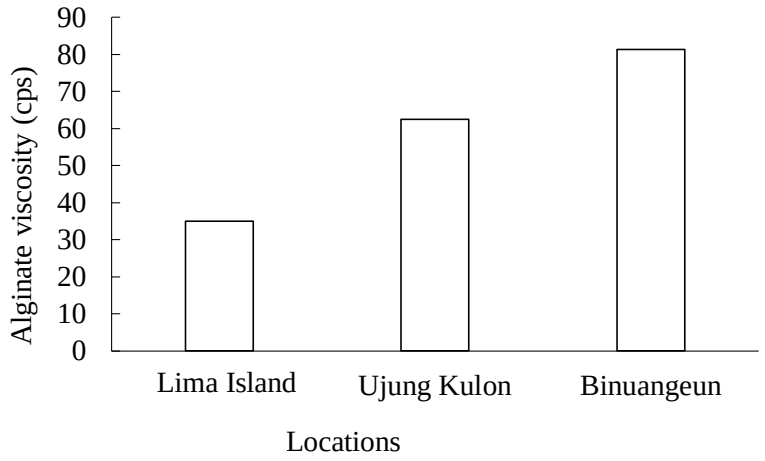

Figure 2. Test results for alginate viscosity at the extraction of *S. polycystum* (cps).

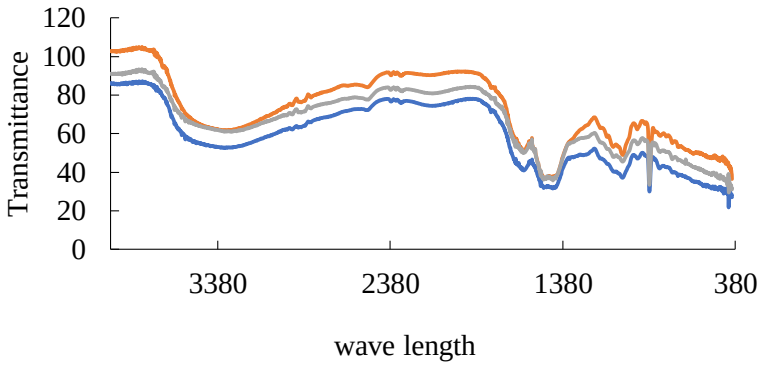

Figure 3. FTIR Curve of Alginate from Lima Island, Ujung Kulon, Binuangeun, Ujung Kulon, Pulau Lima, Binuangen**.**

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 streching
1608.63	50.98	50.29	41.15	C=O
1411.89	38.72	38.49	33.42	bending -C-OH
1091-1093.64	53.62	48.50	41.02	COOH, C-O stretching C-O-C stretching
1170	64.41	56.67	48.21	C-O streching C-C stretching C-C-C bending
1029.99-1033.85	48.94	45.59	37.16	C-O stretching C-O-C stretching
947.05	62.31	54.50	47.11	C-O stretching C-C-H stretching
817.82 -875.68	39.92	35.04	30.96	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).

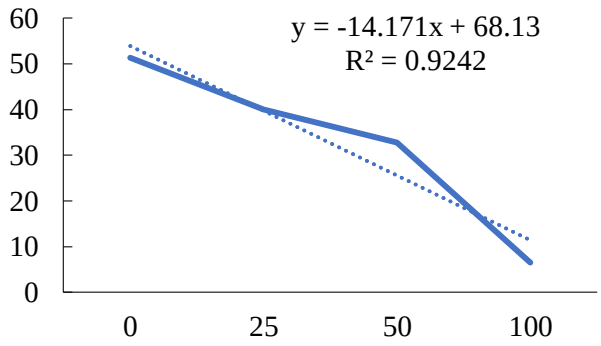

Figure 4. Alginate's Lima Island M/G calibration curve (before pre-concentration).

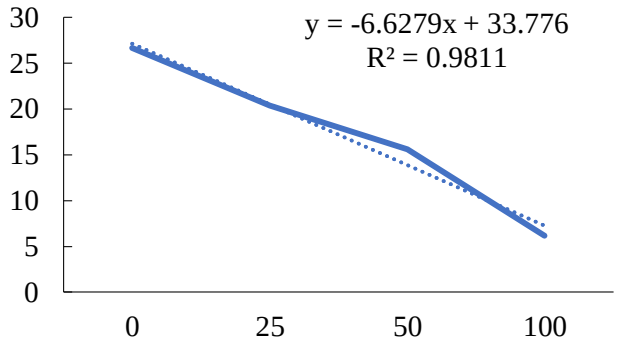

Figure 5. Alginate's Ujung Kulon M/G calibration curve (before pre-concentration).

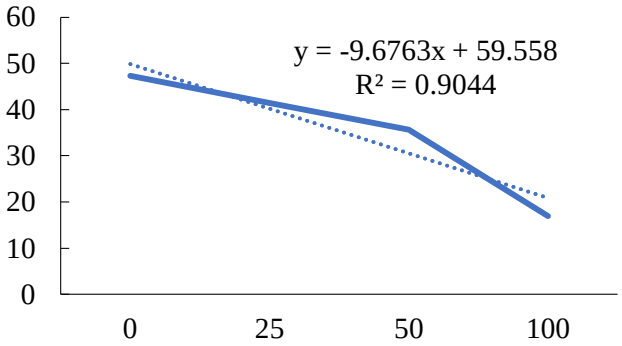

Figure 6. Alginate's Binuangeun M/G calibration curve (before pre-concentration).

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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References

Anggadiredja J, Zatnika A, Purwoto H and Istini S 2008 *Rumput Laut* (Jakarta: Penebar Swadaya)
Clark N J 1976 *Kelco algin: Hydrophilic Derivation of Alginic Acid for Scientific Water Control 2nd ed* (New York: Kelco Division of Merck and Co)

Basmal J, Utomo B S B, Tazwir, Murdiyanah, Wikanta T, Marraskuranto E and Kusunawati R 2013 *Producing alginate from seaweed Sargassum* (Jakarta: Penebar Swadaya)

BPS 2018 *Statistik Indonesia 2018* (Jakarta: Badan Pusat Statistik)

Hamrun N, Thalib B, Tahir D, Kasim S and Nugraha A S 2018 Physical characteristics test (water content and viscosity) of extraction sodium alginate brown algae (phaeophyta) species *Padina* sp. as basic material for production dental impression material *J. Dentomaxillofac. Sci.* **3** 84-87

Kadi A 2005 *Beberapa Catatan Kehadiran Marga Sargassum di Perairan Indonesia* (Jakarta: Bidang Sumberdaya Laut, Puslitbang Oseanologi-LIPI)

Kusumawati R, Basmal J and Utomo B B 2018 Physicochemical characteristics of sodium alginate extracted from *Turbinaria* sp. and *Sargassum* sp. *Squalen Bull. Mar. Fish. Postharvest Biotech*. **13** 79-84

McHugh D J 2008 *Production, properties and uses of alginates* (New South Wales: Australian Defence Force Academy Campbell)

Yamamoto K, Ishikawa C, Katano H, Yasumoto T and Mori N 2011 Fucoxanthin and its deacetylated product, fucoxanthinol, induce apoptosis of primary effusion lymphomas *Cancer Lett*. **300** 225-234