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Nutritional composition and alginate characteristics of *Sargassum polycystum* (C. Agardh, 1824) growth in Sebesi island coastal, Lampung-Indonesia

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Abstract. Macro and micro-nutrient as well as alginate characteristics from *Sargassum polycystum* (C. Agardh, 1824) brown seaweed growth in Sebesi Island were investigated. Gravimetry was used to determine the macro nutrients and Atomic Absorption Spectroscopy (AAS) to identify the micro nutrients. While, alginate extraction followed the acid extraction protocol, Nuclear Magnetic Resonance (NMR) was utilized to determine M/G ratio. The results of this research showed a descending percentage of macro nutrients as follows: Carbohydrate > Ash > Moisture > Crude Fibre > Protein > Fat. The descending presence of micro nutrients was: Manganese (Mn) > Barium (Ba) > Zinc (Zn) > Iron (Fe) > Copper (Cu) > Selenium (Se) > Molybdenum (Mo). The yields of extracted alginate were high (24.18–29.59%) and consisted of high moisture and an ash content of 12.16 ± 0.4 and 24.37 ± 0.5 respectively. The pH was 7.28 ± 0.05 , the viscosity 195.7 ± 8.4 , whiteness and gel strength were about 58.19 ± 0.6 and 60.23 ± 0.7 . The ratio of Maluronate and Guluronate (M/G) blocks varied between 1.04-1.48. Based on the results of the canonical correspondence analysis (CCA), the main character of alginates such as the M/G ratio and the gel strength could be associated with variations in the composition of DO and ammonia, while viscosity was related to variations of nitrate and phosphate. It can be concluded that the growth of *S. polycystum* on the coast of Sebesi Island can be recommended as alginate resource with robust quality.

1. Introduction

Sargassum polycystum is brown macroalgae from the genus of *Sargassum* which can be found in almost all of the Indonesian waters specifically grow in the intertidal zone that formed by hard substrates like dead coral and coral fragments. Based on the study conducted by (1), there were about 58 of *Sargassum* species were found in Indonesian waters but it was only 12 species that well utilized by the people traditionally as the source of direct consumption and alginate (2).

The species of *S. polycystum* has an extensive distribution due to its durable adaptation to high changeable environmental such as a wide range of temperature and salinity (3). This ability supports in the variation of utilization for instance to be the source of alginate that have high economic value. Some studies have been showed that *Sargassum* potentially employed as alginate like the seaweed grown in Alor archipelago, East Nusa Tenggara with the type of *S. fluitans* species (4) dan *S. hystrix* J.



Agardh (5), *S. crassifolium* and *S. polycystum* from Binuangeun waters, Banten (6, 7) and as well as from Gunungkidul waters, Yogyakarta (8).

In general, the main source of alginate as commercial is the species from *Macrocystis*, *Ascophyllum*, *Ecklonia*, *Undaria* dan *Laminaria* spp. (9, 10). However, brown seaweed that grows in tropical regions for example *Sargassum* spp. also have potential as the basis of alginate production (11, 12, 13, 14). Alginate is a polysaccharide found in the walls and the matrix between cells of the brown seaweed. Chemically, it is composed of monomers of β -d-mannuronic acid (M) and α -l-guluronic acid (G) (15). The combination of these monomers (M/G ratio) has implications for the level of viscosity and strength of the gel so that it is commonly used as a thickening agent, gelling agent and emulsifier for food as well as for textile purposes and also for pharmaceutical materials (16, 17).

In addition, the nutritional potential confined in seaweed is also high. The macro and micronutrients content such as protein, fat, carbohydrate, fibre, magnesium, iron, selenium and iodine in *S. polycystum* are quite high (18). However, the presence of these nutrients is highly dependent on the seaweed species, location of growth and seasonal variations (19, 20, 21).

The enormous potential possessed by the *Sargassum* brown seaweed described above becomes an attraction for the deeper exploration. However, research on nutrient content and alginate character in *S. polycystum* brown seaweed have not been done very well especially on small islands with hard substrate domination such as Sebesi. Therefore, this study is aimed to characterize the micro and macronutrient content, the characteristics of alginate and the relationship between environmental conditions and the alginate character of *S. polycystum* seaweed growing in Sebesi Island waters.

2. Materials and methods

2.1. Sample collection

Samples of *S. polycystum* were collected from Sebesi island coast. There were three sampling points, namely at coordinates point of (1) 5°58'05.3"S 105°30'04.0"E; (2) 5°58'04.7"S 105°30'05.2"E dan (3) 5°58'05.7"S 105°30'01.5"E (Figure 1). The sampling time was carried out in April 2019 and at the condition of minimum tidal to ease the sample collection. The seaweed samples were cleaned with clean seawater before being dried under the sunshine in about two days and then transported into a laboratory for alginate extraction. Samples for proximate analysis were in dry form while the wet samples for micronutrient testing were stored in plastic containers and seawater, as well as, ice were added into the box to keep it fresh before the further analysis.

2.2. Water quality of the seaweed habitat

Water quality measurements were conducted on several parameters including temperature, DO, salinity, pH, and turbidity or brightness. The tool used was the 1P67 water quality meter combo 8630 type by in-situ. Water fertility parameters consisted of nitrate, orthophosphate, and ammonia. The instrument used in the determination of the water fertility was Ultra Violet-Visible (UV-VIS) spectrometer with the APHA (American Public Health Association) method (Rice et al. 2017) in the Proling IPB laboratory.

2.3. Macro nutrient analysis

The analysis of water, ash, lipid, protein, and crude fibre content in raw materials was carried out based on the 1990 AOAC method. This proximate assessment was conducted for the dry sample that consists of two samples each sampling point. Analysing of the water content was through the oven method, crude lipid content was through the Soxhlet method, ash content was through Ashing procedure with a furnace at 6000 C, protein content by the Kjeldhal method, and crude fibre by the destruction of samples dissolved in H₂SO₄. The carbohydrate content is presented as by different from the total proximate analysis results.

2.4. Micro nutrient analysis

Fresh seaweed is lifted from the substrate and then washed using clean seawater. Furthermore, the sample was stored in plastic with the addition of a technical ethanol solution that has been diluted with a ratio of 1: 1 which was then a 100 g of the sample diluted in 200 ml of ethanol solution. During transportation, the temperature is kept in low temperature by adding ice to the cool box. The analysis was done using the Perkin Elmer PinAAcle 900H Atomic Absorption Spectro-photometry (AAS) with Flame technique (Acetylene-Air) with the APHA method, 23rd Edition in the Proling IPB laboratory.

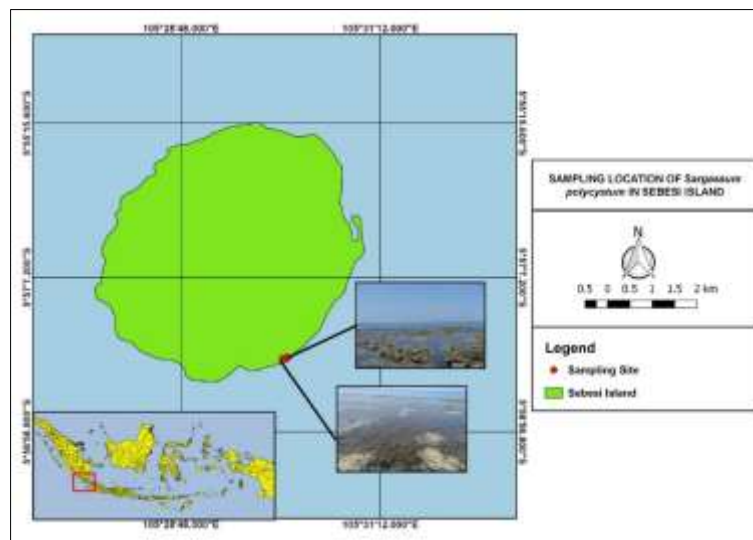


Figure 1. Sampling site

2.5 Alginate extraction

The extraction method was carried out through the modified of Alginic acid pathway that adopted from (15), which consists of soaking of dried seaweed for 1 hour in a 4% HCl solution followed by washing to neutral pH. Furthermore, extraction in 2% Na₂CO₃ solution at 60-70⁰C for 2 hours. Next was filtering using 200 net mess plankton and after that followed by the conversion step to Alginic acid which was used 10% HCl to reach a pH between 2-3. The next step was the conversion of acid into sodium Alginate using NaHCO₃ 10% to reach pH 8-9. Precipitation of the sodium alginate step was conducted by the addition of 99% iso propyl alcohol (IPA) to the solution and kept it for about 12 hours and continued with drying and grinding. All chemicals used were purchased from Mallinckrodt, USA.

2.6 Yield determination

The yield of alginate extraction was calculated based on the percentage of the yield of total dried seaweed as a sample. The yield calculation based on 3 (three) replications where the different sampling points are used as a repetition.

2.7 Alginate pH determination

The pH testing to the alginate extracted from *S. polycystum* was carried out by the following method from (6), where about 3 grams of sample was dissolved into 197 ml of distilled water. The solution was then heated at 80⁰C for 10 minutes until the alginate dissolves completely. After its temperature dropped to 25⁰C then the measurement done by using IONIX pH5S Spear pH Tester.

2.8 Moisture and ash content analysis

Alginate extracted from the seaweed was tested for water and ash content based on the gravimetric method and refers to AOAC 1990 where the determination of water content was using an oven with a temperature of 100⁰ C and ashes content with a Furnace at a temperature of 600⁰ C.

2.9 Viscosity determination

Viscosity analysis carried out using a viscometer (TV-10). About 10 grams of Na₂CO₃ dissolved in 50 mL of distilled water. Alginic acid powder samples were weighed as much as 2.5 g and dissolved in 20% Na₂CO₃ solution at 80°C in a beaker glass. The solutions cooled at room temperature for ± 1 hour. The cooled sample placed into a tubular container after the temperature slowly drops. Spindle number 2 at a speed of 60 rpm was employed to determine the viscosity of the alginate. The results are expressed in centimetres Poise (cP).

2.10 Whiteness degree determination

Whiteness degree analysis of alginic acid powder was carried out using a calibrated color analyzer (Lutron RGB-1002). A sample of ± 4 g was placed on 3x3 cm white paper and measurements were repeated three times. The measurement results appeared on the device in the form of digital numbers which include the values of L *, a *, and b *. Whiteness degree values are expressed in per cent (%). The formula for calculating the value of whiteness degrees, namely:

$$\text{Whiteness degree (\%)} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

2.11 Gel strength determination

Gel strength analysis was carried out refers to (6) which done by using a penetration testing with a Stable Micro System TAXT2 texture analyzer to a depth of 25 mm. Sample preparation prepared by making 20% Na₂CO₃ solution, 18 g Na₂CO₃ was dissolved in 90 mL of water until homogeneous. 10.8 g of alginate powder was dissolved in 90 mL of Na₂CO₃ solution at 70-80°C until it liquefies entirely. The 0.9 g of CaCO₃ was then added to the solution and Glucono Delta Lactone (GDL) around 6 g while stirring was kept as well as the temperature remains at 70-80°C until completely dissolved. The suspension was then placed into a 3 cm diameter tube mould with a height of 3 cm and chilled for 1 hour at room temperature. The solution which begins to thicken then placed in chilling room temperature for about 24 hours. The calculation then carried out by removing the alginate gel formed from the mould on the test equipment. Gel characteristics were observed at the peak of force (g) when the gel breaks divided by contact area (cm²).

2.12 M/G ratio determination

The determination of ratio M/G block begins from the reduction of viscosity value of the alginate. A 100 mg of alginate diluted into 300 ml of distilled water then the pH of the solution was reduced to reach 5.6 by adding 0.1M HCl. After that, the solution heated in a water bath with a temperature of 95⁰ C for 60 minutes. Furthermore, the pH of the solution declined to 3.8 by the addition of the same HCl. Re-heating completed at a temperature of 95⁰ C within 45 minutes. After the temperature dropped, the solution neutralized using 0.1M NaOH and placed it in the oven at 60⁰ C until most of the water evaporates. After that, 1 ml of the solution taken for M/G ratio determination by using NMR.

¹H-NMR JEOL ECS 400 MHz spectrometer was employed to determine the ratio of M/G. 1 ml of alginate solution diluted in 1 ml methanol and followed by centrifugation within 15 minutes. The supernatant then injected to the NMR autosampler after the sample dissolved in D₂O (Deuterium Oxide) which contained TMS (tri-metal silane). The calculation of the ratio conducted based on the reference of (14).

2.13 Data analysis

All data will be presented by displaying standard deviations except the trace-element. The relationship between environmental parameters and Alginate characteristics was analyzed using canonical correspondent analysis. Statistical analysis will be conducted by using Past Statistical Software V4.02 (22).

3. Results and discussion

3.1 The condition of Sebesi island waters

Sebesi Island is a small volcanic island type with coastal contours dominated by rocks and relatively narrow intertidal zones. The research location has a coral substrate so that *S. polycystum* can be firmly attached to the bottom of the water to avoid the influence of strong waves. Based on the results of research on water quality (Table 1), it can be seen that the high Nitrate content may be caused by land activities and enrichment of the sea carried by currents and waves. Nitrate content was high enough in the waters that can be caused by the inclusion of nutrients from the mainland and the activities of various types of biota which are also followed by the decomposition process (23). Water sampling points closed to the mainland may also influence the high value of nitrates (24). The condition of the waters of the research site can be seen in Figure 2.

Table 1. The waters quality of Sebesi island coast

Parameter	Result	Standard*
Nitrate (NO ₃ -N) (mg/l)	0.27±0.1	0.008
Phosphate (PO ₄ -P) + (mg/l)	0.004±0.003	0.015
Ammonia (NH ₃ -N) + (mg/l)	0.27±0.04	0.3
DO (mg/l)	5.6±0.3	>5
pH	7.53±0.2	7-8.5
Temperature (°C)	30.4±0.7	-
Salinity (psu)	32.5±0.4	33-34
Brightness	100%	-

*Sea water quality standards (25)

The coastal water of Sebesi Island has a high richness rate and this condition is perfect to support the growth of seaweed. The seawater nutrient is characterized by an abundance of nutrients including nitrates (NO₃⁻) and phosphate (PO₄). Both nutrients are needed by the organism for N₂ fixation where Nitrate (NO₃⁻) helps the process of metabolism and phosphorus is needed for photosynthesis (26). Optimal absorption of nitrate (NO₃⁻) and Phosphate (PO₄) from seaweed through thallus is significantly in line with stable growth (27). Nitrate (NO₃⁻) requirements are generally more than phosphate (PO₄) in the ratio of 10: 1 (Suthar *et al.* 2019). Physical factors such as hydrodynamics also affect the number of nutrients available so that each area has a different fertility level that causes different types and amounts of biota that live in an area (30, 31).

3.2 Raw materials characteristics

Macro-nutrition characteristics. The results of macronutrient that include moisture, ash, fat, carbohydrate, protein and crude fibre content are presented in Table 2. Carbohydrate and ash levels were detected as high as 47.62 ± 0.22 and 27.74 ± 0.72 of dry weight respectively. These results are not much different from that reported by (31) which was about 42.40 ± 0.41 and (32) of 29.0%. Meanwhile, the moisture content and crude fibre amounted up to 12.95% and 6.93%, respectively. These totals were quite high when compared to the same content in other *Sargassum* species such as *S. oligocystum* with a crude fibre content of 9.4% (33), moisture content of *S. muticum* and *S. polyschides* was 9.65% and 10.88 % correspondingly (Rodrigues *et al.*, 2015) while the fibre in *S. muticum* was much higher at 27.9-44.5% (19). The two components with the smallest percentage were Protein and Fat contents, ranging between 4.45% and 0.31%. These results were not much different from the results of the study from (31) but lower than those found by (32), which was 7.6% of fat and 14.8% of protein content in *S. polycystum* which grows in Tamil Nadu waters, India. Nutrient content in *Sargassum* varies due to environmental conditions of the waters, season, age of seaweed, sample preparation and extraction methods (32, 19). Low-fat content is significantly influenced by

environmental conditions such as the temperature, for an instant, macro algae that grow in subtropical areas contained higher lipid than those that grow in the tropical waters (19).

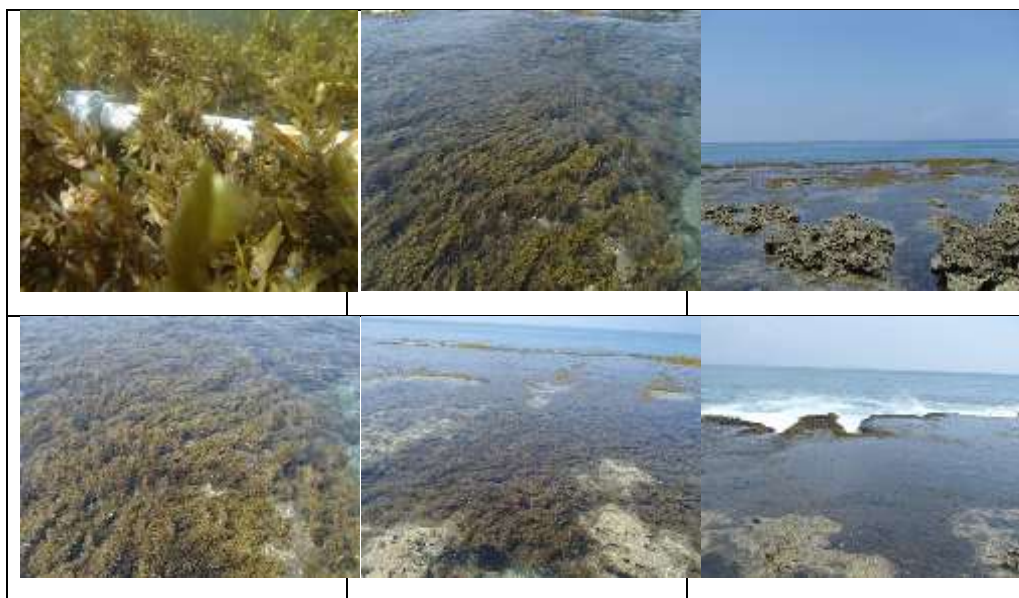


Figure 2. Sampling site condition

Table 2. The macro nutrient content of *S. polycystum* (dry matter).

Parameters	<i>S. polycystum</i> ¹	<i>S. polycystum</i> ²	<i>S. polycystum</i> ³
Moisture (%)	12.95±0.4	-	9.95±0.55
Ash (%)	27.74±0.72	29.0	42.40±0.41
Lipid (%)	0.31±0.02	7.6	0.29±0.01
Protein (%)	4.45±0.43	14.8	5.40±0.07
Crude fibre (%)	6.93±0.34	21.3	8.47±1.21
Carbohydrate (%) (by different)	47.62±0.22	25.0	33.49±1.70

¹This research.

²Perumal *et al.*, 2019.

³Matanjun *et al.*, 2009.

Micro nutrient composition. Seaweed or macroalgae is well known as a source of several micronutrients which are also known as good bioindicators and bioaccumulation in coastal area. The results of micronutrient analysis on *S. polycystum* are presented in Table 3. In general, the number of elements of Manganese (Mn) was the highest of the seven other elements and was followed by Barium (Ba) and Zinc (Zn) as the three highest micronutrients contained on the sample. The descending presence of micronutrients was: Mn> Ba> Zn> Fe> Cu> Se> Mo. Micronutrition has a good function for human health but there is also a risk if the consumption exceeds the maximum limitation such as Iodine (I) which can cause health problems (35).

Manganese (Mn) was the highest microelement found in the sample. Manganese (Mn) is generally found to be accumulating quite large in brown seaweed and its amount is significantly influenced by environmental conditions (35). Furthermore, Barium (Ba) was the second-highest microelement found. This micromineral is needed by humans as well as the macro algae because it has a function as a cofactor of various enzymes that are important for carbohydrate metabolism, protein digestion and defence against free radicals (36). However, the element Ba is also found in other species in high concentrations, such as *Caulerpa racemose*, the green seaweed (37). In order to support growth, seaweed needs micro minerals such as Selenium (Se) which can help in the process of photosynthesis

and antioxidant systems (38). The Se element was also found with high concentrations in *Sargassum fusiforme*, *S. thunbergii* and *S. horneri* (39). On the other hand, the element Iron (Fe) was also reported to have accumulated in the type of *Sargassum* as based on the study of (40) that *Sargassum muticum* contains a high element of Iron (Fe) which was around 0.37 mg/g dry weight.

Some microelements which are accumulated in seaweed can be functioned as indicators of water quality (bioindicator). The presence of Cu (Copper) in seaweed for an instant is usually an indicator of contamination in waters but the amount of concentration contained varies according to season (41). The ability to accumulate Cu was also evidenced by *Sargassum fusiforme* in the study of (42). In fact, the high content of copper elements can inhibit the growth of seaweed because it is proven to interfere with the metabolic process (43). Another element is zinc (Zn) which was quite high, often used as biomonitoring of waters quality (44). However, the presence of Zn as heavy metal in general in brown seaweed was lower than that contained in some species of seaweed that belong to the red and green macroalgae (39). Based on the results of previous studies, it can be explained that the micronutrient content in *S. polycystum* brown seaweed can be varied due to environmental condition such as the level of pollution (45).

Table 3. Micronutrient content of fresh *S. polycystum*

No.	Parameters	Unit	Amount
1	Barium (Ba)	mg/Kg	20,77
2	Selenium (Se)	mg/Kg	0,305
3	Copper (Cu)	mg/Kg	4,25
4	Iron (Fe)	mg/Kg	5,10
5	Manganese (Mn)	mg/Kg	162,22
6	Zinc (Zn)	mg/Kg	13,54
7	Molybdenum (Mo)	mg/Kg	< 5.00

3.3 Alginate characteristics

Sargassum brown seaweed is one of the potential sources of alginate aside from the popular species such as the genus of *Laminaria*, *Saccarina*, *Lessonia*, *Macrocystis* and *Aschophylum*. Commercially, alginate from *Sargassum* has not been able to compete yet from those growths in temperate zone because of the availability of raw materials and quality aspects. The results showed that the yield of alginate from *S. polycystum* in this research was quite high at 28.22%. This result was greater than the yield from the study of (46) which was reached about 27.64% and (14) around 15.85% in *S. polycystum* species. In contrast, the yield is quite high obtained from the *S. aquifolium* species by 39.01% (47), 45.54% from the *S. cristaefolium* species (48) and 40.43% of the *S. latifolium* species (49).

The moisture and ash contents of the alginate were high compared to the commercial. The method used may affect the result since the way to extract alginate plays an important role to produce good quality. The study showed the contents of these two parameters were about 12% and 24%, respectively. Those results were in the standard of commercial alginate that pointed around 12% of moisture and 18-27% of the ash content (50). Another character in the form of pH was found in the range of 7.28, which means that it is still in accordance with commercial standards, namely 6.1-7.8 and similar to the study (14) where the pH of alginate extracted from Malaysian *S. polycystum* was 7.42.

Viscosity is a beneficial characteristic of alginate which formed the ability of a stability and gel performing effect. These characters will be used as references for its utilization in food additives, drink stabilizers and other benefits in the field of nutraceuticals (50). Viscosity test results showed about 195.7 cP where this result was higher than the study of (46) that amounting to 62.4-73.0 cP and (7) accounted in range of 35-81.33 cP on the same species with this study. The differ value of viscosity may be affected by the extraction process, which is at the bleaching phase where the higher levels of bleaching material will reduce the viscosity of the alginate produced (12).

Bleaching is a process on extraction that carried out to produce whiter alginate so it will ease the advance application to any kinds of products. The degree of whiteness is also one of the important parameters of commercial alginate. The percentage of the whiteness of alginate found in this study was 58.19% which means it has a fairly low percentage when compared with *S. hystrix* which was about 75.27% (5). On the other hand, viscosity and degree of white have the opposite effect and also result in varying gel strength of alginate. Gel strength is categorized as high, medium and low in which is affected by the high presence of G block (guluronate) (51). In this study, gel strength was 60.23 g / cm² and was considered low compared to alginate from *S. crasifolium* which was 353.54 (g / cm²) (6).

Table 4. The results of alginate characterisation

No.	Parameters	Results
1	Yield (%)	28.22±2.8
2	Moister content (%)	12.16±0.4
3	Ash content (%)	24.37±0.5
4	pH	7,28±0.05
5	Viscosity (centipoise)	195.7±8.4
6	Whiteness (%)	58.19±0.6
7	Gel strength (g/cm ²)	60.23±0.7
8	M/G ratio	1.24±0.2

In general, alginate characteristics such as gel strength and viscosity are influenced by the composition of Manuronic acid and Guluronic acid blocks, which are usually called the M/G ratio and also by its molecular weight. Species, geographical conditions and seaweed thallus parts are commonly the factors that influence the M/G ratio and molecular weight (52). The M/G ratio of *S. polycystum* alginate that taken from the Sebesi coastal waters was 1.24 where the Manuronic acid block was dominated. Thus, it can be inferred that alginate has more elastic properties with low gel performing/brittle. According to (15), the alginates which can form gels properly have a high content of guluronate acid while those that are more flexible are those that contain higher manuronic acid. The M/G ratio in this study is slightly different from the results of previous studies where *S. turbinarioides* was 0.94 (53), *S. cristaefolium* 0.29 (48) and *S. polycystum* from Malaysian waters amounted to 0.733 (14). The characteristics of alginate from *S. polycystum* brown seaweed and the results of the quantification of the M/G ratio in this study can be seen in Table 4 and Table 5.

Table 5. M/G ratio and its Mono and Diad fraction from of the alginate

Sample	G	M	F _{GG}	F _{MM}	F _{GM}	F _{MG}	M/G Ratio	MM	MG=GM	GG
St 1	2,33	2,42	4,56	4,75	4,65	4,65	1,04	1,44	0,98	1,35
St 2	2,69	3,985	4,51	6,68	5,38	5,38	1,48	2,38	1,605	1,08
St 3	1,045	1,26	1,91	2,3	2,09	2,09	1,21	1,03	0,23	0,81

Based on the results of the Canonical analysis of correspondence to the relationship between environmental parameters and Alginate characteristics (Figure 3), the ratio and gel strength are related to DO and ammonia concentrations in the seawater. On the other hand, the degree of whiteness, moisture content and pH were only related to sea surface temperature.

Separately, the yield was related to salinity, whereas ash content and viscosity were affected by nitrate content of the seawater. These results confirmed that the characteristics of alginate extracted from *S. polycystum* brown seaweed were influenced by environmental conditions. This is consistent with studies by (16, 15, 1) which stated that alginate characteristics are vary according to species, location of growth, extraction method and seasonal variation. The characteristics and quality of

alginate will determine its further application as use for thickeners, stabilizers and gelling agents in the food and pharmaceutical product industries.

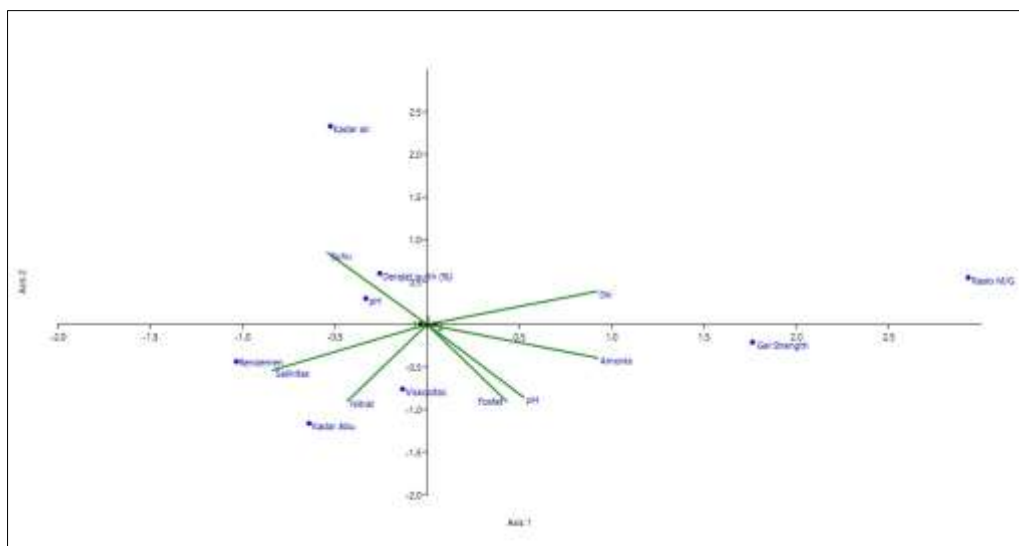


Figure 3. The Canonical correspondence analysis of the relationship between environmental parameters and Alginate characteristics.

4. Conclusion

The *Sargassum polycystum* (C. Agardh, 1824) brown seaweed that grows on the coast of Sebesi island highly contains micro and macronutrients, so that it can be used as a source of alternative nutritional fulfilment. Alginate extracted from this seaweed has almost the same characteristics as commercial alginate with the main characters such as the yield, which was reached $28.22 \pm 2.8\%$, the viscosity up to 195.7 ± 8.4 cP, the gel strength attained 60.23 ± 0.7 (g / cm²), and the ratio of M/G was 1.24 ± 0.2 . Those characteristics were significantly affected by the environmental condition of both the physical and chemical factors surrounding the island waters. Based on the nutritional and alginate characteristics in this study, the utilization of *S. polycystum* seaweed especially those growing on the coast of Sebesi island could be utilized as a source of nutrition and alginate production for the future.

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