The Effect of Alkali Pretreatment and Different Parts of *Sargassum polycystum* Thallus on Alginic Acid Attributes
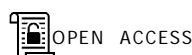
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Abstract

Alginic acid is an intermediate product in the alginate extraction process. In this study, alginic acid was generated from three separate sections of the thallus. The study aimed to define the influence of alkali solvents for pretreatment and the thallus parts from *S. polycystum* to produce alginate. Thus, this study identifies the proper utilization of seaweed as a source of alginate. The extraction procedure to obtain alginic acid was performed using Na_2CO_3 at a 2% concentration. The pretreatment improved the quality before extraction by soaking in KOH as a solvent. The statistical analysis was used to define significant differences in the pretreatment and thallus parts of the *S. polycystum* using ANOVA and the Tukey test as the post hoc. Alkali pretreatment and thallus sections substantially influence alginic acid yield, viscosity, and gel strength. Yield, ash content, moisture, pH, viscosity, whiteness degree, and gel strength of the best alginic acid chosen (pretreatment with KOH 0.90% from the basal section) were around 21.51 %, 10.34 %, 7.04 %, 2.54, 53.37 cP, 57.91 %, and 58.80 g/cm^2, respectively. Except for the ash content parameter, the qualities of the alginic acid generated satisfied the standard. In conclusion, it is a better understanding of the alginate production from different thallus parts, making utilization more effective.

Keywords: thallus parts, alginic acid, quality, KOH solvent

Introduction

Sargassum is a macroalga belonging to the Phaeophyceae, commonly found among five genera distributed in Indonesian waters (Puspita et al., 2020). One of the species belonging to the Sargasaceae family that has a high potential to be utilized is *Sargassum polycystum*. *S. polycystum* is easily found in shallow water and attached firmly to a concrete substrate such as death corals and stones (Baweja et al., 2016). The morphology of the species is distinguished based on the thallus, consisting of a holdfast, stipe, and blade (Camacho et al., 2015). In addition, Widyartini et al. (2017) stated that the *Sargassum* thallus is varied from shape to size depending on their habitats. The thallus also contained various nutrients such as macro-micro-trace elements, polysaccharides, and bioactive compounds according to the season and water quality of the habitat (Reboleira et al., 2018; Saraswathi et al., 2003; Sumandiarsa et al., 2023). Besides, a different part of the thallus also expresses the qualities of the most interesting alginate compound. Kumar & Sahoo

(2017) explained that the young thalli provide alginate in small quantities compared to the old ones.

The existence of abandoned *S. Polycystum* in Indonesian waters was not followed by maximum utilization, so current information about further use is valuable. One of the interesting is alginate obtained from this seaweed with high economic value. Alginates an anionic polysaccharide in salt form (Na⁺, Mg²⁺, dan Ca²⁺), which contains β-D-mannuronic acid (M) and α-L-guluronic acid (G) as well as formed in strong and flexible bonds. β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomer tied up by β-(1→4)/α-(1→4) glycosidic bonds which will form three different sequences based on the proportion between the two monomers bonded, they are MM, GG, and MG blocks (Fawzy et al., 2017; Jensen et al., 2015; Peteiro, 2018; Rhein-knudsen et al., 2017; Zhang et al., 2020) were stated that the mannuronate (M) and guluronate (G) acid composition has an important effect on gel performance quality because the two monomers have different structures.

Alginate extraction generates variability in yield due to the condition of the sample. Some research found that thallus condition and its parts influenced the quantity and quality of alginate. Saraswathi et al. (2003) explained that the axis, holdfast, and blade of thallus provide different alginate yields. Kumar & Sahoo (2017) proved that the highest alginate was obtained from the axis (31.5%) compared to the whole, old, and young thallus of *S. wightii*, around 30%, 28 and 26%, respectively. Besides the yield percentage, viscosity is a more comprehensive parameter determined as a quality standard due to its function as an attribute needed for product enrichment, such as stabilizer and thickener. More viscous alginate is often required for food or non-food applications.

The solvent used for pre-extraction and extraction is decisive in obtaining high alginate quality. NaOH and Na_2CO_3 are common solvents with varying concentrations and functionalities, such as cellulose and alginate separation in brown seaweed cells (Peteiro, 2018). According to Ardalan et al. (2018), the optimal extraction method for obtaining alginate quality was to employ Na_2CO_3 2% solvent for 2 hours at 90 °C. The alginate parameters such as yield, viscosity, M/G ratio, and molecular weight were dramatically altered by pretreatment extraction in the form of soaking seaweed in acidic and alkaline chemical solvents (Anwar et al., 2013; Borazjani et al., 2017; Diharningrum & Husni, 2018; Fawzy et al., 2017; Gao et al., 2018).

Alginic acid is an intermediate product of the alginate extraction process frequently generated as a semi-product before further processing. Alginic acid may be generated by adding a certain quantity of HCl to seaweed filtrate (Anwar et al., 2013; Fawzy et al., 2017). According to Diharningrum & Husni (2018), adding HCl to the filtrate produces alginic acid with a fine texture on the surface of the filtrate; however, the texture is difficult to filter, which reduces the yield.

Currently, alginate extraction methods have been widely explored and published. Still, the extraction of alginic acid from seaweed *S. polycystum* by alkaline pretreatment and the influence of the thallus component on alginic acid properties is negligible. According to the description, the research is aimed to define the influence of different alkali solvent concentrations as pretreatment and the thallus parts from *S. polycystum* to produce alginate.

Material and Methods

Materials

The brown seaweed *S. polycystum* was collected from Sebesi waters, Lampung Province, Indonesia (5°55'26.0 "S, 105°29'02.8 "E) during the dry and wet seasons (June and September). Seaweed was taken from the intertidal zone, which is exposed during the low tide and hit by strong waves during the high tide. The seaweed samples were kept in plastic bags and stored on ice during transportation to the laboratory. It was washed thoroughly with fresh water to remove debris and other unwanted materials, stored frozen at -23 °C and thawed by fresh water at room temperature before extraction. The chemical used for alginate extraction includes aquadest, formaldehyde 0.4%, KOH (0.5%, 0.7%, 0.9%), NaOH 40%, selenium 0.25 g, Na_2CO_3, HCl 10%, HCl 0.1N, NaOCl 4%, isopropyl alcohol (IPA), H_3BO_3 2%, concentrated H_2SO_4, NaOH 40%, H_3BO_4 2 %, and Brom Cresol Green-Methyl Red indicator. All chemicals were obtained from Sigma-Aldrich, Germany. Before extraction, all samples were washed thoroughly and divided based on the basal, center/middle, and apical/upper parts of the thallus (Figure 1). The size of the whole thallus used is between 45-50 cm and each part measured around 10-15 cm long as extraction sample. Alginate acid
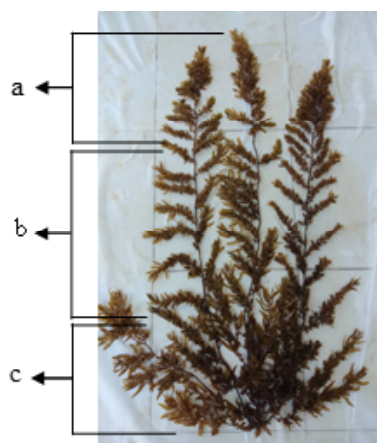

Figure 1. *S. polycystum* thallus parts: (a) apical, (b) central, (c) basal.

extraction was conducted in the Research Center for Marine and Fisheries Product Processing and Biotechnology, Jakarta. Remain analysis was carried out at IPB University, Bogor.

Methods

Proximate Content Analysis

The AOAC (2005) technique determined the moisture, protein, ash, lipid, and seaweed. Moister content was evaluated using gravimetric methods in an oven at 105 ºC using 2 g of samples. The crude ash concentration was determined by incineration, which involved heating 2 g of samples in a furnace at 600 ºC for 8 hours until a consistent weight was reached. The soxhlet procedure was executed with n-hexane as a solvent to get crude lipid from 2 g of samples. After 2 hours of oven drying at 105 ºC, the total crude lipid was measured gravimetrically. The Kjeldahl technique determined the protein content, which converted nitrogen to protein using 6.25 conversion factors. 0.25 g samples were destructed for 1 hour with 0.25 g selenium and 3 ml H_2SO_4. The crude fiber was measured by diluting 1 g of sample in 100 ml of 1.25% H_2SO_4 and 1.25% NaOH. The residue was dried in an oven for 2 hours at 105 ºC before being ashed in a muffle furnace for 30 min at 600 ºC. The weight of the crucible containing the sample after drying and ashing was measured, and the crude fiber content was estimated. The carbohydrate content was calculated using the weight difference between the protein, fat, fiber, and ash values.

Alginate Extraction

The extraction consisted of 3 phases of the process: pretreatment, extraction, and precipitation, respectively, as shown in Fig. 02. A method from Barquilha et al. (2019) was modified for the pretreatment of the *S. polycystum* thallus. The thallus pieces were soaked in 4500 ml formaldehyde 0.4% for 6 h with a 1:30 (w/v) ratio between the samples and solution. After soaking, the samples were washed thoroughly to become free formaldehyde and followed by a second soaking with KOH in different concentrations, which are 0.5%, 0.7%, and 0.9% in the 1:30 (w/v) ratio for 30 minutes.

The extraction procedure used to get alginic acid,. The procedure of extracting alginate is a modified version of the one employed by Rhein-Knudsen et al. (2017). A 1:30 seaweed to solvent ratio is used for extraction and boiled for two hours at 60-70 ºC using 2% Na_2CO_3 solvent. After one hour of boiling, 3 g of diatomaceous earth are added. Once the filtrate temperature reaches room temperature, proceed to filter through a 200-mesh plankton net.

Precipitation was the last phase, which was done by using 180 ml NaOCl 4% in 4500 ml filtrate to generate a bright color of alginate. Alginate acid was performed by adding slowly 470 ml HCl 10% until the pH reached 2-3. The alginic acid was then filtered with plankton net 200 mesh and continued by purification through the isopropyl alcohol with a 1:1 ratio. Finally, the plankton net 200 mesh was used to separate alginic acid from isopropyl alcohol to get pure alginic acid.

Alginate yield was defined as the percentage of alginate powder weight divided by sample mass. Acidic degree or pH value was measured using a pH meter (HM-20J/Japan) from 1 g alginate diluted in 50 ml aquadest. Viscosity was measured using a TV-10 viscometer (Japan) with spindle no. 2 at 60 rpm speed. The viscosity value is counted at centi Poise (cP). A Lutron RGB-1002 colour analyzer (Taiwan) was employed to determine the whiteness degree of the alginate powder. A stable Micro System TAXT2 texture analyzer (Germany) with a 25 mm penetration depth is used to find the gel strength. The characteristics of the gel were observed at the peak of force (g) when the gel broke, divided by the contact area (cm^2). pH, viscosity, whiteness degree, and gel strength are determined according to the Joint FAO/WHO Expert Committee on Food Additives (FAO, 2011). On the other hand, the moister and ash content of the alginate powder were determined according to the AOAC (2005) method.

Data Analysis

All data were expressed as mean ± standard deviation. The effect of different thallus parts and alkali pretreatment were analyzed using a two-way analysis of variance (ANOVA) after normality was performed. Tukey test was employed to determine a pairwise comparison of each treatment. The data analysis was performed by the Minitab 18.1 program.

Results and Discussion

Chemical Composition

The chemical composition of dried *S. polycystum* changed from apical to the middle to base thallus (Table 01). The apical had high quantities of fat, protein, and carbohydrates, with values of 0.39, 5.29, and 49.59%, respectively. On the other hand, the basal section had high amounts of moisture, ash, and crude fiber of around 11.25, 28.86, and 8.56%, respectively. Surprisingly, the intermediate values of all indicators
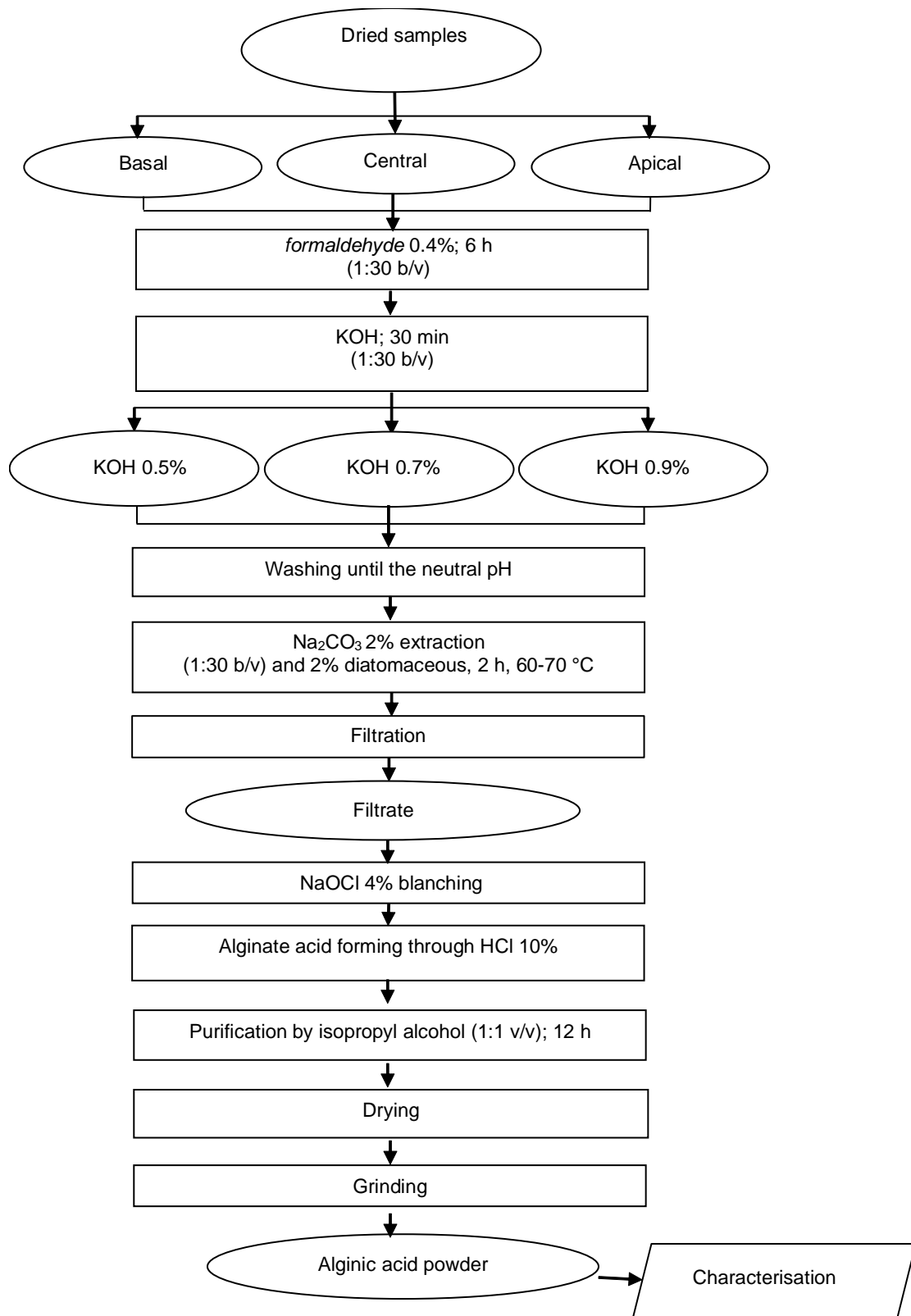
Figure 2. Extraction scheme to obtain alginic acid from the *S. polycystum* Alginate acid characteristics determination.

studied were accounted for by the central part of the thallus. Except for crude lipid content, different parts of the *S. polycystum* thallus significantly impacted the chemical composition of moisture, ash, protein, crude fiber, and carbohydrate ($p<0.05$).

The thickness of the thallus altered the moisture content, which was nevertheless rather considerable and considerably varied between treatments. A thicker thallus has more difficulty evaporating water than a thinner thallus. Regarding moisture, the parts that get closer to the bottom have greater moister due to their thicker features. This condition is affected by the part closed to the root-like (holdfast) that can hold water more effectively. According to Kumar et al. (2015), the physical condition also affects the ash content because the position of the thallus in direct contact with the substrate has a greater mineral content and thus produces high ash. The thallus best absorbs minerals towards the base and close to the substrate. Mineral nutrient absorption occurs throughout the whole surface of the thallus; the quantity of mineral nutrients taken affects the ash content of the seaweed tissue, causing the ash content to rise (Balboa et al., 2016; Kumar et al., 2015). The moisture and ash content of the *S. polycystum* seaweed reported were equivalent to those discovered in prior investigations, such as *S. ilicifolium* has 10.4% moisture and 29.9% ash (Rohani-Ghadikolaei et al., 2012), *S. wightii* has 22.4% moisture and 25% ash (Syad et al., 2013), *S. polycystum* has 9.95% moisture and 42.4% ash (Matanjun et al., 2009), whereas *S. muticum* contains 13.2-30.% ash (Balboa et al., 2016).

The food storage mechanism is carbohydrates, so the fat level in *Sargassum* or other varieties of seaweed that grow in the tropics is relatively low. This differs from seaweed that grows in temperate locations, such as *S. horneri* in Hokkaido waters, with a total fat content of 4.49-10.12% (Nomura et al., 2013). In addition, *Laminaria ochroleuca*, *Saccharina latissima*, and *Saccorhiza polyschides* had 39.64 to 63.09 mg/g

dry weights, respectively (Fernandes et al., 2018). However, the protein, crude fiber, and carbohydrate contents were high and comparable to widely known brown macroalgae in Indonesia, such as *Turbinaria conoides*, which has 5.56% protein, 5.56% crude fiber, and 59.84% carbohydrate content, and *Padina australis*, which has 3.9 % protein, 2.14% crude fiber, and 65.58% carbohydrate content (Siahaan et al., 2018). Muraguri et al. (2016) discovered that carbohydrates' nutrient composition in nitrogen-free extract dominated *Sargassum* type *S. oligocystum* at 71.42 %. Composition varies across species and seasons and between parts of the thallus due to the growth phase, photosynthetic process, and age of the seaweed (Kumar et al., 2015; Murakami et al., 2011).

Extract and Characterization of Alginic Acid

Yield: The alginic acid yield ranged from 20.78 to 28.27 %. The largest alginic acid synthesis was discovered in alkaline pretreatment with a concentration of 0.5% in the central thallus of 28.27%. In comparison, the lowest was found in alkaline pretreatment with a concentration of 0.9% in the apical of the thallus of only 20.78 %. Figure 3 depicts the alginate acid yield value obtained in the research.

The yield of alginic acid generated tends to decrease as the concentration of KOH utilized increases. The results indicated that the alkali pretreatment, the talus portion, and the interaction between the alkali pretreatment and the talus part all had a significant ($p<0.05$) effect on the alginic acid production. According to a prior study, pretreatment using KOH led to a decreased yield in *Sargassum duplicatum* and *Padina* sp. with concentrations of 0.3, 0.5, and 0.7%, with 33.63, 29.23, 23.06 %, and 30.54, 27, and 24.65% of yield, respectively (Anwar et al., 2013; Mirza et al., 2013). Alkali is thought to boost yield, but only at a concentration of no more than 0.6 % at a temperature of no upwards of 80 °C (Mohammed et al., 2020a).

Table 1. Chemical composition of different thallus parts.

Parameter	Thallus parts		
	Apical	Central	Basal
Moisture (%)	10.81±0.02[b]	11.13±0.08[a]	11.25±0.06[a]
Ash (%)	27.06±0.18[b]	27.52±0.45[b]	28.86±0.07[a]
Crude lipid (%)	0.39±0.06[a]	0.34±0.02[a]	0.30±0.04[a]
Crude protein (%)	5.29±0.05[a]	4.51±0.08[b]	3.57±0.06[c]
Crude fibre (%)	6.89±0.26[b]	8.26±0.25[a]	8.56±0.55[a]
Carbohydrate (%)	49.59±0.18[a]	48.25±0.69[ab]	47.47±0.35[b]

Notes: Numbers followed by different superscript letters (a, b, c) in the same line show a significant difference ($p<0.05$).

Based on the Box-Behnken experimental design, research by Fawzy et al. (2017) discovered that combining the usage of alkali, temperature, and extraction period was able to maximize the yield of alginate generated from *Sargassum latifolium*. In general, the yields obtained were comparable to Dharmayanti et al. (2019), Sugiono et al. (2019), and Sumandiarsa et al. (2020), which were 12.09-25.77%, 8.50-45.54%, and 24.18-29.59%, correspondingly.

Additionally, the alginate yield depends on extraction and the condition of the samples, such as growth phase, maturity, age, and parts of the thallus. Kumar & Sahoo (2017) stated that the axis had the highest alginate percentage, and the thallus of young blades had the lowest alginate content. The study resulted in the highest alginate in the axis of 31.50% and the lowest in the young blade thallus, which was 26.47% obtained from *S. wightii*. The older thallus tends to have more food reserves and alginate content due to the different accumulation of food reserves according to seasons (Setyawidati et al., 2018; Zailanie et al., 2001).

Moisture Content

The water content analysis tries to identify the moisture content in a material stated in percent (%), which might affect the alginate's quality and durability. The resultant alginic acid had a water content ranging from 7.04 to 10.66%. The maximum water content was achieved using an alkaline pretreatment concentration of 0.5 to 10.66%. The lowest water content was obtained using an alkaline pretreatment concentration of 0.9%, which was 7.04%. Figure 4 depicts the value of the water content of alginic acid generated in the research.
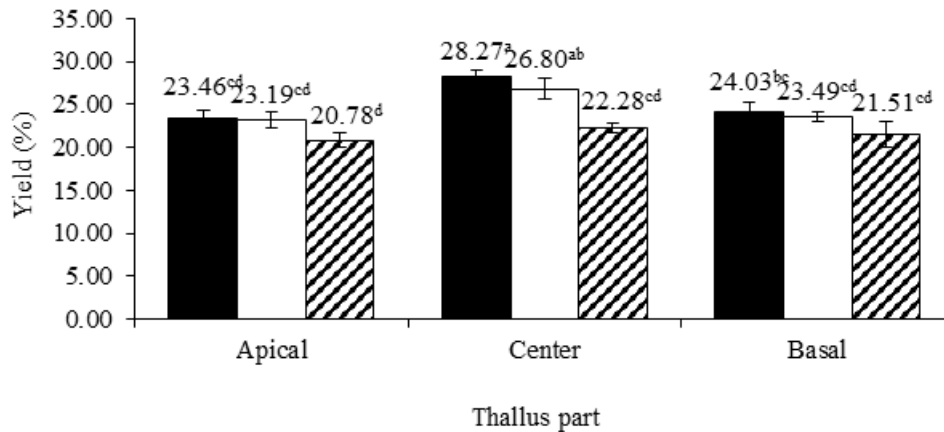
Figure 3. Alginate acid yield from different thallus parts and concentration of the solution (■) KOH 0.5%, (□) KOH 0.7%, and (▨) KOH 0.9%. The values preceded by distinct superscript letters (a, b, c, d) demonstrated a significant difference (p<0.05) in the interaction of the thallus component with solution concentration.
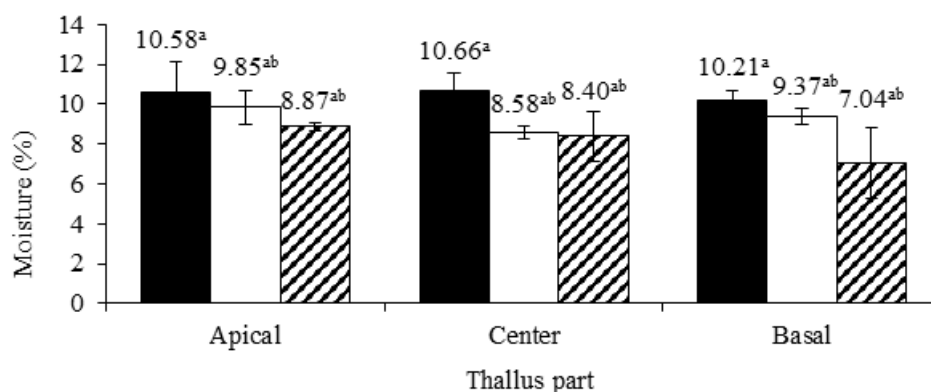
Figure 4. Alginic acid's moisture content on different thallus parts and solution concentrates (■) KOH 0.5%, (□) KOH 0.7%, dan (▨) KOH 0.9%. The values preceded by distinct superscript letters (a, b, c, d) demonstrated a significant difference (p<0.05) in the interaction of the thallus component with solution concentration.

According to the analysis, the concentration of the solution had a significant ($p<0.05$) influence on the water content of alginic acid. However, the thallus part and the interaction between the solution concentration and the talus portion had no significant effect ($p>0.05$). As the alkali concentration increases, the water content of the resultant alginic acid decreases. The decreasing water content of alginate was also found by (Mirza et al., 2013) in the extraction of *S. polycystum* that employed KOH with KOH concentration as a pretreatment of 0.3%, 0.5% and 0.7% and produced water content of 15.31%, 14.05%, and 13.78%, respectively. Septiani et al. (2017) discovered a decreasing water content of alginate employing *Padina* sp. with a KOH concentration of 0.6 % and 0.8 %, producing an alginate water content of 10.93 % and 10.87 %, respectively. The varying water content in this investigation was driven by the kind of seaweed and the different alkali concentrations. In addition, according to the alginate commercial product standard stated by Hernández-Carmona et al. (2013), the water content must be less than 12%, so the result of this research is equivalent to the commercial standard.

Alginate's physicochemical qualities might vary depending on the climate, harvesting season, and sample processing procedure (Zhang et al., 2020). A review by Hernández-Carmona et al. (2013) discovered that the commercial-use moisture property criterion must be less than 12%. Other studies revealed that the moisture content of alginate obtained from *S. polycystum* growth in Banten, Indonesia was 10.23-13.31% (Dharmayanti et al., 2019), *S. polycystum* growth in Sebesi island was 12.16 %% Sumandiarsa et al. (2020), and *Sargassum fluitans* growth in Alor

waters, East Nusa Tenggara was low at 9.35% (Maharani et al., 2017).

Ash Content

The ash content of a product is an important characteristic to understand since it may assess the purity level from undesirable components. Ash is a material that remains as a byproduct of combustion in the form of inorganic compounds in the form of minerals. The alginic acid ash content ranged from 9.34 to 11.33 %. Figure 5 depicts the value of the alginic acid ash content produced in the research.

Figure 5 indicates the maximum ash content achieved in alkaline pretreatment at 0.5 % concentration at the basal and the lowest ash content obtained in alkali pretreatment at 0.9 % concentration at the apical. The solution concentration and the talus parts had a significant ($p<0.05$) influence on the ash content of alginic acid. Still, the interaction between the concentration and the talus parts had no significant effect ($p>0.05$). In this investigation, alginate ash content decreased with increasing concentration of KOH utilized and rose as the talus section extracted was older. Anwar et al. (2013) created decreasing alginate ash content by treating seaweed *S. duplicatum* with KOH concentrations of 0.3, 0.5, and 0.7% and produced ash content of 32.03, 30.85, and 27.82%, respectively. Mirza et al. (2013) discovered reducing alginate ash content by using KOH concentrations of 0.3, 0.5, and 0.7% and producing ash content of 32.08, 30.57, and 30.08%, correspondingly in *the S. polycystum* sample. Overall, the ash contents met the
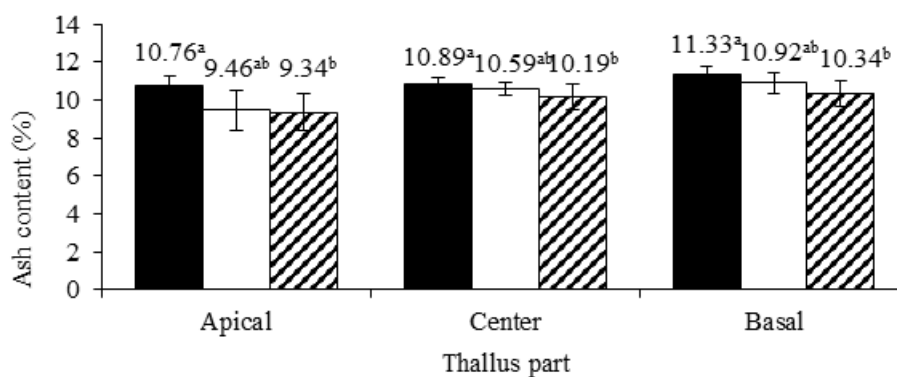

Figure 5. Alginic acid ash content in different parts of the thallus with the concentration of the solution (■) KOH 0.5%, (□) KOH 0.7%, and (▨) KOH 0.9%. There was a significant difference (p<0.05) between the values followed by distinct superscript letters (a, b) in alkaline concentration and (p, q, r) in the talus parts.

commercial standards of 18-27% (Hernández-Carmona et al., 2013).

pH Value

A physical factor determining a product's quality is its degree of acidity or pH value. The alkaline pretreatment with a concentration of 0.9% generated the greatest pH value. Figure 6 shows the pH value of alginic acid synthesized in this investigation. The derived alginic acid tends to rise in pH value as the concentration of alkali applied increases.

The results indicated that the solution concentration had a significant ($p<0.05$) influence on the pH value of alginic acid. However, the thallus parts and their interaction with the solution concentration had no significant effect ($p>0.05$). The increase in alginate pH value as alkaline concentration increases is similar

to the findings of Septiani et al. (2017), who employed a KOH concentration of 0.6–1 % to create an alginate pH value of 8.17-8.39 after converting it to sodium alginate. Alginic acid isolated from *S. wightii* has a pH of roughly 2.35, according to (Sivagnanavelmurugan et al., 2018), even though alginate in the form of sodium, should have a pH range of 6.1-7.8 (Hernández-Carmona et al., 2013).

Viscosity

The main determinant in the properties of the alginate that is closely connected to the quality of the alginate produced is viscosity. The alginate viscosity values obtained in this investigation varied from 24.67 to 53.37 cP. The maximum viscosity was obtained in alkaline pretreatment at the basal thallus with a concentration of 0.9%. Moreover, the lowest viscosity was obtained in alkali pretreatment at the apical thallus with a
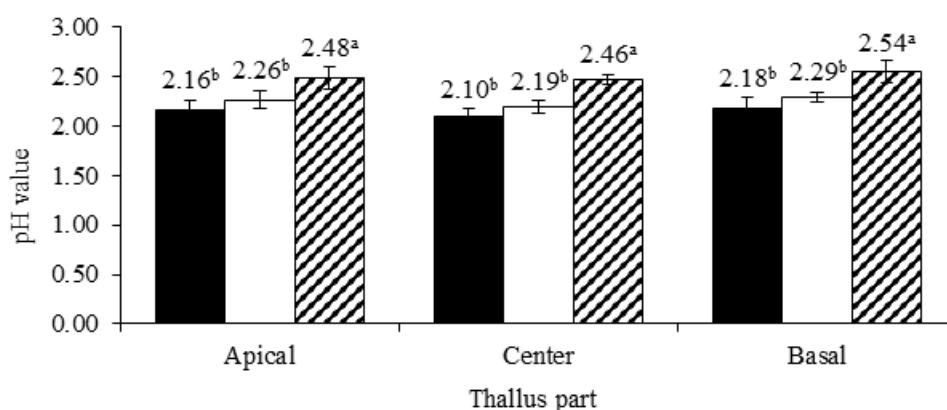
Figure 6   pH value of alginic acid in different thallus parts with concentrations of (▪) KOH 0.5%, (☐) KOH 0.7%, and (▨) KOH 0.9%. Different superscript letters (a, b) indicate a significant difference (p<0.05) at each concentration of the solution.
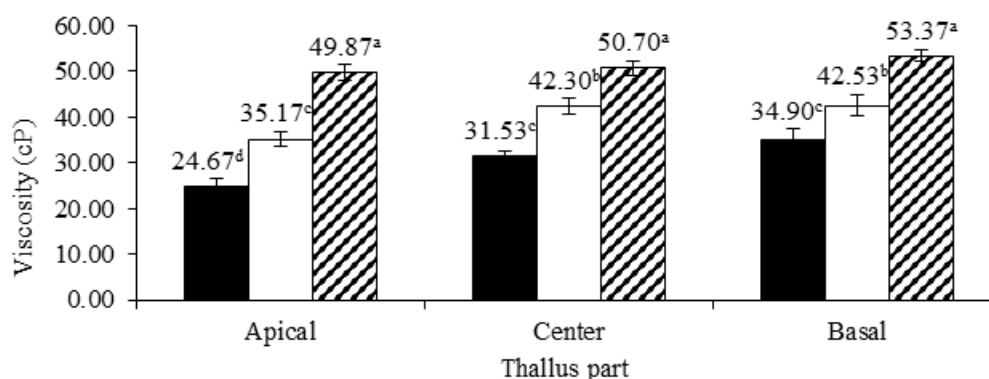
Figure 7   Alginic acid viscosity in different parts of the thallus with the concentration of the solution (▪) KOH 0.5%, (☐) KOH 0,7%, and (▨) KOH 0.9%. Different superscript letters (a, b, c, d) indicate a significant difference (p<0.05) in the interaction between thallus parts and different concentrations.

concentratsion of 0.5%. Fig. 07 indicates the value of the viscosity of the alginate developed in this investigation.

The concentration of the solution, the thallus part, and the interaction of the solution concentration with the thallus portion all had a significant ($p<0.05$) effect on the viscosity value of alginic acid, except the viscosity between center and basal has no significant different in the used of 0.9% solution. The alginic acid generated in this study tends to increase viscosity as alkali concentration increases. Anwar et al. (2013) discovered a rising viscosity value using KOH concentrations of 0.3, 0.5, and 0.7%, producing 14.3 cP, 20.3 cP, and 23.3 cP, respectively. The result indicated that the alginic acid was in a low viscosity level (21-60 cP) according to FAO and commercial standards (FAO, 1987; Hernández-Carmona et al., 2013). According to Mirza et al. (2013), the greater the concentration of KOH used (0.3, 0.5, and 0.7%), the mineral salts present in seaweed decrease, causing the viscosity to rise (17.3 cP, 20.6 cP, and 23.6 cP). According to Saraswathi et al., (2003), the greatest bond between guluronic acid (GG block) was identified in the axis, which ranged from 46.04-53.7% and was continued by the leaf, which ranged from 42.04-49.66%, such that alginate extracted from the seaweed axis provided the maximum viscosity value. The maximum viscosity value on the axis, 13.70 cP, was also obtained in a prior investigation by (Zailanie et al., 2001).

The amount of mannuronic acid (M) and guluronic acid (G) in alginate is linked to its viscosity. The greater the linkages between guluronic acid (GG block) in alginate, the greater the viscosity value achieved (Fu et al., 2011). According to Saraswathi et al. (2003), the greatest bond between guluronic acid (GG block) was identified in the axis, which ranged from 46.04-53.7% and was continued by the leaf, which ranged from 42.04-49.66%, such that alginate extracted from the seaweed axis provided the maximum viscosity value. The maximum viscosity value on the axis, 13.70 cP, was also discovered in a prior investigation by Zailanie et al. (2001). The thallus near the base has the largest ash concentration (Table 1), indicating that it contains many minerals. According to Kumar et al. (2015), the minerals found in Sargassum seaweed include sodium (Na), iron (Fe), and magnesium (Mg), with potassium (K) and calcium (Ca) predominate. Since the talus contains the most calcium, the presence of ionized calcium (Ca^{2+}) can bind to alginate monomers. In the "egg-box" concept, divalent cations such as Ca^{2+} will be bonded in the bond between guluronic acid (GG block); the more Ca^{2+} bound, the stronger the gel and the greater the viscosity (Bhujbal et al., 2014; Mørch et al., 2006).

According to the data, the alginate generated in this study has a viscosity value that is a poor grade. It is lower than previous studies, such as Dharmayanti et al. (2019), with 81.33 cP from *S. polycystum* grown in the western part of Java, but higher than those Anwar et al. (2013), with 14.33 to 23.33 cP from *S. duplicatum* grown in Jepara, Central Java, and only 14.10 cP and 17.22 cP from *Caribbean Sargassum* and *Macrocystis pyrifera*, respectively (Mohammed et al., 2020b; Paul et al., 2020). Furthermore, "Draget et al., 1994" produced an alginic acid viscosity of 14.8 cP in the stipe thallus and 14.2 cP in the leaves using *Laminaria hyperborea* seaweed. The kind of seaweed utilized for extraction, the solution used for soaking before extraction, and the extraction temperature all contributed to the variance in alginate viscosity. Low viscosity of alginate can be used as a food product, such as in addition to ice cream and edible film that need low viscous of alginate (Kok & Wong, 2018; Mulyani et al., 2017)

Whiteness Degree

The degree of whiteness is a parameter used to determine the product's color level. The higher the whiteness value, the higher the quality of the alginate. In this investigation, the whiteness value of alginic acid generated varied from 45.79 to 59.88%. The maximum degree of whiteness was obtained at the apical with 0.9% in alkali pretreatment, while the lowest was obtained at the basal with 0.5% alkali pretreatment. The alginic acid (Figure 8) becomes whiter as the alkali concentration increases. The concentration of the solution and the talus part had a significant ($p<0.05$) influence on the whiteness value. However, the solution concentration and the thallus part interaction showed no significant effect ($p>0.05$). The apical of the thallus has the most whiteness, while the basal has the least. This result also implies that the greater the quantity of KOH and the longer the soaking period, the more the pigments in the seaweed dissolve in the alkaline solution. Seaweed steeped in a 0.9% alkaline solution releases more pigment, causing the alginate produced to turn dark brown.

The age of the thallus (upper thallus as the youngest part), which is older than the talus at the tip and center, accounts for the low degree of whiteness at the base. The pigment content of the thallus increases with age, making the filtrate darker (Basmal et al., 2002). This corresponds with the results of the filtrate in the research, which produced a dark brown and thick filtrate near the basal, the oldest section of the thallus. Herdianto & Husni (2019) explained that the extraction temperature (30, 40, 50, 60, and 70 ºC) is one of the factors that affect the white degree value; the higher
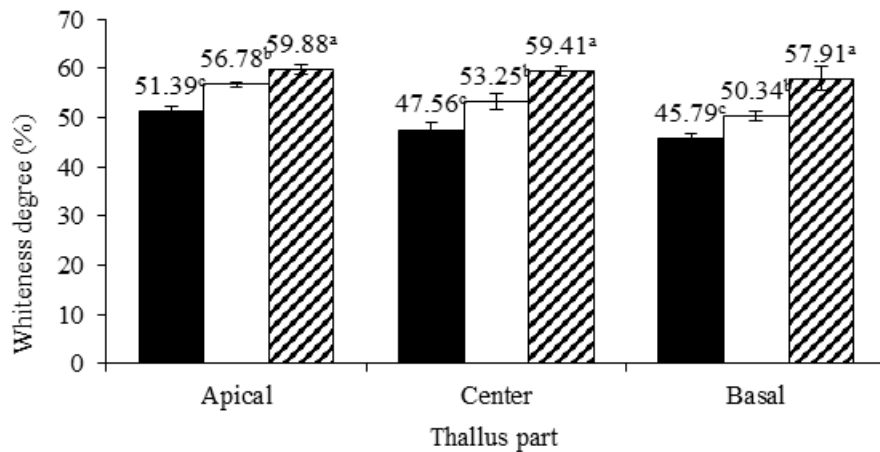
Figure 8. Whiteness degree of the alginic acid from different thallus parts and concentration of (■) KOH 0.5%, (□) KOH 0.7%, and (▨) KOH 0.9%. The numbers followed by different superscript letters (a, b, c) in the alkali concentration and (p, q, r) in the talus section showed a significant difference (p<0.05).

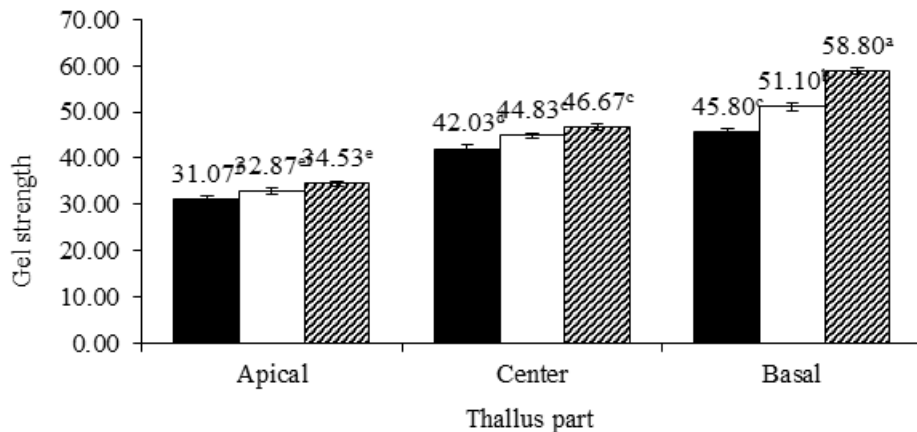**Figure 09.** The alginic acid gel strength from different thallus parts and solution concentration of (■) KOH 0.5%, (□) KOH 0,7%, and (▨) KOH 0.9%. The values preceded by different superscript letters (a, b, c, d, e, f) revealed a significant difference (p<0.05) in the interaction of the talus portion with solution concentration.

the extraction temperature, the higher the white degree value obtained (32.3, 32.8, 32.9, 37.4, and 42.2%). The bleaching agent concentration can also influence the color of the alginate generated. According to Basmal et al. (2001), the higher the concentration of the bleaching agent employed (3-9 %), the more oxidized the chromophore groups in the pigment becomes, increasing the whiteness of the alginate generated (53.37-87.3%). The whiteness value of alginate in this study is typically lower when compared to Diharningrum & Husni, (2018) 's research, which resulted in the whiteness of alginate being 75.27 %. The type of seaweed utilized also contributes to the variance in whiteness.

Gel Strength

The gel strength study assesses alginate's capacity to form a gel. The alginic acid gel formed in this investigation varied from 31.07 to 58.80 g/cm2. The maximum gel strength value was obtained in alkaline pretreatment at the basal part with a concentration of 0.9%, and the lowest was produced in alkali pretreatment at the apical of the thallus with a concentration of 0.5%. Fig. 09 shows the strength of the alginate gel created in this investigation. The thallus part, the solution's concentration, and the thallus parts' interaction with the solution concentration all had a significant (p<0.05) effect on the alginic acid gel

strength value. Gel strength is tightly connected to pH value, and it is achieved in a carefully controlled environment by lowering the pH below the pKa of the uronic acid (Pawar & Edgar, 2012). In addition, the strength of the alginic acid gel generated in this investigation was considerably more significant than that of (Draget et al., 1994), who obtained strength of 15-20.4 g/cm2 from the seaweed *Laminaria hyperborean*, despite the commercial standard is only standardize the viscosity rather than gel strength due to the functional of alginate is for gel performing (Hernández-Carmona et al., 2013).

The ratio of mannuronic acid (M) to guluronic acid (G) in alginate influences the strength of the resultant alginate gel. Since the two monomers have such a distinct structure, the amount of the two monomers in a bond determines the properties of the ensuing gel (Fertah, 2017; Kok & Wong, 2018; Zhang et al., 2020). According to (Qin, 2008), the bond between guluronic acid blocks (GG blocks) may produce a gap between the monomer units, allowing it to bind greater calcium ions. Furthermore, the bond between mannuronic acid blocks (MM blocks) has a poorer calcium ion binding ability. M/G ratios of 0.4, 1.6, and 1.8 were obtained in his research, with gel strengths of 85.1, 32.5, and 25.7 g/cm2, respectively. Additionally, Saraswathi et al. (2003) reported that the highest bond between guluronic acid (GG block) was found in the axis (0.46-0.53) and continued by the leaf (0.42-0.49), which correlated with the results of the study that obtained the highest gel strength value in alginates extracted from the basal of the talus.

Conclusion

The optimum treatment for alginic acid is soaking *S. polycystum* in 0.9% KOH. The thallus component has a considerable impact on the properties of the alginate generated, with the basal having noticeable properties in terms of optimum viscosity and gel strength. As a result, using an alkaline solution as a pretreatment and selecting the thallus parts might be an alternative for creating good-quality alginic acid.

Acknowledgments

Supplementary Materials

Supplementary materials is not available for this article

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