

# Optimization of alginate alkaline extraction technology from *Sargassum polycystum* and its antioxidant properties

*by Yudiati Et Al.*

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## Optimization of alginate alkaline extraction technology from *Sargassum polycystum* and its antioxidant properties

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## 27 Optimization of alginate alkaline extraction technology from *Sargassum polycystum* and its antioxidant properties

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<sup>1</sup> **Abstract.** The quality of alginate may differ considerably depending on several aspects. The aims of this research were to identify, characterize the alginate, examine the quality and determine the antioxidant properties. Identification was done phenotypically. Characterization was done by FT-IR spectroscopy by comparing the samples to standard alginate (Sigma, USA). The extraction was done in two treatments: soaked with 5 % NaOCl, extraction of Na<sub>2</sub>CO<sub>3</sub> (3.0; 5.0; 7.0 and 9.0 %)/EDTA, KCl and precipitated with ethanol (T1) and dried. T2 was pretreated with KOH, HCl, boiled by Na<sub>2</sub>CO<sub>3</sub> (3.0; 5.0; 7.0 and 9.0 %), depigmented, HCl and NaOH addition precipitated with ethanol absolute and then sundried. Antioxidant properties test were done by DPPH and NBT radical scavenging assays. Phenotypic identification showed that the species was *Sargassum polycystum*. There were similarities in signal vibration between the samples and the standard. <sup>39</sup> highest yield was produced from 7.0 % Na<sub>2</sub>CO<sub>3</sub>, while the average yield of T1 (37.56 %) was significantly higher ( $P \leq 0.05$ ) than T2 (21.77 %). The level of dynamic viscosity was correlated with alkali concentration. Indication in T1 results showed that the higher alkali concentration, the better yield. The best yield was the lowest viscosity, produced the strongest antioxidant activity.

### <sup>8</sup> 1. Introduction

<sup>8</sup> Alginates are a group of hydrocolloids, localized in the cell wall and matrix of brown seaweeds and play an important structural role in providing mechanical resistance and flexibility to seaweeds against currents [1]. This natural biomaterial has some excellent properties, such as non toxicity, biodegradability, reproducibility, and biocompatibility. It has a wide range of applications in many fields can be applied in cosmeceutical, foods application [2, 3, 4], nutraceutical and drugs as well as immunostimulants in marine culture [5, 6].

Antioxidants decrease the oxidative damage mediated by free radicals [7]. Due to the severe side effects of many synthesized chemicals which act as a free radical scavenger [8], nowadays, researchers are trying to use antioxidants with the natural origin, including alginates. Researchers have also shown that alginate polymer has antioxidant properties and this property increases by breaking the polymer chain into oligosaccharide [9, 10, 11]. Antioxidants have widespread applications in medical and food industry [12].

The chemical composition and molecular mass of the alginate may differ considerably depending on several factors, such as the algal species, ecological and seasonal variation, or on the age and type of tissue sampled [13, 14]. On the other hand, the extraction and further processing conditions may greatly influence the structure and the composition of the isolated alginate and subsequently its considerable practical utility. The main step in the extraction protocol used in the alginate industry is



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the alkaline treatment. Based on our previous study, compared to acid and calcium alginate, the alkaline alginate produced the highest yield [6].

The brown algae *Sargassum polycystum* abundant in North Sea of Java, Indonesia and still unexploited. The simple and cheap extraction methods of this local tropical alga will pursue some new information regarding methods of extraction with different alkaline concentration, yield, alginates characterization, viscosity and molecular weight as well as its superoxide radical scavenger and DPPH activity.

## 2. Materials and Methods

### 2.1. Identification of brown seaweed

In fact, *Sargassum* genus from Phaeophyta is more than 400 species all over the world [15], including Indonesia [16]. The key of phenotypic characters of brown algae was determined by the main axis, vesicle, receptacle and thallus [17].

### 2.2. Preparation of sodium alginate of *S. polycystum*

*S. polycystum* was collected from the seabed of Panjang Island, Jepara Regency, Central Java, Indonesia. The sodium alginate extract was prepared by two treatments: treatment 1 (T1) [18] and treatment 2 (T2) [19]. The sodium alginate from T1 was collected by overnight magnetic stirrer extraction with 3.0; 5.0; 7.0; and 9.0 %  $\text{Na}_2\text{CO}_3/50 \mu\text{M}$  EDTA. Prior to this, the dried alga was soaked with 5 % NaOCl for 30 min. The pellet was filtered and 0.13 M KCl was added and followed by 96 % ethanol in 1:1 volume and stirred 41 l. Centrifugation was performed at 3,500 rpm for 5 min. Finally, the Na-alginate was collected and then dried overnight in the oven at 60 °C.

The sodium alginate of T2 was prepared by immersing the algae with 0.7 % KOH (1:30) w/v, rinsed with tap water and then soaked with HCl 5 % for one night. After tap water rinsing, the alga were then boiled with  $\text{Na}_2\text{CO}_3$  (3.0 %; 5.0 %; 7.0 % and 9.0 %) (1:20) w/v for 2 h, filtered and discoloured by 4 % NaOCl. The extraction was then added with 10 % HCl (pH 2.8–3.2), followed by NaOH 10 % addition (pH7), precipitated with 96 % ethanol (1:20) w/v and then centrifugated. The Na-alginate was collected and then sun-dried. The two treatments of alginate yield were then determined by comparing the dried weight before and after extraction (%).

### 2.3. Physicochemical properties of sodium alginate

2.3.1. *Water content.* The water content was done following AOAC [20]. The samples of alginate were dried at 105 °C.

2.3.2. *Ash content.* The ash content was obtained following AOAC [20]. This was done by burning the sample in a hot air oven at 200 °C.

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2.3.3. *FT-IR spectroscopy.* The characterizations of alginates were determined spectrophotometrically by signal vibration using Fourier Transformed-Infrared. The preparation was done by mixing the samples with KBr in pellets formation (10 % w/w). The signal was recorded in the 400–500  $\text{cm}^{-1}$  region using a Thermo Nicolet 380 FTIR (Germany).

### 2.4. Viscosity

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The viscosity examination was done with a simple device by comparing the flow times of two liquids of known density [21].

## 2.5. Antioxidant activity

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2.5.1. *Superoxide radical-scavenging property.* This assay is based on the ability of the alginate to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT), in the riboflavin-light-NBT system [22]. The alginate solutions were prepared in 50 mM potassium dihydrogen phosphate buffer (pH 7.8). In brief, reaction mixture composed of 13 mM methionine, 2 µM riboflavin, 100 µM TA, 75 µM NBT and various concentrations of samples (0.05; 1:2 w/v). The reaction mixtures were kept in front of a fluorescent light for 40 min [23], then absorbance was measured using 96-well plate at 560 nm by microplate spectrophotometer (R-Biopharm Well Reader, Germany). Unilluminated identical tubes containing the reaction mixture were used as blanks (control). The relative superoxide radical-scavenging activity was calculated using the equation:

$$\text{Relative activity (\%)} = \frac{As}{Ac} \times 100$$

Where: Ac is the absorbance of the control and As is the absorbance of the sample.

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2.5.2. *DPPH radical scavenging activity assay.* The concentration of samples was one w/v. An aliquot of each sample (100 µL) was mixed with 100 µL of 0.1 mM DPPH (prepared with ethanol) and then followed by incubation for 30 min. [24]. The absorbance of each sample was read at 515 nm using a microplate reader (R-Biopharm Well Reader, Germany). The percentage of scavenged DPPH was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \left[ \frac{(Ac - As)}{Ac} \right] \times 100$$

Where: Ac is the absorbance of the control (100 µL of ethanol with 100 µL of the DPPH solution) and As is the absorbance of the sample. BHA was used as a positive reference.

## **12** 2.6. Statistical analysis

All data were subjected to one-way and two-way analysis of variance (ANOVA) at the level of significance of 0.01 and 0.05. A multiple comparison (LSD) test was used to examine significant differences among treatments using IBM SPSS Statistics 20 computer software.

## **36** 3. Results and Discussion

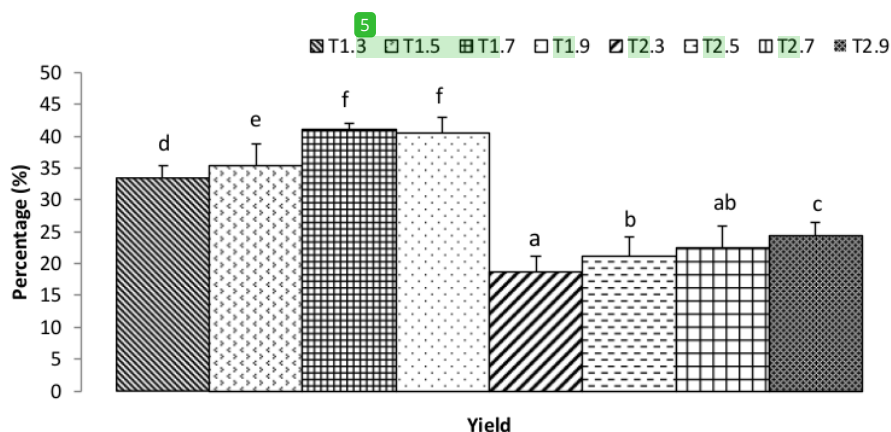
### 3.1. Brown algae identification

The morphology of this species are thallus of variable size from 10 cm in shallow exposed habitats to 1.5 m deeper areas; holdfast discoidal or conical; main axis cylindrical and warty, bearing up to 10 stolon-like branches, 0.5–1 cm long; stolon-like branches cylindrical or flattened, smooth or with few spines, exhibiting sometimes short leaf-like lateral or secondary haptera; secondary axes cylindrical, distichous and bearing numerous “y” shaped spine-like protuberances, up to 0.2 cm in diameter; leaves linear to lanceolate or oblong and straight in lateral view, apex acute or obtuse, margins irregularly serrate, petioles short, basecuneate, midribs percurrent; cryptostomata small, abundant and scattered irregularly on the leaf surface, up to 3 cm long and 0.8 cm wide; vesicles spherical or slightly oval, smooth or bearing prominent cryptostomata and a short spine-like mucro up to 0.25 cm in diameter, petioles cylindrical and shorter than the vesicles; receptacles unisexual; female receptacles simple or branched, cylindrical or slightly flattened, lanceolate, warty, bearing coarse spines; male receptacles simple or bifid, cylindrical, lanceolate, smooth or bearing a few fine spines, possibly mixed with vesicles. Based on the phenotypic characters, it was concluded that the species of this brown seaweed is *Sargassum polycystum*.

The classification is subgenus *Sargassum*, section *Polycystae*, type locality from Sunda Strait, Indonesia. Geographical distribution is worldwide, especially Indo-Pacific region and lives in shallow reef flats and rocky bottom habitat.

### 3.2. Alginate extraction and yield

The yield of *S. polycystum* sodium alginate of T1 and T2 is showed in figure 1. This *S. polycystum* was locally from northern waters area of Java, Indonesia. Based on the result, T1 treatment was much simple and easy method when compared to T2. In fact, the yield obtained from T1 alginates was higher than T2 alginate. The EDTA addition in T1 extraction reached the highest yield at 7.0 % and 9.0 % alkaline extraction. EDTA (*ethylenediaminetetraacetic acid*) has been known as a chelating agent and this, consequently, was improved the extract yield. Similar data were reported from experiment conducting with *S. siliquosum* from the southern sea of Java, Indonesia [6] and the *Sargassum* sp. extract from Madagascar [25]. The other experiment found that in absence of EDTA, the alginate yield is lower (10–13 %) [26].



**Figure 1.** The yield of sodium alginate in different methods (T1 and T2) of *S. polycystum* at 3.0; 5.0; 7.0 and 9.0 %  $\text{Na}_2\text{CO}_3$  concentration. Data at the same sampling time with different letters indicate the significant difference ( $p < 0.05$ ).

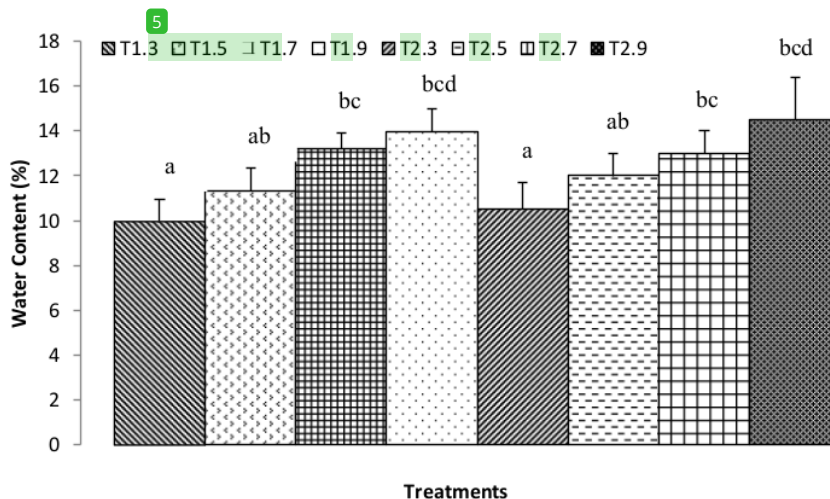
Application of  $\text{Na}_2\text{CO}_3$  during extraction enabling the separation of cellulose from alginate which contained in alginofit cell walls. Therefore, this process increased the yield [27, 28]. The higher  $\text{Na}_2\text{CO}_3$  concentration, the higher yield was obtained. We used 96 % ethanol for precipitation. This influenced the yield due to its ability to bind water from alginate solution, therefore the sodium alginate was separated.

The condition of bleaching in T1 treatment was administered in the initial ground algae, while T2 treatment was administered after alkaline extraction. The T1 resulted in better yield than T2, supported by the similar data by another researcher [26]. Moreover, instead of low alginate yield, the T2 treatment might lead the occurrence of chain degradation.

Some researchers found that *Sargassum* sp. is a potential source of alginate. The best yield of alginate from *Sargassum tenerrimum* from Tuticorin Coast was reported to obtain 32.57 % yield [13]. Similar yield (31 %) of *Sargassum* sp. was reported by previous study [25]. Up to now, the best alginate yield (40 %) was obtained by *Sargassum siliquosum* from the south coast of Java [6] and *Sargassum polycystum* from this research, which originated from the north coast of Java. Both species were the local species of Indonesia.

### 3.3. Water content

The water content was influenced by precipitation of ethanol. Ethanol has an ability to bind water from alginate solution, which reduces the water content [29]. This present study showed the similar water content of T1 (12.13 %) and T2 (13 %) with the earlier report by other researchers [13, 29]. The water content that permitted by Food Chemical Codex (FCC) was less than 15 % [30]. The average of water content for *S. polycystum* in this research was shown in figure 2. This water content has already fulfilled the permitted condition by FCC.

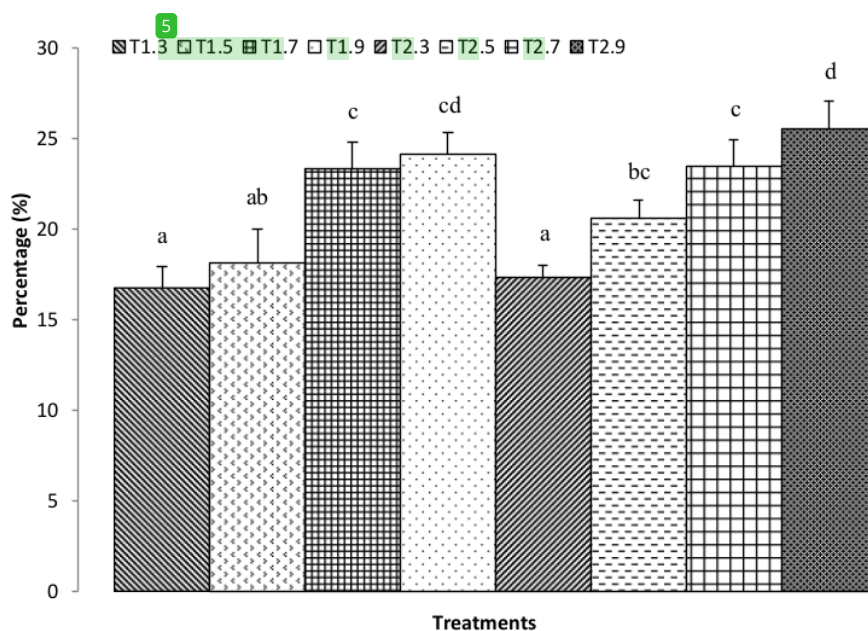


**Figure 2.** The water content of sodium alginate in different methods (T1 and T2) of *S. polycystum* at 3.0; 5.0; 7.0 and 9.0 %  $\text{Na}_2\text{CO}_3$  concentration. Data at the same sampling time with different letters indicate the significant difference ( $p < 0.05$ ).

### 3.4. Ash content

The ash content shows the mineral salt. The composition of alginates directly depends on the season and on the ecological conditions of algae growth [13, 14]. Mineral salt could be found on the surface and in thallus. Conditions of hydrology and hydrochemistry on the habitat also influence the ash content [29]. The average of ash content for *S. polycystum* in this research was shown in figure 3.

In native state, alginates exist as an insoluble salt form of mixed counter ions found in seawater, especially  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  [31]. The addition of  $\text{Na}_2\text{CO}_3$  in different concentration in T1 and T2 treatments have proven that the higher concentration, the higher ash content. This indicates that the extraction process of alkaline was succeeded. The extraction of alginates is based on the conversion from the insoluble to their soluble form using the  $\text{Na}^+$  counter ions slightly basic conditions [26]. The ash content in this research was 16.75 % at a minimum level and 25.56 % at maximum level which was similar to 21.43 % recorded ash content in *S. tenerrimum* [13] and around 20 % in *S. polycystum* in the southern waters area of Java [29].



**Figure 3.** Ash content of sodium alginate in different methods (T1 and T2) of *S. polydactylum* at 3.0; 5.0; 7.0 and 9.0 %  $\text{Na}_2\text{CO}_3$  concentration. Data at the same sampling time with different letters indicate the significant difference ( $p < 0.05$ ).

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### 3.5. FT-IR spectroscopic analysis

The FT-IR spectra from T1 and T2 in 3.0; 5.0; 7.0 and 9.0 % of the  $\text{Na}_2\text{CO}_3$  concentration of alginate compared to the standard alginate (Sigma, USA) can be seen in Figure 4 a, b, c and d the vibration signal is shown in table 1. There was a wide band at  $3,400 \text{ cm}^{-1}$  shows the signal of O-H stretching vibration, while the signal at  $2,900$  and  $1,600 \text{ cm}^{-1}$  was interacted with C-H stretching vibration and O-C=O carboxylate bound asymmetrically. The absorbance around  $1,401 \text{ cm}^{-1}$  is correlated to the deformation vibration of C-OH, which is the contribution of O-C-O symmetrically stretching vibration from carboxylate group [32, 33]. It is shown that the overall spectral pattern was not change by different alginate alkaline methods. The additional bands did not appear.

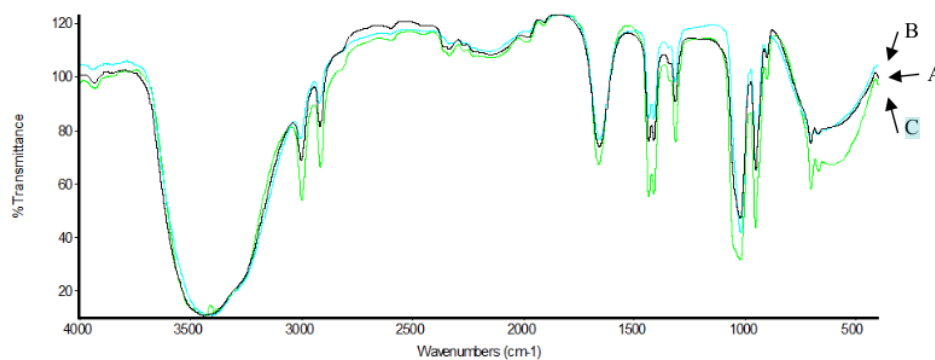
The observed band at around  $1,300 \text{ cm}^{-1}$  was predicted from deformation of C-C-H (and O-C-H) attributes. Furthermore,  $1,095$  bands was the stretching from C-O vibration at pyranose ring. The stretching formation from C-C vibration was measured at  $1,033 \text{ cm}^{-1}$ . The indication of uronic acid which formatted by the C-O group was observed at  $946 \text{ cm}^{-1}$  wavelength number [34, 35]. Moreover, the recorded signal at around  $900 \text{ cm}^{-1}$  shows the existence of asymmetric  $\alpha$ -L-gulopyranuronate vibration ring [32, 34].



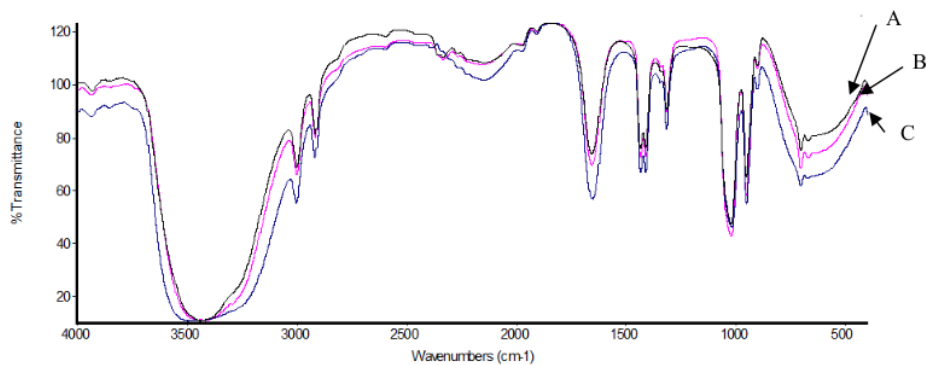
**Table 1.** The vibration signal on fingerprint area of T1 and T2 treatments Sodium Alginate of *S. polycystum*.

Type of Alginate	Wave number (cm <sup>-1</sup> )
T1-3.0 %	952.59
T1-5.0 %	952.93–902,53
T1-7.0 %	933.55
T1-9.0 %	954.02
T2-3.0 %	954.37–901.75
T2-5.0 %	953.44
T2-7.0 %	953.36–902.65
T2-9.0 %	953.26
Standard alginate (Sigma, USA)	941.26–902.96

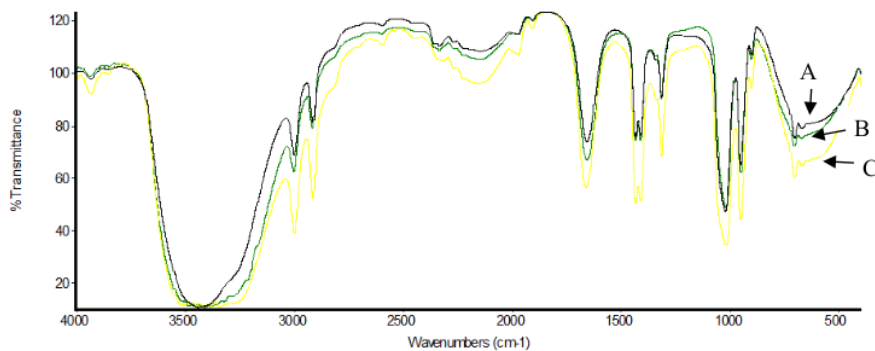
The fingerprint area at 950–750 cm<sup>-1</sup> [32] has been mostly discussed. The spectrum band of three types of alginate at 930–940cm<sup>-1</sup> is referred to C-O stretching of uronic acid residue [36]. The present study of FT-IR analysis showed that spectra of three different types of alginate were fit with that of the standard alginate (Sigma), and positively fingerprinted at a specific alginate wave number (950–750 cm<sup>-1</sup>), though the intensity was varied. Based on figure 4a, b, c and d below, though the overall spectral pattern was similar, some differences were observed in the height and shape of certain absorption bands. The peak at 1,600 cm<sup>-1</sup> and the characteristic broad absorbance in the region of fingerprint area, 1,000 cm<sup>-1</sup> and around 1,300 cm<sup>-1</sup> increased remarkably with the increase in alkaline ex-40tension. This is associated with uronic acid residue, the formation of carboxylate and deformation of C-C-H and O-C-H attributes.



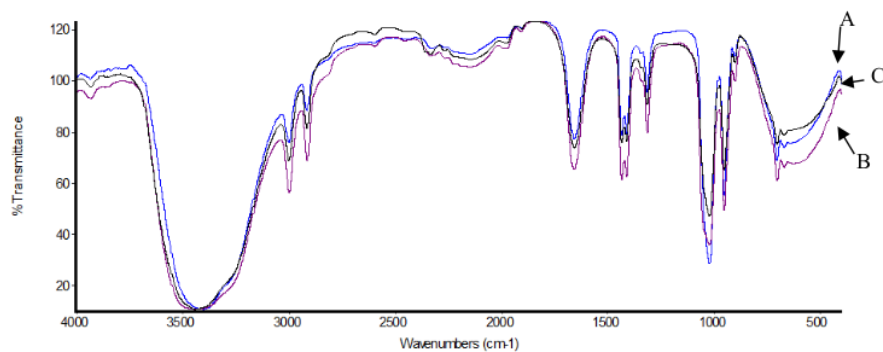
**Figure 4a.** The FT-IR spectra of Standard (A), T1-3.0 % (B), T2-3.0 % (C) sodium alginate of *Sargassum polycystum*.



**Figure 4b.** The FT-IR spectra of Standard (A), T1-5.0 % (B), T2-5.0 % (C) sodium alginate of *Sargassum polycystum*.



**Figure 4c.** The FT-IR spectra of Standard (A), T1-7.0 % (B), T2-7.0 % (C) sodium alginate of *Sargassum polycystum*.

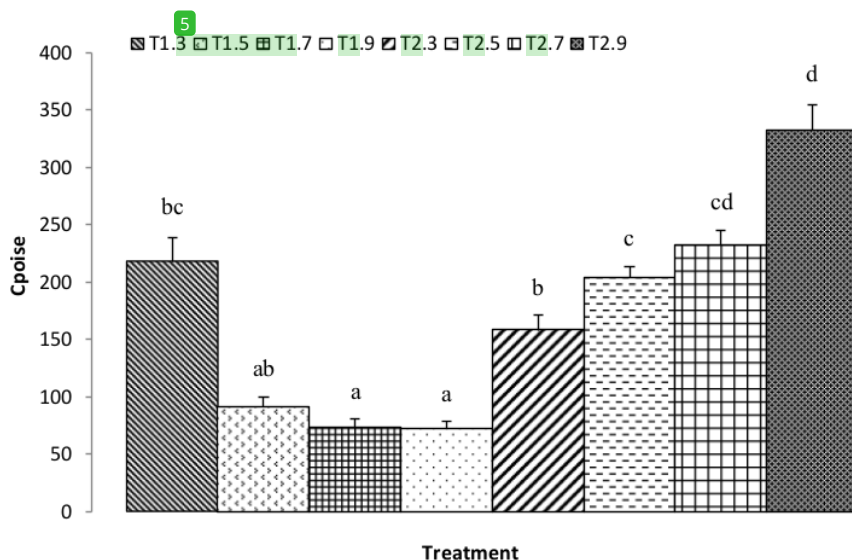


**Figure 4d.** The FT-IR spectra of Standard (A), T1-9.0 % (B), T2-9.0 % (C) sodium alginate of *Sargassum polycystum*.

### 3.6. Viscosity

The viscosity of T1 and T2 sodium alginates in different  $\text{Na}_2\text{CO}_3$  concentration from *S. polycystum* is shown in figure 5. As the results show, by different methods and alkaline concentration, the viscosity in T2 was increased as the increment of alkaline concentration. In contrast, the viscosity of T1 is reduced by the reduction of alkaline concentration. Viscosity related to the molecular weight, the higher viscosity and the higher molecular weight [7]. The viscosity of T2 was classified as medium viscosity (300–1000 cPs) and the viscosity of T1 was classified as low viscosity (< 100 cPs) [37].

Nowadays, according to the consumer preference for natural food ingredients, the researchers tend to enhance the antioxidant activity from natural sources such as alginates. Based on this study, T1 sodium alginate have a potency to be the good source of low viscosity alginates in terms of the extraction methods and the source of tropical alginate from Indonesian habitat.

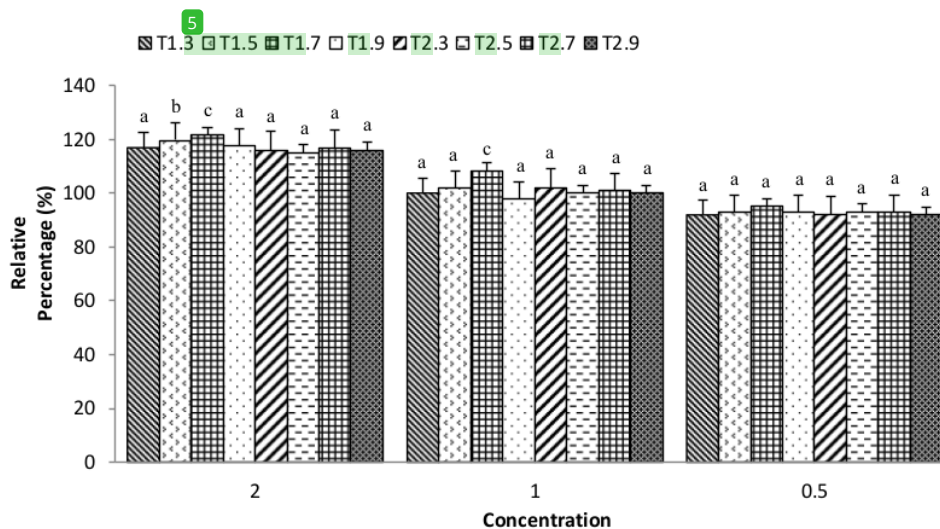


**Figure 5.** The viscosity of sodium alginate in different methods (T1 and T2) of *S. polycystum* 3.0; 5.0; 7.0 and 9.0 %  $\text{Na}_2\text{CO}_3$  concentration. Data at the same sampling time with different letters indicate the significant difference ( $p < 0.05$ ).

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### 3.7. Antioxidant activity

**3.7.1. Superoxide radical-scavenging property.** The ability of T1 and T2 alginates in different  $\text{Na}_2\text{CO}_3$  concentration to scavenge  $\text{O}_2^-$  radical was studied by superoxide radical ( $\text{O}_2^-$ ) scavenging assay in the presence of the riboflavin-light-NBT system (figure 6). As the results show, by applying alkaline extraction, superoxide radical ( $\text{O}_2^-$ ) scavenging activity increased in a concentration and time-dependent manner. These results confirmed that T1-7.0 % alginate in three concentrations had better antioxidant activity compared with others. The increase in antioxidant activity caused by the depolymerization of these polysaccharides. Some studies revealed the low molecular of sodium alginate increased the bioactivity, including the immunity of fish [38] as well as the antioxidant activity [7].

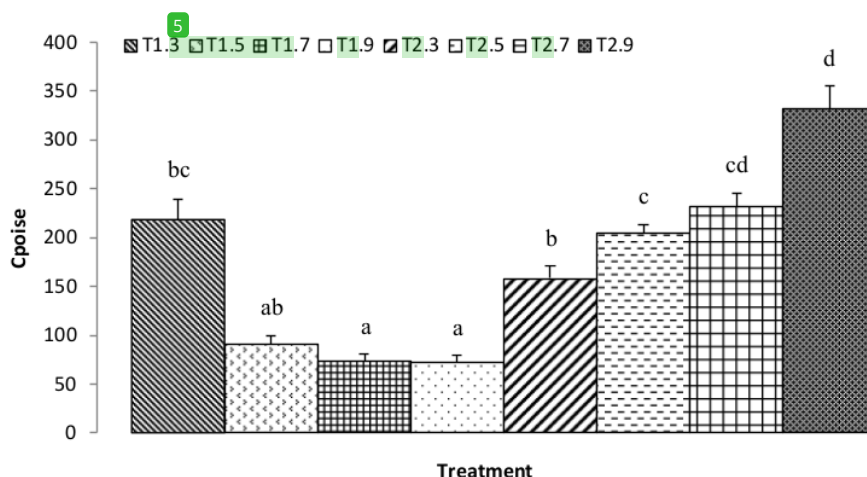


**Figure 6.** Three concentrations (0.05; 1:2 w/v) of relative percentage superoxide radical scavenging activity of sodium alginate in different methods (T1 and T2) of *S. polycystum* at 3.0; 5.0; 7.0 and 9.0 %  $\text{Na}_2\text{CO}_3$  concentration. Data at the same sampling time with different letters indicate the significant difference ( $p < 0.05$ ).

**3.7.2. DPPH radical scavenging activity assay.** DPPH has been extensively used as a free radical to evaluate antioxidant substances that reduce DPPH by donating hydrogen to form the non-radical DPPH-H. The DPPH radical scavenging activity of T1 and T2 sodium alginates in different  $\text{Na}_2\text{CO}_3$  concentration from *S. polycystum* and BHT as positive control are shown in figure 6. The employment of different treatment and  $\text{Na}_2\text{CO}_3$  concentration produced alginates with diverse DPPH scavenging activities. The results exhibited a concentration-dependent antiradical activity. The order of DPPH assay measurements was according to that of NBT assay.

The highest antiradical activity (17.48 %) was reached by T1 at 7.0 %  $\text{Na}_2\text{CO}_3$  concentration. The ability to scavenge free radical is presumably because the donate electrons or hydrogen atoms to inactivate this radical action [9]. In general, the antiradical activity of T2 was lower than T1. This due to the fact that T2 drying technique was sundried and this allowed the degradation of the hydrochemical compound. The drying technique of T1 used the oven at stable temperature (60 °C), therefore the water content was lower than T2. Furthermore, the administration of bleaching reagent after  $\text{Na}_2\text{CO}_3$  extraction caused chain degradation, though promote the yield of extraction, slightly [9]. The antiradical activity of these treatments was much lower than BHT. Based on DPPH and superoxide scavenging assay, T1-7.0 %  $\text{Na}_2\text{CO}_3$  treated alginates displayed best antioxidant activity.

Further efforts have to enhance this antioxidant activity. Researchers have also shown that alginate polymer has antioxidant properties and this property increases by breaking the polymer chain [7, 9, 10, 11]. These include the purification of sodium alginate, producing the oligosaccharide alginate by different techniques i.e. irradiation [11, 39], enzyme application [1, 9, 10] or simply by heating the alginate powder in certain temperature [7].



**Figure 7.** Percentage inhibition of sodium alginate in different methods (T1 and T2) of *S. polycystum* at 3.0; 5.0; 7.0 and 9.0 %  $\text{Na}_2\text{CO}_3$  concentration and BHT as a positive control. Data at the same sampling time with different letters indicate the significant difference ( $p < 0.05$ ).

There was a strong relationship between superoxide radical scavenging activity, DPPH activity and viscosity. Low viscosity indicated the low molecular weight. Similarly to other researchers [7, 38], antioxidant measurements confirmed antioxidant activity of alginate increased upon a decrease in molecular weight.

#### 4. Conclusion

The *S. polycystum* from Panjang Island Coast of Jepara, Indonesia had a high yield of sodium alginate (41.08 %), low viscosity and high antioxidant potency. The FT-IR analysis shows that all types of alginate had a similar character compared with standard Alginate (Sigma, USA). The high yield of alginate from Indonesia promising good opportunities concerning the application of alginate in food, biomedical as well as immunostimulants in marine culture.

#### Acknowledgement

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