Characterizing the Three Different Alginate Type of *Sargassum siliquosum*

Ervia Yudiati[1]*and Alim Isnansetyo[2]

[1]*Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Jl. Prof. H. Soedarto, S.H, TembalangSemarang, Indonesia 50275*
[2]*Department of Fisheries,Faculty of Agriculture, Gadjah Mada University, JL. Flora, Bulaksumur, Caturtunggal, Yogyakarta, Indonesia55281*
Email: eyudiati@gmail.com

Abstract

This research was aimed to identify the brown seaweed, to characterize the acid, sodium and calcium alginate, and to examine the alginate yield. The identification was done phaenotypically. The extraction method was pretreated by ethanol depigmentation, followed by the extraction of Na_2CO_3/EDTA and$CaCl_2$ and presipitated with absolute ethanol. The characterization of alginate was done by FT-IR spectroscopy and Thin Layer Chromatographyby comparing the samples with standard alginate (Sigma, USA). The key of identification showed that the species was Sargassum siliquosum. There are similarities in signal vibration and TLC spots among the samples and the standard. The TLC test was also showed that those alginates contain mannuronic and guluronic acid. The highest yield was produced by Sodium alginate (40.34% ± 0,21), followed by Acid alginate (11.51% ± 0,15) and Calcium alginate (4.8% ± 0,09).

Keywords: *alginate, characterization, Sargassum siliquosum, yield*

Introduction

Biopolymers, that originally come from marine natural products, has been studied very progressively lately (Fawzy *et al*., 2017). Moreover, those application in industry have been devepoled very well. Alginate is one of the biopolymer which can be applicated in cosmetics, foods, drugs (Falkeborg *et al*., 2014) as well as in marine culture (Isnansetyo *et al*., 2014; Yudiati *et al*., 2016). These biopolymers was kept in the brown seaweed cell wall such as *Turbinaria* sp*., Sargassum* sp., and *Padina* sp.

Alginate is a linear polysaccharide which constructed as α-L-guluronate (G) dan C5 epimer β-D-mannuronate (M), homopolymeric blocks (polymannuronate and polyguluronate), and threaded as heteropolymeric blocks (alternative GM blocks or G/M blocks). Guluronate and mannuronate are uronate with carboxyl groups at C5, every configuration shows the difference between two pyranose (Pawar & Edgar, 2011). Alginate is an insolute part of algae (Draget & Taylor, 2011) and take place in intercellular matrix as a gel which consist Natrium, Calcium, Magnesium, Strontium and Barium ions. The ionic composition was determined by the balance of ionic exchange and seawater (Pawar & Edgar, 2011).

Alginate have a high abundant of free hydroxil and carboxyl group bonds which isdistributed along the backbone of their polymers chain. These two functional groups is enable to modify and shifting differently from the original coumpound (Yang *et al*., 2011). Na_2CO_3 is an alcalic compounds which can modify the carboxyl groups, so therefore, shift the conformation. $CaCl_2$ which is added in the Calcium Alginate extraction will build an “egg box” for more stabilized alginate (Yang *et al*., 2009). Both compounds, will be expected producing more yield by EDTA addition (Jork *et al*., 2000).

Alginate is commonly used for food industry as thickening agent due to the ability of gel formation. In marine culture, alginate plays a role in immunostimulating grouper *Epinephelus* spp. (Chiu *et al,* 2008; Harikrishnan *et al*, 2011) and also penaeid shrimps (Cheng *et al*., 2005;Liu *et al*., 2006;Yeh *et al*., 2009; Chung *et al*., 2011). As an anti inflammatory agent (Kezia *et al*., 2013), anti tumor (Cong *et al*., 2014), and as an Herpes Simplex Virus therapy (Sinha *et al*., 2010).

The brown algae *Sargassum siliquosum* is abundant in South Sea, Gunungkidul, Yogyakarta, Indonesia and still unexploitated. The simple and unexpensive extraction methods of this local n tropical algae will pursue some new information by targetting the higher yield of alginate and the

*) Corresponding author

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prosperous of Indonesian alginate industry in the future.

Materials and Methods

Identification of Brown Seaweed

The key of phaenotypic characters of brown algae was determined by the main axis, vesicle, receptacle, and thallus (Nguyen, 2015).Sargassum genus from Phaeophyta is one of the largest species all over the world (Nguyen *et al*., 2013). Consequently, this species is ecologically important in the marine ecosystem including Indonesia. Is has been reported that there was around 400 species of Sargassum spread over the world and 14 of these have been found in Indonesia : *Sargassum duplicatum, S. histrix*, *S. echinocarpum*, *S. gracilimun, S. obtusifolium, S. binderi, S. policystum, S. crassifolium, S. microphylum*, *S. aquofilum, S. vulgare, S. polyceratium dan* S. *siliquosum* (Rachmat, 1999). Due to the morphological plasticity of Sargassum, the genus identification is slighly difficult (Stiger & Payri., 1999).

Extraction ofAcid, Sodium andCalcium Alginate of S. siliquosum

S. siliquosum was collected from Sundak Coast, Gunungkidul, Yogyakarta, Indonesia. The collection were then brought to the laboratory for cleaning up andwas rinsed with tapwater dan then dried up in room temperature without sun light exposure.

The acid, calcium and sodium alginate extract was prepared based on the methods of Jork *et al*. (2000), Davis *et al*. (2004), and Kim *et al*. (2004),respectively. Prior to the extraction, the dried seaweed was depigmented with 85% ethanol until colourless. The preparation of acid alginate was done by 2 hr waterbath extraction of *S. siliquosum* with 2% Na_2CO_3at 70^oC. Filtration was then administered, continued byprecipitation of HCl at pH<1, and followed by centrifugation at 3.500 rpm for 20 min. The supernatant was then discharged and the pellet was washed in absolute ethanol (1:1) and then filtered. The sodium alginat was prepared by overnight magnetic stirrer extraction with 5% Na_2CO_3/ 50mM EDTA. The pellet was then filtered, 0.13 M KCl was added and followed by 96% ethanol in 1:1 volume, stirred well. Centrifugation was then performed at 3.500 rpm for 5 min. The extraction of Calcium alginate was prepared by 60^oC waterbath immersion with 0,2 N HCl for 2 hrs. The pellet was then filtered at pH=7, followed by washing with absolute ethanol and then centrifugation was administered at 3000 rpm for 15 min and added with aquadest until pH=2. Finally, the all types ofalginate were collected and then dried overnight in the oven at 60^oC.The three types of alginate yield were then determined by comparing the dried weight before and after extraction (%).

FT-IR spectroscopy

The characterization of alginates were determined spectrophotometrically by signal vibrationusing Fourier Transformed-Infra Red.Preparation was done by mixing the samples with KBr in pellets formation (10% w/w). It was then recorded at the 4000–500 cm^{-1} region using a *Thermo Nicolet 380 FTIR* (Germany).

Thin Layer Chromatography

Prior to this, the three type of alginate extracts as well as the standard alginate (Sigma, USA) were hydrolised by aquadest dilution and added with TFA (*Triflouroacetic acid*) and then heated up to100^oC for six hours.

The hydrolised alginate were then spotted to the TLC plate (silica gel as static phase and isopropanol, ethyl acetate and aquadest 7:2:1 vol/vol as mobile phase). Aniline was used as visualisation. The plate was then heated up to 105^oC for five minutes. The alginate and monosaccharide compunds was appeared and the Rf value were then counted.

Results and Discussion

Brown Algae Identification

The key phaenotypic characters of this brown algae showed that the main axis is silindrical, the leaves periphere isserrated and sharp, the vesicula was ovally without wings. The receptacle was compact and grouped, cilindrically and without spines. The thallus was ovale, and up to 1 cm in long. Based on the phaenotypic characters, it was concluded that the species of this brown seaweed is *Sargassum siliquosum.*

Alginate Extraction and Yield

Both, acid and sodium alginates, were extracted in Na_2CO_3. Based on the result the yield obtained from sodium and acid alginates was higher than calcium alginate (Table 1). The addition of EDTA in sodium alginate extraction even reached the highest yield. EDTA (*ethylendiaminetetraacetic acid)* has known as a chelating agent and this, consequently, was improved the extract yield more than three times. Rahelivao *et al*. (2013) reported that EDTA addition in three alga species *Sargassum* sp., *Turbinaria* sp. dan *Hormophysa* sp from

Madagscar gave the highest yield. The addition of $CaCl_2$ will build some calcium mats called egg box which is the interaction chain mediated by Ca^{2+} (Yang *et al*., 2009). Based on the molecular density, it has also been reported that $CaCl_2$ was also improve the gelling properties (Yang *et al*., 2011). Eventhogh, the yield of Calcium alginate was the lowest one.

Based on Table 2, the yield from different Sargassum in differrent origin habitats, reported by different researchers are varied. It had proven, that *Sargassum siliquosum*, originally from Indonesia resulted the best yield compare to others.

The commercial alginate is extracted from *Ascophyllum nodosum*, *Laminaria* spp, *Lessonia nigrescens*, *Ecklonia maxima*, *Macrocystis pyrifera* dan *Durviella antartica* and give 40% yield (Draget and Taylor, 2002; Rinaudo, 2007). The conducting research used *Desmarestia distans* showed the highest result (56.4%) when compared to *Lessonia flavicans* (41.3%) dan *Desmarstia ligulata* (47.1%). Acid alginate extraction *Lessonia vadosa*from Chile in spring time only gave 17.6% yield (Chandia *et al*., 2004). The extraction of *S. filipendula* was done by formalin 0.4% (w/w) maseration, Na_2CO_3 addition and acid precipitation. The formaline maseration was aimed to eliminate the phenolic compound and produced the brighter colour (Bertagnolli *et al*., 2014). Hernandez-Carmona *et al*. (2002) have done the extraction of acid to sodium alginate of *Macrocystis pyrifera* brown seaweed and resulted 14.3% in yield. The extraction temperature and the sample size had influenced the sodium alginate yield (44.01–51.8%) of *Laminaria digitata*. The highest result was reached in 40^{o}C and < 1 mm sample size (Fertah *et al*., 2014).

Up to now, 23 tonnes of world commercial alginate is produced from 85 tonnes dries algae. Based on the table above, in fact, the highest yield was produced from this research by *Sargassum siliquosum* from local tropical Indonesian coast.

The ability of alginate into gel formation is a great potency of being a biomaterial product as well as the matrix of supporting the renewable and regeneration of human tissue. Moreover, the alginates have an ability as a biocampatible, biodegradable, non-antigenic dan chelating agent. There are some application of alginate in biomedical has been reported includes the tissue improvement (Chandika *et al*., 2015), drugs wrapping, also useful for the enrichment of the cancer stem cells (CSCs) (Xu *et al.,* 2014). Mutia *et al*. (2011), has also reported that alginate is a fine primary dressing for wound bandage. This due to the fact that of the absorbant strength, so, therefore, the wound become sealed and humid, easy to use, elastic, antibacterial agent, non toxic, hypoallergenic, non carcinogenic, biodegradable and biocompatible and easily degrades into monoscachharides and absorbed by the human body. Furthermore, the ability of alginate as immunostimulants both in fish (Chiu *et al.,* 2008; Harikrishnan *et al*., 2011; Isnansetyo *et al*., 2014) and shrimp (Cheng *et al*., 2005; Liu *et al*., 2006, Yeh *et al*., 2009; Chung *et al*., 2011; Yudiati *et al*., 2016) was quite interested to conduct some expreriments and counteract the problem on marine culture.

Table 1. Yield of three different type of alginate of *S. siliqosum*

Type	Yield (%)
Acid alginate	11.51 ± 0.15
Sodium alginate	40.34 ± 0.21
Calcium alginate	4.8 ± 0.09

FT-IR Spectroscopic Analysis

The FT-IR spectra from three different type of algiante compared to the standard alginate (Sigma, USA) can be seen in Figure 1 and the vibration signal is shown in Table 3. There is a wide band at 3400 cm^{-1} shows the signal of O-H stretching vibration, while the signal at 2900 and 1600 cm^{-1} is interacted to C-H stretching vibration and O-C-O carboxylate bound assymetrically. The absorbance around 1401 cm^{-1} is correlated to the deformationvibration of C-OH, which is the contribution of O-C-O symetrically stretching vibration from carboxylate group (Mathlouti & Keoning, 1986; Silverstein & Webster, 1991).

The obeserved band at around 1300 cm^{-1} was predicted from deformation of C-C-H (dan O-C-H) attributes. Furthermore, 1095 band was the stretching from C-O vibration at pyranose ring. The stretching formation from C-C vibration was measured at 1033 cm^{-1}. The indication of uronic acid wich formated by the C-O group was observed at 946 cm^{-1} wavelength number (Chandia *et al*., 2001; 2004). Moreover, the recorded signal at around 900 cm^{-1} shows the existency of assymetric α-L-gulopyranuronate vibration ring. The maruronic acid residue was observed at 815 cm^{-1} (Mathlouthi & Koenig, 1986; Chandia *et al*., 2001).

The fingerprint area at 950-750 cm^{-1} (Tul'chinsky *et al*., 1976; Mathlouthi *et al*., 1986) has been mostly discussed. The spectrum band of three types of alginate at 930-940 cm^{-1} is referred to

Table 2. The alginate yield in different species of Sargassum

Species	Yield (%)	Reference
S. vulgare	30.2	Behairy & El-Sayed (1983)
S. polycystum	17.1 – 27.6	Saraswathi *et al.* (2003)
S. dentifolium	3.3	Larsen *et al*. (2003)
S. oligocystum	16.3 – 20.5	Davis *et al*. (2004)
S. vulgare	16.9	Torres *et al*. (2007)
Sargassum sp.	17.3 – 30.5	Andriamanantioanina & Rinaudo, (2010)
S. turbunarioides	10	Fenoradosoa *et al*. (2010)
S. tenerrimum	32.57	Parthiban *et al*. (2012)
S. filipendula	15.1 – 17.2	Bertagnolli *et al*. (2014)
Sargassum sp.	31	Rahelivao *et al*. (2013)
S. siliquosum	4.8 – 40.34	This research

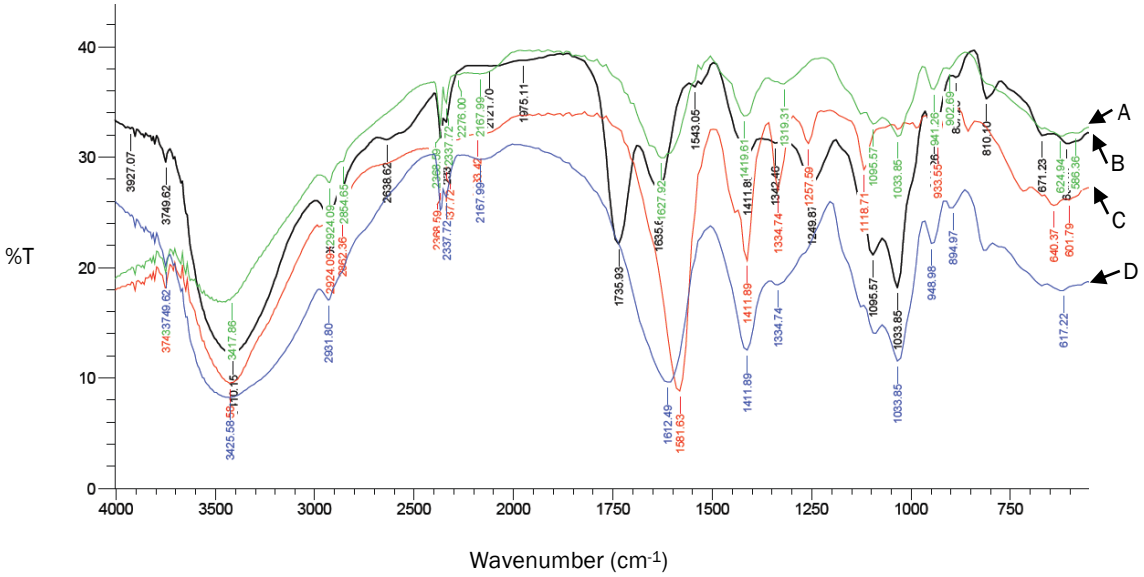

Figure 1. The FT-IR spectra of Standard (A), Acid (B), Calcium (C) and Sodium (D) alginate of *Sargassum siliquosum*

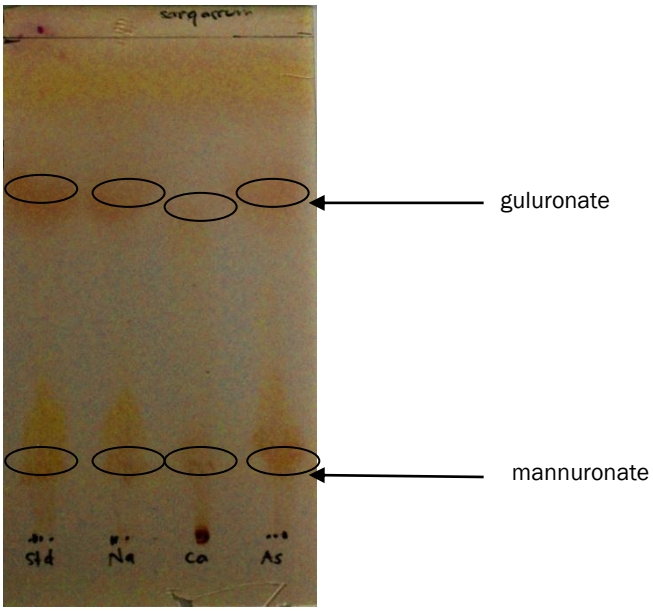

Figure 2. The comparison of Sodium, Calcium and Acid from *S. siliquosum* alginate compounds by Standard Alginate

Table 2. The vibration signal of Acid, Calcium and Sodium alginate of *S. siliquosum*

Type of Alginat	Wavenumber (cm^{-1})
Acid alginate	941,26
	887,26
	810,10
Sodium alginate	948,98
	894,97
Calcium alginat	933,55
Standard alginate (Sigma, USA)	941,26
	902,96

C-O stretching of uronic acid residue (Leal *et al*., 2008). Meanwhile, the band formation at 880-890 cm^{-1} shows the signal vibration of C1-H deformation at β-mannuronic acid. Wave no at 810.10 $cm^{-1}$is the characterisation of mannuronic acid existence (Chandia *et al*., 2001; 2004). 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 finger printed at a specific alginate wave number (950-750 cm^{-1})

Thin Layer Chromatography Analysis

The monosaccharide compund was analysed using Thin Layer Chromatograpy (TLC) Methods. The TLC analysis showed that there were two spots appeared in Acid, Sodium and Cacium and Standard Alginate (Sigma, USA) compounds. The Rf of standard alginate hydrolisate spot was 0.18 and 0.69. On the other hand, the Rf samples were similar to the Standard alginate, except the Calcium alginate (0,18 dan 0.64). TLC results is shown in Figure 2.

TLC is objected to test the extract purity by comparing the extract's Rf and samples' Rf. The simmilar Rf value shows that the compound is similar. Analysis by Zhang *etal.* (2006) based on the HPTLC of guluronic acid and mannuronic acid (silica gel as static phase and *n*-buthanol/ formic acid/ aquadest 4:6:1 (vol/vol) as mobila phase) showed that the Rf value of guluronate acid is higher than marruronic acid. Furthtermore, 0,69 of standard Rf value dan 0.69; 0.64 and 0.69 of Sodium, Calcium and Acid Alginate are guluronic acid. On the other hand, 0.18 is a standard, Sodium, Calcium and Acid Alginate Rf value and these indicate as mannuronic acid. The TLC analysis is shown in Figure 2. By this TLC analysis, it is clearly confirmed that the three alginates samples consist of two monosaccharides ie. guluronic and mannuronic acid.

Conclusion

The *S.Siliquosum* from Sundak Coast of Gunungkidul, Indonesia have a high yield of the sodium (40.34%), followed with Acid (11.51%) and Calcium alginate (4.8%), respectively. The FT-IR and TLC analysis showed that all three types of alginate have a similar characters compared with standard Alginate (Sigma, USA). The high yield of alginate from Indonesia promising a good opportunities concerning the application of alginate in food, industry, biomedical/pharmacy as well as immunostimulants in marine culture.

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