Characterizing the Three Different Alginate Type of Sargassum siliquosum

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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 Na2CO3/EDTA and CaCl2 and precipitated with absolute ethanol. The characterization of alginate was done by FT-IR spectroscopy and Thin Layer Chromatography by 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 deveopled very well. Alginate is one of the biopolymer which can be appilicated 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-gulurionate (G) dan C5 epimer β-D-mannurionate (M), homopolymeric blocks (polymannurionate and polygulurionate), and threaded as heteropolymeric blocks (alternative GM blocks or G/M blocks). Guluronate and mannurionate 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). Na2CO3 is an alcalic compounds which can modify the carboxyl groups, so therefore, shift the conformation. CaCl2 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., 2014). 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 tropical algae will pursue some new information by targetting the higher yield of alginate and the
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. microphyllum, S. aquofilum, S. vulgare, S. polyceratium dan S. siliquosum (Rachmat, 1999). Due to the morphological plasticity of Sargassum, the genus identification is slightly difficult (Stiger & Payri., 1999).

Extraction of Acid, Sodium and Calcium 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 and 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% Na₂CO₃ at 70°C. Filtration was then administered, continued by precipitation 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 alginate was prepared by overnight magnetic stirrer extraction with 5% Na₂CO₃/ 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°C 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 3,000 rpm for 15 min and added with aquadest until pH=2. Finally, the all types of alginate were collected and then dried overnight in the oven at 60°C. The three types of alginate yield were then determined by comparing the dried weight before and after extraction (%).

FT-IR spectroscopy

The characterization of alginate were determined spectrophotometrically by signal vibration using Fourier Transform-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 hydrolysed by aquadest dilution and added with TFA (Trifluoroacetic acid) and then heated up to 100°C for six hours.

The hydrolysed 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°C 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 ovaly 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 Na₂CO₃. 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 (ethylenediaminetetraacetic 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
Madagascar gave the highest yield. The addition of CaCl₂ will build some calcium mats called egg box which is the interaction chain mediated by Ca²⁺ (Yang et al., 2009). Based on the molecular density, it has also been reported that CaCl₂ was also improve the gelling properties (Yang et al., 2011). Even though, the yield of Calcium alginate was the lowest one.

Based on Table 2, the yield from different Sargassum in different 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 Durvillaea antarctica 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 flaviicans (41.3%) dan Desmarshia ligulata (47.1%). Acid alginate extraction Lessonia vadosafom 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, Na₂CO₃ 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°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 alginate has an ability as a biocompatible, 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 monosaccharides 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; Yudiatin et al., 2016) was quite interested to conduct some experiments and counteract the problem on marine culture.

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

<table>
<thead>
<tr>
<th>Type</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>Acid alginate</td>
<td>11.51 ± 0.15</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>40.34 ± 0.21</td>
</tr>
<tr>
<td>Calcium alginate</td>
<td>4.8 ± 0.09</td>
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</tbody>
</table>

**FT-IR Spectroscopic Analysis**

The FT-IR spectra from three different type of alginate 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 CH stretching vibration and O-C-O carboxylic bond asymmetrically. The absorbance around 1401 cm⁻¹ is correlated to the deformation vibration of C-OH, which is the contribution of O-C-O symmetrically stretching vibration from carboxylic group (Mathlouthi & Keoning, 1986; Silverstein & Webster, 1991).

The observed 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 formed 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 asymmetric α-L-gulopyranurons vibration ring. The marunoric acid residue was observed at 815 cm⁻¹ (Mathlouthi & Koening, 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Yield (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. vulgare</td>
<td>30.2</td>
<td>Behairy &amp; El-Sayed (1983)</td>
</tr>
<tr>
<td>S. polycystum</td>
<td>17.1 – 27.6</td>
<td>SARASWATHI et al. (2003)</td>
</tr>
<tr>
<td>S. dentifolium</td>
<td>3.3</td>
<td>LARSEN et al. (2003)</td>
</tr>
<tr>
<td>S. vulgare</td>
<td>16.9</td>
<td>TORBRE END et al. (2007)</td>
</tr>
<tr>
<td>Sargassum sp.</td>
<td>17.3 – 30.5</td>
<td>ANDRIAMANAINTOANINA &amp; RINAUDO, (2010)</td>
</tr>
<tr>
<td>S. turbinarioides</td>
<td>10</td>
<td>FENOAROSOA et al. (2010)</td>
</tr>
<tr>
<td>S. tenerrimum</td>
<td>32.57</td>
<td>PARTHIBAN et al. (2012)</td>
</tr>
<tr>
<td>S. filipendula</td>
<td>15.1 – 17.2</td>
<td>BERTAGNOLLI et al. (2014)</td>
</tr>
<tr>
<td>Sargassum sp.</td>
<td>31</td>
<td>RAHELIVAO et al. (2013)</td>
</tr>
<tr>
<td>S. siliquosum</td>
<td>4.8 – 40.34</td>
<td>This research</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Type of Alginate</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Acid alginate</td>
<td>941.26 887.26</td>
</tr>
<tr>
<td></td>
<td>810.10</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>948.98 894.97</td>
</tr>
<tr>
<td>Calcium alginate</td>
<td>933.55</td>
</tr>
<tr>
<td>Standard alginate (Sigma, USA)</td>
<td>941.26 902.96</td>
</tr>
</tbody>
</table>

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 C=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 Chromatography (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 hydrolysate 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 simillar Rf value shows that the compound is similar. Analysis by Zhang et al. (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 guluronic acid is higher than mannuronic acid. Furthermore, 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 alginites samples consist of two monosaccharides i.e. 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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Characterizing the Three Different Alginate (E. Yudiati and A. Isnansetyo)

