Improving The Antioxidant Activity of Sodium Alginate from *Sargassum* sp. by Thermal Heating and Chemical Methods

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Abstract

The relationship between molecular structure and bioactivity was evaluated for sodium alginates obtained under different degradation treatment (raw alginates, heat-treated, and chemical-treated) from Sargassum sp. This study was conducted to identify the antioxidant activities of the degraded sodium alginate from Sargassum sp. compared to raw extract. Raw alginate as the control treatment was dried overnight at 60 °C, while heat-treated was heated raw alginate at 140◦C in a laboratory oven (4.5 hours). Two chemical-treated were applied. Raw alginate added hydrogen peroxide and raw alginat with hydrogen peroxide plus ascorbic acid. These treatments were replicated three times. All the parameters were evaluated spectrophotometrically. The spectroscopy results from the degradation methods showed a new absorbance spectra pattern. The FT-IR spectrum revealed that treatment affects the structure of the alginates. Heat treated and chemical treated sodium alginates showed non significantly different on DPPH radical scavenging activity. Meanwhile, the combination of alginate and hydrogen peroxide treatment was at the lowest scavenging ability. Therefore, alginate oligosaccharides (AOS) produced by heating or adding chemical reagents could be considered as a stronger antioxidant than raw alginate, which may be applied in the industry and biomedical.

Keywords: *antioxidant activity, Sargassum, sodium alginate, degradation*

INTRODUCTION

Alginates consist of (1,4) linked β-D-mannuronic acid (M) with ^4_1C ring formation and α-L-guluronic acid (G) with ^1_4C ring conformation (Fenoradosoa *et al.*, 2010). Alginates, commonly derived from brown algae (Phaeophyceae), have been widely investigated by many researchers for a possible new alternative in the food industry and medical approach (Cherng, 2018). The recent studies revealed that polysaccharides from *Sargassum* sp. possess multiple functions, such as antitumor (Chen, 2017), immunomodulatory (Borazjani *et al.*, 2017), emulsifying agents (Fawzy *et al.*, 2017), antioxidant activity (Sellimi *et al.*, 2015). Due to the excellent functions, research continues to develop the optimal results of alginate polysaccharides. There have been many research results related to decrease in molecular mass alginate using depolymerization (Sari-Chmayssem *et al.*, 2016; Liu *et al.*, 2019; Dodero *et al.*, 2020). Alginate which has undergone a depolymerization process called alginate oligosaccharides (AOS) (Addina *et al.*, 2020). AOS has been shown to have antioxidant properties (Kelishomi *et al.*, 2016; Chen *et al.*, 2016; Yudiati *et al.*, 2018).

The antioxidant properties are the ability of a compound to inhibit and catch on reactive free radicals in the body by neutralizing the free radical molecules (Kale & Nabunome, 2019). Research conducted by Chen *et al.* (2016) and Yudiati *et al.* (2018) proved that the depolymerization process can improve the ability of AOS antioxidants. Alginate oligosaccharides, oligomers containing 2 to 25 monomers, can be produced by splitting of the glycosidic bonds. AOS can be obtained via physical or chemical methods, enzymatic methods, fermentation, organic synthesis, and biosynthesis (Liu *et al.*, 2019).

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Referring to the AOS obtained using chemical methods, as has been done by Zhang *et al.* (2013) and Chen *et al.* (2016), stated that hydroxyl radical ($HO^{\bullet}$) as a free radical can degrade polysaccharides by attacking and separating glycosidic bonds into powerful oxidants with short-lived. The reaction is could be obtained from H_2O_2 with a reduced transition metal ion. Ascorbic acid is also able to reduce hydrogen peroxide to produce hydroxyl radicals in the presence of trace metals. Therefore, the combination of hydrogen peroxide with ascorbic acid has attracted interest in the degradation of polysaccharides. On the other hand, AOS uses the heating method is reported by Yudiati *et al.* (2018), which is also able to improved antioxidants of *Sargassum polycystum* in 4.5 h treatment (20.07%).

Refers to the simplest and availability of tools and materials for degradation methods, researchers used physical (heat-treated) and chemical (H_2O_2; H_2O_2+ascorbic acid) method. Besides, these two methods are more cost-effective and accessible compared to the other methods (Kelishomi *et al.,* 2016). Herein, the antioxidant activity of the degraded sodium alginate from *Sargassum* sp. is compared with raw sodium alginate extract.

MATERIALS AND METHODS

Raw sodium alginate extraction

The dried and powdered materials (20 g) of *Sargassum* sp. were extracted with Na_2CO_3 5% solution in 500 mL aqueous volume and pH was neutralized to 7 using HCl. The sample was kept and macerated at room temperature for 24 h with magnetic stirrer extraction. Then, the solution was filtered, 0.13 M KCl was added and followed by 96% ethanol in 1:1 volume, stirred well. Centrifugation was then performed for 7 min at 3500 rpm. After centrifugation, the pellet was collected and dried overnight at 60 °C (Yudiati & Isnansetyo, 2017). This method was subjected to Treatment A.

Degradation of raw sodium alginate

There were two types of degradation. The first was used for thermal degradation. The raw sodium alginate powder was heated using an oven at 140 °C for 4.5 h (Yudiati et al 2016). This subjected to treatment B. The later was used chemical compound. The raw alginate (1.0 g) was dissolved in 200 mL distilled water, then added H_2O_2 (Treatment C) and ascorbic acid plus H_2O_2 as Treatment D (molar ratio 1:1) directly into the solution. Afterward, the solution was heated at 51 °C for 1.6 h. The solution was filtered and removed the pellets. The sample solution was precipitated by adding 96% ethanol (ratio 1:3, V/V). Next, the solution was then centrifuged for 10 min at 8000 rpm (Chen *et al.,* 2016). This treatment were replicated three times and aimed to reduce the molecular weight of alginate to facilitate the dissolution process and increase the ability of antioxidants.

UV-visible spectroscopy

The depolymerization process was detected by UV-visible spectroscopy. Aqueous solutions of alginate samples were prepared with distillation water at a concentration of 0.01 (w/v). UV-visible spectroscopy of raw alginate, heat-treated, and chemical-treated alginates were performed by Shimadzu UV-1601 UV-VIS Spectrophotometer in 200-400 nm range (Yudiati *et al.,* 2018).

Fourier-Transform IR (FT-IR) spectroscopy

The FT-IR spectra of alginates were recorded in the 4000–500 cm-1 region using a *Thermo Nicolet 380 FTIR* (Germany). Preparation was done by mixing the samples with KBr in pellets formation (10% w/w) (Yudiati *et al.,* 2018).

Determination of DPPH maximum absorbance

DPPH was weighed for 2 mg and dissolved with 50 mL of ethanol (0.1 mM). The solution is homogenized and incubated for 30 min in a dark place. The solution of DPPH 0.1 mM was pipetted at 4 mL. Absorbance was measured by Shimadzu UV-1280 UV-VIS spectrophotometer in 400-800 nm wavelength range (Chen *et al.,* 2016).

The assessment of antioxidant activity

An antioxidant activity using the method of Chen *et al*. (2016) with

modification of concentration and incubation time. The alginates were diluted with a concentration of 10, 20, 30, 40, 50, and 100 ppm. Each sample was taken 2 mL and added a DPPH solution (2 mL, 0.1 mmol/L). The solution was incubated for 120 min and absorbance was measured by Shimadzu UV-1280 UV-VIS spectrophotometer at the maximum wavelength of DPPH (Chen *et al.,* 2016).

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics 16 computer software at the level of significance of 0.05.

RESULT AND DISCUSSION

FT-IR Spectroscopic Analysis

Figure 1 showed FT-IR spectra from raw (A), heat-treated (B), chemical-treated ie. alginate+H_2O_2 (C), and H_2O_2+ascorbic acid (D) alginate compared to the standard alginate (Sigma, USA). The characteristic bands for the OH stretching vibration were observed at 3431, 3420, 3420, and 3419 cm^{-1}, respectively. It is worth mentioning that bands appearing in the region of 3400 cm^{-1} belong to all types of hydrogen-bonded OH groups. The bands appeared at 1656, 1657, 1652, 1656 cm^{-1} and 1437, 1436, 1436, 1436 cm^{-1} belong to the asymmetric and symmetric – COO^- stretching vibrations, respectively. The main bands revealed were positioned around 1315, 1021, 953 cm^{-1} which could be attributed to deformation vibration of the C-C band, stretching vibration of C-O-C, and O-H bending vibration of alginate. The band about 1315 cm^{-1} could be attributed to the C-OH stretching vibration, while 1021 and 953 cm^{-1} to O-H bending vibration of alginate (Safi *et al.,* 2007; Xiao *et al.,* 2014).

Overall, there were not change in the spectra pattern and no additional bands appeared with the different degradation methods. However, there were some differences could be observed in the height and shape of each absorbed band. The peak broad absorbance band approximately at 3400 cm^{-1} wave number was reduced by the heating method and decreased by the chemical method. Visually, in the combined of alginate with hydrogen peroxide method was a slightly expanded in the wavenumber of 3400 cm^{-1}, which is quite visible compared to raw and other degradation methods, also related to the O-H bonding. This method enables the bonding of O-H tends to be

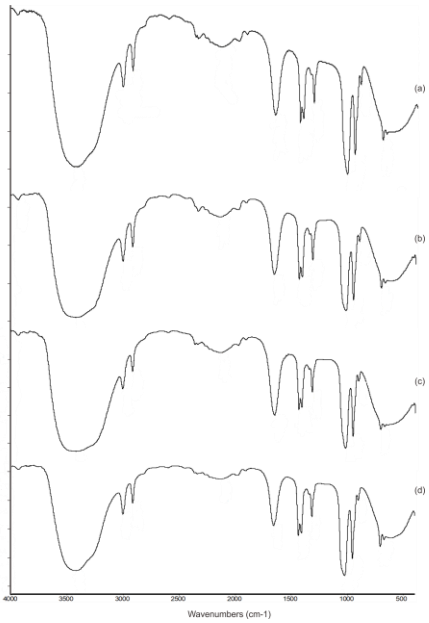

Figure 1. The FT-IR spectra of raw (A), 4.5h heat-treated (B), alginate+H_2O_2 (C), alginate+H_2O_2+ascorbic acid (D) from *Sargassum* sp.

unstable for antioxidant properties than other methods. At the 3400 cm^{-1} wavenumbers, the transmittance of raw alginate and the degradation methods were reduced (35, 25, 15, 10%) respectively. These characteristics reveal the fact that the antioxidant activity is largely contributed by hydrogen cations which play a role in inhibiting free radicals. Furthermore, there is a marked difference in absorbance intensity of about 1600 cm^{-1} and slightly different characteristics in the fingerprint area (750-950 cm^{-1}) but in the same wave range, sodium alginate category (948; 894 cm^{-1}) (Yudiati & Isnansetyo, 2017).

UV-visible Spectroscopic Analysis

Figure 2 shows the UV absorption spectra from raw, heat-treated, and two chemical-treated. The raw alginate showed a sloping spectra pattern. The combination method of alginate and hydrogen peroxide was formed a new pattern at about 250 nm, followed by a combination of the alginate method plus hydrogen peroxide and ascorbic acid.

The heating method begins to form peaks and valleys ranging from 270 and 250 nm accompanied by increasing absorption intensity. It was explained this peak can be ascribed to double bonds formed after the main chain scission of the polymer followed by the ring-opening (Nagasawa *et al.,* 2000). It is consistent with previous results where two peaks were observed and ascribed to carbonyl and carboxyl groups (Ulański & Rosiak, 1992).

DPPH radical scavenging activity

DPPH is a stable free radical compound. In this study showed maximum absorbance of DPPH at 516 nm (Figure 3). It was commonly used in assessing antioxidant activity. The DPPH assays were expressed in IC_{50} values. The concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was determined (Johnson et al 2019). Therefore, the lower value of IC_{50} indicates a higher antioxidant activity (Sivaraman *et al.,* 2013). The DPPH scavenging effect of alginate with the degradation method was higher than raw alginate, however, both showed lower scavenging activity than ascorbic acid (Chen *et al.,* 2016). The IC_{50} values of Treatment A, Treatment C were, 40.86 ppm, 196.15 ppm, respectively. The alginate structure of Treatment C may be damaged due to the destructive properties of hydrogen peroxide which is a strong oxidizing agent. This is confirmed by Zhang *et al.* (2013), in that method, there was only one degradation reagent, H_2O_2, which was unstable and unrepeatable. Therefore, in this study we tried to combined H_2O_2 and ascorbic acid to be

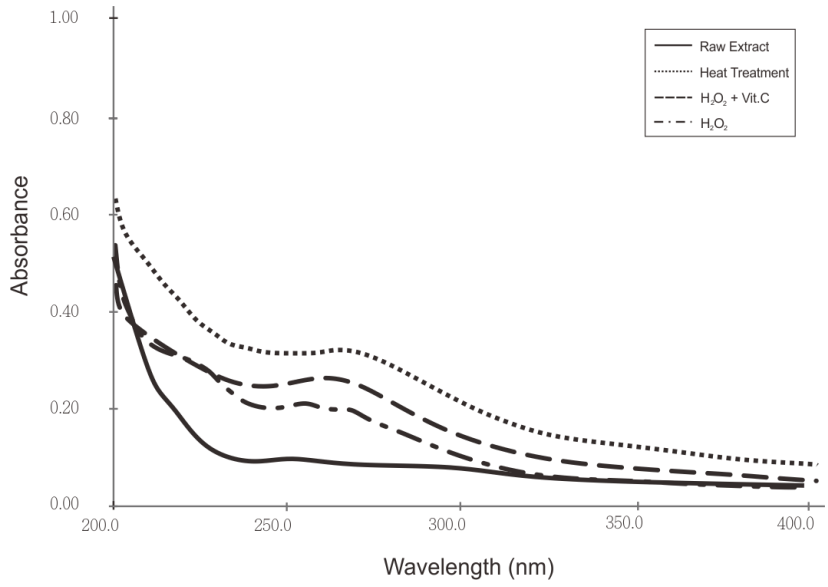

Figure 2. UV absorption spectra for raw alginate, heat-treated and two chemical-treated

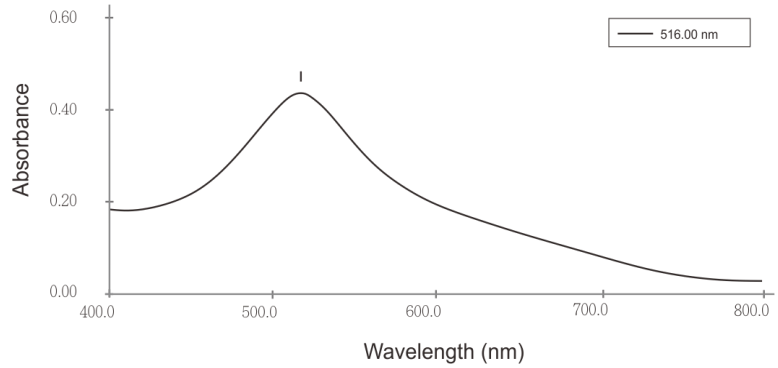

Figure 3. The spectrum of DPPH solution and the peak spectra (516 nm)

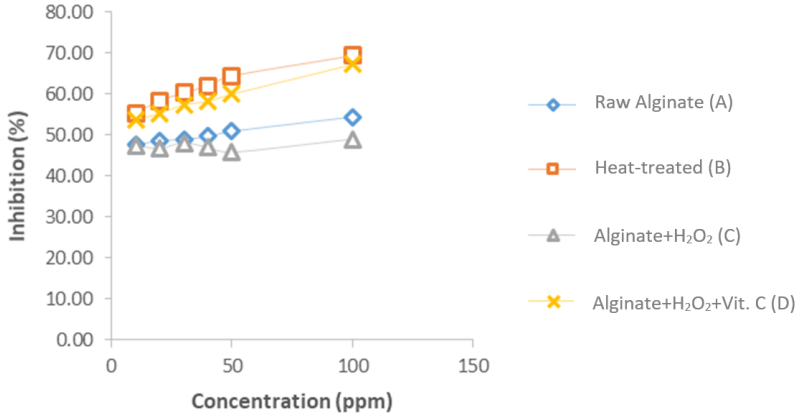

Figure 4. The antioxidant activities of *Sargassum* sp. in different degradation techniques

the degradation reagent, referring to the ability of ascorbic acid which can reduce H_2O_2 to produce hydroxyl radicals in the presence of trace metals (Chen *et al.,* 2016).

Also, the scavenging effect of Treatment B and Treatment D was determined to be 55.39% and 53.71% even at 10 ppm. Research from Yudiati *et al.* (2018) and Chen *et al.* (2016) supported a similar phenomenon. The heat treatment and combined H_2O_2 plus ascorbic acid alginates are showed higher the DPPH radical scavenging activity than the raw alginates. The results suggest that oligosaccharides alginate may expose more active moieties, which could serve as hydrogen donors to scavenge DPPH radicals.

CONCLUSION

Efforts to increase the ability of antioxidants by adding H_2O_2+ascorbic acid for alginate degradation showed from the raw alginate sample (40.86 ppm). These results were not significantly different ($p>0.05$) from the heating method. The combination of alginate and hydrogen peroxide showed the lowest activity (196.15 ppm) ($p<0.05$). Efforts to develop oligosaccharide alginate can be potential in the fields of medicine, cosmetics, and food production.

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