Accelerating The Physilogical Properties of Sodium Alginate Paste by Thermal Method and Microwave Irradiation

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Accelerating The Physilogical Properties of Sodium Alginate Paste by Thermal Method and Microwave Irradiation

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Abstract. Breaking down the polysaccharide of sodium alginate powder to enhance the bioactivity has been done by several methods. This study was aimed to enhance the physiological properties by drying up the sodium alginate paste with cool dry, oven dry, low and high intensity of microwave irradiation. Cool dry technique was done in refrigerated laboratory at 4ºC for 24 hr. The drying technique was done in oven laboratory at 60ºC for 24 hr. The microwave was set up at minimum intensity (2 Ghz, 450 watt, 13 mins) and maximum intensity (4 Ghz, 450 watt, 5 mins) for the low and high microwave irradiation. The assessment of molecular weight, UV-visible and FT-IR spectroscopic studies were applied. The UV-visible spectroscopic studies pointed that there was a new absorption band around 260-270 nm in all treatments. Comparing to the standard alginate, the spectra of FT_IR analysis show that there were some variation in certain bands and fingerprint area. The antiradical scavenging activity (DPPH) of cool and oven dry, low and high microwave irradiation were 24.68%, 26.54%, 40.43% and 45.44%, respectively. The highest antioxidant activity was exhibited from the lowest molecular weight at high intensity of microwave irradiation treatment ($P<0.05$). The simple and practical treatment of high microwave irradiation accelerated the antioxidant activity and reduced the ability of donate electrons or hydrogen atoms to inactivate this radical action.

1. Introduction

Brown macroalgae (Phaeophyceae) including Sargassum spp. contain a sustainable, a soluble carbohydrates known as alginates. Alginate is a linear polysaccharide composed of variable proportions of b-D-mannuronic acid and a-L-guluronic acid linked by 1–4 glycosidic bonds [1]. Sodium alginate is a polyelectrolyte with negative charges on its backbone [2].

Alginates play an important role as gelling and emulsifying agent in food industry as well as in pharmaceutical and medical fields [3]. Alginates were reported for their antioxidant acitivity [4, 5, 6,7,8]. Supplementation of alginate in feed, orally, have managed to boost the immunosystem for fish [9] and shrimp culture [10]. All these properties of alginates are highly influenced by the variation of their molecular weights, M/G ratios, also their molecular conformations [11] and this correlated to their antioxidant activity.

Antioxidants inhibit or prevent oxidation of a substrate, and evolve to protect biological systems against damage induced by ROS (reactive oxygen species). Free radicals are the free electrons which are highly reactive in nature and cause deleterious changes in our body. The main sources of ROS generation are environmental pollution, harmful ultraviolet rays, and metabolism, phagocyte cells etc [13]. Some effort were conducted to increase the antioxidant activity by breaking the polymer chain into oligosaccharide [5].

Depolymerization of polysaccharide has occurred by cleavage of the glycosidic bonds [14]. The glycosidic bonds of algnates are susceptible to various degradation mechanisms such as acidic [15], alkaline [16], enzymatic [17], as well as by reducing [15] and oxidizing [18]. Other methods were also done by thermal heating [8, 18, 19, 20, 21] microwave [19], x-ray [22] and UV irradiation [23]. Some efforts reported was pointed to the sodium alginat powder. In fact, the drying process of alginate from paste to powder is still limited. The objective of this study, was depolymerized the alginate paste from local seaweed by thermal heating and microwave irradiation. The determination of molecular weight and size the alginate powder were done by viscometry. The confirmation of new functional groups were determined by UV–visible and Fourier Transform-Infra Red spectroscopies. The percentage inhibition of antioxidant activity was determined by DPPH superoxide-radical scavenging assay.

2. Materials and Methods

2.1. Extraction and dried up treatment of sodium alginate from Sargassum spp.

Sargassum spp. was originally from Teluk Awur Bay, Jepara, Central Java, Indonesia were collected. The macroalgae were then cleaned, rinsed with fresh water and then dried up in room temperature for three days.

The extraction of sodium alginate was prepared based on [24]. The alginate was then extracted with 5% Na2CO3, over night and then filtered. The supernatant was added with 0.13 M KCl and precipitated with 96% ethanol, stirred well. The extract were then followed by centrifugation at 3.500 rpm for 5 min. The alginate were collected and then dried up in different methods. Cool dry methods was done by placing the alginate extract in refrigerator and using a fan for optimization. Oven dry was done by overnight heating in the oven at 60^{o}C. The microwave irradiation was done in low and high intensity. The low intensity of microwave irradiation was prepared by set up the microwave (Panasonic NN SM322M) at lowest intensity (2 Ghz, 450 watt, 13 mins). On the other hand, the high intensity was done by set up the microwave at highest density (4 Ghz, 450 watt, 5 mins).

2.2. Determination of molecular weight

Molecular weight of cool, oven, low and high microwave irradiation alginates were calculated from the Mark–Houwink equation below:

$$[\eta]_{int} = kM_v^a$$

where Mv and [η]int are the molecular weight of the polymer and the intrinsic viscosity. The constants ‘‘k” and ‘‘a” for alginate are 7.3x10^3 and 0.92, respectively [25].

Oswald Viscometer was used to determine specific viscosities (η_{sp}) of diluted alginate solutions in each treatment [26]. The intrinsic viscosity ([η] $_{int}$) was determined by extrapolating the [η_{sp}]/C vs. C curve to zero.

2.3. Study on Spectroscopy

The determination of depolymerization process by different drying methods were examined by UV–visible spectroscopy. Aqueous solutions of alginate samples were prepared with distillation water. Concentration of the solution was 0.01 (w/v). UV–Visible spectroscopy of different treatments of sodium alginate were performed by Carry 100 Bio spectrophotometer at 200–400 nm wavelength.

2.4. Fourier-Transform IR (FT-IR) spectroscopy

The sodium alginates characterization were determined spectrometrically by signal vibration using Fourier Transformed-Infra Red. The pellets form were made by mixing the sample of alginate with KBr at 1:20 w/w. The pellet was then recorded at the wavelength region of 4000–500 cm-1 using a Thermo Nicolet 380 FTIR (Germany).

2.5. Antioxidant activity

The assay used for the antioxidant activity was DPPH radical scavenging activity. The concentration of samples were 1 w/v. The DPPH solution was prepared in absolut ethanol. An aliquot of each sample (100 µL) was mixed with 100 µL of 0.1 mM DPPH. The mixture was shaken and left to stand for 30 min at room temperature. The absorbance of each sample was examined at 517 nm using a microplate reader (R-Biopharm Well Reader, Germany). The DPPH radical scavenging activity was estimated from the difference in the absorbances for the samples and the blank and expressed as a percentage of DPPH scavenging.

2.6. Statistical analysis.

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

3. Research method

3.1. Molecular Weight Determination

The molecular weight of different drying methods of sodium alginate is shown if Fig. 1. It is clearly proofed that different drying methods on alginate paste, resulted the shorter polymer with different molecular weight ($P<0.05$). Similar to our research before [8] , reduction of molecular weight is probably caused by shortening the polymer chain due to the fact of glycosidic bond breakage. Choi et al (2010) also reported that microwave treatment was managed to degrade high molecular microwave-hyaluronic acid to low molecular weight- hyaluronic acid . The cool and oven dry treatment resulted the similar molecular weight. This probably due to the fact that the breakage of glycosidic bond, which resulted the alginate depolimerisation have not occurred.

Figure 1. The molecular weight of different drying methods of sodium alginate

3.2. Study on Spectroscopy

3.2.1. FT-IR analysis on spectroscopy

The FT-IR spectra of cool dry (B), oven dry (C), mirowave low (D) and microwave high intensity (E) alginate paste compared to the standard alginate Sigma, USA (A) can be seen on Fig. 2.

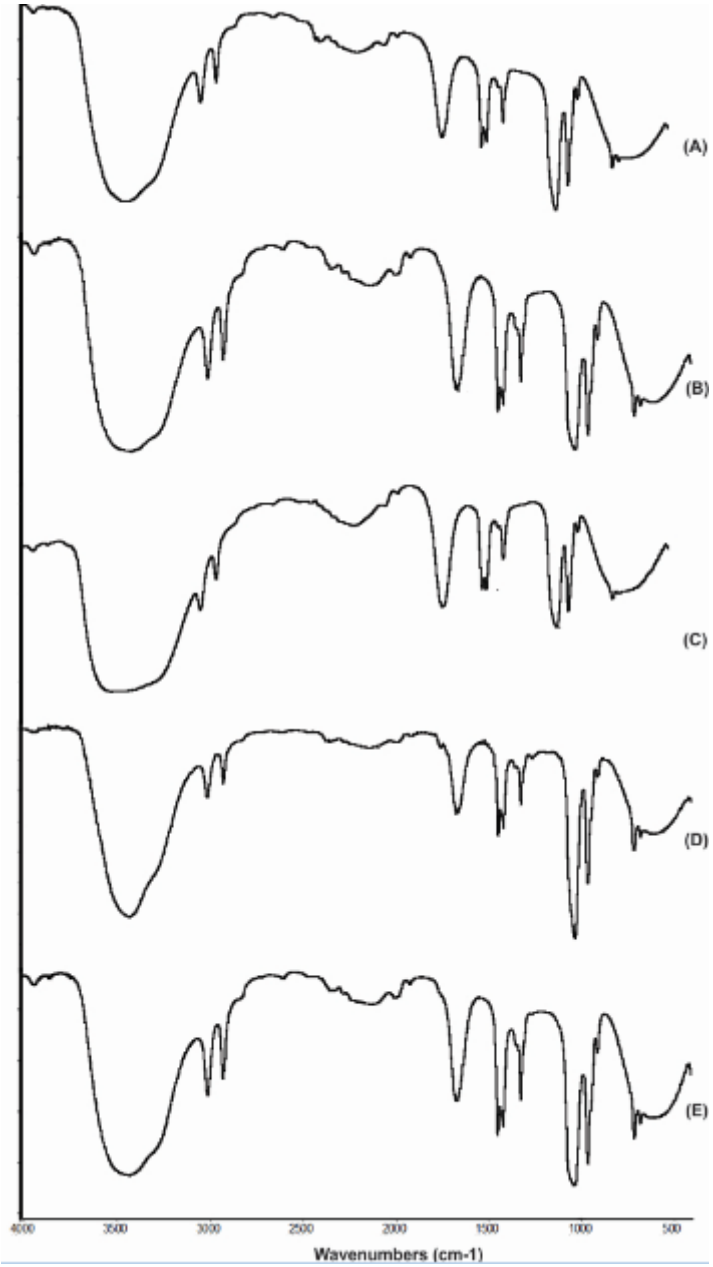

Figure 2. The FT-IR spectra of cool dry (B), oven dry (C); low microwave intensity (D), high microwave intensity (E) and standard alginate Sigma, USA (A) alginate from *Sargassum spp.*

Generally, spectra at 3400 cm^{-1} shows the signal of O-H stretching vibration. C-H stretching vibration and O-C-O assymetric carboxylate bound is shown at 2900 and 1600 cm^{-1}. The observation of band around 1300 cm^{-1} is predicted from deformation of C-C-H (O-C-H) attributes, while 1095 band is pointing the stretching from C-O vibration at pyranose ring. The antisymmetric vibrations of free and hydrogen-bonded COO^{-} groups is appeared around 1500 cm^{-1} and the symmetric bands seems to be appeared at 1400 cm^{-1}. The spectra around 1401 cm^{-1} is signalling the deformation vibration of C-OH, which contributes of O-C-O symetrically stretching vibration from carboxylate group [27, 28]. 1033 cm^{-1} is indicated the stretching formation from C-C vibration. The indication of uronic acid wich formated by the C-O group was observed at 946 cm^{-1} wavelength number of fingerprint area [29, 30]. In addition, the recorded signal at around 900 cm^{-1} shows the existency of assymetric α-L-gulopyranuronate vibration ring [27, 29].

Fig. 2 shows the FTIR spectra in the range of 1200-960 cm^{-1} as a function of different drying methods. The absorbance of cool dry methods is dereased. On the other hand, the absorbance values for the spectra gradually increased with three other methods, which can be attributed to the increase signals from C-C, C-O and C-O-C vibrations associated with the six membered (pyranose) ring of alginate. The absorbance bands within the region from 1800 to 1500 cm^{-1} provide the information of water and carboxylic group of alginate. The knowledge about the skeletal modes of pyranose ring of alginate have also to be determined in the region from 1200 to 960 cm^{-1}. This finding is sinchronized to the research reported by [31] related to their study on alginate drying process.

3.2.2. UV-visible analysis on spectroscopy

Fig. 3. shows the UV spectra of different drying methods of cool, oven, high and low microwave intensity of alginate paste. The oven dried reached the highest intensity. On the other hand, the cool dry was the lowest one. This suggested that the lower temperature, resulted in lower intensity. Dried up the alginate paste at 0^{o}C in the cool refrigerator gave the lowest intensity compared ro 60^{o}C in the oven. [8] and [14] suggested that the peak intensity increased according to the heat treatment.

Based on Figure 3, there was a new absorption band around 250 nm. The formation of peaks around 240 nm spectral region was attributed to the formation of carbonyl groups. Previous studies on depolymerizing of chitosan [32] alginate [33], hyaluronic acid [22, 23] by gamma and x-ray irradiation, assigned these peaks to the same functional groups. Moreover, on their research, [19] used thermal treatment and microwave irradiation to degrade hyaluronic acid. Similarly, [14] used alginate and they reported the presence of the similar band peaks. So, therefore, it was indicated the final products of thermal treatment (cool and oven dry) were similar to the products of the microwave irradiated treatment of alginate. In these treatments, it is postulated that producing the carbonyl groups were potential. This phenomenon was in agreed to our previous reasearch [8]. In addition, absorbance at 234 nm indicates the formation and existence of double bonds between C-4 and C-5 in the pyranose rings [14, 17].

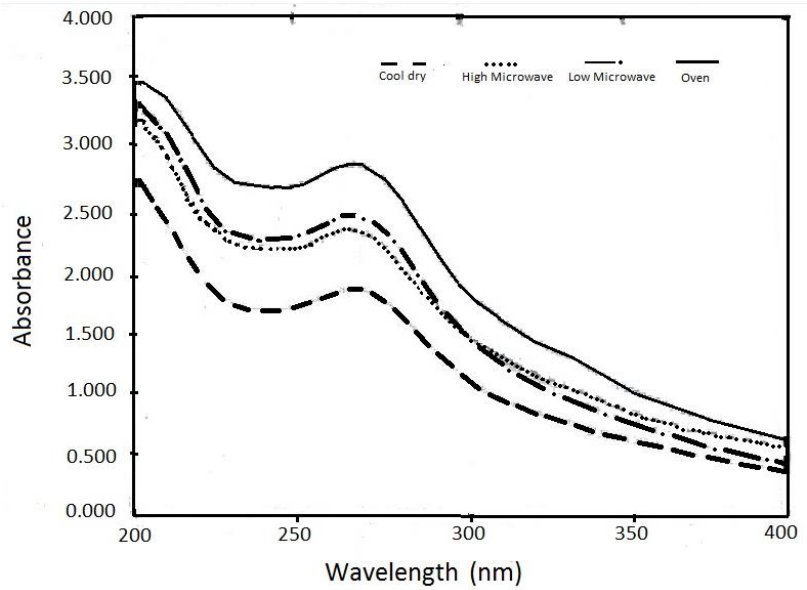

Figure 3. UV spectra of cool, oven, high and low microwave intensity of sodium alginate paste of *Sargassum* spp.

3.3. Antioxidant activity

Diphenyl Picryl Hydrazil-DPPH has been extensively used as a free radical. This to evaluate the antioxidant substances that reduce DPPH by donating hydrogen atoms to form the non-radical DPPH-

H. The DPPH radical scavenging activity of different drying methods of alginate paste treatments are shown in Fig. 4. The application of different drying treatment produced alginates with diverse DPPH scavenging activities. The results exhibited a signficant antiradical activity.

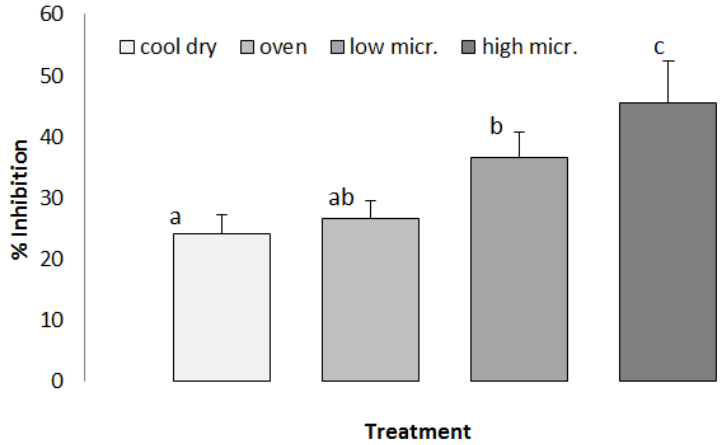

Figure 4. Percentage inhibition of sodium alginate paste at cool, oven, low and high microwave intensity of *Sargassum* spp. Data with different letters indicate the significant difference ($p < 0.05$).

All of the Low Molecular Weight of Sodium Alginate displayed an increased DPPH radical scavenging ability after degradation, especially high intensity microwave treatment (45.44%). Similar to our findings before [8], proofed by spectroscopy study in Fig. 3, a double bond between C-4 and C-5 in the pyranose rings was formed. This double bond was essential for reducing toxicity of the radicals (ROO-, HO-) [19]. Alternatively, the ability to scavenge free radical is presumably because the donate electrons or hydrogen atoms to inactivate this radical action [17]. The weak antioxidant properties of sodium alginates at cool dried methods values may be attributed to their insoluble form. The browning of MW-treated may also be due to a double bond formation and this could lead to an increase in the DPPH radical scavenging activity [19, 34].

4. Conclusion

Producing the low molecular weight of sodium alginate by dried up in microwave treatments was simple and practicle methods. This lower sodium alginate molecular weight lead to enhance the antioxidant activity.

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