

Biophysical-chemistry Characterization of Alginate in Brown Algae Species *Sargassum dupplicatum*

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Abstract: Alginate is a high-value biopolymer, exists in brown algae, and applied widely in numerous fields, for example, food, functional foods, and pharmaceuticals. The study focused on the biophysical-chemistry characterization of alginate in brown algae species *Sargassum dupplicatum* grown commonly in Vietnam under the effect of NaCl, KCl, MgCl₂, chitosan, carrageenan, and ethanol in different physical condition. Antioxidant activity of alginate and their compound was also studied. The results showed that Na_{Alg}, K_{Alg}, and Mg_{Alg} disperse the net style in the water. Ca_{Alg}, chitosan_{Alg}, and Carrageenan_{Alg} absorbed water and swelled for forming a sphere, yarn, and yarn in the water, respectively. Chitosan_{Alg} and carrageenan_{Alg} precipitated faster in ethanol, compared to Na_{Alg}, K_{Alg}, and Mg_{Alg}. 20% of ethanol did not cause the precipitation of Alginate salt, Chitosan_{Alg}, and carrageenan_{Alg} for 30 minutes. Alginate salt, Chitosan_{Alg}, and Carrageenan_{Alg} were full precipitated for 30 minutes when using ethanol concentration was more than 80%. Chitosan_{Alg} and Carrageenan_{Alg} occurred the precipitation in 20% of ethanol for 30 minutes. Total antioxidant activity and reducing power activity of chitosan_{Alg} got the highest value (a and b, respectively), compared to Carrageenan_{Alg}, Na_{Alg}, K_{Alg}, and Mg_{Alg}. The antioxidant activity difference in a group of Na_{Alg}, K_{Alg}, and Mg_{Alg}, and a group (Chitosan_{Alg} and Carrageenan_{Alg}) did not happen. The difference only occurred between the two groups.

Keywords: Alginate, Chitosan, Carrageenan, Antioxidant, Ethanol

1. Introduction

Alginate is calcium, magnesium, and sodium salts of alginic acid found commonly in the cell wall of brown algae. Alginate is an unbranched biopolymer consisting of (1→4)-linked β-d-mannuronic and α-l-guluronic acid residues. The structure, the bioactive, and the content of alginate, as well as mannuronate to guluronate ratio in alginate, depended on various algae species, habitation, and season. The mannuronate/guluronate ratio of alginate in species *Cystoseira barbata* [1], *Laminaria digitata* [2], *Macrocystis pyrifera*, and *Laminaria hyperborea* [3] was 0.59 (37%: 63%, mannuronic acid, and guluronic acid, respectively), 1.2, 1.6, and 0.45, respectively. The viscosity

and average molecular weight of alginate varied from 31 to 5,500 (cm³/g) and 5,100 to 2,700,000 g/mol, respectively [4]. They can absorb water taking up 200–300 times, compared to their weight for forming the gelation that depends on alginate concentration [5] and temperature [6]. Therefore, they are useful in the food industry, medicine, cosmetics, and biotechnology. Different types of alginate play various roles, for example, emulsifiers, gelling agents, thickener, stabilizer, coating agent, and membrane agent. Alginate is also used in the immobilization of enzyme and pigment, in inks, in material (bioplastics, dentistry, prosthetics, and lifecasting).

Alginate possesses value-high bioactive consisting of cytotoxicity, antibacterial, antioxidants [2, 7], heartburn [8], and gastroesophageal reflux disease [9-11]. Their bioactive

was improved when they combined to active polymer, for example, chitosan [12], carrageenan [13], polyphenol [14], and drugs [15]. Previous studies show that free radicals and oxidation stress are one of the reasons leading to the disease of reflux esophagitis, Barrett's esophagus [16; 17], and inflammation [18] in humans. Therefore, antioxidant activity is one of the values and desirable activities of alginate.

Brown algae species *Sargassum duplicatum* found commonly grown in numerous areas in the world, for example, Pacific ocean [19], Indian ocean [20]. The previous studies show the content and characterization of alginate extracting from the species, but mainly the extraction yields [21], typical alginate, the morphology, chemical element, and functional groups [22]. The non-notices presented alginate of the species in Vietnam.

Thus, the study presented the characterization of physical, chemical, and biological (total antioxidant activity, and reducing power activity) of alginate extracting from *Sargassum duplicatum* commonly grown in Vietnam.

2. Material and Methods

2.1. Material

Alginate (Alg) from brown algae *Sargassum duplicatum* commonly found in Vietnam was extracted in HCl solution (pH 2) for 24 hours and filtering through the membrane. The residues were soaked in Na₂CO₃ solution (pH 9) at 50°C for 4 hours according to the solvent to algae of 40/1 (v/w) and filtering the membrane. Alginic acid in the filtrate was precipitated in HCl solution (pH 2) and filtered. Alginic acid was continuously neutral cleaned by using Na₂CO₃ solution, precipitated by using 96% ethanol and dried at 60°C for 30 minutes.

κ – carrageenan was extracted from red algae *Kappaphycus alvarezii* at 80°C for 2 hours with aqueous according to the aqueous to red algae ratio of 30/1 (v/w). After extraction, the supernatant was filtered through the membrane, precipitated in 96% ethanol, and dried at 60°C for 20 minutes for collecting κ – carrageenan. The viscosity and gel strength of κ – carrageenan corresponded to 67.5 mPa/s and 218.9 g/cm². The SO₄²⁻ content of κ – carrageenan was 21.4±0.2%.

Chitosan was prepared according to the enzyme-assisted chemical method as the description in [23] with a light adjustment. Shrimp heads waste was macerated in the distilled water at 50°C for 2 hours and collecting the residue through the filtration. The residue was then soaked with the enzyme/residue ratio of 2% (pH 8) at 55°C for 8 hours. The mixture was continuously adjusted pH by using 1N NaOH and stopped the reaction by the heat at 90 oC for 05 minutes. The protein in the residue was continuously removed by 2% of (w/v) NaOH at room temperature with the solid/liquid ratio of 1/5 (w/v) for 12 hours. After the protein movement, the demineralization in the deproteinized residue was by soaking at room temperature for 12 h in 4% HCl solution with the solid/liquid ratio of 1/5 (w/v) for collecting the chitin. Chitin was soaked in 50% (v/v) NaOH at 65°C for 20 hours and

purified by dissolving in 1% lactic acid, and the precipitation of chitosan was by 4% NaOH at pH 10. The purified chitosan was finally cleaned by using 96% ethanol and dried by the freeze-drying method.

All chemicals using in the analysis were from Sigma - Aldrich, except for distilled water and 96% ethanol of Vietnam.

2.2. Sample Preparation

10 g of alginate was in turn dissolved in difference solutions, for example, 5% of NaCl, 5% of KCl, 5% of CaCl₂, 5% of MgCl₂, 1% of chitosan, 1% of carrageenan for forming the mixture of sodium alginate (Na_Alg), potassium alginate (K_Alg), magnesium alginate (Mg_(Alg)), chitosan alginate (chitosan_Alg), and carrageenan alginate (carrageenan_Alg), respectively. These mixtures were filtered and evaluated on the characterization of physic chemistry and biology (antioxidant activity). For the evaluation of the precipitation of Alg mixture in ethanol, the adjustment of ethanol concentration in these mixtures corresponded to 20%, 40%, 60%, 80% và 100%, respectively.

2.3. Evaluation of Physical Characterization

The phenomenon observation occurred in the reaction between alginate, different salts (sodium, potassium, magnesium, and calcium), and polymers (chitosan and carrageenan) for evaluating physical characterization (morphology).

2.4. Evaluation of Chemical Characterization

The sugar component determination was by using the machine HPLC-UV (Agilent 1100 Series).

The functional group analysis was on the equipment IR Equinox 50, Bruker.

The determination of the precipitated Alg mixture ratio in ethanol for 30 minutes was to base on alginate content that existed in the solution and not precipitated.

2.5. Quantification of Alginate Content

One ml of 0.8 M sodium hydroxide mixed 01 ml of alginate solution (1mg/1ml) and after 5 minutes, neutral by 120 ml of 2.25 M citric acid. The mixture then added to 40 ml of DMMB reagent (Dimethylmethylene Blue Assay), vortexed, and kept for 45 minutes at room temperature. The absorbance measurement of the mixture was at the wavelength of 520 and 650 nm for the calculation of alginate content based on the ratio of 520:650 nm [24] that used in the determination of non-precipitating alginate ratio in ethanol.

2.5. Evaluation of Biological Characterization

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2.5.1. Total Antioxidant Activity

Total antioxidant activity was determined as in [25], basing to the metabolism of Mo^{6+} to Mo^{5+} with ascorbic acid standard, and the absorbance measurement at the wavelength of 695 nm.

2.5.2. Reducing Power Activity

Reducing power activity was determined to base to the metabolism of Fe^{3+} to Fe^{2+} with FeSO_4 standard, described in [26]. The absorbance measurement was at 655 nm.

2.6. Data Analysis

All experiments were in triplication ($n=3$). Statistic analysis was by using the software MS. Excel 2010.

3. Results and Discussion

3.1. Physical Characterization of Alginate

K_Al, Na_Al, and Mg_Al dispersed and formed a distributing network in the water leading the viscosity characterization of the solution that was dependent on the concentration of ion metal (Na, K, and Mg). The viscosity increased following the non-linear model as the concentration increase of Na, K, and Mg. Ca_Al in Ca^{2+} solution, Alg in 1% of chitosan at pH 4.5, and Alg in 1% of carrageenan at pH 7 formed the fibrous or spherical shapes that depended on the injection tip. Alg, Chitosan_Al, and Carrageenan_Al did not disperse in the water but absorbed and swelled in the water. The absorbency tendency of the Na^+ hydrogels in salt solutions was stronger than Ca^{2+}

hydrogels and K^+ hydrogels, as in [27]. Moreover, the LD_{50} of Na_Al (mg/kg body weight) was higher than Ca_Al and K_Al. Therefore, different Alg salts led the various applications, for example, Na_Al in the beverage, the food, and the pharmaceuticals, Ca_Al in material, and K_Al in the mask and the cosmetics.

3.2. Chemical Characterization of Alginate

3.2.1. The Precipitation of Alg Mixture in Ethanol

The table is as follows: All Alg mixture (Na_Al, K_Al, Mg_Al, and Ca_Al) did not precipitate in 20% of ethanol, except for chitosan_Al and carrageenan_Al that formed the precipitation in 20% of ethanol. When the ethanol concentration increased to 40%, the weight of Na_Al, K_Al, Mg_Al, Ca_Al, chitosan_Al, and carrageenan_Al precipitated corresponding to 1/6, 1/6, 1/6, 1/5.5, 1/5, and 1/5 of their weight. 2/3 weight of Alg mixture (Na_Al, K_Al, Mg_Al, and Ca_Al) was precipitated in ethanol when ethanol concentration increased to 60% (Table 1). At the condition same, $\frac{3}{4}$ weight of chitosan_Al and carrageenan_Al precipitated in ethanol. The whole weight of the Alg mixture consisting of Na_Al, K_Al, Mg_Al, Ca_Al, chitosan_Al, and carrageenan_Al was precipitated in ethanol more than 80%. The ethanol concentration was proportional to the precipitation of the Alg mixture. The higher the ethanol concentration, the greater the water separation out of the Alg mixture. ionic cross-links in the presence of various divalent cations, e.g. Ca^{2+} , Mg^{2+} , by cross-linking the carboxylate groups of the guluronate groups on the polymer backbone.

Table 1. The precipitation weight of Alg mixture in ethanol for 30 minutes.

Interaction factor with Alg	Formed mixture	Ethanol (%)				
		20	40	60	80	100
NaCl	Na_Al	None	1/6	2/3	All weight	
KCl	K_Al	None	1/6	2/3	All weight	
CaCl_2	Ca_Al	None	1/5.5	2/3	All weight	
MgCl_2	Mg_Al	None	1/6	2/3	All weight	
Chitosan	CS_Al	PO	1/5	3/4	All weight	
Carrageenan	Ca_Al	PO	1/5	3/4	All weight	

Note: None: Non precipitation; PO: Precipitation appears.

3.2.2. Functional Groups of Alginate in *Sargassum duplicatum*

The IR spectrum of Alginate extracting from brown algae *Sargassum duplicatum* had the peaks at a wavenumber of 3264 cm^{-1} , 2900 cm^{-1} , 1723 cm^{-1} , 1600 cm^{-1} , 1409 cm^{-1} , 1322 cm^{-1} , 1190 cm^{-1} , and 1027 cm^{-1} (Figure 1). The peak of 3264 cm^{-1} showed O-H bonds stretching vibrations that described in the range of $3000\text{--}3600\text{ cm}^{-1}$ in [28] and $3,130\text{ cm}^{-1}$ in [29]. The peak at 2900 cm^{-1} in rang of $2920\text{--}2850\text{ cm}^{-1}$ and the peak at 1190 cm^{-1} s belonged to the stretching vibrations of aliphatic C-H and the C-O stretching vibration of pyranosyl ring, respectively, described in [28]. The absorption band at $1,612\text{ cm}^{-1}$ corresponding to the C-C stretch [29], the stretching vibrations of carboxylate anions at around 1610 cm^{-1} [30] and $1620\text{--}1598\text{ cm}^{-1}$ [28]

showed the peaks at wavenumber 1600 cm^{-1} in the current study can be due to the carboxylate anions stretching vibrations. Reference [31] showed that the peak at 1406 cm^{-1} related to the deformation vibration of the C-OH group and O-C-O symmetric stretching vibration, and in the current study, the IR spectrum of alginate appeared the peak at wavenumber 1409 cm^{-1} . The peak at 1322 was due to the C-N bending vibration, as in 1327 cm^{-1} in [32]. The peak at 1027 cm^{-1} may be due to the group of C-C-H and O-C-H, as in the band at $1,089\text{ cm}^{-1}$ [29] and 1030 cm^{-1} [31]. According to [28], the ingredients of alginate polymer are sodium homopolymannuronate that presented at the band of $1100\text{--}1010\text{ cm}^{-1}$. The difference in the signal of functional groups of alginate between the current study and the previous studies can be due to the brown algae species and the extraction method.

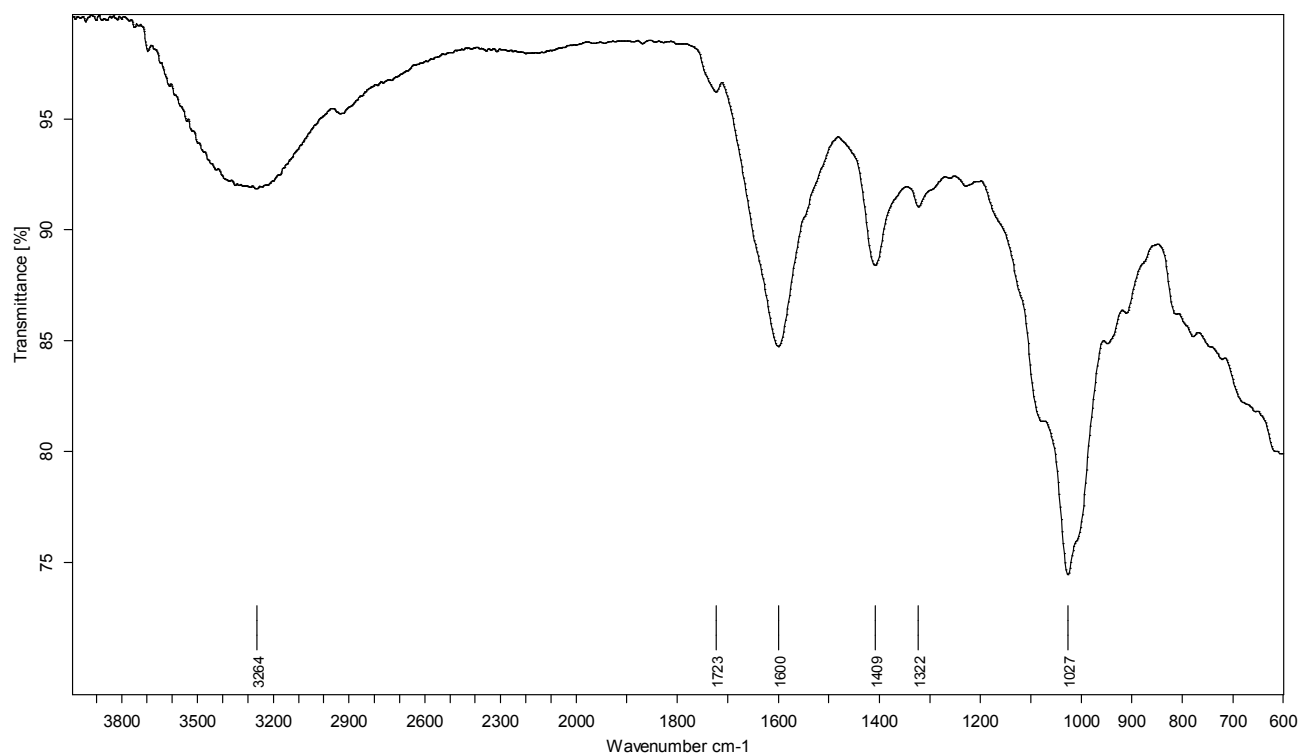


Figure 1. IR spectrum of Alginate.

3.2.3. Sugar Composition of Alginate in *Sargassum duplicatum*

Table 2. The precipitation weight of Alg mixture in ethanol for 30 minutes.

Targets	Units	Results
Galactose	mg/g	32.00
Rhamnose	mg/g	50.15
Manose	mg/g	41.74
Glucose	mg/g	61.41

The figure 2 is as follows: Alginate in brown algae *Sargassum duplicatum* contained different sugar compositions, for example, galactose (32 mg galactose equivalent/g alginate), rhamnose (50.15 mg galactose equivalent/g alginate), mannose (41.74 mg galactose equivalent/g alginate), and glucose (61.41 mg galactose equivalent/g alginate) (Figure 2). Mannuronic acid and guluronic acid are uronic acid derived from mannose and gulose (C-3 epimer of galactose), respectively. Galactose, rhamnose, mannose, and glucose are basic sugar constituents of brown algae joining the formation of alginate.

3.3. Biological Characterization of Alginate

Biological characterization of alginate in the current study focused on the antioxidant activity consisting of total antioxidant activity and reducing power activity. The highest antioxidant activity (35.24 ± 1.14 mg ascorbic acid equivalent/g DW) belonged to chitosan_Alg, compared to other Alg mixtures. The followings were carrageenan_Alg, Mg_Alg, K_Alg, and Na_Alg. The antioxidant activity of Ca_Alg could not analyze because of the too high noise in

the analysis and non-disperse Ca_Alg in water.

The insignificant difference in the antioxidant activity of chitosan_Alg and carrageenan_Alg appeared ($p > 0.05$), and was found similar in Mg_Alg, K_Alg, and Na_Alg. The difference was significantly occurring between the Alg salts groups (Mg_Alg, K_Alg, and Na_Alg) and the polymer Alg groups (chitosan_Alg and carrageenan_Alg) ($p < 0.05$).

3.3.1. Total Antioxidant Activity

Total antioxidant activity of various Alg mixtures was 23.62 ± 17.52 , 23.54 ± 17.01 , 23.66 ± 16.79 , 35.24 ± 23.54 , and 33.17 ± 21.86 mg ascorbic acid equivalent/g DW, corresponding to Na_Alg, K_Alg, Mg_Alg, chitosan_Alg, and carrageenan_Alg, respectively.

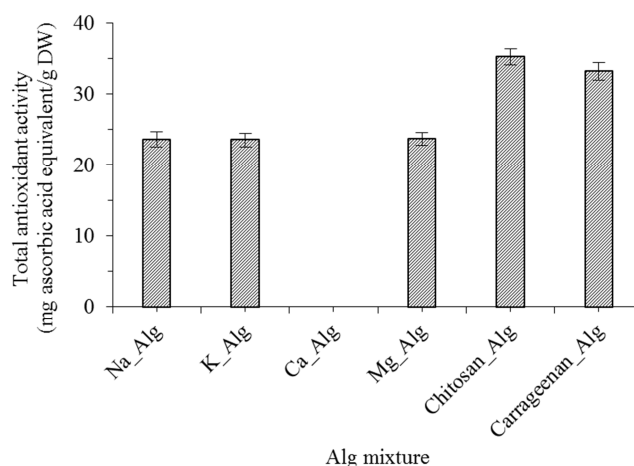


Figure 2. Total antioxidant activity of Alg mixture.

The average total antioxidant activity of polymer Alg groups was 1.45 times of Alg salts (Figure 2). Ca_Alge did exhibit antioxidant activity, suitable for the previous studies. Ca_Alge was useful in the forming of calcium alginate hydrogels, for example, encapsulation of lemon balm antioxidants [33], encapsulation of yerba mate polyphenols [34], and calcium alginate hydrogels consisting of antioxidants, alginate, and chitosan or starch. Chitosan_Alge and carrageenan_Alge exhibited antioxidant activity, suitable for the previous notices on antioxidant activity of alginate [1], carrageenan [35], and chitosan [36].

3.3.2. Reducing Power Activity

Reducing power activity of Alg mixture was in rang of 16.79 ± 0.92 and 23.54 ± 1.19 mg FeSO₄ equivalent/g DW, corresponding to Mg_Alge and chitosan_Alge, respectively. Reducing power activity of Alg was arranged in the decreasing order as follows: Mg_Alge, K_Alge, Na_Alge, carrageenan_Alge, and chitosan_Alge. The difference between the Alg salts group and polymer Alg group was significant ($p < 0.05$), but did not occur in the group ($p > 0.05$). Reducing power activity of Alg salts varied from 16.79 ± 0.92 to 17.52 ± 1.12 mg FeSO₄ equivalent/g DW, corresponding to Mg_Alge and Na_Alge, respectively (Figure 3). Ca_Alge did not possess reducing power activity. The average reducing power activity of polymer Alg mixture was 17.11 mg FeSO₄ equivalent/g DW and was 1.33 times of Alg salts mixture. Therefore, metal iron did not play a role in the exhibition on reducing power activity of Alg mixture that depended on the appearance of Alg and bioactive polymer (chitosan and carrageenan).

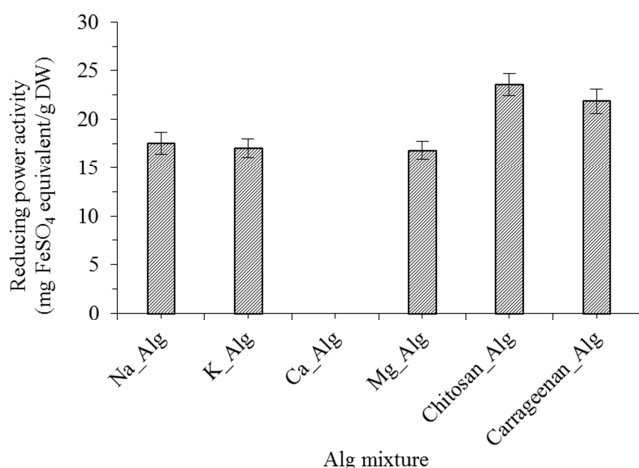


Figure 3. Reducing power activity of Alg mixture.

4. Conclusion

Alginate mixture was precipitated for 30 minutes in ethanol concentration by more than 80%. Na_Alge, K_Alge, and Mg_Alge precipitated slower in ethanol, compared to chitosan_Alge and carrageenan_Alge. Alginate mixture possessed antioxidant activity, except for Ca_Alge. The difference in antioxidant activity between a group of Alg salts (Na_Alge, K_Alge, and Mg_Alge) and polymer Alg (Chitosan_Alge and Carrageenan_Alge) happened, but not in

the group. The disperse of Na_Alge, K_Alge, and Mg_Alge in the water was the net style. Ca_Alge, chitosan_Alge, and carrageenan_Alge swelled good in the water and existing under a sphere, yarn, and yarn, respectively.

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