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# **The Content, Antioxidant Activity, and Structural Characteristics of Sodium Alginate Extracting from**  *Sargassum polycystum* **Grew in Vietnam: Effect of Various Extraction Conditions**

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#### *Authors' contributions*

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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#### **ABSTRACT**

**Introduction:** Alginate is a biopolymer commonly in brown algae, high content, diversity in structure and bioactivity. They are applied to other fields such as food, functional food, pharmaceuticals, and heavy industry and extracted from Sargassum, Laminarin, Tubinaria and *Sargassum polycystum* species commonly grow in the world than another genus. The content, the antioxidant activity, and the physical chemistry properties of alginate extracting from the species did not exhibit in the previous studies.

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**Methods:** The study focused on the impact of various extraction conditions (temperature (40 to 90,<sup>o</sup>C), time (01 to 06, hours), the solvent-to-material ratio (10/1 to 40/1, v/w), solvent pH (8, 9, 10), and numbers of extraction (1, 2, and 3 times) on alginate content and its antioxidant activity, also evaluated sugar compositions, average molecular, viscosity, and 13C-NMR spectrum characteristics of alginate.

**Results:** The results showed that the suitable condition for the extraction of alginate was for 4 hours at 60°C with the ratio of Na<sub>2</sub>CO<sub>3</sub> (pH 9)-to-material (40/1, v/w) and one number of extraction. The highest alginate content was 176.22 mg uronic acid equivalent/g DW, corresponding to antioxidant activity (188.54 mg ascorbic acid equivalent/g DW). Alginate possessed the intrinsic viscosity (271.86, n) and the average molecular weight (78.6, kD). Different sugar compositions of antioxidant alginate were fructose, fucose, mannose, galactose, rhamnose, mannose, and glucose. The 13C-NMR spectrum of antioxidant alginate exhibited chemical shifts of C sites of D-mannuronic (M) and L-guluronic (G), corresponded to  $C_1$ ,  $C_2$ ,  $C_6$ , and  $C_2$ ,  $C_3$ ,  $C_4$ ,  $C_5$ ,  $C_6$ , respectively.

**Conclusion:** Alginate is non-toxic, therefore, antioxidant alginate extracting from brown algae *Sargassum polycystum* grown in Vietnam is a potential in functional food and pharmaceuticals.

*Keywords: Alginate; antioxidant; extraction; Sargassum polycystum; structure.*

#### **1. INTRODUCTION**

Alginate is a polysaccharide commonly in brown algae with basic units such as D-mannuronic (M) and L-guluronic (G) that contained various single sugars [1]. Alginate content was up to 52 (%, DW) that depended on the genus, species, and environmental conditions [2,3]. Alginate of different brown algae species possesses various bioactivities, described by the previous notices [4]. The difference depends on the  $M_w$ , viscosity, and  $SO_2^4$  numbers of the group in their molecular structure [5,6]. Different bioactivities of alginate were known as antioxidant [7], antibacterial [8], antibiotics [9], diabetes, adiposity [5], and stomach protection [10,11]. Numerous studies showed that alginate occurs in other fields, for example, textiles, food industry, agri-foods (slow-release fertilizer), pharmaceuticals (drug release, encapsulation, stomach-treated support, and cell immobilization), paper, and medical supplies, among others [12], cosmetics [13], functional food [14]. Therefore, alginate is usually found, extracted, purified, and evaluated on its bioactivities.

Nowadays, there are many methods of alginate extraction, for example, the assistance of enzymes, microwave, and ultrasound, pressurized liquid extraction [5], maceration [7]. Different extracting methods caused the difference in alginate content, bioactivities, structure characteristics,  $M_w$ , and viscosity [6]. The notices on the impact of various extraction conditions according to the maceration such as solvent pH, time, temperature, the solvent-tomaterial ratio, and numbers of extraction on

alginate content, also the correlation between alginate content and its antioxidant activity,  $M_w$ , viscosity, and structural characteristics of alginate extracting from brown algae *Sargassum polycystum* grown in South-Centre of Vietnam did not find. While the species commonly distribute in the world with high reserves and contains numerous bioactive substances.

Therefore, the current study focused on the over problems for the orientation of alginate application into functional food and pharmaceuticals.

# **2. MATERIALS AND METHODS**

#### **2.1 Sample Preparation**

Brown algae *Sargassum polycystum* grown in the South-Center of Vietnam was selected, cleaned, and stored under  $10^{\circ}$ C for transferring to the laboratory. The elimination of the salts and the impurities of the seaweed was performed continuously by using fresh water and then dried until  $19±1$  % of humidity in the laboratory. After drying, the seaweed grinding was to the powder that stored under  $10^{\circ}$ C for further studies.

#### **2.2 Experience Design**

#### **2.2.1 Effect of extracting temperature**

The soaking of brown algae powder was for 04 hours with the  $Na<sub>2</sub>CO<sub>3</sub>$  (pH 10)-to-powder of 40/1 (v/w) at the temperature (40, 50, 60, 70, 80, and  $90^{\circ}$ C) and then filtered for the filtrate collection servicing the survey of the extraction temperature.

#### **2.2.2 Effect of extracting time**

For the survey of the extraction time, the keeping of brown algae powder was in  $Na<sub>2</sub>CO<sub>3</sub>$  solution ( $pH$  10) at  $50^{\circ}$ C with the solvent-to-material ratio of 40/1 (v/w) for 01, 02, 03, 04, 05, and 06 hours, respectively.

# **2.2.3 Effect of solvent pH**

Brown algae powder added with the ratio solvent (pH of 8, 9, and 10)-to-material (40/1, v/w) and kept at 50°C for 04 hours, respectively, and filtered for the solvent pH survey.

# **2.2.4 Effect of the solvent-to-material ratio**

For the collection of the solvent-to-material ratio, the soak of brown algae powder in  $Na<sub>2</sub>CO<sub>3</sub>$ solution was with the ratio  $Na<sub>2</sub>CO<sub>3</sub>$  (pH 9)-tomaterial (10/1, 20/1, 30/1 and 40/1, v/w) at  $50^{\circ}$ C for 04 hours, respectively, and filtered for the filtrate.

# **2.2.5 Effect of the number of the extraction**

For the survey of the extractions number, brown algae powder was macerated in  $Na<sub>2</sub>CO<sub>3</sub>$  (pH 9) solution with the ratio solvent-to-material (40/1,  $v/w$ ) at  $50^{\circ}$ C for 04 hours and repeated triplication and filtered for the filtrate. The analysis of alginate content and antioxidant activity for all filtrates in various extraction conditions. Alginate extracted in a suitable condition was continuously purified for the evaluation of the structural characteristics.

# **2.3 Purification of Antioxidant Alginate**

The conversion of antioxidant alginate to antioxidant calcium alginate was by using 10%  $CaCl<sub>2</sub>$  solution (the CaCl<sub>2</sub>-to-alginate ratio of 2.0/1.0). Then, filtering and washing the precipitate with clean water was five times. The colour elimination of antioxidant calcium alginate was effectuated continuously by using 20-30 mL chlorine (1 % chlorine solution/100 g antioxidant calcium alginate) for 30 minutes and then the movement of the chlorine out the residue with fresh water. The residue was soaked in a solution (pH 2.0) for the formation of antioxidant alginic acid. Finally, the preparation of antioxidant purified sodium alginate was by using the maceration of antioxidant alginic acid in  $Na<sub>2</sub>CO<sub>3</sub>$  solution with the  $Na<sub>2</sub>CO<sub>3</sub>$ -to-alginic acid ratio of 0.35/1 (w/w). Finally, the precipitation of antioxidant sodium alginate and the cleaning of them was with 96% ethanol. Antioxidant purified alginate was cool dried and evaluated for  $M_{w}$ , viscosity, sugar compositions and structural characteristics.

# **2.4 Quantification of Alginate Content**

200 mL alkaline extract in section 2.2 added 538.5 mL of 96 % ethanol to reach 70 % ethanol in the mixture. After which the mixture is filtered and the residue was collected and dried until a constant weight was achieved. One mL of residue solution (01 mg/ 01 mL) was mixed 01 mL of 0.8 M sodium hydroxide and vortexed. After 5 minutes, the mixture was neutralized by adding 120 mL of 2.25M citric acid and then added to 40 mL of DMMB reagent (Dimethylmethylene Blue Assay). The mixture was continuously vortexed and kept at room temperature for 45 minutes. Alginate content and the non-precipitating alginate ratio in ethanol were calculated based on the mixture absorbance at the wavelength of 520 and 650 nm and the 520:650 nm ratio [15].

# **2.5 Determination of Antioxidant Activity**

The antioxidant activity of alginate was determined based on the description of Dang et al. (2020) [7]. The method is the reaction between alginate and  $Mo^{6+}$  ion for forming  $Mo^{5+}$ in the acid environment. The absorbance measurement was at the wavelength of 655 nm with the standard of ascorbic acid.

# **2.6 Determination of Viscosity and Average Molecular Weight**

The intrinsic viscosity parameter of sodium alginate was determined based on the machine Viscometers - AMETEK Brookfield and used for calculating the average molecular weight of sodium alginate.

# **2.7 Determination of Sugar Composition of Alginate**

Sugar composition of antioxidant alginate was measured using the GC - FID method on Agilent's 6890 N gas chromatograph (USA). Chromatography conditions: Agilent's 6890 N gas chromatograph (USA) included automatic sample injector, injection chamber, column furnace, flame ionization detector (FID), and HP5 MS column (30 m x 0.25 m, x 0.25 m). Injection chamber temperature 280 $\degree$ C, line split ratio of

 $50/1$ , program temperature column at  $100^{\circ}$ C, increase to 325°C at a rate of 20 °C/min, keep the heat for 10 minutes. The probe temperature was at 300°C. Carrier gas speed was 01 mL/min. Derivation process: The hydrolysis of the sample (m, g) was in 1.5 M HCl, makeup to 10 mL for reacting with acetic anhydride and finally injected into the GC system.

# **2.8 Determination of Structure Characteristics**

NMR spectra were recorded on Bruker AVANCE Neo 600 MHz instrument at  $70^{\circ}$ C, using  $D_2$ O solvent with 01 %  $CD_3COOD$  and DSS as an internal standard with water reduction standard with water measurement technique.

# **2.9 Data Analysis**

Data was expressed under the average ± standard deviation (Mean  $\pm$  SD) and removing unnormal values using the Duncan method. The analysis of statistics, ANOVA, and regression was using MS. Excel 2010.

# **3. RESULTS AND DISCUSSION**

# **3.1 Effect of Various Extraction Conditions**

# **3.1.1 Effect of extraction time**

The extraction time affected alginate content and their antioxidants (*p*<0.05). Alginate content increased from 96.75 to 109.87 mg uronic acid equivalent/g DW as the extraction time increase from 01 to 02 hours. Alginate content continuously increased and got the highest value of 162.17 mg uronic acid equivalent/g DW at 04 extraction hours when the extraction time increased from 02 to 04 hours. Alginate content changed according to the non-linear model of level 2 and decreased as the extraction time was more than 04 hours (decreased to 150.88 mg uronic acid equivalent/g DW). At the extraction time of 6 hours, the antioxidant activity got the highest value of 192.71 mg ascorbic acid equivalent/g DW (Fig. 1). Antioxidant activity increased the linear model as the extraction time increase from 01 to 06 hours. Antioxidant activity corresponded to 174.28 ascorbic acid equivalent/g DW as alginate content of 162.17 mg uronic acid equivalent/g DW. The lowest alginate content was 96.75 mg uronic acid equivalent/g DW, their antioxidant activity

corresponded to 118.62 mg ascorbic acid equivalent/g DW. Alginate content and its antioxidant activity was a strong correlation  $(R<sup>2</sup>=0.9534)$  and interacted according to the nonlinear equation (y = -0.0227x<sup>2</sup> + 6.8502x -333.16). Generally, the longer the extraction time, the higher the efficiency of the compounds. However, the effect of extraction time on alginate content and their antioxidants depends on the material characteristics (size, moisture content, growth time, harvest method). Long extraction time will consume more energy and other costs. In the current study, the extraction time of more than 04 hours caused the full destruction of brown algae structure and forming the paste mixture that was difficult during the filtering process. The extraction time of alginate depended on the genus, species such as *Sargassum* sp. (2.5 hours) [16], *Macrocystis pyrifera* (120 minutes) [17], *Sargassum mangarevense, Turbinaria ornata* (4 hours), *Sargassum wightii* (12 hours) [2].

# **3.1.2 Effect of the extraction temperature**

The extraction temperature affected alginate content and antioxidant activity (*p*<0.05). The current study was suitable for the notice of Tores et al. (2007) on the impact of temperature on the extraction yield of alginate [18]. When the temperature increased from 40 to  $50^{\circ}$ C, alginate content strongly changed from 132.17 to 162.17 mg uronic acid equivalent/g DW and continuously increased at 60 $\degree$ C (164.22 mg uronic acid equivalent/g DW) (Fig. 2). Alginate content decreased from 164.22 down 151.08 mg uronic acid equivalent/g DW as the extraction  $temperature$  increase to  $90^{\circ}$ C. Alginate content changed according to the level 2–model with the maximum peak at 50°C. The difference in alginate content as extracting at 50 and  $60^{\circ}$ C was not significant (*p*<0.05). The thing was also suitable for the description of Gholamipoor et al. (2013) on the extraction temperature of alginate from Sargassum angustifolium [19]. As the extraction at 50  ${}^{6}C$ , antioxidant activity corresponded to 174.28 mg ascorbic acid equivalent/g DW, while the extraction at  $90^{\circ}$ C, antioxidant activity decreased to 130.24 mg ascorbic acid equivalent/g DW. Alginate content got 164.22 mg uronic acid equivalent/g DW, corresponded to the antioxidant activity of 177.48 mg ascorbic acid equivalent/g DW. As alginate content of 160.05 uronic acid equivalent/g DW, the antioxidant activity got 170.03 mg ascorbic acid equivalent/g DW. Alginate content correlated to antioxidant activity according to the

non-linear model of level 2 (y =  $0.1166x^2$  – 33.681x + 2568.4) with strong adjusted  $R^2$ (79.28%). Under the impact of time and temperature, alginate still played an important role in exhibiting antioxidant activity.

#### **3.1.3 Effect of solvent pH**

Solvent pH impacted alginate content and antioxidant activity of various extracts from brown algae *Sargassum polycystum* (*p*<0.05). Alginate content strongly increased to 169.75 mg uronic acid equivalent/g DW, corresponded to 111.55 as pH increase from 8 to 9. %). Under the impact of time and<br>rature, alginate still played an important<br>exhibiting antioxidant activity.<br>**Effect of solvent pH**<br>the pH impacted alginate content and<br>dant activity of various extracts from brown<br>Sargass

Alginate content decreased by 4.47% when solvent pH increased over 9 (pH 10). At pH solvent of 9, antioxidant activity was 1.15 and 1.02 times, compared to pH 8 and 10, respectively (Fig. 3). The current study was suitable for the notices of Hernández-carmona et al. (1999) on alginate content that depended on the concentration of an alkaline solution (exhibit via pH index) at the extraction step [20]. The alkaline extraction process converts the alginic acid from its insoluble form to the soluble sodium alginate form and diffuses out of the solution. The concentration of  $Na<sup>+</sup>$  is a decisive factor in the conversion of alginic acid to alginate. Therefore, the alginic acid has not fully converted to sodium alginate form as the low pH, and then alginic acid still exists in the seaweed residue in the form of insoluble, not diffused to the outside. With higher pH, the concentration of Na<sup>+</sup> increases, the reaction process takes place solvent of 9, antioxidant activity was 1.15 and<br>1.02 times, compared to pH 8 and 10,<br>respectively (Fig. 3). The current study was<br>suitable for the notices of Hernández-carmona et al. (1999) on alginate content that depen<br>the concentration of an alkaline solution<br>via pH index) at the extraction step [2]<br>alkaline extraction process converts the<br>acid from its insoluble form to the soluble concentration of Na<sup>+</sup> is a decisive factor in conversion of alginic acid to alginate.<br>efore, the alginic acid has not fully converted<br>dium alginate form as the low pH, and then<br>c acid still exists in the seaweed residue

better, the alginic acid content in the seaweed turns to more and more soluble alginate and diffuses out of the solution at the time. Alginate was cleavage that caused the tends of the alginate content as solvent pH continuously increase [21]. phtent in the seaweed<br>is soluble alginate and<br>in at the time. Alginate<br>ied the tends of the<br>vent pH continuously

#### **3.1.4 Effect of the solvent-to-material ratio material**

The increase of alginate content and antioxidant activity and the filter processing was comfortable The increase of alginate content and antioxidant<br>activity and the filter processing was comfortable<br>when the  $\text{Na}_2\text{CO}_3$  solution-to-material ratio increased. Alginate content as the extraction with increased. Alginate content as the extraction with<br>the solution-to-material ratio (20/1, v/w) was 89.8, 93.23, and 118.13%, compared to the ratio 89.8, 93.23, and 118.13%, compared to the ratio<br>of solvent-to-material (40/1, 30/1, and 10/1, v/w). The antioxidant activity got the highest value as the extraction with 40/1 (v/w) and the lowest value with 10/1 (v/w) (143.68 mg ascorbic acid equivalent/g DW. Alginate content was 176.22 mg uronic acid equivalent/g DW, its antioxidant activity corresponded to 188.54 mg ascorbic acid equivalent/g DW (Fig. 4). As alginate content of 152.28 mg uronic acid equivalent/g DW, antioxidant activity only was 169.73 mg ascorbic acid equivalent/g DW. ANOVA analysis showed that the change of the solution-to impacted alginate content and its antioxidant activity (*p*<0.05). The variation of antioxidant alginate content under the impact of the solvent ratio did not appear in the previous studies that mainly focused on temperature, extraction time [17], and the  $Na<sub>2</sub>CO<sub>3</sub>$ concentration [16]. v got the highest value as<br>  $v/1$  (v/w) and the lowest<br>  $(143.68 \text{ mg}$  ascorbic acid<br>
nate content was 176.22<br>
lent/g DW, its antioxidant<br>  $v$  188.54 mg ascorbic acid<br>
4). As alginate content of<br>
acid equivalent/g DW,<br>
v content and its antioxidant<br>the variation of antioxidant<br>der the impact of the solvent<br>appear in the previous<br>y focused on temperature,<br>[17], and the  $Na<sub>2</sub>CO<sub>3</sub>$ 



**Fig. 1. Alginate content and antioxidant activity for different extraction time Alginate times**



**Fig. 2. Alginate content and antioxidant activity at different extraction temperatures**



**Fig. 3. Alginate content and antioxidant activity at different solvent pH**



**Fig. 4. Alginate content and antioxidant activity at different the solvent**

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**Fig. 5. Alginate content and antioxidant activity at different numbers of extraction**





**Table 1. Chemical shifts of standard alginate via 13C NMR spectrum**



**Fig. 7. 13C NMR spectrum of alginate extracting from brown algae C NMR** *Sargassum polycystum*

#### **3.1.5 Effect of the numbers of the extraction**

The alginate content of the extract was the inverse ratio to the number of extraction. Alginate content got the highest value (176.22 mg uronic acid equivalent/g DW) for the  $1<sup>st</sup>$  extraction. Alginate content for the  $2^{nd}$  and  $3^{rd}$  extraction corresponded to 33.33 and 13.14 %, compared to the 1<sup>st</sup> extraction. Therefore, the extraction yield of alginate for the  $1<sup>st</sup>$  extraction got 68.27  $\%$ . For the 1<sup>st</sup> extraction, the antioxidant activity got the highest value and corresponded to 2.179 and 1.28 times, compared to the  $3^{rd}$  and  $2^{nd}$ extraction. For the  $2^{nd}$  extraction, antioxidant activity was the average level, corresponding to 147.21 mg ascorbic acid equivalent/g DW, compared to the  $1<sup>st</sup>$  and  $3<sup>rd</sup>$  extraction (Fig. 5). Alginate content, antioxidant activity, and the yield of alginate extraction in the current study were higher than the notice of Yudiati et al. (2018) [22]. Under the same extraction conditions, in the first extraction, the alginic acid content in the seaweed changed to highly soluble alginate and diffused out of the solution, so the obtained alginate content would be high. The destruction of the seaweed structure and the alginate cleavage occur more strongly in the following extractions, leading to a decrease in the obtained alginate with an increasing number of extraction times.

#### **3.2 Physico-chemistry Characteristics of Antioxidant Alginate**

Intrinsic viscosity ( $\eta$ ) and M<sub>w</sub> (kD) of antioxidant alginate corresponded to 271.86 and 78.6, respectively. The thing exhibited that alginate extracting from brown algae *Sargassum polycystum* possessed a molecular weight lower than the previous notices [18]. The analysis of alginate using GC-FID showed the diversity of antioxidant alginate on sugar composition. For example, fructose, fucose, rhamnose, mannose, glucose, and galactose (Fig 6.). The thing could form the diversity of the structure of antioxidant alginate extracted from brown algae *Sargassum polycystum*, also bioactivities of alginate that depended on its structure characteristics [23,24]. The <sup>13</sup>C-NMR spectrum of antioxidant alginate occurred 12 signals, corresponding to 12 carbon atoms belonging to 02 monome (L-guluronic and D-mannuronic) that was basic units forming the alginate structure. Chemical shifts of different sites such as  $C_1$ ,  $C_2$ , and  $C_6$  was 103.28, 72.64, and 177.46, respectively, in D-mannuronic (M) structure. The chemical shift of  $C_2$ ,  $C_3$ ,  $C_4$ ,  $C_5$ , and  $C_6$  sites was 67.80, 71.79, 82.56, 69.95, and

177.65, respectively, in L-guluronic (G) structure (Tab. 1 and Fig. 7). The significant difference in the chemical shifts of carbon atoms in the current study and the previous reports on the NMR of alginate did not appear because the differences were lower than 0.05 ppm [25], describled by Hans et al. (1977) [26] and Peter (1988) [27]. The absorbance of the British standard solution (1mg/ml) is D=0.473. Meanwhile, the absorbance of the alginate sample in this study is D=0.47. Thus, it could identify that the purity of the obtained sample was greater than 98 %.

#### **4. CONCLUSION**

Various extraction conditions impacted alginate content and antioxidant activity, almost according to the non-linear model of level 2, except for extraction numbers and the solvent-to-material ratio. Alginate extracting from brown algae *Sargassum polycystum* grown in South-Centre of Vietnam played a primary role in antioxidant activity with an intrinsic viscosity and the average molecular weight lower than the previous studies. Sugar compositions of antioxidant alginate are similar to the noticed alginate (glucose, mannose, galactose, fructose, rhamnose, fucose, and mannose). The interaction in antioxidant alginate structure was different, so its  $13^{\circ}$ C-NMR spectrum only exhibited chemical shifts of C sites  $(C_1, C_2,$  and  $C_6$ ) of D-mannuronic (M) and  $(C_2, C_3, C_4, C_5,$  and  $C_6$ ) of L-guluronic (G).<br>Alginate of brown algae Sargassum Alginate of brown algae *Sargassum polycystum* is potential in functionalantioxidant food and antioxidant pharmaceuticals.

#### **CONSENT**

It is not applicable.

#### **ETHICAL APPROVAL**

It is not applicable.

#### **DISCLAIMER**

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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