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.

Article Information
DOI: 10.9734/JPRI/2021/v33i41A32318
Editors:
(1) Dr. Barkat Ali Khan, Gomal University, Pakistan.
(2) Salama K. Ibrahim, Bayero University Kano, Nigeria.
Reviewers:
(1) Salama K. Ibrahim, Bayero University Kano, Nigeria.
(2) Ratan Sadhukhan, University of Arkansas for Medical Sciences, USA.
Complete Peer review History: https://www.sdiarticle4.com/review-history/72634

Received 06 June 2021
Accepted 10 August 2021
Published 17 August 2021

Original Research Article

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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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 Mₜ, viscosity, and SO₂⁻ 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, paper, medical, 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, Mₜ, and viscosity [6]. The notices on the impact of various extraction conditions according to the maceration such as solvent pH, time, temperature, the solvent-to-material ratio, and numbers of extraction on alginate content, also the correlation between alginate content and its antioxidant activity, Mₜ viscosity, and structural characteristics of alginate extracting from brown algae Sargassum polycystum grown in 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°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°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 Na₂CO₃ (pH 10)-to-powder of 40/1 (v/w) at the temperature (40, 50, 60, 70, 80, and 90°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 Na₂CO₃ solution (pH 10) at 50°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 Na₂CO₃ solution was with the ratio Na₂CO₃ (pH 9)-to-material (10/1, 20/1, 30/1 and 40/1, v/w) at 50°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 Na₂CO₃ (pH 9) solution with the ratio solvent-to-material (40/1, v/w) at 50°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% CaCl₂ solution (the CaCl₂-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 Na₂CO₃ solution with the Na₂CO₃-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 Mₚ, 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 DMBB reagent (Dimethylmethylen 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 Mo⁶⁺ ion for forming Mo⁷⁺ 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 °C, line split ratio of
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 increased 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 mg 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 (R²=0.9534) and interacted according to the non-linear equation (y = -0.0227x² + 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°C, alginate content strongly changed from 132.17 to 162.17 mg uronic acid equivalent/g DW and continuously increased at 60°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°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°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°C, antioxidant activity corresponded to 174.28 mg ascorbic acid equivalent/g DW, while the extraction at 90°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 mg 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 R² (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.

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⁺ 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⁺ increases, the reaction process takes place 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 tendency of the alginic content as solvent pH continuously increase [21].

3.1.4 Effect of the solvent-to-material ratio

The increase of alginate content and antioxidant activity and the filter processing was comfortable when the Na₂CO₃ solution-to-material ratio increased. Alginate content as the extraction with the solution-to-material ratio \((20/1, v/w)\) was 89.8, 93.23, and 118.13%, compared to the ratio of solvent-to-material \((40/1, 30/1, \text{and } 10/1, v/w)\). The antioxidant activity got the highest value as the extraction with \(40/1\) (v/w) and the lowest value with \(10/1\) (v/w) \((143.68 \text{ mg ascorbic acid equivalent/g DW})\). 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-material ratio impacted alginate content and its antioxidant activity \((p<0.05)\). The variation of antioxidant alginic 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 Na₂CO₃ concentration [16].

![Fig. 1. Alginate content and antioxidant activity for different extraction times](image-url)
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-to-materia ratio
Fig. 5. Alginate content and antioxidant activity at different numbers of extraction

Fig. 6. Sugar composition of alginate extracting from brown algae *Sargassum polycystum*: a) Fucose spectrum; b) Xylose spectrum; c) Rhamnose, manose, glucose, galactose, and fructose spectrum

Table 1. Chemical shifts of standard alginate via $^{13}$C NMR spectrum

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-mannuronic (M)</td>
<td>103.23</td>
<td>72.64</td>
<td>74.01</td>
<td>80.62</td>
<td>78.72</td>
<td>177.46</td>
</tr>
<tr>
<td>L-guluronic (G)</td>
<td>102.22</td>
<td>67.81</td>
<td>71.79</td>
<td>82.56</td>
<td>69.95</td>
<td>177.65</td>
</tr>
</tbody>
</table>

Fig. 7. $^{13}$C NMR spectrum of alginate extracting from brown algae *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 1st extraction. Alginate content for the 2nd and 3rd extraction corresponded to 33.33 and 13.14 %, compared to the 1st extraction. Therefore, the extraction yield of alginate for the 1st extraction got 68.27 %. For the 1st extraction, the antioxidant activity got the highest value and corresponded to 2.179 and 1.28 times, compared to the 3rd and 2nd extraction. For the 2nd extraction, antioxidant activity was the average level, corresponding to 147.21 mg ascorbic acid equivalent/g DW, compared to the 1st and 3rd 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 (n) and \( M_w \) (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 \(^{13}\)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], describied 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 \(^{13}\)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). Alginate of brown algae Sargassum polycystum is potential in functional-antioxidant 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.
ACKNOWLEDGEMENTS

Thankful for the project of UDSPTM.04/20-21 and VAST03.08/20-21 are under the VAST, chaired by Associate Professor Dang Xuan Cuong and Dr Nguyen Dinh Thuat, for funding to contribute to the implementation of the study. Thankful for the project of antioxidant lignin-alginate pharmaceutical material of the Ministry of Industry and Trade, chaired by Associate Professor Nguyen Xuan Hoan funded to contribute to the implementation of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/72634