EFFECTS OF CELLULASE ENZYME AND MICROWAVE ON POLYSACCHARIDE EXTRACTION FROM *Enteromorpha* sp. ALGAE

Ha Thi Minh Thu, Nguyen Ngoc Thu, Hoang Thi Ngoc Nhon*

Ho Chi Minh City University of Industry and Trade

*Email: *nhonhtn@huit.edu.vn*

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ABSTRACT

This study focused on the extraction of polysaccharides (PS) from *Enteromorpha* using two methods: enzyme-assisted extraction (EAE) and microwave-assisted extraction (MAE). The findings determined that the optimal conditions for MAE were a microwave power of 420 W, an extraction time of 3 min, and a solid-to-liquid ratio of 1:20 w/v. For EAE, the best results were obtained with a solid-to-liquid ratio of 1:20 w/v, pH of 5.0, an extraction temperature of 50 °C, and an extraction time of 4h. The polysaccharide yields were 205.4 ± 0.64 mg/g dry matter for MAE and 147.9 ± 0.13 mg/g dry matter for EAE.

Keywords: Enzyme, microwave, polysaccharide, *Enteromorpha* sp.

1. INTRODUCTION

Algae are widely acknowledged as a rich source of bioactive compounds, encompassing lipids, proteins, peptides, polysaccharides, carotenoids, and phenolic compounds [1]. The three main types of algae-green, brown, and red, classified as *Chlorophyta*, *Phaeophyta*, and *Rhodophyta*-contain a wealth of health-promoting substances like alginates, agars, carrageenan, ulvans, and fucoidans. These compounds are widely used in the food and pharmaceutical sectors [2]. *Enteromorpha* sp., a green algae, is known for its chlorophyll b content and essential minerals like magnesium, iron, and calcium. Edible species of *Chlorophyta* have been reported to contain 12.4-18.7% ash, 16-22.1% protein, and 43.4-60.2% carbohydrates in their dry matter [3]. Various extracts from *Enteromorpha* species have been recognized for their bioactivities, such as antitumor, antimicrobial, antithrombotic, antimutagenic, anti-inflammatory, immunomodulatory, etc [4-6].

Polysaccharides, which are abundant in marine macroalgae, are valuable natural biopolymers with numerous potential applications in fields such as nutrition, biomedicine, and cosmetics [7, 8]. It is composed of 10 or more monosaccharides via glycoside linkage. Polysaccharides are generally composed of linear or branched chains, with molecular weights varying from tens of thousands to several millions. Depending on the chemical structure, type of bond, and composition of the monomers, polysaccharides will have different functions, such as: providing energy (starch), and cell wall structure (cellulose) [9]. Thanks to their structural diversity, polysaccharides also have many different biological functions such as signal recognition, communication between cells, regulation of the immune system, and disease inhibition. The extraction of polysaccharides from certain green algae has been associated with a wide range of biological activities, including antioxidant, anti-tumor, antiviral, anti-inflammatory, cardioprotective, and anti-mutagenic effects [10-12]. In recent years, advanced technologies such as microwave-assisted extraction (MAE) based on electromagnetic radiation and enzyme-assisted extraction (EAE) have been considered compatible, safe methods with inexpensive solvents. This process is used in the extraction of bioactive compounds from plant materials by disrupting the cell wall structure to release the desired components. This study investigated the effects of enzyme-assisted extraction (EAE) and microwave-assisted extraction (MAE) on the extraction of polysaccharides from *Enteromorpha* sp.

2. MATERIALS AND METHODS

2.1. Materials

Enteromorpha sp. was gathered from Thoi Binh commune in Ca Mau province and transported to the laboratory under cooled conditions during daytime hours. Upon reaching the lab, the algae underwent rigorous washing to remove impurities, followed by drying at 60 °C until its moisture content fell below 10%. Once dried, it was ground, sieved through a 0.5 µm mesh, and stored in a frozen state for subsequent experimental use.

The cellulase enzyme used in this study was supplied by Biogreen Pharmaceutical Chemistry and Biotechnology Joint Stock Company, Vietnam, with an enzyme activity of 2000 IU/g. The enzyme has a shelf life of two years from the production date and should be stored in a cool, dry environment, away from direct light, with a temperature range of 4-25 °C.

The equipment used included a UV-VIS spectrophotometer (Model V-730, Japan), a thermostat tank (WNB 14, Germany), a microwave oven (Sharp 23 liter R-370VN-S, China), a drying oven (Venticell, Germany), and a centrifuge (Hermle Z206A, Germany).

2.2. Methods

2.2.1. Materials treatment

The algae was treated to remove lipids and pigments by the Soxhlet method for 6 hours. Fat removing finished by observing the traces on the surface of the filter paper after the dropped extracting solvent is dried. Then, the residue was dried at 60 °C to under 10% moisture content and stored for the following experiments.

2.2.2. Effects of EAE on polysaccharide extraction from Enteromorpha sp.

Cellulase enzyme 2% was used in all experiments during polysaccharide extraction. 1 g of sample (calculated by weight of dry content), pH 5.0 [13]. The solid-to-liquid ratios used were 1:10 w/v, 1:15 w/v, 1:20 w/v, 1:30 w/v, and 1:40 w/v, while extraction temperatures were set at 40, 50, 60, and 70 °C. Extraction times varied between 1, 2, 3, 4, and 5 hours. During the final step, the enzyme was deactivated by heating the solution to 90-100 °C, followed by immediate cooling. The samples were then centrifuged at 5000 rpm for 15 minutes and filtered to obtain the polysaccharide-rich supernatant.

2.2.3. Effects of MAE on polysaccharide extraction from Enteromorpha sp.

One gram of the sample (calculated on a dry weight basis) was combined with distilled water at a solid-to-liquid ratio of 1:20 w/v, as guided by prior research studies [14, 15]. The experiments utilized different microwave power levels, including 140W, 280W, 420W, 560W, and 700W, combined with treatment durations of 1, 2, 3, 4, and 5 minutes. Following the extraction process, the mixtures were centrifuged at 5000 rpm for 15 minutes to separate the supernatant. This supernatant was subsequently analyzed for polysaccharide content using the phenol-sulfuric acid method.

2.3. Phenol-sulfuric acid method

D-glucose served as the reference compound for constructing a calibration curve, with standard concentrations prepared at 12, 24, 36, 48, and 60 µg/mL. For each concentration, 2 mL of the standard solution was transferred into separate test tubes and combined with 1 mL of a 4% phenol solution, followed by the addition of 5 mL of concentrated sulfuric acid (H_2SO_4). The test tubes were securely sealed and gently agitated to ensure homogeneity of the mixture. Subsequently, the tubes were incubated at 40 °C for 30 minutes to facilitate the reaction. After incubation, they were rapidly cooled by immersion in ice water for 5 minutes. Absorbance measurements were then taken at a wavelength of 493 nm. Control (blank) samples were prepared under identical conditions. The polysaccharide concentration in the experimental samples was determined by leveraging the calibration curve, wherein the standard solutions were replaced with the respective sample solutions.

2.4. Data analysis

All experiments were conducted in triplicate, and the data obtained were processed using Microsoft Excel 2016. The determination of differences and the selection of optimal parameters were carried out based on analyses performed with Minitab software. Statistical evaluation was conducted through an ANOVA test with a significance level of 0.05. The results are expressed as the mean values derived from three independent experiments.

3. RESULTS AND DISCUSSION

3.1. Effects of EAE on polysaccharide extraction from *Enteromorpha* sp.

3.1.1. Effects of solid-liquid ratio on polysaccharide extraction

The effects of solid-liquid ratios (1:10 w/v,1:15 w/v, 1:20 w/v, 1:30 w/v, and 1:40 w/v) on the obtained polysaccharide content at static immersion extraction conditions at 50 °C in 1 hour and pH 5.0. The results were shown in Figure 1.

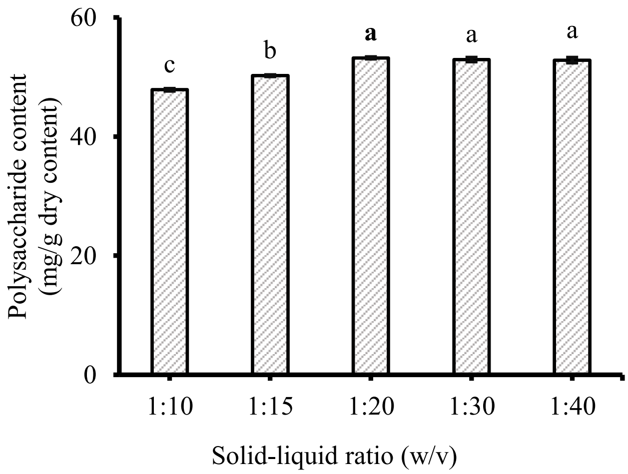

Fig. 1. Effects of solid-liquid ratios on polysaccharide extraction

Fig. 1 shows that the highest content of polysaccharide was 53.21 ± 0.18 mg/g of dry content at the ratio of solid-liquid of 1:20 w/v. Based on the results of ANOVA analysis, there was no significant difference when increasing the ratio of solid-liquid increased to 1:20 w/v, 1:30 w/v, and 1:40 w/v (values in the columns with different exponents represent the significantly different at the α= 0.05 level). The extraction efficiency does not increase when the solvent is increased beyond a certain point because the material has a limited number of substances. Even if more solvent is used, the amount of extracted substance cannot be increased. Therefore, it is crucial to select the appropriate solid-liquid ratio. In this experiment, a solid-liquid ratio of 1:20 w/v is the most suitable.

3.1.2. Effects of temperature on polysaccharide extraction

Temperature is also a factor that directly affect the polysaccharide extraction by EAE due to the sensitivity of enzymes at high temperatures. Therefore, in this experiment, 4 different temperatures of 40 °C, 50 °C, 60 °C, and 70 °C with the fixed ratio of solid-liquid are 1:20 w/v, pH 5.0, extraction time 1 hour, and static immersion extraction. The right temperature yielded the best polysaccharide content. The results are shown in Figure 2.

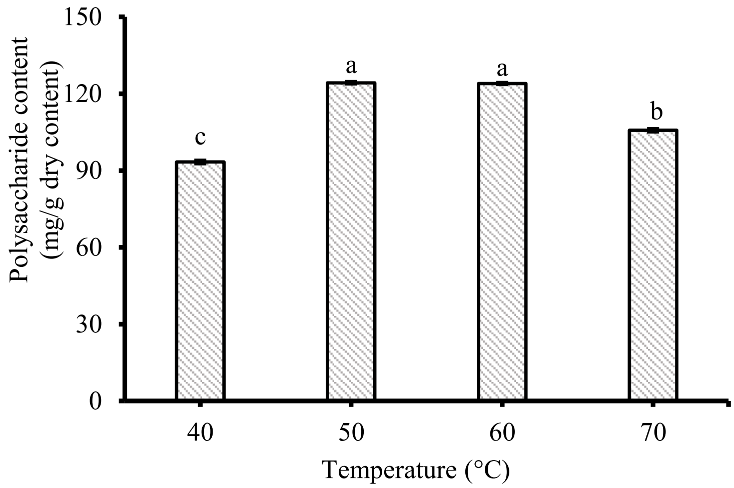

Fig. 2. Effects of temperature on polysaccharide extraction

Enzyme-assisted extraction is garnering attention due to its high efficiency, enzymatic hydrolytic activity that breaks down and weakens cell walls, and the subsequent release of intracellular compounds such as polysaccharides. The use of cellulase enzymes enhances the breakdown of cell walls, facilitating the release of intracellular polysaccharides and thereby increasing the yield of these compounds. Each enzyme functions best within a specific temperature range. A study conducted by Pardon et al. found that the optimal temperature for cellulase system activity is between 50 and 55 °C [16]. This study determined that cellulase enzymes perform optimally at a temperature of around 50 °C, yielding a polysaccharide content of 124.25 ± 0.46 mg/g of dry material. ANOVA analysis indicates no significant difference between the two tested temperature levels, 50 °C and 60 °C, when evaluated at the α = 0.05 level. This outcome can be attributed to the fact that temperatures exceeding the enzyme's optimal range may disrupt its active site, hindering its ability to bind effectively with the substrate and consequently reducing the efficiency of polysaccharide extraction from raw algae. Additionally, excessively high temperatures can lead to the thermal decomposition of polysaccharides, increased energy consumption, accelerated solvent evaporation, and a rise in extraction impurities. Based on these findings, 50 °C is identified as the most suitable temperature for efficient polysaccharide extraction.

3.1.3. Effects of enzyme treatment time on polysaccharide extraction

The efficiency of polysaccharide extraction also depends on the treatment time. In fact, excessive prolongation of extraction time can cause changes in polysaccharide molecular structure. Therefore, the extraction time survey should be conducted with 5 different time levels: 1 hour, 2 hours, 3 hours, 4 hours and 5 hours with a fixed temperature of 50 °C, the ratio of solid-liquid is 1:20 w/v, and static immersion extraction. The results are shown in Figure 3.

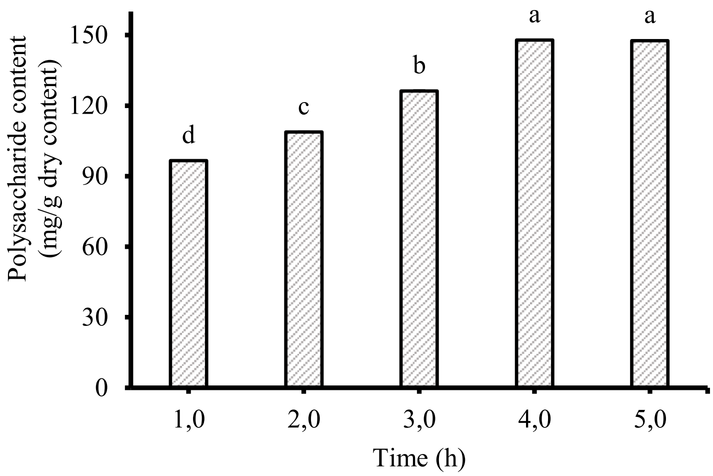

Fig. 3. Effects of treatment time on polysaccharide extraction

Enzymes play a supporting role in weakening the cell wall of materials, promoting the extraction of polysaccharides. If the enzyme contact time with the sample is not enough, the ability to destroy the cell wall will not achieve good results, thereby affecting the polysaccharide extraction efficiency of the experiment. And this is proved through the data from the survey results, it can be seen that, at 4 hours, the obtained polysaccharide content is the highest with the data of 147.9 ± 0.13 mg/g dry content. Results Figure 3 also showed that the polysaccharide content increased since the extended extraction time from 1 hour to 4 hours. After this point, there is no difference between 4 hours and 5 hours. The most suitable time for polysaccharide extraction is 4 hours according to this experiment.

3.2. Effects of MAE on polysaccharide extraction from *Enteromorpha* sp.

3.2.1. Effects of microwave power on polysaccharide extraction

In microwave-assisted polysaccharide extraction, microwave power is a factor that has a direct impact on the process. The essence of microwave-assisted extraction is the use of non-ionizing electromagnetic radiation that disrupts hydrogen bonds and the movement of dissolved ions. This allows the solvent to penetrate the cell matrix and facilitates the extraction of compounds of interest. The survey was conducted with 5 microwave power levels 140W, 280W, 420W, 560W, and 700W and the results are shown in Figure 4.

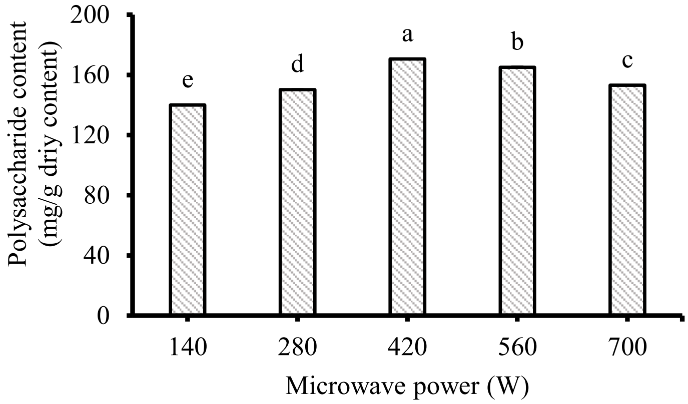

Fig. 4. Effects of microwave power on polysaccharide extraction

With the results of ANOVA analysis, there was a significant difference at the microwave power 420W, the obtained polysaccharide content was 170.58 ± 0.28 mg/g dry content. The extracted polysaccharide content was significantly different at 170.58 ± 0.28 mg/g dry content (at 420W) and 165.08 ± 0.62 mg/g dry content (at 560W). It can be understood that an increase in microwave power leads to an increase extraction efficiency of polysaccharides from starting material. However, if the microwave power is too high, it also leads to undesirable effects, that is, causing high temperatures to affect the chemical changes of the components in the algae, resulting in a significant reduction in the content of the compounds obtained after extraction, specifically here is the polysaccharide. According to the survey results in this study, the suitable microwave power is 420W.

3.2.2. Effects of microwave treatment time on polysaccharide extraction

Microwave time is one of the investigated factors affecting the extraction of polysaccharides, more or less time has a negative influence on the extraction process. Conducting a microwave time survey is necessary with 5 different time levels of 1, 2, 3, 4 and 5 min respectively, and the results are presented in Figure 5.

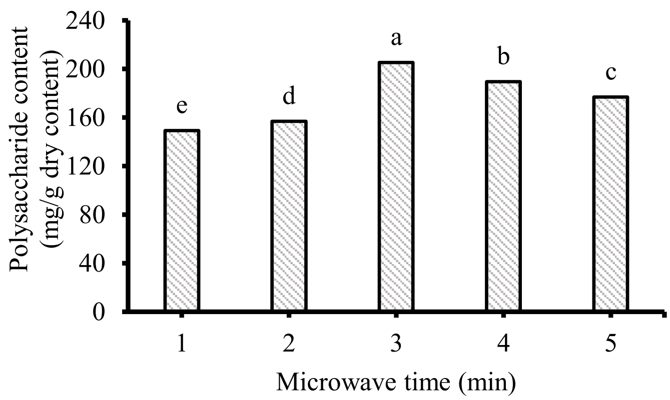

Fig. 5. Effects of microwave treatment time on polysaccharide content

Based on the results of ANOVA analysis showing that there is a difference between different microwave time levels, 3 min is the most promising survey level in obtaining the highest polysaccharide content with 205.4 ± 0.64 mg/g dry content. The content of polysaccharide decreased when the microwave time exceeded 3 minutes according to the survey results in Figure 5. During the initial extraction phase, longer time, and higher capacity accelerated mass transfer of intracellular substances. However, the time of extraction of more than 3 min may cause the denaturation of polysaccharides and reduce extraction efficiency. The results of the study on obtaining polysaccharides under microwave support at 420W power and 3 min microwave time are the appropriate parameters selected in this study and suitable for the extraction of polysaccharides from *Enteromorpha* sp. algae.

4. CONCLUSION

In this study, the extract methods supported by enzymes and extract methods supported by the microwave have been used for polysaccharides extract from *Enteromorpha* sp. Effects of extraction temperature, solid-liquid ratio, extraction time, microwave power, and other factors for polysaccharide extraction speed. The sample is added to the water with a solid-liquid ratio of 1:20 w/v, the extraction temperature supports the enzyme is 50 °C, the extraction is done in 4 hours, the pH is 5.0 and the extract is supported by conditional microwave assist is 420W in 3 minutes. Polysaccharide content is 147.9 ± 0.13 mg/g of dry content (EAE) and 205.4 ± 0.64 mg/g of dry content (MAE). These research results provide a theoretical basis for larger-scale research on polysaccharide extract from *Enteromorpha* sp.

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REFERENCES

1. Michalak I., & Chojnacka K. - Algae as production systems of bioactive compounds. Engineering in Life Sciences **15** (2) (2015) 160-176. https://doi.org/10.1002/elsc.201400191
2. Mirea M., Negreanu-Pirjol T., & Sirbu R. - Bioactive compounds from three green algae species along Romanian Black Sea Coast with therapeutically properties. European Journal of Medicine and Natural Sciences **3** (1) (2020) 67-86. https://doi.org/10.26417/720mnv73y
3. Mamatha B., Namitha K., Senthil A., Smitha J., & Ravishankar G. - Studies on use of Enteromorpha in snack food. Food Chemistry **101** (4) (2007) 1707-1713. https://doi.org/https://doi.org/10.1016/j.foodchem.2006.04.032
4. Sukatar A., Karabay-Yavaşsoglu N.U., Ozdemir G., & Horzum Z. - Antimicrobial activity of volatile component and various extracts of *Enteromorpha linza* (*Linnaeus*) J. Agardh from the coast of Izmir, Turkey. Annals of Microbiology **56** (3) (2006) 275-279. https://doi.org/10.1007/bf03175018
5. Ouahid E.A., Mohamed R., & Soufiane F. - Green Seaweed Polysaccharides Inventory of *Nador Lagoon* in North East Morocco. Polysaccharides: Properties and Applications (2021) 163-175. https://doi.org/10.1002/9781119711414.ch8
6. Cao S., He X., Qin L., He M., Yang Y., Liu Z., & Mao W. - Anticoagulant and antithrombotic properties *in vitro* and *in vivo* of a novel sulfated polysaccharide from marine green algae *Monostroma nitidum*. Marine Drugs **17** (4) (2019) 247. https://doi.org/10.3390/md17040247

7. Gawade R.P., Chinke S.L., & Alegaonkar P.S. - Polymers in cosmetics. Polymer science and innovative applications (2020) 545-565. https://doi.org/10.1016/B978-0-12-816808-0.00017-2
8. Saeed M., Arain M.A., Ali Fazlani S., Marghazani I.B., Umar M., Soomro J., & Alagawany M. - A comprehensive review on the health benefits and nutritional significance of fucoidan polysaccharide derived from brown seaweeds in human, animals and aquatic organisms. Aquaculture Nutrition **27** (3) (2021) 633-654. https://doi.org/10.1111/anu.13233
9. Song E.H., Shang J., & Ratner D.M. - Polysaccharides. Polymer Science: A Comprehensive Reference **9** (2012) 137-155. https://doi.org/10.1016/b978-0-444-53349-4.00246-6
10. Rajasekar P., Palanisamy S., Anjali R., Vinosha M., Elakkiya M., Marudhupandi T., & Prabhu N.M. - Isolation and structural characterization of sulfated polysaccharide from *Spirulina platensis* and its bioactive potential: In vitro antioxidant, antibacterial activity and *Zebrafish* growth and reproductive performance. International Journal of Biological Macromolecules **141** (2019) 809-821. https://doi.org/10.1016/j.ijbiomac.2019.09.024
11. Li H., Lu X., Zhang S., Lu M., & Liu H. - Anti-inflammatory activity of polysaccharide from *Pholiota nameko*. Biochemistry (Mosc) **73** (6) (2008) 669-75. https://doi.org/10.1134/s0006297908060060
12. Cheong K.L., Yu B., Chen J., & Zhong S. - A comprehensive review of the cardioprotective effect of marine algae polysaccharide on the gut microbiota. Foods **11** (22) (2022). https://doi.org/10.3390/foods11223550
13. Sun Y., Miao L., Yang F., Sun W., Peng Z., & Li Y. - Comparative study of the extracting polysaccharides from traditional chinese medicine *Astragali radix* in the field of new biological cellulase-assisted enzymes technology. Bulgarian Chemical Communications **53** (B) (2021). https://doi.org/10.34049/bcc.53.B.0079
14. Zhong K., Lin W., Wang Q., & Zhou S. - Extraction and radicals scavenging activity of polysaccharides with microwave extraction from mung bean hulls. International Journal of Biological Macromolecules **51** (4) (2012) 612-7. https://doi.org/10.1016/j.ijbiomac.2012.06.032
15. Gu C., & Pan S. - The comparison and analysis of three extraction methods for polysaccharides in Purslane. Journal of Food and Nutrition Research **2** (7) (2014) 401-405. https://doi.org/10.12691/jfnr-2-7-12
16. Pardo A.G., & Forchiassin F. - Influence of temperature and pH on cellulase activity and stability in *Nectria catalinensis*. Revista Argentina de microbiologia **31** (1) (1999) 31–35.

TÓM TẮT

NGHIÊN CỨU ẢNH HƯỞNG CỦA ENZYME CELLULASE VÀ VI SÓNG ĐẾN THU NHẬN POLYSACCHARIDE TỪ RONG *Enteromorpha* sp.

Hà Thị Minh Thư, Nguyễn Ngọc Thư, Hoàng Thị Ngọc Nhơn*

Trường Đại học Công Thương Thành phố Hồ Chí Minh

*Email: *nhonhtn@huit.edu.vn*

Trong nghiên cứu này, trích ly có sự hỗ trợ của enzyme (EAE) và trích ly có hỗ trợ vi sóng (MAE) là các phương pháp được sử dụng để trích ly polysaccharide (PS) từ rong bún *Enteromorpha* sp. Hàm lượng polysaccharide được xác định bằng phương pháp phenol-sulfuric acid. Kết quả chỉ ra rằng các điều kiện của phương pháp MAE được xác định như sau: công suất vi sóng là 420W, thời gian trích ly là 3 phút và tỷ lệ nguyên liệu-dung môi là 1:20 w/v. Các điều kiện của phương pháp EAE gồm tỷ lệ nguyên liệu-dung môi là 1:20 w/v, pH 5.0, nhiệt độ 50 °C và thời gian chiết là 4 giờ. Hàm lượng của PS lần lượt là 205.4 ± 0.64 mg/g chất khô (MAE) và 147.9 ± 0.13 mg/g chất khô (EAE).

Từ khóa: Enzyme, vi sóng, polysaccharide, *Enteromorpha* sp.