COMPARISION OF LIPID EXTRACTION EFFECTIVENESS FROM *Sargassum oligocystum* ALGAE BY ENZYME AND ULTRASOUND METHODS

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ABSTRACT

This study aimed to examine the effects of enzyme and ultrasound on lipid extraction from *Sargassum oligocystum* algae. The enzyme-assisted extraction conditions of lipid with the enzyme included: enzyme ratio (0, 0.5, 1, 1.5, and 2 v/w), pH (3.5, 4, 4.5, 5, and 5.5), temperatures (35, 40, 45, 50, and 55 ℃) and extraction time (60, 90, 120, 150, and 180 min). The investigation of lipid extraction parameters from *S. oligocystum* algae using ultrasonic waves, such as material/solvent ratio (1/5, 1/10, 1/15, 1/20, and 1/25 w/v), time (5, 10, 15, 20, and 25 min). The results showed that the suitable conditions for lipid extraction with Viscozyme L were an enzyme concentration of 1.5%, pH 4.5, at 50 ℃ for 150 min. The results show that the recovery efficiency of lipid extraction from *S. oligocystm* algae using shaking method combined with Viscoenzyme L treatment (48.17 ± 0.91%) was higher than the that of ultrasound method (46.88 ± 1.03%).

Keywords: Enzyme, lipid, *Sargassum oligocystum*, ultrasound.

1. INTRODUCTION

Some brown seaweed species have a high total lipid content of 10-20% of the dry weight. The lipid layer is mainly glyceroglycolipids, containing a lot of stearidonic acid (18:4n-3), eicosapentaenoic acid (20:5n-3) and arachidonic acid (20:4n-6), the lipids of brown seaweed contain many biologically active substances such as omega-3, PUFA, omega-6, etc. Therefore, the lipids in brown seaweed represent a vital functional lipid source, which contains valuable nutrients and bioactive components, some of which are not found in terrestrial plants [1].

Sargassum oligocystum is a specie of brown algae in the family *Sargassaceae* grown commonly in shallow coastal waters, often clinging to objects such as corals, rocks, etc. [2]. Several previous reports showed that *S. oligocystum* contains a high volume of minerals, glucides, proteins, and lipids, including unsaturated fatty acids, a potential source of foodstuff and functional food industries [3]. It is found that *S. oligocystum* had a high amount of omega-3 on average, about 20%, omega-9 accounts for about 15%, and omega-6 accounts for about 9% [4].

Enzyme-assisted extraction is considered the green and effective method for obtaining active ingredients from natural sources. It can catalyze and break down plant cell walls to facilitate solute entry into the solvent, increasing extraction efficiency. This is a low-cost and safe method [5]. In addition, ultrasound-assisted extraction is based on the movement of an ultrasonic wave that vibrates the air bubbles to form a heat flow inside the liquid and create gas cavitation, which helps to release the target compounds. The reaction, thanks to the presence of free ions, breaks chemical bonds, can break the cell membrane of raw materials,

making the solvent's permeability easy and improving the extraction efficiency. This is considered a short-time, effective way to extract bioactive compounds [6]. Ultrasound and enzymes were used to extract lipids from some seaweed species such as *Sargassum muticum, Osmundea pinnatifida*…[7]. In this study, the effects of ultrasound and enzyme on lipid extraction from *S. oligocystum* were examined. The extraction efficiency of both methods was also compared. This study aimed to compare the lipid extraction efficiency from *S. oligocystum* with the help of enzymes and ultrasound.

2. MATERIALS AND METHODS

2.1. Materials

S. oligocystum seaweed was collected in Son Hai 1 village, Phuoc Dinh commune, Thuan Nam district, Ninh Thuan province. After harvesting, the seaweed was pre-washed, drained, and transported to the laboratory, where the seaweed was washed with tap water to remove unwanted parts such as sand, shells, snails, etc., then dried at a temperature of 50 ^{o}C until under 10% moisture content, ground into a powder of 0.3 mm size and stored in the freezer compartment for use for the experiments.

Chemicals: $CHCl_3$ (99%), CH_3OH (≥ 99.5%), NaCl (≥ 99.5%), H_3PO_4 (85%), NaOH (99%), ethanol (99.5%), Vanillin, H_2SO_4 (99.5%), phosphate buffered (99%) (Xilong, China) During the pH investigation, the use of phosphate buffer (Xilong, China) still maintained the stability of the solution. Therefore, this chemical is still used to carry out this study, Viscoenzyme L (Novozymes, Denmark) and once time distilled water. The chemicals used in the study met the technical requirements of the analytical chemicals, including sample preparation, experimental analysis, quality control, and data pre-processing.

Equipment: UV-VIS spectrophotometer (model V-730, Jasco brand, Japan), Vortex-ZX Classic shaker (Velp company, Italy), WNB 14 type thermostat (Memmert, Germany), ultrasonic washing tank (Branson brand, Mexico).

2.2. Methods

2.2.1. Experimental method

- Effect of raw material/solvent ratio

5 g of raw materials (calculated by weight of dry matter) was weighed in a 250 mL - beaker, and extract samples with the ratio of materials/solvent investigated 1/5, 1/10, 1/15, 1/20, 1/25 (w/v) at 360 W for 40 seconds. The residue is then removed by filtration to obtain the liquid fraction mixture. Carry out lipid quantification based on phospho-vanillin.

- Effects of enzyme on lipid extraction from S. oligocystum

Enzyme Viscozyme L is a lignocellulose-specialized multi enzymatic complex. It contains a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase. Viscozyme L aids in releasing oil bodies and proteins from cells by degrading the hemicellulose, cellulose, and pectin molecules, and by breaking the cell wall components. Viscozyme L has d = 1.2 g/mL, 100 FBG activity, optimal pH 4.5 - 5.5, optimum temperature 40-50 ºC, operating temperature is 25-55 ºC. The selected pH and temperature parameters are completely consistent with the optimal parameters of the enzyme [8].

5 g (based on the dry matter) of algae powder was soaked in 75 mL of distilled water into a 250 mL beaker, adjusting pH with 1 N NaOH and 1 N HCl. Then, the enzyme would be added and inactivated at 80 ºC for 2 to 3 min [7]. After treatment with the enzyme, the residue

was collected by filtering and dried at 45 – 50 ^{o}C until the moisture content was 16% for lipid extraction at 50 ℃ in a thermostatic tank with chloroform: methanol (2:1) solvent, the ratio of raw materials to solvent 1/20 w/v for 4 hours [6]. The mixture was centrifugated to obtain the supernatant for lipid quantification based on the phospho-vanillin reaction, to measure lipids [9]. The survey elements include:

Experiment 1: Effects of enzyme concentration with 0; 0.5; 1.5; 2% Viscoenzyme L. The fixed parameters were: temperature 45 °C, time 90 min, pH = 4.5.

Experiment 2: Effects of pH at values of 3.5; 4; 4.5; 5; 5.5. The fixed parameters were: temperature 45 °C, 90 min, and enzyme concentration (The result of Experiment 1).

Experiment 3: Effects of temperatures 35, 40, 45, 50, 55 ℃. Fixed parameters were: time 90 min with enzyme concentration and pH as the results of Experiment 1 and Experiment 2.

Experiment 4: Effect of treatment time of 60, 90, 120, 150, 180 min. The fixed parameters were: enzyme concentration, pH, and temperature conditions selected from the results in Experiment 1, experiment 2, and Experiment 3.

- *Effects of ultrasonic waves on lipids extraction from S. oligocystum*

5 g of material powder (calculated on a dry basis) was weighed in a 250 mL beaker. An ultrasonic washing tank device was used as an ultrasonic generator with 20 kHz and chloroform: methanol (2:1). Then, the residue was removed, and collected the supernatant to quantify lipids based on phospho-vanillin reaction [10]. The survey elements include:

Experiment 1: Effects of solvent ratios at the values of 1/5, 1/10, 1/15, 1/20, 1/25 (w/v). The fixed parameters were: time 15 min and ultrasonic power 30%. In this experiment, the solvent ratio was not investigated because the solvent ratio was investigated in the above test. The solvent ratios were reused in this test.

Experiment 2: Effects of treatment time at the intervals of 5, 10, 15, 20, 25 min. The fixed parameters were: ultrasonic power 30% and solvent ratio taken from the results in Experiment 1.

2.2.2. Analytical method

- *Method of determination of moisture:*

Determination of moisture by infrared drying method.

- *Protein quantification method*

Protein quantification method according to TCVN 10034:2013 (ISO 1871:2009).

- *Lipid quantification method*

Lipids were quantified by measuring absorbance at 530 nm using a spectrophotometer.

2.3. Analytical method by measuring absorbance at 530 nm using a spectrophotometer

Lipid calibration curve: Simply rapeseed oil (product of CALOFIC Co., Ltd.) prepared in chloroform, the standard lipid concentration (120 - 420 ppm) was performed in a clean test tube with a stopper [11]. The test tubes were incubated in a bath at 90 °C for 15 min to completely evaporate the chloroform. 2 mL of concentrated sulfuric acid was added and continued to incubate in a 90 °C bath for 10 min, then cooled the tubes for 5 min in a 1000 mL beaker filled with ice. 5 mL of phospho-vanillin reagent was added and incubated at 37 ℃ for 5 min. Then the sample was shaken continuously for 10 min with a vortex shaker. Samples were measured using a UV-VIS spectrophotometer with a wavelength of 530 nm to determine the total lipid content according to the equation: $y = 0.002x – 0.115$, with Correlation coefficients $R^2 = 0.982$. This demonstrates the high level of reliability of the Sulfo-phospho-

vanillin method in lipid quantification [10]. The lipid content % dry matter (DM) was calculated according to the formula:

$$A = \frac{C \times n \times V}{m \times 10000} \times 100\%$$

A: the lipid content (% DM).

C: the lipid concentration from the calibration curve (g/mL).

n: the dilution factor

V: the extract volume (mL)

m: the sample weight according to the dry matter (g)

10000: the µg to g conversion value

2.4. Data analysis

Each experiment was repeated three times, and the results are presented as mean ± standard deviation. Statistical analysis of experimental data was performed using Microsoft Excel 2020. Evaluation of significant differences between experimental samples was performed by the statistical method ANOVA (with t-test) on JMP software. The results were considered to be statistically significant with $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effects of enzyme on lipid extraction from *S. oligocystum*

The effects of the ratio of material and solvent on lipid extraction was shown in Fig. 1.

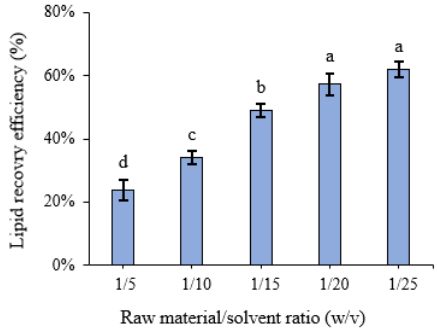

Fig. 1. Effects of material/solvent ratio on lipid extraction efficiency

Note: Values followed by the same letter in the same column are not significantly different at the 5% level.

The results showed that the lipid recovery efficiency increases with increasing solvent ratio. However, the material/solvent ratio 1/20 and 1/25 (w/v) resulted in no significant difference in the recovery efficiency (57.34 ± 3.44% and 62.14 ± 2.52%). Therefore, to save solvent, the ratio of raw materials/solvent of 1/20 (w/v) was chosen for further experiments.

The enzyme used is Viscoenzyme L to hydrolyze the cell wall layers of seaweed, helping the cells in the seaweed to be more soluble in solvents [11]. The effects of enzyme concentration, pH, temperature, and extraction time were examined and the results were shown in Fig. 2A-D.

Fig. 2A showed that the hydrolysis reaction rate increased with increasing enzyme concentration. Hydrolysis causes a lot of damage to the cell wall and allows the solute to dissolve easily with the solvent. In the pH 3.5 - 4.5 range, the lipid content reached the highest value of 44.85 ± 1.61% at pH 4.5 (Fig. 2B). The operating temperature range of Viscozyme L enzyme was 35-55 °C. And the lipid content was obtained at 50 °C (47.75 ± 1.84%) (Fig. 2C). Longer treatment time resulted in lower enzyme activity, reducing the extraction efficiency.

The lipid content was almost unchanged after 150 min and 180 min extraction (Fig. 2D). Therefore, in this study, the Viscozyme L enzyme treatment time for lipid extraction was chosen to be 150 min, and the selected enzyme concentration for lipid extraction from *Sargassum* algae was 1.5%. The obtained lipid in this study was lower than that of Tran Thi Ngoc Mai *et al.*, with a recovery efficiency of 7.192 ± 0.213% [7].

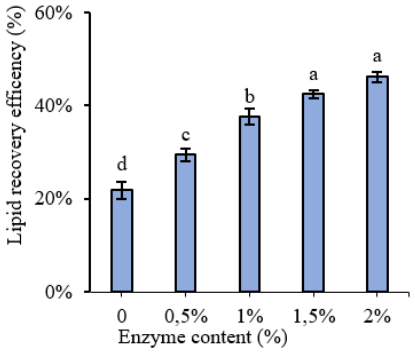

Fig. 2A. Effects of enzyme concentration on lipid recovery efficency

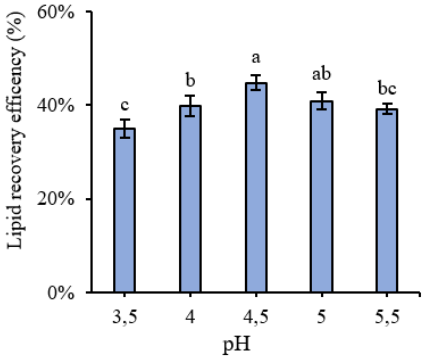

Fig. 2B. Effects of pH on lipid recovery efficency

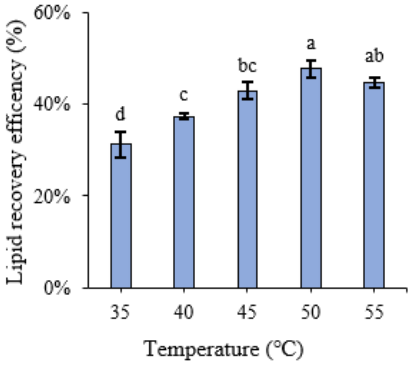

Fig. 2C. Effects of temperature on lipid recovey efficency

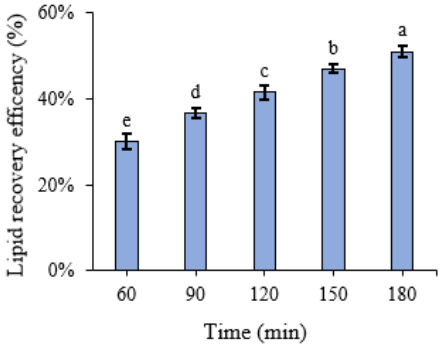

Fig. 2D. Effects of time on lipid recovey efficency

Note: Values followed by the same letter in the same column are not significantly different at the 5% level.

3.2. Effects of ultrasound on lipid extraction from *S. oligocystum*

The effects of solvent ratio, and ultrasonic treatment time on lipid extraction from *S. oligocystum* seaweed were presented in Fig. 3A-B.

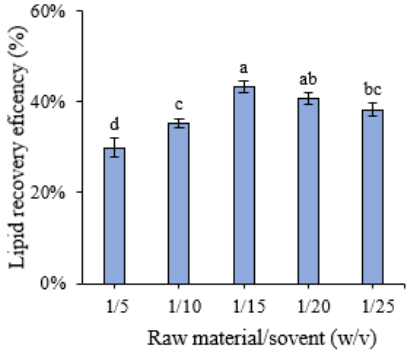

Fig. 3A. Effects of solvent ratio on lipid recovery efficency

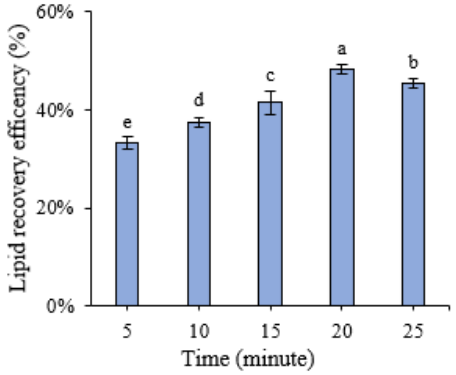

Fig. 3B. Effects of ultrasound treatment time on lipid recovery efficency

Note: Values followed by the same letter in the same column are not significantly different at the 5% level.

The results in Fig. 3A and Fig. 3B showed that the material/solvent ratio and ultrasonic treatment time significantly impacted on the lipid extraction from *S. oligocystum*. The lipid content increased gradually from 29.87 ± 2.05% - 43.24 ± 1.25% with the material/solvent ratio from 1/5 to 1/20 w/v and decreased at the material/solvent ratio of 1/25 w/v. However, the differences in lipid content at the material/solvent ratios 1/15 and 1/20 w/v were not significant statistics (Fig. 3A). Ultrasound treatment times of 15 min and 25 min resulted in no difference in lipid content ($p < 0.05$) and the highest lipid content of 46.88 ± 1.03% at 20 min. Thus this study selected the material/solvent ratio of 1/20 w/v and a treatment time of 20 min was selected for lipid ultrasound-assisted extraction. Transmitting ultrasonic waves from a liquid medium to a solid medium (algae raw material) would increase pressure and temperature and break the cell structure. From there, the components in the cell would dissolve into the solvent more easily. But the extraction time must be long enough, and the ratio of ingredients must ensure that the substances in the raw materials would completely dissolve into the solvent. In addition, the raw material/solvent ratio also helped to increase the efficiency of the extraction process. The larger the raw material/solvent ratio, the larger the contact area between the raw material and the solvent, leading to the greater solubility of the ingredients in the raw material. Therefore, the higher the raw material/solvent ratio, the higher the solute content. At the same time, at a certain rate, the solute content will stop, so the more solvents are used, the more solvents are wasted, and it will take longer to process the extract. The previous of Rodrigues D. *et al.* also demonstrated this rule.

The extract obtained in this study was lower than that of Rodrigues *et al*., using ultrasound in lipid extraction in *Sargassum muticum, Osmundea pinnatifida*, and *Codium tomentosum* species, showed inhibitory activity against α-glucosidase (38-49%) [12]. In this study, the material/solvent ratio of 1/20 w/v and treatment time of 20 min was selected for lipid ultrasound-assisted extraction.

3.3. Comparison of lipid extraction efficiency from *S. oligocystum* with the support of enzyme and ultrasound

From the selected conditions of two methods of enzyme and ultrasound-assisted extraction (sections 3.1 and 3.2). The experiments were conducted under the selected conditions. The obtained lipid content from the two methods was shown in Fig. 4.

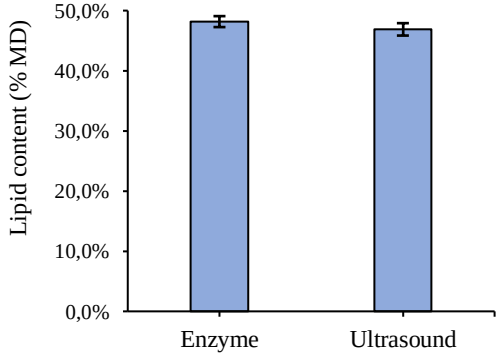

Fig. 4. Lipid content from *S. oligocystum* in enzyme and ultrasound-assisted extraction

From Fig. 4, the lipid content in the extraction method with enzyme treatment is higher than that in the extraction method with ultrasound. The lipid content reached the highest value of 48.17 ± 0.91% at pH 4.5 when using shaking method combined with Viscoenzyme L higher than the study on extracting lipids from some brown seaweeds by Tran Thi Ngoc Mai *et al.* from 3.649 ± 0.131% to 8.373 ± 0.122% [13]. When using ultrasound method, sample processing time at 150 minutes, lipid recovery efficiency 46.88 ± 1.03%. The results were

higher than the study on obtaining Sargassum brown seaweed extract by Tran Thi Ngoc Mai, 7.192 ± 0.213% [13]. However, *S. oligocystum* also contained a significant lipid content. And these two extraction methods can be carried out on a larger scale, which is potential for further studies and applications.

4. CONCLUSION

The study indicated that enzyme-assisted extraction with Viscozyme L and ultrasound-assisted extraction significantly effected on the lipid extraction efficiency from *S. oligocystum*. The results showed that Viscozyme L enzyme gave the lipid content of 48.17 ± 0.91% ± 0.91% of dry matter at conditions of enzyme concentration of 1.5%, pH 4.5 at 50 ^{o}C in 150 min treatment. Besides, the lipid content was 46.88 ± 1.03% dry matter in ultrasonic treatment conditions of the material/solvent ratio of 1:15 w/v in 20 min. Thus, the enzyme-assisted extraction was considered more effective than the ultrasound-assisted extraction for lipid extraction from *S. oligocystum*. This study offers the primary platform for further studies on acquiring and applying biologically active lipids in functional food or foodstuff industries.

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TÓM TẮT

SO SÁNH HIỆU QUẢ CHIẾT XUẤT LIPID TỪ RONG *Sargassum oligocystum* BẰNG PHƯƠNG PHÁP ENZYME VÀ SIÊU ÂM

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Nghiên cứu này nhằm khảo sát ảnh hưởng của enzyme và siêu âm đối với việc chiết xuất lipid từ rong *Sargassum oligocystum*. Các điều kiện chiết xuất enzyme hỗ trợ lipid với enzyme bao gồm: tỷ lệ enzyme (0; 0,5; 1; 1,5 và 2 v/w), pH (3,5; 4; 4,5; 5 và 5,5), nhiệt độ (35; 40; 45; 50 và 55 °C) và thời gian chiết xuất (60, 90, 120, 150, 180 phút). Các thông số nghiên cứu là tỷ lệ dung môi (1/5; 1/10; 1/15; 1/20; 1/25 w/v), thời gian (5, 10, 15, 20, 25 phút). Kết quả cho thấy điều kiện thích hợp để chiết xuất lipid bằng Viscozyme L là nồng độ enzyme 1,5%, pH 4,5, ở 50 °C trong 150 phút. Kết quả cho thấy, hiệu quả thu nhận thu lipid từ rong *S. oligocystm* bằng phương pháp lắc kết hợp xử lý Viscoenzyme L (48,17 $\pm$ 0,91%) cao hơn so với phương pháp sử dụng sóng siêu âm (46,88 $\pm$ 1,03%).

Từ khóa: Enzyme, lipid, *Sargassum oligocystum*, siêu âm.