Optimization of Total Protein Extraction from Caulerpa Lentillifera Based on Response Surface Methodology

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Abstract. Response surface methodology (RSM) was applied to obtain the optimal extraction yield of total protein from Caulerpa lentillifera. The effects of liquid-material ratio, crushing grinding power, crushing grinding time, and reaction temperature on the extraction yield of protein were investigated, respectively. Based on single factor tests, RSM with four factors and three levels was used to determine the extraction yield of total protein. Results showed that the optimal extraction conditions were as follows: 30.47:1 of liquid-material ratio, 51.15 Hz of crushing grinding power, 10.78 min of crushing grinding time and 58.62℃ of reaction temperature. The extraction yield of total protein was 35.95% under optimal conditions with the relative error 0.28%. Results showed that RSM could be used to predict extraction yield of total protein from Caulerpa lentillifera with satisfactory result.

1. Introduction
Caulerpa lentillifera is an edible seaweed that grows in tropical waters. It is known as sea grape because it looks like clusters of sparkling green grapes [1]. The taste of sea grape is quite similar with salmon eggs, but it is fresh and fragrant, without fishy smell of fish eggs. Therefore, it is compared to the "green caviar" in plants, and it is a perfect match for sushi. It is very popular on Japanese tables. Sea grape belongs to the family chlorophyllaceae, which mainly distributed in Japan, Okinawa, Indonesia, Vietnam and other Asian countries [2]. It is rich in a variety of nutrients required by the human body, including amino acids, unsaturated fatty acids and minerals, as well as constant or trace elements such as P, Ca, Mg, Cu and Se [3]. Studies have shown that the protein content is relatively high (10.4%-14.7%) [4]. Sea grape is a kind of food with certain health function. In recent years, Qingdao as a representative of the marine city has been introduced and is vigorously promoting the application of aquaculture, there are small-scale aquaculture in a few areas in Fujian province. However, there are few studies on the extraction technology and application of protein from sea grape. In this paper, response surface methodology (RSM) was applied to obtain the optimal extraction yield of total protein from Caulerpa lentillifera. The effects of liquid-material ratio, crushing grinding power,
crushing grinding time, and reaction temperature on the extraction yield of protein were investigated, respectively. The optimization of the extraction processed of protein from sea grape, can further promote the development and utilization of sea grape, which would bring inestimable economic value.

2. Materials and methods

2.1. Materials and Apparatus
KQ-100DE ultrasonic extraction device (Kunshan Ultrasonic Instrument co., LTD., China); UV-visible spectrophotometer (UV-1100, Shanghai Meipuda Instrument co., LTD., China); DHG-9416A electro-thermostatic blast oven (Shanghai Jinghong Experimental Equipment co., LTD., China); microcentrifuge (Sorvall Legend Micro17, Thermo Scientific, USA); electronic scale (PR124ZHE, Aohaosi Instruments co., LTD., China); freeze dryer (TF-SFD-5, Shanghai Tianfeng Instrument co., LTD., China); Tissuelyser (TISS-48, Shanghai Jingxin Instrument co., LTD., China).

2.2. Methods

2.2.1. The extraction of total protein. Sea grapes were washed with distilled water, then the water was sucked up with filter paper. They were dried at -40°C overnight using a freeze dryer. After grounded with liquid nitrogen, the sea grape powder was dehydrated using a freeze dryer until constant weight. The dried samples were passed through 60-mesh sieve (aperture size 0.25 mm) for use. 0.010 g of the powder was accurately weighed, and a certain amount of extracting agent was added, and ultrasonic assisted extraction was conducted at a specific temperature. The system was centrifuged at 2500 rpm for 10 minutes, and the supernatant was taken to be tested.

2.2.2. The determination of total protein. The content of total protein was determined by Coomassie brilliant blue method [5]. When the brown-red Coomassie brilliant blue colorant was added to the standard protein solution or sample, the anion in the Coomassie brilliant blue dye combined with the -NH₃⁺ in the protein molecules to make the solution blue. In 3.0 ml of Coomassie brilliant blue solution, 0.05 ml of twice distilled water, standard protein solution (0.5630 g L⁻¹) or the tested samples were added. They were blank, standard solution or tested samples. After being mixed homogeneously and stood still for 10 minutes, they were tested at the wavelength of 595 nm. By using the formula (1), the concentration of the protein was calculated. The content of total crude protein was tested by Kjeldahl method[6], and the extraction yield of protein was obtained from formula (2).

\[
\text{Content of total protein (g/L)} = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{standard}} - OD_{\text{blank}}} \times C_{\text{standard}} \tag{1}
\]

\[
\text{Extraction yield of protein (\%)} = \frac{\text{content of total protein}}{\text{content of total crude protein}} \times 100\% \tag{2}
\]

2.2.3. Single factor experiment. The effects of liquid-material ratio (20:1, 25:1, 30:1, 35:1 and 40:1), crushing grinding power (40, 45, 50, 55 and 60 Hz), crushing grinding time (1, 6, 11, 16 and 21 min), and reaction temperature (50, 55, 60, 65 and 70°C) on the extraction yield of protein were examined, respectively. Each experiment was carried out for three times and the average values were calculated as the results.

2.2.4. Response face experiment. Based on the single factor process in Section 2.2.3, the extraction yield of protein from sea grape was taken as the response value. According to the design principle of Box-Behnken experiment, a four-factor and three-level experiment was designed for liquid-material ratio (A), crushing grinding power (B), crushing grinding time (C) and reaction temperature (D). The factors coding and levels were shown in table 1.
Table 1. Code table of factors and levels.

| A | B | C | D |
|---|---|---|---|
| liquid-material ratio | crushing grinding power (W) | crushing grinding time (min) | reaction temperature (°C) |
| -1 | 25:1 | 45 | 6 | 55 |
| 0  | 30:1 | 50 | 11 | 60 |
| 1  | 35:1 | 55 | 16 | 65 |

2.3. Data statistics and analysis
Excel 2017, Origin 9.0 and Design-Expert 8.05 were used for data statistics, analysis and chart making.

3. Results

3.1. Single factor test results
The effects of liquid-material ratio (20:1, 25:1, 30:1, 35:1 and 40:1), crushing grinding power (40, 45, 50, 55 and 60 Hz) of the tissuelyser, crushing grinding time (1, 6, 11, 16 and 21 min), and reaction temperature (50, 55, 60, 65 and 70°C) on the extraction yield of total protein were investigated, respectively. Results show that the optimum ratio of liquid to material was 30:1 (figure 1), the optimum crushing grinding power of the tissuelyser was 50 Hz (figure 2), the optimum crushing grinding time was 11 min (figure 3) and the optimum reaction temperature was 60°C (figure 4).

![Figure 1. Effect of liquid-material ratio on extraction yield of total protein.](image1)

![Figure 2. Effect of crushing grinding power on extraction yield of total protein.](image2)
3.2. Response surface test and result analysis

3.2.1. The regression model. Based on the results of single factor tests, the liquid-material ratio (A), crushing grinding power (B), crushing grinding time (C) and reaction temperature (D) were selected as the investigated factors, according to the Box-Behnken principle, the results of response surface are shown in Table 2. The data were fitted by multiple regression using Design-Expert 8.05, the regression equation was: 

\[ Y = 35.95 + 0.27A + 0.078B + 0.24C - 0.46D - 0.22AB - 0.085AC - 0.81AD - 0.17BC + 0.62BD - 0.38CD - 2.61A^2 - 1.79B^2 - 0.75C^2 - 1.19D^2. \]

| NO. | A    | B    | C    | D    | Yield (%) |
|-----|------|------|------|------|-----------|
| 1   | -1   | -1   | 0    | 0    | 30.61     |
| 2   | 1    | -1   | 0    | 0    | 31.88     |
| 3   | -1   | 1    | 0    | 0    | 30.96     |
| 4   | 1    | 1    | 0    | 0    | 31.36     |
| 5   | 0    | 0    | -1   | -1   | 33.73     |
| 6   | 0    | 0    | 1    | -1   | 34.79     |
| 7   | 0    | 0    | -1   | 1    | 33.28     |
| 8   | 0    | 0    | 1    | 1    | 32.82     |
| 9   | -1   | 0    | 0    | -1   | 32.64     |
| 10  | 1    | 0    | 0    | -1   | 33.47     |
| 11  | -1   | 0    | 0    | 1    | 33.09     |
| 12  | 1    | 0    | 0    | 1    | 30.67     |
| 13  | 0    | -1   | -1   | 0    | 33.51     |
| 14  | 0    | 1    | -1   | 0    | 33.91     |
| 15  | 0    | -1   | 1    | 0    | 33.88     |

Table 2. Design and results of response surface methodology.
3.2.2. The effect of various factors on the extraction yield of total protein. The response surface graphs shown in figure 5-10 are surface graphs composed of response value to each test factor. From the response surface graphs, interaction among parameters and the optimal conditions could be observed easily.

**Figure 5.** Response surfaces of liquid-material ratio and crushing grinding power on extraction yield of total protein.

**Figure 6.** Response surfaces of liquid-material ratio and crushing grinding time on extraction yield of total protein.

**Figure 7.** Response surfaces of liquid-material ratio and reaction temperature on extraction yield of total protein.

**Figure 8.** Response surfaces of crushing grinding power and crushing grinding time on extraction yield of total protein.
3.2.3. Determination and verification of the optimal extraction conditions. Analyzed by Design Expert 8.05, the best conditions were as follows: 30.47:1 of liquid-material ratio, 51.15 Hz of crushing grinding power, 10.78 min of crushing grinding time and 58.62 ℃ of reaction temperature. After three parallel experiments, the extraction yield of total protein was 35.95% under the optimal condition, with no significant difference from the theoretical extraction yield.

4. Conclusions
The factors which influenced the extraction yield of protein from sea grapes were analyzed by single factor experiments. Then the optimum extraction conditions were determined, and the extraction processes by tissuelyzer were optimized by response surface methodology. Results showed that the order of the factors influencing the extraction yield of total protein from sea grapes was: reaction temperature, liquid-material ratio, crushing grinding time and crushing grinding power. The optimal extraction conditions were as follows: 30.47:1 of liquid-material ratio, 51.15 Hz of crushing grinding power, 10.78 min of crushing grinding time and 58.62 ℃ of reaction temperature. In actual, after three parallel determination experiments, the extraction yield was 35.95%, while the predicted yield was 36.05%, the relative error was 0.28%. These showed that the regression equation obtained by RSM could be used to forecast the extraction yield of total protein from sea grapes with satisfactory result.

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