Extraction Process Optimization of Fucoidan from Dealginated Kelp Waste

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Abstract. In order to optimize the extraction process of fucoidan from dealginated kelp waste. On the basis of single-factor experiments, Box-Behnken design was evaluated to study different variables of pH, extraction temperature and extraction time on fucoidan yield. By solving the regression equations and analyzing 3-D plots, the optimum conditions were at pH4.9, liquid/material ratio of 20:1 mL/g, extraction temperature of 73°C, and extraction time of 4 h. Under these conditions, the fucoidan yield of dealginated Kelp waste was 1.63 ± 0.07%, which were in good agreement with the predicted values. It is the first report of fucoidan extraction from dealginated kelp waste and the results provides a basis for further purification and application of fucoidan.

1. Introduction
Polysaccharides are one of the main active ingredients of kelp (Laminaria japonica), mainly including three types of alginate, laminarin, and fucoidan. The structure and properties of these polysaccharides are quite different [1]. Alginates generally refers to alginic acid, which are abundant in kelp, in which the alginate contents are around 30% of their dry weight [2]. Sodium alginate is the most common and widely used form of kelp polysaccharide in industry, with structural monomers similar to glucose monomers [3,4].

Fucoidans are L-fucose polysaccharides containing sulfate groups and minor monosaccharides, such as D-galactose, D-xylose, D-glucose, D-mannose and D-uronic acid [5,6]. Studies have shown that fucoidan had no toxic side effects and exhibited a broad range of biological activities such as antitumor [7], anticoagulant [8], antiangiogenic [9], antiviral [10], anti-inflammatory [11], antioxidant [12], antibacterial [13], antihyperlipidemic [14], antihyperglycemic [15], immunomodulatory [16] and wound healing properties [17].

Dealginated kelp waste is the solid industrial residues at the end of alginate production, the major producer of cultivated kelp in the world. Nowadays, a substantial amount of kelp waste is often directly discarded or sold at a very low price for biological fertilizer, which has brought problems such as waste of resources and environmental pollution and has greatly restricted the healthy development of the kelp industry [2]. Dealginated kelp waste still contains a certain amount of fucoidan. Therefore, using dealginated kelp waste to carry out research on the production of fucoidan has high economic and social benefits. The objective of this study is to optimize the extraction process of fucoidan from
dealginated kelp waste. The optimization process was carried out by integrating single-factor experiments, Box-Behnken design (BBD), and response surface methodology (RSM), with a view to providing a basis for the industrial production of fucoidan from dealginated kelp waste.

2. Materials and Methods

2.1. Materials and Chemicals
Dealginated kelp waste was provided by Jiangsu Zhongda Biotechnology Group Co., Ltd (Lianyung, China); L-fucose was purchased from Hefei Bomei Biotechnology Co., Ltd (Hefei, China); Cysteine hydrochloride, citric acid, sodium citrate, and sodium chloride were of analytical grade and purchased from Sinopharm Group (Shanghai, China).

2.2. Single-Factor Experiments
Dealginated kelp waste were dried to a constant weight, crushed and passed through a 40-mesh sieve. 1.0 g of kelp waste powder was accurately weighed, and distilled water was used as an extraction solvent. The extraction was performed at different pH values (adjusting the pH value with 1 mol/L HCl), extraction temperature, extraction time, and liquid/material ratio. After the extraction was completed, the supernatant was obtained by vacuum filtration, and the content of fucoidan in the supernatant was measured to calculate the yield. The factors and levels of the single-factor experiments are shown in Table 1.

| Level | pH | Temperature (°C) | Time (h) | Liquid/material ratio (mL/g) |
|-------|----|------------------|----------|-----------------------------|
| 1     | 3  | 60               | 2        | 10                          |
| 2     | 4  | 70               | 3        | 20                          |
| 3     | 5  | 80               | 4        | 30                          |

2.3. Box-Behnken Design
Based on the results of the single-factor experiments, pH value, extraction time, and liquid/material ratio were used as the independent variables, which were represented by $X_i$, $X_j$ and $X_k$, and the levels of each variable were represented by $-1$, $0$, and $+1$. The fucoidan yield ($Y$) was the response value, tri-factors and tri-levels Box-Behnken design (BBD) was performed to optimize the extraction conditions. The factors, levels and results of BBD are shown in Table 2. Design Expert 7.0.0 statistical software (Stat-Ease Inc., USA) was used to analyze the experimental data and build a model [18].

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{j=k}^{3} \sum_{j=k}^{3} \beta_{ij} X_i X_j$$ (1)

where $Y$ represents the predicted response, $\beta_0$, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ represent the constant coefficients, while $X_i$ and $X_j$ are the independent variables.

2.4. Determination of Fucoidan Yield
The cysteine-sulfuric acid method was used to determine the fucoidan content in the extract using L-fucose as the standard [19]. L-fucose was prepared into 4% standard solution with deionized water. The standard solutions of 0.00, 0.20, 0.40, 0.60, 0.80 and 1.00 mL was accurately drawn and the deionized water was added to the total volume of 1.00 mL in the tubes, then 4.50 mL of 87% sulfuric acid solution was added in an ice water bath. After 1 min, the tubes were heated for 10 min in a boiling water bath, then quickly cooled to room temperature. 0.10 mL of 3% cysteine hydrochloride solution was added, well mixed and kept at 37°C for 90 min. The absorbances at 396 nm and 427 nm of the solutions were measured, respectively. The difference in absorbance was used as the ordinate, the standard solution concentration (µg/mL) was used as the abscissa and the standard regression equation is obtained as $Y = 0.0103X + 0.0434 (R^2 = 0.9998)$. 

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1.00 mL of the sample solution was operated 3 times in parallel using the above method and measured to obtain the absorbances at 396 nm and 427 nm. The fucose content equivalent to the absorbance of the sample was calculated according to the standard regression equation, and the fucoidan yield was obtained according to the following formula: \[ Y = \frac{nCV}{m} \times 100\% \]
where \( n \), \( C \), \( V \), and \( m \) represent the dilution multiple, the concentration of sample, the volume of sample, and the mass of material, respectively.

3. Results and Discussion

3.1. Single-Factor Experiments

The effects of different pH values on the fucoidan yield under the conditions of liquid/material ratio of 20:1 mL/g, extraction temperature of 60°C, and extraction time of 2 h are shown in Fig. 1. It can be seen from Fig. 1 that the fucoidan yield initially increased with the increase of pH value, and then decreased after exceeding pH 5, which indicated that a suitable pH value is beneficial to the leaching of fucoidan from kelp residue and improves the yield. Therefore, 5.0 is used as the center point of pH value in BBD.

As can be seen from Fig. 2 that with the increase of extraction temperature, the fucoidan yield also increases, and it tends to decrease after 70°C, which indicated that too high temperature is not
conducive to fucoidan leaching. Thus, 70°C as the center point of extraction temperature in BBD. Other factors were pH 5.0, liquid/material ratio of 20:1 mL/g, and extraction time of 2 h.

The effects of different extraction time on the fucoidan yield under the conditions of pH 5.0, extraction temperature of 70°C, and liquid/material ratio of 20:1 mL/g are shown in Fig. 3. With the increase of extraction time, the fucoidan yield gradually increased, and reached the maximum at 4 h, so 4 h was used as the central point of extraction time in BBD.

The effects of different liquid/material ratio on the fucoidan yield under the conditions of pH 5.0, extraction temperature of 70°C, and extraction time of 4 h are shown in Fig. 4. It can be seen that with the increase of the liquid/material ratio, the fucoidan yield reached the maximum at 20 mL/g, and then it became stable. From the perspective of energy conservation and comprehensive cost-effectiveness, the liquid/material ratio was set at 20 mL/g.

3.2. Modelling the Extraction of Fucoidan

Based on the results of the single-factor test, three factors, pH ($X_1$), extraction temperature ($X_2$), and time ($X_3$), which have significant effects on the fucoidan yield, were selected as the independent variables. Box-Behnken design and response surface methodology were used to determine the optimal process conditions for fucoidan extraction from dealginated kelp waste. The results are shown in Table 2. With the fucoidan yield ($Y$) as the response value, Design Expert 7.0.0 software was used for multiple regression fit analysis. The effects of the independent variables on the response can be expressed by the following multiple quadratic regression equation:

$$Y = 1.63 - 0.034X_1 + 0.036X_2 + 0.008X_3 - 0.15X_1X_2 + 0.008X_1X_3 + 0.11X_2X_3 - 0.35X_1^2 - 0.098X_2^2 - 0.21X_3^2$$

The results of the analysis of variance of the model are shown in Table 3. From the model's P value < 0.0001, it can be seen that the model is very significant, and the lack of fit value is not significant, indicating that the equation fits the experiment well [18]. It is also known from Table 3 that the primary terms pH and temperature are significant, the interaction terms pH and temperature, temperature and time, and the quadratic terms are very significant. The $R^2$ value of the model is 0.9889, indicating that 98.89% of the experimental data can be explained by the model [20].

| No. | $X_1$ pH | $X_2$ Temperature (°C) | $X_3$ Time (h) | Yield (%) |
|-----|----------|------------------------|---------------|-----------|
| 1   | −1 (4.0) | −1 (60)                | 0 (4)         | 1.05      |
| 2   | +1 (6.0) | −1 (60)                | 0 (4)         | 1.25      |
| 3   | −1 (4.0) | +1 (80)                | 0 (4)         | 1.42      |
| 4   | +1 (6.0) | +1 (80)                | 0 (4)         | 1.02      |
| 5   | −1 (4.0) | 0 (70)                 | −1 (3)        | 1.09      |
| 6   | +1 (6.0) | 0 (70)                 | −1 (3)        | 1.04      |
| 7   | −1 (4.0) | 0 (70)                 | +1 (5)        | 1.08      |
| 8   | +1 (6.0) | 0 (70)                 | +1 (5)        | 1.06      |
| 9   | 0 (5.0)  | −1 (60)                | −1 (3)        | 1.38      |
| 10  | 0 (5.0)  | +1 (80)                | −1 (3)        | 1.23      |
| 11  | 0 (5.0)  | −1 (60)                | +1 (5)        | 1.18      |
| 12  | 0 (5.0)  | +1 (80)                | +1 (5)        | 1.48      |
| 13  | 0 (5.0)  | 0 (70)                 | 0 (4)         | 1.66      |
| 14  | 0 (5.0)  | 0 (70)                 | 0 (4)         | 1.58      |
| 15  | 0 (5.0)  | 0 (70)                 | 0 (4)         | 1.59      |
| 16  | 0 (5.0)  | 0 (70)                 | 0 (4)         | 1.63      |
| 17  | 0 (5.0)  | 0 (70)                 | 0 (4)         | 1.69      |
3.3. Validation of the Optimized Conditions

According to the regression equation, the optimal process conditions for fucoidan extraction from dealginated kelp waste predicted by the model are: pH 4.9, temperature 73°C and time 4 h, and the predicted yield is 1.64%. Validation tests were performed under the above-mentioned optimal extraction conditions. The practical fucoidan yield was 1.63 ± 0.07% (n = 4), which was not significantly different from the theoretical predicted value. Therefore, the optimized extraction parameters obtained based on response surface methodology are accurate and reliable, and have practical value.

Extraction of fucoidans dates back to 1913, whereas Kylin describes the use of a diluted acid solution to extract fucoidan from Laminaria digitate [1]. After that, it is of great interest to extract and characterize fucoidans with the continuous expansion of the bioactivities. Many studies have reported the use of different extraction techniques, such as hot water extraction [21], acid water extraction [6], ultrasound- [22], microwave- [23], and enzyme-assisted extraction [24], to extract fucoidans from different biological resources. These studies are all based on fucoidan as the main or sole target of the organisms, and this study is to extract fucoidan using the dealginated kelp waste, focusing on the comprehensive and full utilization of resources and reduce environmental pollution. To the best of our knowledge, this is the first report of fucoidan extraction from dealginated kelp waste using acidic solution.

Table 3. Analysis of variance for the effects of pH, temperature and time on the extraction rate of fucoidan using the quadratic response surface model.a

| Source | Sum of Squares | df | Mean Square | F Value | Prob > F | Sig. |
|--------|----------------|----|-------------|---------|----------|------|
| Model  | 0.97           | 9  | 0.11        | 69.01   | < 0.0001 | **   |
| $X_1$  | 0.009          | 1  | 0.009       | 5.84    | 0.0463   | *    |
| $X_2$  | 0.011          | 1  | 0.011       | 6.74    | 0.0357   | *    |
| $X_3$  | 0.0005         | 1  | 0.0005      | 0.29    | 0.6079   |      |
| $X_1X_2$ | 0.09         | 1  | 0.09        | 57.67   | 0.0001   | **   |
| $X_1X_3$ | 0.0002        | 1  | 0.0002      | 0.14    | 0.7154   |      |
| $X_2X_3$ | 0.051         | 1  | 0.051       | 32.44   | 0.0007   | **   |
| $X_1^2$ | 0.51           | 1  | 0.51        | 325.78  | < 0.0001 | **   |
| $X_2^2$ | 0.04           | 1  | 0.04        | 25.65   | 0.0015   | **   |
| $X_3^2$ | 0.19           | 1  | 0.19        | 124.71  | < 0.0001 | **   |
| Lack of Fit | 0.0023     | 3  | 0.0008      | 0.36    | 0.7859   |      |

*a *p < 0.05, **p < 0.01.

4. Conclusion

The extraction process of fucoidan from dealginated kelp waste was optimized through integrating the single-factor experiments, Box-Behnken design, and response surface methodology. The optimal extraction conditions were at pH 4.9, liquid/material ratio of 20:1 mL/g, extraction temperature of 73°C, and extraction time of 4 h, the fucoidan yield of dealginated Kelp waste was 1.63 ± 0.07%, which were in good agreement with the predicted values. The results provided a basis for further purification and application of fucoidan from dealginated kelp waste.

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