Application of star point design method in enzymatic hydrolysis of paphia undulate

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Abstract. The effects of microwave–acid hydrolysis on enzymatic hydrolysis reaction of Paphia undulata were studied, and conditions of microwave–acid hydrolysis to prepare small molecular peptides from Paphia undulate were optimized by central composite design–response surface methodology to determine the best conditions of microwave–acid hydrolysis and enzymatic hydrolysis for this species Paphia undulata. Based on experimental results, to prepare small molecular peptides from Paphia undulate by enzymatic hydrolysis with peptide yield as the effect size, the optimal microwave-acid hydrolysis conditions are a solid-to-liquid ratio of 1:3, use of 6.4 mol/L hydrochloric acid, and microwaving at a power of 480 W for 210 s. The yield of small molecular peptide was 85.67%, which was similar to the peptide yield derived from the response surface model (86.62%).

1. Introduction
Corrugated not clam (p. undulata), are mainly distributed in Guangdong, guangxi, fujian and other coastal areas in China, is a kind of high protein low fat, delicious Marine shellfish, if simply to digestion, is difficult to satisfy the market demand efficiently the ripple of the clams small molecular peptide, this is because the digestion process is quite harsh conditions, system of pH, temperature, ultraviolet light, heavy metal ion, etc will affect this enzyme which affects the activity of the enzyme, in order to make the smooth progress of the enzymatic reaction generally requires strict control of temperature, pH and other factors, with a long cycle and enzymolysis.

Central composite design (CCD) is an experimental design and optimization method often used to investigate the influence of multiple factors on the results at the same time. It is characterized by good predictability, simple experiment and high efficiency [1]. Compared with orthogonal experiment and uniform design, star point design is not limited by the selected linear model, and the extreme value of the experiment can be obtained. The deviation between the predicted value and the measured value of the effect under the best conditions is small, which overcomes the defects of orthogonal experiment and uniform design [2]. At present, star point design is mostly applied in the pharmaceutical research field [3-5], while the application of star point design-effect surface method in the enzymatic hydrolysis of small molecular peptides is rarely studied.
2. Experiment part

2.1. Raw material
Wavy paphia clams were purchased from shanzi market, chancheng district, foshan city, identified by
sun huili, a researcher from South China Sea institute of oceanology, Chinese academy of sciences.

2.2. Experimental method
The hydrolyzing process flow chart of corrugated paphia acid is as follows in Figure 1.

![Hydrolyzing process flow chart of corrugated paphia acid](image)

**Figure 1.** Hydrolyzing process flow chart of corrugated paphia acid

2.3. Determination of peptide yield
In reference [6], the peptide yield was determined by the combination of TCA precipitation method and
formaldehyde titration method. After hydrolysis, 50mL 20% TCA was added to the corrugated paphia
sinensis, mixed and allowed to stand for 20min. The solution and precipitation in the beaker were shaken
well and folded into the centrifuge tube. After centrifugation, the supernatant was collected, and the
volume was fixed to 250mL. 10mL clear liquid was taken, and the obtained clear liquid was denoted as
supernatant liquid (TCA). The total nitrogen content and ammonia-nitrogen content in the supernatant
were determined by kjeldahl method and formaldehyde titration method, respectively. Then the amount
of peptide nitrogen and peptide yield were calculated according to the following in Formula 1:

\[
\text{Peptide nitrogen} = \frac{\text{Supernatant}^{\text{TCA}} \times \text{Peptide nitrogen}}{\text{Total protein nitrogen of raw material}} \times 100\%
\]

(1)

3. Results and discussion

3.1. Star design experiment of acid hydrolysis
The substrate concentration was selected for acid hydrolysis as the solid-liquid mass ratio of 1:3, and
the peptide yield (Rp’) was taken as the effect value. The experimental factors included the type of acid
(nitric acid, hydrogen peroxide, hydrochloric acid, phosphoric acid, sulfuric acid). The acid
concentration (C) was (5 mol/L ~ 7 mol/L). The microwave time (t) was (180s ~ 240 s). The microwave
power (W) is (119w ~ 595 w).

A regression model was established to predict the peptide yield of parafil wavelets based on the
influence of four main factors on the yield of parafil wavelets including acid species, acid concentration,
microwave time and microwave power. The correlation between the peptide yield in hydrolysate and the factors can be predicted by a quadripartite quadratic polynomial shown in Formula 2:

\[
Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{14}X_1X_4 + a_{23}X_2X_3 + a_{24}X_2X_4 + a_{34}X_3X_4 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{44}X_4^2
\]  

(2)

In this equation, \(X_1, X_2, X_3\) and \(X_4\) are the independent variables. Whether the established regression model fits the actual situation is jointly evaluated by the \(R^2\) determination coefficient and the analysis of variance.

3.2. Establishment of regression model

According to the principle of quadratic orthogonal rotation combination test design, the relationship between the original variables and coding variables determined in this test (centrifugal speed 4000r/min, centrifugation time 10min) is shown in table 1. The structure matrix is designed by the combination of quadratic orthogonal rotation of three factors, as shown in table 1-2.

### Table 1. Coded factor versus coded level.

| Level | \(X_1\) (acid species) | \(X_2\) (acid content/mol L\(^{-1}\)) | \(X_3\) (microwave duration/s) | \(X_4\) (microwave power/W) |
|-------|------------------------|---------------------------------|-------------------------------|-----------------------------|
| -2.00 | H\(_2\)SO\(_4\)         | 5.0                             | 120                           | 119                         |
| -1.00 | H\(_2\)O\(_2\)          | 5.5                             | 150                           | 238                         |
| 0.00  | HCl                    | 6.0                             | 180                           | 357                         |
| 1.00  | H\(_3\)PO\(_4\)         | 6.5                             | 210                           | 476                         |
| 2.00  | HNO\(_3\)              | 7.0                             | 240                           | 595                         |

### Table 2. CCD scheme and results.

| No. | Experiment Codes | Experimental Scheme |
|-----|------------------|---------------------|
|     | \(X_1\) \(X_2\) \(X_3\) \(X_4\) | \(X_1\) \(X_2\) \(X_3\) \(X_4\) |
| 1   | 2 0 0 0           | H\(_2\)SO\(_4\) 6.0 180 357 |
| 2   | -2 0 0 0          | HNO\(_3\) 6.0 180 357  |
| 3   | 0 -2 0 0          | HCl 5.0 180 357     |
| 4   | 0 2 0 0           | HCl 7.0 180 357     |
| 5   | 0 0 -2 0          | HCl 6.0 120 357     |
| 6   | 0 0 2 0           | HCl 6.0 240 357     |
| 7   | 0 0 0 -2          | HCl 6.0 180 119     |
| 8   | 0 0 0 2           | HCl 6.0 180 595     |
| 9   | 1 -1 -1 -1        | H\(_2\)O\(_2\) 5.5 150 238 |
| 10  | 1 -1 -1 -1        | H\(_2\)O\(_2\) 5.5 150 476 |
| 11  | 1 -1 1 -1         | H\(_2\)O\(_2\) 5.5 210 238 |
| 12  | 1 -1 1 1          | H\(_2\)O\(_2\) 5.5 210 476 |
| 13  | 1 1 -1 -1         | H\(_2\)O\(_2\) 6.5 150 238 |
| 14  | 1 1 -1 1          | H\(_2\)O\(_2\) 6.5 150 476 |
| 15  | 1 1 1 -1          | H\(_2\)O\(_2\) 6.5 210 238 |
| 16  | 1 1 1 1           | H\(_2\)O\(_2\) 6.5 210 476 |
| 17  | 1 -1 -1 -1        | H\(_3\)PO\(_4\) 5.5 150 238 |
Test results in Table 2 were processed by SAS 9.1 software (Cary, NC, USA), resulting in relational expressions of hydrolysate peptide yield $R_p$ versus factors to constitute a regression model:

$$Y = -1356137.97 + 52.29065X_1 + 43.67484X_2 + 315.18432X_3 + 574.07149X_4 - 30.71590X_1X_2 - 32.64593X_1X_3 - 5.06801X_1X_4 - 14.55609X_1^2 - 521.75132X_2^2 - 5.0270X_3^2 - 1.50049X_4^2$$

(Type 2-2)

(Note: $X_i$ is acid species, and coded values represent corresponding acid species)

### 3.3. Analysis of regression model

The regression equations used for ANOVA are shown in Table 3.

#### Table 3. Regression equations for ANOVA.

| Source of variance | Degree of freedom | Sum of squares | Mean sum-of-squares | F Value | P Value | $R^2$ |
|--------------------|------------------|----------------|---------------------|---------|---------|-------|
| Regression model   | 14               | 7587.91        | 541.99              | 9.85    | <0.0001 |       |
| Error              | 5                | 11.54          | 2.31                |         |         |       |
| Total              | 29               | 8413.60        |                     | 0.9019  |         |       |

#### Table 4. Quadratic regression model parameters.

| Source of variance | Degree of freedom | Parameter estimate | Standard error $t$ Value | $P$ value | Significance |
|--------------------|------------------|-------------------|------------------------|-----------|--------------|
| Model              | 14               |                   |                        |           |              |
| Constant term      | 1                | -1356137.97       | -1.78                  | <0.0001   |              |
| $X_1$              | 1                | 52.29065          | 2.01                   | 0.0954    |              |
| $X_2$              | 1                | 43.67484          | 1.91                   | 0.0052    |              |
| $X_3$              | 1                | 315.18432         | 1.24                   | 0.0213    |              |
| $X_4$              | 1                | 574.07149         | -0.00                  | 0.0016    |              |
| $X_1^2$            | 1                | -14.55609         | -9.72                  | <0.0001   |              |
| $X_2^2$            | 1                | -521.75132        | -2.40                  | 0.0299    |              |
| $X_3^2$            | 1                | -5.0270           | -2.13                  | 0.0504    |              |
| $X_4^2$            | 1                | -1.50049          | -2.00                  | 0.0639    |              |
| $X_1X_2$           | 1                | -30.71590         | -1.27                  | 0.0905    |              |
| $X_1X_3$           | 1                | -32.64593         | -1.92                  | 0.0746    |              |
| $X_1X_4$           | 1                | 5.06801           | 0.42                   | 0.6816    |              |
| $X_2X_3$           | 1                | 12.11415          |                       |           |              |
| $X_2X_4$           | 1                | 17.03390          |                       |           |              |
| $X_3X_4$           | 1                | 10.76760          |                       |           |              |

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Table 3 is the analysis of variance of quadratic model designed by star points, and table 4 is the parameters of quadratic regression model. When P value is less than 0.05, the difference between the influence factors and the effect value is significant; when P value is less than 0.01, the difference is highly significant; when P value is less than 0.001, the difference is extremely significant. As can be seen from table 3, the F value of the model is 9.85, indicating that the model is highly significant, and P < 0.001 indicates that the quadratic equation model is extremely significant. The $R^2$ was determined to be 0.9019, suggesting a significant regression effect. The goodness of fit was 90.19%.

4. Conclusion
(1) The star-point design method has been well applied in the enzymatic hydrolysis of corrugated paphia.
(2) The regression equation obtained from the quadratic orthogonal rotation combination test design fits well with the actual situation, so the regression equation can describe the real relationship between each factor and the effect value well, and the optimal condition can be determined by using this equation.

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