Application of effect-surface method in enzymatic hydrolysis of corrugated *Paphia undulata* by microwave acid

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Abstract. Application of effect-surface method in enzymatic hydrolysis of corrugated *Paphia undulata* by microwave acid was studied. In this experiment, the process was optimized, and the influence of microwave acid combined treatment on enzymatic hydrolysis of corrugated *Paphia undulata* was explored by means of star-point design and effect-surface analysis. The experimental results show that the acid type and concentration of acid, acid type and power of microwave, acid concentration and microwave power, microwave power and microwave time affect peptide yield, under the optimal process conditions, peptide yield can reach 85.67%, the value and the response surface model prediction is very close to 86.62%, star design - response surface model can be applied to small molecular peptides hydrolyzed well optimization of process conditions.

1. Introduction

*Paphia undulata* (*p. undulata*) is a delicious Marine shellfish with high protein and low fat. After enzymatic hydrolysis, free amino acids and small molecular peptides can be obtained from *p. undulata*. These small molecular peptides have anti-fatigue effect [¹] and anti-skin aging effect [²].

Microwave energy is much bigger than ordinary radio waves, microwave high speed vibration effect can destroy the protein disulfide bond, the structure of the protein becomes loose, the original at the molecular internal exposure to some non-polar groups in the molecular surface, to prevent the accumulation of the protein molecules so as to effectively reduce the protein molecular weight [³-⁴]. Microwave heating speed is fast, all parts of the object are usually able to evenly permeate electromagnetic waves; Compared with conventional heating, microwave uniformity is greatly improved, easy to control and operate, and is safe.

This experiment by corrugated not clam as raw materials, application of star design - response surface method, the microwave and acid treatment of the effects of the ripple, the clam protease solution to explore the kinds of acid, acid concentration, time of microwave and microwave power on the utilization of protein, poly peptide yield, etc. The change law of the optimal value, seek the best experimental conditions, for corrugated the high value of exploitation and utilization of the clams and other protein hydrolysis technology research provided the scientific basis.
2. Experiment

2.1. Treatment of raw materials
Soak fresh corrugated Paphia clams in light salt water to spit sand, shelled and gutted, clean, drain and set aside.

2.2. Acid hydrolysis treatment
According to references [5], the homogenized shell meat was placed in a 250 mL conical flask, and the acid with a preset concentration was added. The acid was hydrolyzed for 3 h in a water bath at a constant temperature of 90°C. During the whole process of acid hydrolysis, constant oscillation was needed to ensure the full reaction.

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, 50 ml 20% TCA was added to the corrugated Paphia sinensis. After mixing, 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 250 mL. 10 mL 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 formula:

\[
\text{Peptide yield (Rp')} = \frac{\text{Peptide nitrogen content of supernatant}^{\text{TCA}}}{\text{Total nitrogen content of material}} \times 100\% \quad (1)
\]

3. Results and discussions

3.1. Influence of acid type and acid concentration on peptide yield
Pursuant to software operating specifications, acids used in the experiment were coded (-2, -1, 0, 1, and 2 represent H₂SO₄, H₂O₂, HCl, H₃PO₄, and HNO₃, respectively), and the regression model was analyzed (Figure 1). Among the five acid species, HCl had the greatest impact on peptide yield. The peptide yield was lowest in the case of HNO₃, mainly because *P. undulate* protein contains tyrosine, phenylalanine, and tryptophan, which carry benzene rings. The nitric acid and benzene ring undergo nitrification to generate a yellow nitro aromatic compound, and protein was denatured to produce nitrobenzene derivatives, leading to low peptide yield. Furthermore, during acid hydrolysis in a temperature-controlled bath, the slurry gradually turned yellow and produced a specific odor. When H₂SO₄ served as the material for acid hydrolysis, the protein in *P. undulate* homogenate was partially carbonized, which lowered the peptide yield. Among these five acids, HCl influenced peptide yield to the greatest extent, and it increases with higher acid concentration, until it reached 6.0 mol/L, when the peptide yield begins to decrease.
3.2. Influence of acid type and microwave power on peptide yield

The acid concentration and microwave power were fixed to 6.0 mol/L and 357 W, respectively, and the regression model was analyzed by MATLAB (Figure 2). Peptide yield was high in the presence of HCl and tended to increase with longer microwave duration.

Figure 1. Effects of acid species and concentration on peptide yield.
3.3. Influence of acid concentration and microwave time on peptide yield

When acid concentration and microwave duration were fixed to 6.0 mol/L and 180 s, respectively, the maximum peptide yield appeared at a microwave power of 480 W (Figure 3).
Figure 3. Effects of acid species and microwave power on peptide yield.

3.4. Effects of acid concentration and microwave power on peptide yield
The regression model results for HCl microwaved for 180 s are shown in Figure 4.
3.5. Influence of microwave power and microwave time on peptide yield

The acid species (H$_2$SO$_4$, H$_2$O$_2$, HCl, H$_3$PO$_4$, and HNO$_3$) were analyzed for an acid concentration of 6.0 mol/L in MATLAB (Figure 5). Higher microwave power and longer duration would cause *P. undulate* meat to be charred or gelatinized, making it difficult to obtain good yield. Our results indicate that it is particularly important to select appropriate microwave duration and power settings, which is in agreement with literature [7].

**Figure 4.** Effects of acid concentration and microwave power on peptide yield.
Figure 5. Effects of microwave power and microwave duration on peptide yield.

3.6. Parameter settings for optimal hydrolysis process
Figures 1 and 5 show obvious interactions of acid species versus concentration and of acid concentration versus microwave power. Circular contours indicate insignificant between-factor interaction, as can be
seen in Figure 3, and the interaction of acid species versus microwave power were distinctly circular, indicating that between-factor interaction was not significant.

Based on our analysis, peptide yield was highest when \( X_1 = 0.0 \), \( X_2 = 6.0 \), \( X_3 = 212 \), and \( X_4 = 480 \). The response surface results were optimized by software. As \( X_i \) denotes acid species and cannot be expressed with a specific number, and \( X_1 = 0.0 \) corresponds to the highest peptide yield, \( X_1 = 0.0 \) was substituted into Eq. (1) to obtain Eq. (2). To maximize \( Y \), the first partial derivative of \( Y \) with respect to \( X_2 \), \( X_3 \), and \( X_4 \) should be zero, that is, the following equation:

\[
Y = -1356137.97 + 43.67484 X_2 + 315.18432 X_3 + 574.07149 X_4 + 1.64816 X_2 X_3 + 13.15577 X_2 X_4 + 3.7248 X_3 X_4 - 521.75132 X_2^2 - 5.0270 X_3^2 - 1.50049 X_4^2, \tag{2}
\]

The first partial derivatives were taken as follows:

\[
\begin{align*}
\frac{\partial Y}{\partial X_2} &= 43.67484 - 1043.50264 X_2 + 1.64816 X_3 + 13 \tag{3} \\
\frac{\partial Y}{\partial X_3} &= 315.18432 + 1.64816 X_2 - 10.054 X_3 + 3.729 \tag{4} \\
\frac{\partial Y}{\partial X_4} &= 574.07149 + 13.15577 X_2 + 3.7248 X_3 - 3.00 \tag{5}
\end{align*}
\]

and were substituted into \( X_1 = 212, X_4 = 480 \) and \( X_2 = 6.0, X_3 = 212 \) to obtain \( X_1 = 6.4 \). Peptide yield was the highest at an HCl concentration of 6.4 mol/L, microwave power of 480 W, and microwave duration of ~210 s.

### 3.7. Testing of regression models

In order to verify the accuracy of the regression model, four parallel experiments were carried out on the paphiellal undulate under the condition of hydrochloric acid concentration \( X_2 = 6.4 \) mol/L, microwave time \( X_3 = 210 \) s and microwave power \( X_4 = 480 \) w at the solid-liquid mass ratio of 1:3. The results showed that the peptide yield of Rp ’ in the hydrolysate was \( Y_1 = 84.33\% \), \( Y_2 = 85.06\% \), \( Y_3 = 85.67\% \) and \( Y_4 = 84.85\% \), respectively. It is very close to the value of \( Y \) modulus \( Y_{mod} = 86.62\% \) calculated by the regression model. This fully reflects that the model has a strong analytical ability. This experiment is different from the experimental method adopted by Chen Xianggang et al. [8]. It is close to the peptide yield (82.21%) obtained by acid hydrolysis of paphiella undulate, and the time spent is about 5 h. Compared with 69.31% obtained by Chen Xin et al. [9], the peptide yield of 86.62% obtained after the treatment of *Paphia undulate* by microwave and acid was increased by 1.2 times, which was more significant.

### 4. Conclusion

The star design - response surface analysis to explore the microwave acid combined processing of corrugated and not clam enzyme solution and optimization process, the influence of the optimal process condition, the peptide yield can reach 85.67%, the value and the response surface model prediction is very close to 86.62%, star design - response surface model can be applied to small molecular peptides hydrolyzed well optimization of process condition.

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