Affecting factors on the enzymatic hydrolysis of *paphia undulate* in Marine shellfish

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**Abstract.** In this experiment, active peptide was extracted from the common clams corrugated *paphia sinensis* with high yield on the basis of predecessors. Research of enzymatic hydrolysis dynamics is mainly study the enzymatic hydrolysis rate and its influencing factors, the inherent law between the protein enzymatic hydrolysis dynamics model is deduced, and eventually to the industrialized production dynamic model to guide practice, dynamic model of Marine low-temperature enzyme digestion for understanding the mechanism of action of enzymes, enzymatic hydrolysis process condition control and optimization of reactor type and so on all is of great significance.

1. **Introduction**

   The 21st century is the century of ocean and the century of sustainable development of ocean economy. Marine natural active substances are the focus of modern biology research, and natural Marine active peptides are an important part of Marine active substances research. All kinds of active substances in Marine life, is to develop the study of Marine biological drugs and functional health food raw materials base, rich Marine biological resources for peptide protein preparation provides abundant species resources and resources, and Marine biological protein source of high quality, rich content of amino acid balance shows the erogenous protein sources are far superior to and replaced by [1]. The enzymatic hydrolysis products of many proteins have a variety of short peptides with physiological activities, so people begin to study the development of active short peptides from abundant, high-quality and inexpensive Marine proteins [2-4]. Since the 1970s, millions of new compounds have been isolated from Marine life, including peptides, proteins, polysaccharides and alkaloids. Among the bioactive substances in Marine life, peptides are the most abundant type of compounds, amounting to tens of thousands of species [5, 6].

2. **Experiment**

2.1. **Experimental materials**

   Fresh corrugated *paphia* clams were purchased from shanzi market, foshan, identified by sun huili, a researcher from South China Sea institute of oceanology, Chinese academy of sciences.
2.2. Enzymatic hydrolysis process of paphia corrugated
Raw material corrugated paphia paphia → pretreatment → adding water to homogenize → adjusting pH value → heat preservation → determining the degree of hydrolysis in the enzymatic hydrolysis process.

2.3. Enzymatic hydrolysis treatment
Frozen clam meat of a certain quality was taken, defrosted, and a certain proportion of distilled water was added, which was placed in a constant temperature water bath at a certain temperature for enzymatic hydrolysis. The whole enzymatic hydrolysis process required constant stirring to ensure full reaction.

2.4. Determination of various indexes of the paphia undulata

2.4.1. Determination of total nitrogen content in paphia undulata. The standard method (GB5009.5-85) was used to determine Semi-trace kjeldahl determination of nitrogen [7].

2.4.2. Determination of free amino acids in enzymatic hydrolysate. The content of free amino acids was determined by potentiometric titration.

3. Results and analysis
The initial concentration of low-temperature Marine enzyme was 2250 u/g, and the initial substrate concentration was set at 6%, 9%, 12%, 15% and 18%, respectively. The reaction was carried out at a constant temperature of 30°C and solution pH10, and the hydrolysis degree at different hydrolysis times was recorded. The values of hydrolysis degree and time obtained are shown in table 1.

| t (min⁻¹) | 6% DH  | 9% DH  | 12% DH | 15% DH | 18% DH |
|-----------|--------|--------|--------|--------|--------|
| 5         | 0.6571 | 1.2804 | 0.7983 | 0.683  | 0.9603 | 0.6124 |
| 10        | 1.7042 |        | 1.1478 | 1.067  | 1.324  | 1.435  |
| 15        | 2.134  |        | 1.8025 | 1.689  | 2.21   | 2.347  |
| 20        | 2.3474 |        | 2.5608 | 2.4541 | 3.158  | 3.206  |
| 40        | 2.8275 |        | 3.0943 | 3.6687 | 4.4334 | 4.1613 |
| 60        | 3.7345 |        | 3.5211 | 4.2953 | 5.06825| 4.9082 |
| 80        | 4.268  |        | 3.6278 | 5.335  | 5.4417 | 5.175  |
| 100       | 4.4417 |        | 4.2147 | 5.8685 | 5.9752 | 5.4417 |
| 120       | 4.492  |        | 4.6414 | 5.8635 | 6.1886 | 6.0819 |
| 140       | 4.7132 |        | 5.8993 | 6.4553 | 6.5087 |

The relationships between the DHs and time are shown in Figure 1.
Figure 1 shows the progress of hydrolysis under different initial substrate concentrations. The initial rate of hydrolysis was faster when the initial substrate concentration was lower. As the initial substrate concentration increased, the DH and hydrolysis rate also increased; however, these parameters also tailed off at higher concentrations. The hydrolysis curves for initial substrate concentrations of 15% and 18% are similar because the enzyme and substrate were saturated, and further increases in substrate concentration were unable to increase the rate of hydrolysis. At a given initial substrate concentration, the rate of hydrolysis initially increased with time but later tailed off. Therefore, after some time, changes in the rate of hydrolysis tended to level out. Additionally, the hydrolysis rate tended to decrease as the initial substrate concentration increased.

3.1. Influence of initial enzyme concentration on enzymatic hydrolysis reaction

To investigate the effect of initial enzyme concentration on hydrolysis, the initial substrate concentration was fixed at 10%, and initial quantities of 1,250 U/g, 2,500 U/g, 3,750 U/g, 5,000 U/g, and 7,250 U/g of MLAP were used in separate reactions. The enzyme reactions were performed at a constant temperature of 30°C in solutions of pH 10, and the DHs were recorded at different times. The DHs and times are shown in Table 2.

Table 2. Degrees of hydrolysis at different time points

| t (min⁻¹) | 1,250 U/g | 2,500 U/g | 3,750 U/g | 5,000 U/g | 7,250 U/g |
|-----------|-----------|-----------|-----------|-----------|-----------|
| 5         | 0.519     | 0.873     | 0.936     | 0.798     | 1.356     |
| 10        | 1.024     | 1.456     | 1.764     | 1.588     | 2.582     |
| 15        | 1.623     | 2.034     | 2.821     | 2.591     | 3.175     |
| 20        | 2.1143    | 2.568     | 3.7345    | 3.3077    | 4.001     |
| 40        | 2.6675    | 2.774     | 4.1546    | 3.5211    | 5.228     |
| 60        | 2.774     | 2.987     | 4.4613    | 3.7211    | 6.455     |
| 80        | 2.8809    | 3.201     | 4.668     | 4.42805   | 6.882     |
| 100       | 2.9343    | 3.467     | 4.961     | 4.96155   | 7.469     |
| 120       | 2.9352    | 3.475     | 5.121     | 6.2028    | 7.566     |
| 140       | 2.936     | 3.461     | 5.113     | 6.2283    | 7.621     |
The relationships between the DHs and time are shown in Figure 2:

![Figure 2. Effect of initial enzyme concentration on the DHs](image)

3.2. Influence of enzymatic hydrolysis time on enzymatic hydrolysis reaction

Figures 1 and 2 show that the DH increased over time and then tended to stabilize for different concentrations of both the initial substrate and of MLAP. The rate of hydrolysis was initially rapid but clearly slowed after 40 min and tended to stop after 140 min. Therefore, the hydrolysis rate decreased as the reaction time and DH increased. The reasons for this are as follows: 1) as enzymatic hydrolysis progresses, the peptide-bond substrate concentration decreased; 2) the increase in product concentration inhibited the enzyme reaction; and 3) the denaturation and inactivation of the enzyme may occur during the reaction process.

3.3. Discussion on the mechanism of enzymatic hydrolysis reaction

To investigate why the DH decreased as the reaction progressed, a substrate concentration of 10% was hydrolyzed with 2,250 U/g of MLAP at 30°C and pH 10. After 35 min and 75 min, 0.5× the initial enzyme and 0.5× the initial substrate concentration were added to the reaction system in two separate experiments. The relationships between the DHs and time are shown in Figures 3 and 4.

![Figure 3. Effect of fresh substrate addition on the DH](image)
If the overall decrease in the hydrolysis rate were due to the decrease in the concentration of peptide bonds during the reaction, the rate of hydrolysis should have increased rapidly when new substrate was added. However, Figure 3 shows that, when new substrate was added, the hydrolysis rate did not increase. Therefore, the decreasing concentration of the peptide bonds was not the major reason for the overall decrease in the hydrolysis rate.

Figure 4. Effect of fresh enzyme addition on the DH

If the overall decrease in the hydrolysis rate were due to the denaturation and inactivation of enzyme, the rate of hydrolysis should have increased when more enzyme was added. Figure 4 shows the effect of adding enzyme to the system at 35 min. After a short time, the hydrolysis rate increased significantly, indicating that enzyme inactivation occurred during the reaction and that there were still many peptide bonds available for hydrolysis after 50 min.

4. Conclusion

(1) With different initial enzyme concentrations, the degree of hydrolysis and the hydrolysis rate of the system were enhanced with the increase of the initial enzyme concentration at the same treatment time.

(2) At different initial substrate concentrations and low-temperature Marine enzyme concentrations, the degree of hydrolysis increased with the extension of reaction time and reached a stable value.

(3) As the reaction progresses, the decreasing concentration of the enzymatically soluble peptide bond is not the main reason for the decrease of the overall hydrolysis rate of the system.

(4) There was enzyme inactivation in the reaction, and there were still many enzymatically soluble peptide bonds in the system when the reaction was carried out for 50 min.

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