Composition and high performance liquid phase analysis of paphia undulate in Marine shellfish

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Abstract. Study of Marine shellfish ripple and the composition of the clams and HPLC analysis of the ripple, the clam, by high performance liquid technical analysis corrugated and the composition of the clam, the experimental results show that the high performance liquid chromatographic separation of small molecular peptides in the mass spectogram corresponding molecular weight range of 120-896, mixed small peptide molecular weight below mainly concentrated in the 1000 u, meet the needs of the preparation of small molecular peptides.

1. Introduction
Clams undulate, belonging to the phylum mollusca, class flavibranchiae, order papyriformes, family papyriformidae, clams papyriformes. Mainly distributed in tropical and subtropical coastal areas, mainly distributed in Japan, Thailand, Oman, the Philippines, Australia and other places abroad, mainly distributed in zhejiang, fujian, guangdong, Hong Kong, beibu gulf, hainan and other places.

Marine shellfish contain a large amount of protein, polypeptide, amino acids and other active substances, the fermentation engineering, enzyme engineering and other modern biological engineering technology, Marine shellfish protein resource of high value, utilization and ecological development, has become one of the important ways of shellfish resources comprehensive utilization at home and abroad, is widely used in dressing, health food, nutrition, food, medicine and other fields [1].

The enzyme used in this experiment is Marine alkaline protease 894 extracted from Marine organisms. At present, the research on Marine cryogenic enzymes is still in the exploratory stage, and no similar literature has been published on the study on the kinetics of enzymatic hydrolysis of shellfish. The composition of corrugated paphia sinensis was analyzed by high performance liquid technique.

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. Experimental equipment

**Table 1.** Experimental equipment

| Device name                        | The manufacturer                          |
|------------------------------------|-------------------------------------------|
| E2695/2695 high performance liquid chromatograph | Suzhou sato precision instrument co. LTD  |

When each component dissolved in the mobile phase passes through the fixed phase, due to the difference in size and strength of the interaction (adsorption, distribution, ion attraction, exclusion, affinity) with the fixed phase, the retention time in the fixed phase is different, so it flows out of the fixed phase successively. The difference between high performance liquid chromatography and classical liquid chromatography is that the packing particles are small and uniform, requiring high pressure transport of mobile phase.

2.3. 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.

2.4. enzymatic hydrolysis treatment

Take a certain quality of clam meat, frozen thawed, add a certain percentage of distilled water, homogenate five times, each time 10 s, mix slurry into the digestion in the bottle, adjust well in 6 mol/L NaOH solution slurry pH value of 10, in constant temperature water-bath water heating (or refrigeration) in ice water bath to set the enzyme solution temperature, stay clam meat paste 30 ℃, the temperature reaches the preset value add quantitative enzyme, within a certain temperature of constant temperature water-bath water in timing for digestion, the enzymatic hydrolysis process need to fully stir constantly so as to ensure the reaction.

2.5. establishment of experimental conditions

2.5.1. influence of initial substrate concentration on enzymatic hydrolysis reaction. The experimental conditions were: the dosage of protease was 2250 u/g, the enzymatic hydrolysis temperature was 30 ℃, the pH was 10, and the substrate concentration was 6%, 9%, 12%, 15% and 18%, respectively.

2.5.2. influence of initial enzyme concentration on enzymatic hydrolysis reaction. The experimental conditions were: substrate concentration of 10%, enzymatic hydrolysis temperature of 30℃, enzymatic hydrolysis time of 140min, pH of 10, and the dosage of proteases were: 1250 U/g, 2250 U/g, 3160 U/g, 4100 U/g, 5250 U/g, and 6050 U/g, respectively.

3. Results and discussions

3.1. Composition of the corrugated paphia clam

The composition of the corrugated paphia clams is shown in table 2.
Table 2. Composition analysis of amino acids in *Paphia undulata*

| Amino acid     | Abbreviation | Content (mg/100 g raw meat) |
|----------------|--------------|-----------------------------|
| Aspartic acid  | Asp          | 49.2                        |
| Threonine      | Thr          | 14.4                        |
| Serine         | Ser          | 18.5                        |
| Glutamic acid  | Glu          | 113                         |
| Proline        | Pro          | 0                           |
| Glycine        | Gly          | 580.2                       |
| Alanine        | Ala          | 187                         |
| Cysteine       | Cys          | 14.4                        |
| Valine         | Val          | 14.9                        |
| Methionine     | Met          | 12.7                        |
| Isoleucine     | Ile          | 10.6                        |
| Leucine        | Leu          | 15.8                        |
| Tyrosine       | Tyr          | 0                           |
| Phenylalanine  | Phe          | 45.1                        |
| Lysine         | Lys          | 24.5                        |
| Histidine      | His          | 8.4                         |
| Arginine       | Arg          | 330.5                       |
| Taurine        | Tau          | 815                         |
| **Total quantity of amino acids** | **Total** | **2264.7** |

In order to test whether there is inhibition of enzyme activity in the system, this study compared the changes of hydrolysis degree of the system with time at the initial stage of hydrolysis reaction under the same initial enzyme concentration (2250 U/g) and different initial substrate concentration (figure 1). You can see from figure 1, hydrolysis rate is not increased with the increase of initial concentration of the substrate and a linear increasing trend, but with the increase of initial concentration of the substrate peak after the fall, that limited hydrolysis of the substrate and at the same time there is a dual function of promoting reaction and inhibition of enzyme activity, this was confirmed in the study of Darby and [2]. The arginine content in paphia corrugated is relatively rich, and the protease hydrolysis of the arginine-lysine residues in the substrate will produce some inhibitory peptides that can bind to enzymes [3], and the binding of inhibitory peptides to enzymes can regulate the efficiency of the enzymes [2]. Therefore, the nonlinear variation trend of the hydrolysis process curve with reaction time in this experiment may be caused by the competitive combination of the substrate and the inhibitory peptide to the active site of the enzyme. Its mechanism theory is in the process of hydrolysis reaction, the system can be dissolved in inhibiting peptide concentration increasing and combined with the enzyme's active site, which could inhibit the substrate degradation to the system after the release of the amount of free amino acid, thus the system of free amino acid in net increment is zero, the hydrolysis of dynamic balance, formaldehyde titration method is used to measure the degree of hydrolysis is zero, so the system of the overall reaction rate than the reaction performance has a significant decline in the initial stage.

Based on the above, it can be inferred that for the limited enzymatic hydrolysis of the paphia undulate protein, the inhibitory peptide produced in the reaction process may be the main reason for the denaturation and inactivation of the enzyme, and its degree of inactivation is related to the initial substrate concentration, but its specific mechanism of action remains to be confirmed.

3.2. Analysis of enzymatic hydrolysate by high performance liquid chromatography

In the experiment, the initial substrate concentration of 10 % (meat/water ratio), the initial protease concentration of 2250 u/g, the temperature of 30 °C, pH 10, and the enzymatic hydrolysis time of 120 min were selected. The upper enzymatic solution was centrifuged for liquid chromatography analysis, and the results were shown in figure 1.
In figure 1, after the enzymatic hydrolysate was injected into the column, the peak of ion chromatogram (a) appeared at 9.791 min, the peak of cationic mass spectrogram (b) appeared at 10.191 and 15.443 min, and the peak of anion mass spectrogram (c) appeared at 6.972 min, indicating a substance with a high content in the sample. The mass spectrogram corresponding to the substance at the peak of the three different ion diagrams is shown in figure 2 below.

3.3. Ion mass spectrometry analysis of enzymatic hydrolysate
As can be seen from figure 2, the molecular ion to charge ratio corresponding to the peak value of cationic mass spectrometry at 9.791 min is 148.1, and the fragment ion abundance with the mass to charge ratio of 148.1 is the highest. Figure 1 (b) cationic mass spectrometry: the mass/charge ratios of molecular ions corresponding to the peaks at 10.191 and 15.443 min were 120.2 and 132.2, respectively, with the highest fragment ion abundance in the two groups.

4. Conclusion
The molecular weight range of the small molecular peptides separated by high performance liquid chromatography in mass spectrometry was 120-896, and the molecular weight of the mixed small peptides was mainly below 1000u, meeting the requirements of preparing small molecular peptides.

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