Optimization of the Temperature and Time for the Autolysis and Enzymolysis of Perna Viridis Protein

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Abstract. To determine the optimal conditions for autolysis, the autolysis of mussel (Perna viridis) protein was studied. Using P. viridis meat as the raw material and the degree of hydrolysis as the index, the effects of hydrolysis temperature and hydrolysis time, were researched. Single-factor experiments of autolysis of P. viridis were conducted, taking the degree of hydrolysis as the index. Keeping the pH, time, and solid/liquid ratio unchanged, the temperature was controlled at 30, 40, 50, 60, and 70 °C to study the effects of temperature on the autolysis of P. viridis protein. When the enzymatic hydrolysis temperature was 30 °C, the degree of protein hydrolysis was very low. The results showed that: with increasing temperature up to 50 °C, the degree of hydrolysis increased, reaching a maximum of 15.84%. Keeping the pH, temperature, and solid/liquid ratio unchanged, hydrolysis times of 2, 3, 4, 5, and 6 h were tested to determine the effect of time on the autolysis of P. viridis protein. The results showed that: hydrolysis occurred between 2 and 5 h, when the degree of hydrolysis increased from 13.62% to 15.31%, exhibiting a marked change in the extent of hydrolysis. The continuous increase in hydrolysis could be explained by the fact that P. viridis increased the amino nitrogen content in the hydrolysate via enzymatic hydrolysis and microbial degradation of endogenous enzymes. Therefore, the optimum conditions obtained in these experiments were a temperature of 50 °C, time of 5 h.

1. Introduction

The Asian green mussel (Perna viridis) is found widely in Indonesia, Singapore, Malaysia, and the southern part of the East China Sea [1], P. viridis is an important marine shellfish, which has a strong reproductive capacity, fast growth rate, and large yields, and is commonly consumed in many countries. It can be consumed directly after steaming or cooking, and it can be used to make delicious cooked dishes with other vegetables. It can also be made into a dried product, which is easy to store and consume. In addition, mussel meat is an important Chinese herbal medicine. It is rich in protein, glycogen, and a variety of amino acids and vitamins. It can nourish yin and kidney, and nourish the blood for regulating menstruation. It has beneficial effects in improving blood lipids [2], resisting fatigue [3,4], and improving immunity and other functions. To remedy the limitations affecting sales and promote the development of mussel aquaculture, processing and preservation of mussels must be improved. The currently available processed products of P. viridis are jade mussel oil, canned steamed emerald mussels, and canned cashmere mussels, but their processing methods are too simple to adapt to the developments of the food industry and to fast-paced modern life. In addition, freezing mussel
meat may cause spoilage, so it cannot be adequately preserved. Therefore, it is necessary to conduct additional studies on *P. viridis* processing to maximize its economic and medicinal values.

However, in recent years, because processing technologies have failed to keep up with improvements in breeding, and because of competition with other newly cultured species (e.g., bay scallops), *P. viridis* has experienced poor sales and its production has declined in many areas. In the main farming areas, it is usually used as fresh bait for shrimp, and human consumption only accounts for a small proportion; therefore, its development is limited. In China, most *P. viridis* is sold as fresh and live aquatic products, with a limited sales range and low processing rate. In addition, the harvest period of *P. viridis* has seasonally and regionally unique characteristics. In coastal areas, excess amounts of *P. viridis* are marketed, resulting in the supply exceeding the demand; however, it is rarely marketed in inland areas. Moreover, the period for staying fresh is so short that *P. viridis* frequently spoils, so the time frame for selling these fresh products is small, which often causes slow sales and affects fishery production.

The enzymatic hydrolysate of mussel meat is rich in a variety of amino acids that are beneficial to the human body. Using enzymatic hydrolysis technology to treat mussel meat can effectively improve its resource utilization and added value. At present, studies on the enzymatic hydrolysis of mussel meat have mainly focused on the hydrolysis conditions and functional characteristics of enzymatic hydrolysates. For example, Zhang et al. used orthogonal experiments to optimize the hydrolysis process of *P. viridis*.

In the present study, we characterized the autolysis parameters of *P. viridis* protein. Endogenous protease was used for autolysis of *P. viridis* protein, and the hydrolysis time, hydrolysis temperature, initial pH, and solid/liquid ratio were optimized.

2. Experimental materials and methods

2.1 Experimental Materials
Mussel meat was purchased from the Huangsha aquatic products market (Guangzhou, China) and was identified by Sun Huili, an investigator at the South China Sea Institute of Oceanology, Chinese Academy of Sciences. After screening and cleaning, the mussel meat was removed after 2 days of seawater culture. After removing the shells, the viscera and mussel meat were separated, and all tissue was cleaned and separately homogenized using a blender. The viscera and mussel meat were weighed separately and the viscera/mussel meat mass ratio was calculated. In subsequent experiments, these tissues were used according to this mass ratio. The viscera and mussel meat, which were evenly homogenized, were stored separately in several portions at −5 °C in fresh-keeping bags.

2.2. Experimental Instruments
The HH-2 digital display constant temperature water bath was from the Jintan Fuhua Instrument Co. (China). The PHSJ-3F laboratory pH meter was from the Shanghai Precision Scientific Instrument Co. (Shanghai, China). The Midea juice mixer and TDL-50 electric centrifuge were obtained from the Jintan Medical Instrument Factory (Jintan, China). The KDN-102C nitrogen analyzer was from the Shanghai Fiber Inspection Instrument Co. (Shanghai, China). The HYP-1004 digestion furnace was from the Shanghai Fiber Inspection Instrument Co. (Shanghai, China). The electronic balance was from the Jinan Shangdi Electronic Technology Co. (Jinan City, China).

2.3. Autolytic hydrolysis of mussel meat

2.3.1. Effect of temperature on the autolysis of *P. viridis* protein. First, 10 g of mussel meat was weighed and put into a 50-mL beaker, and 10 g of distilled water was added to each beaker. The pH was then adjusted to 7, the beakers were sealed with plastic wrap, and then placed in thermostat water baths at 30, 40, 50, 60, and 70°C and hydrolyzed for 5 h. After denaturing the enzyme and centrifuging, the supernatant was used to determine the amino nitrogen content.
2.3.2. Effect of time on the autolysis of *P. viridis* protein. First, 10 g of mussel meat was weighed and put into 50-mL beakers, and 10 g of distilled water was added to each beaker. The pH was then adjusted to 7, and the beakers were sealed with plastic wrap and placed in thermostat water baths at 50 °C and hydrolyzed for 2, 3, 4, 5, and 6 h. After denaturing the enzyme and centrifuging, the supernatant was used to determine the amino nitrogen content.

3. Experimental results and analysis

3.1. Effect of temperature on the autolysis of *P. viridis* protein

Keeping the pH, time, and solid/liquid ratio unchanged, the temperature was controlled at 30, 40, 50, 60, and 70 °C to study the effects of temperature on the autolysis of *P. viridis* protein (Table 1, Figure 1).

| Mussel meat (g) | pH | Temperature (°C) | Solid/liquid ratio (m:m) | Hydrolysis time (h) | Degree of hydrolysis (%) |
|----------------|----|------------------|--------------------------|---------------------|--------------------------|
| 10             | 7  | 30               | 1:1                      | 5                   | 14.34                    |
| 10             | 7  | 40               | 1:1                      | 5                   | 15.32                    |
| 10             | 7  | 50               | 1:1                      | 5                   | 15.84                    |
| 10             | 7  | 60               | 1:1                      | 5                   | 15.32                    |
| 10             | 7  | 70               | 1:1                      | 5                   | 14.74                    |

![Figure 1](image-url)  
**Figure 1.** Effects of temperature on the autolysis of *Perna viridis* protein.

When the enzymatic hydrolysis temperature was 30 °C, the degree of protein hydrolysis was very low. With increasing temperature up to 50 °C, the degree of hydrolysis increased, reaching a maximum of 15.84%. However, when the temperature exceeded 50 °C, the degree of hydrolysis began to decrease. This could be explained by the fact that, at the optimum temperature, the endogenous
enzyme activity reached a maximum, promoting the maximum velocity of enzyme-promoted autolysis of *P. viridis* protein. Within a suitable temperature range, the rate of enzyme-promoting reactions can be increased by 1–2 times for every 10 °C increase in temperature. However, excessively high or low enzymatic hydrolysis temperatures reduce the catalytic efficiency of endogenous enzymes, resulting in a reduced velocity of the enzyme-promoting reaction and inhibition of the autolytic enzymatic hydrolysis of the *P. viridis* protein. Based on these considerations, the optimum temperature for endogenous enzymes of *P. viridis* protein was 50 °C. This is consistent with the optimum temperature of endogenous enzymes in aquatic animals (20–52 °C) determined in previous studies [5].

### 3.2 Effect of time on the autolysis of P. viridis protein

Keeping the pH, temperature, and solid/liquid ratio unchanged, hydrolysis times of 2, 3, 4, 5, and 6 h were tested to determine the effect of time on the autolysis of *P. viridis* protein (Table 2, Figure 2).

#### Table 2. Effect of time on the autolysis of *Perna viridis* protein.

| Mussel meat (g) | pH | Temperature (°C) | Solid/liquid ratio (m:m) | Hydrolysis time (h) | Degree of hydrolysis (%) |
|----------------|----|------------------|-------------------------|---------------------|--------------------------|
| 10             | 7  | 50               | 1:1                     | 2                   | 13.62                    |
| 10             | 7  | 50               | 1:1                     | 3                   | 13.64                    |
| 10             | 7  | 50               | 1:1                     | 4                   | 13.84                    |
| 10             | 7  | 50               | 1:1                     | 5                   | 15.31                    |
| 10             | 7  | 50               | 1:1                     | 6                   | 14.86                    |

![Figure 2](image)

*Figure 2. Effect of time on the autolysis of *Perna viridis* protein.*

Hydrolysis occurred between 2 and 5 h, when the degree of hydrolysis increased from 13.62% to 15.31%, exhibiting a marked change in the extent of hydrolysis. The continuous increase in hydrolysis could be explained by the fact that *P. viridis* increased the amino nitrogen content in the hydrolysate via enzymatic hydrolysis and microbial degradation of endogenous enzymes [6]. However, when the enzymatic hydrolysis time exceeded 5 h, the amino nitrogen in the hydrolysate did not increase, and
the degree of hydrolysis was unchanged. The autolysis of the *P. viridis* protein achieved the best effect, and an increased enzymatic hydrolysis time reduced the amino nitrogen content in the hydrolysate. This may have been because the long-term heat action inactivated heat-exposed endogenous enzymes of *P. viridis*, thereby affecting protein hydrolysis [7]. Based on these results, to save time and reduce the effects of excessive hydrolysis time on protein hydrolysis, an optimal hydrolysis time of 5 h should be used.

4. Conclusions
Single-factor experiments of autolysis of *P. viridis* were conducted, taking the degree of hydrolysis as the index. The optimum conditions obtained in these experiments were a temperature of 50 °C, time of 5 h.

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