Research on olid/liquid ratio and initial pH for Perna Viridis protein reacting

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Abstract. Taking the degree of hydrolysis as the index, single-factor experiments of autolysis of P. viridis were conducted. The solid/liquid ratio and initial pH on the degree of autolysis were chosen, and the optimum conditions obtained in these experiments were liquid/solid material ratio of 1:3, and pH of 7.0.

1. Introduction

Jadeite mussel is an important mariculture shellfish with strong reproductive ability, fast growth rate and large yield. Jadeite mussel is also popular seafood, can be steamed, boiled food, also can be peeled off after mixing with other vegetables Fried, delicious taste, can also be made into dry goods namely mussel, storage and consumption are very convenient. Jadeite mussel meat is a kind of important medicinal herbs, rich in protein, glycogen and many kinds of amino acids and vitamins, can ziyin kidney, nourishing blood regulate the menstrual function, to improve blood lipid [1], anti-fatigue [2, 3] has prominent effect, enhance immunity function such as the study of jadeite mussel meat in antitumor function and zengzhi fuelling function can significantly help [4]. Due to its nutritional value and unparalleled price advantage, the development prospect of mussels is very broad. First, its nutritional value is high, with the continuous improvement of processing technology and the vast number of consumers of its nutritional value, the consumer market will continue to expand; Secondly, due to the low technical requirements, rapid growth and low cost of mussel culture, in the current Chinese people are not too rich, there is a price advantage, easy to be accepted by the majority of consumers; In addition, the safety and health of mussel jadeite is basically good. But in recent years, because of jadeite mussel processing technology has failed to keep pace with the development of aquaculture, and subject to breeding new varieties, such as the bay scallop, etc.), the impact of jadeite mussel purchase, in many areas has been as an object of compressed output dropped significantly, in the main breeding area mainly used for prawns and other fresh bait, people's consumption accounts for a small proportion, limiting its development. China's jadeite mussel mainly live fresh sales, sales range is narrow, deep processing rate is low. At present, the processing of jadeite mussel products are oil of jadeite mussel, steamed smoked jadeite mussel canned canned jadeite mussel and oily be soiled, but their processing method is simple and can not adapt to the development of food industry and the modern quick jun play of life, especially frozen mussels meat, frozen and can make the jadeite mussel protein denaturation, long-term preservation of inconvenience. Therefore it is necessary to carry out jadeite mussel deeper step research, better play its economic value and medicinal value.
In Republic of Korea, Taiwan, and the South China Sea [5], the Asian green mussel (*Perna viridis*) is found widely. Studies have shown that the meat has anti-tumor properties, and aids in the physiological and mental development of humans [6]. Because of its high nutritional value and relatively modest price, *P. viridis* has numerous advantages for human consumption. First, its nutritional value is high, and the consumer market can continue to expand with continued improvements in processing technologies and consumers’ understanding of its nutritional value. Second, *P. viridis* farming requires minimal technology; at the same time, it grows rapidly and sells for a modest cost, making it an important nutritional source for the Chinese population with a modest income. Third, *P. viridis* is safe to consume.

As such, relying on fresh sales is insufficient, and it is necessary to further process *P. viridis*. In addition to fresh sales, *P. viridis* is sometimes dried or frozen. Because these products are made using traditional methods, they are not processed in a cost-effective manner. 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.

Meanwhile, Deng and collaborators [7] established the double enzyme hydrolysis method for *P. viridis*, and further determined the optimal process parameters of double enzymatic hydrolysis. However, there has been no systematic study on the enzymatic parameters and product characteristics of *P. viridis* protein when treated with endogenous proteases. Preparing amino nitrogen via the autolytic enzymatic hydrolysis technique does not introduce foreign enzymes, and the viscera are used as the raw material for self-decomposition to obtain amino nitrogen [8]. Moreover, mussel meat is rich in endogenous proteases, which can be used for enzymatic decomposition without introducing external enzymes, saving resources and improving the utilization of ocean resources.

Finally, the optimal conditions for the autolysis of *P. viridis* were determined using orthogonal experiments to achieve the optimal hydrolysis effect, which provided basic data for the high-value utilization of this material.

2. Experimental materials and methods

2.1. Experimental Reagents

Potassium sulfate was obtained from the Guangdong Chemical Reagent Engineering Technology Research and Development Center (Guangzhou, China). Copper sulfate was obtained from the Tianjin Yongda Chemical Co. (Tianjin, China). Concentrated sulfuric acid was obtained from the Guangzhou Donghong Chemical Plant (Guangzhou, China). Bromocresol Green was obtained from the Tianjin Tianxin Fine Chemical Development Center (Tianjin, China). Methyl red was obtained from the Tianjin Guangfu Technology Development (Tianjin, China). Sodium hydroxide granules were obtained from the Tianjin Zhiyuan Chemical Reagent Co. (Tianjin, China). All reagents were of analytical grade.

2.2. Experimental methods

(1) Effect of the solid/liquid ratio on the autolysis of *P. viridis* protein

First, 10 g of mussel meat was weighed and put into 150-mL beakers, using solid/liquid ratios of 1:1.5, 1:2, 1:2.5, 1:3, and 1:3.5 (g/g), and then 15, 20, 25, 30, and 35 g of distilled water, respectively, was added into each breaker. Then, the pH was adjusted to 7, the beakers were sealed with plastic wrap, and then placed in thermostat water baths at 50°C and hydrolyzed for 5 h. After denaturing the enzyme and centrifuging, the supernatant was used to determine the amino nitrogen content.

(2) Effect of pH 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. After adjusting the pH of the samples to 5.0, 6.0, 7.0, 8.0, and 9.0 with 1 M sodium hydroxide and 1 M hydrochloric acid solutions, they were sealed with plastic wrap and hydrolyzed at 50°C for 5 h at a constant temperature. 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 the solid/liquid ratio on the autolysis of P. viridis protein

Keeping the pH, temperature, and time unchanged, the solid/liquid ratio was varied in the range of 1:1.5, 1:2, 1:2.5, 1:3, and 1:3.5 (g/g) to test the effect of the solid/liquid ratio on the autolysis of P. viridis protein (Table 5, Figure 1). The optimal solid/liquid ratio ranged from 1:1.0 to 1:3, which resulted in an increase in the degree of hydrolysis from 18.20% to 22.13%. This increasing trend of hydrolysis was similar to that observed in the effect of temperature on the autolysis of P. viridis protein. However, at solid/liquid ratios beyond 1:3, the degree of hydrolysis began to decrease. In these experiments, the amount of endogenous enzyme was constant, but the substrate concentration decreased. In the early reaction, the degree of hydrolysis increased; however, in the later reaction, the degree of hydrolysis decreased. In this enzymatic hydrolysis reaction, even if the enzyme concentration was sufficiently high, the velocity of the enzyme-promoting reaction did not increase and was even inhibited at higher substrate concentrations. This was because high concentrations of substrate lowered the effective concentration of water, leading to decreased molecular diffusion, and resulting in a decreased velocity in the enzyme-promoting reaction. In addition, excess substrate accumulated on the enzyme molecule, producing an inactive intermediate product, and the enzyme molecule could not be released because it was surrounded, thereby reducing the reaction velocity. At the end of the experiment, the hydrolysis curve began to decline, indicating that the substrate concentration had reached a maximum value. At low substrate concentrations, the velocity of the enzyme-promoting reaction decreases with decreases in the substrate concentration [14]. Therefore, in the case of a constant pH, temperature, and time, a ratio of 1:3 was the optimal solid/liquid ratio to achieve the maximum enzymatic hydrolysis.

| Mussel meat (g) | pH | Temperature (°C) | Solid/liquid ratio (m:m) | Hydrolysis time (h) | Degree of hydrolysis (%) |
|-----------------|----|------------------|--------------------------|---------------------|-------------------------|
| 10              | 7  | 50               | 1:1.5                    | 5                   | 18.20                   |
| 10              | 7  | 50               | 1:2                      | 5                   | 19.80                   |
| 10              | 7  | 50               | 1:2.5                    |                     | 20.94                   |
| 10              | 7  | 50               | 1:3                      |                     | 22.13                   |
| 10              | 7  | 50               | 1:3.5                    |                     | 21.95                   |
Figure 1. Effect of the solid/liquid ratio on the autolysis of *Perna viridis* protein.

3.2. *Effect of pH on the autolysis of P. viridis protein*

Keeping the temperature, time, and solid/liquid ratio unchanged, the pH was controlled at 5, 6, 7, 8, or 9. Under these conditions, the effect of pH on the autolysis of *P. viridis* protein was determined (Table 2, Figure 2). From pH 5 to 7, the degree of hydrolysis increased from 14.20% to 16.08%, showing that increasing the pH enhanced *P. viridis* protein autolysis. However, increasing the pH beyond 7 resulted in a decrease in the degree of hydrolysis of the hydrolysate from 16.08% to 14.24%, indicating that autolysis of the *P. viridis* protein was inhibited. This may have been because the activity of endogenous enzymes of *P. viridis* was directly related to pH. When the pH was 7, the endogenous enzyme activity of *P. viridis* reached a maximum, showing the optimum enzymatic self-hydrolysis effect of endogenous enzymes. However, excessively high or low pH levels changed the charged state of the substrate and enzyme molecules, thereby affecting the binding of endogenous enzymes and substrates. Unsuitable pH affected the stability of endogenous enzymes, thereby irreversibly inhibiting the autolysis of *P. viridis* protein [11]. Based on our results, the optimum pH for endogenous enzymes of *P. viridis* protein was 7.

Table 2. Effect of pH 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             | 5  | 50               | 1:1                     | 5                   | 14.20                   |
| 10             | 6  | 50               | 1:1                     | 5                   | 14.96                   |
| 10             | 7  | 50               | 1:1                     | 5                   | 16.08                   |
| 10             | 8  | 50               | 1:1                     | 5                   | 14.85                   |
| 10             | 9  | 50               | 1:1                     | 5                   | 14.24                   |
4. Conclusions
Taking the degree of hydrolysis as the index, single-factor experiments of autolysis of *P. viridis* were conducted. The optimum conditions obtained in these experiments were liquid/solid material ratio of 1:3, and pH of 7.0.

Acknowledgments
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