

Optimization of the Process of Compound Enzymatic Hydrolysis of Soluble Protease Preparation

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Abstract: soluble protease preparations, including trypsin, papain and other preparations, are widely used in various fields, such as medicine, agriculture, industry and so on. Based on this, this paper takes the soluble protease as the main research object, and uses the experimental method of compound enzymolysis to observe and analyze the utilization of egg membrane protein, so as to improve the utilization rate of egg membrane protein.

1 Introduction

Egg foods are most common in people's daily diets. Both egg whites and egg yolks contain very rich substances and are the main source of raw materials for the chemical industry. Generally, the proteins contained in egg foods are of a hard nature, and protease are difficult to dissolve.

Therefore, in the study of this paper, the original enzyme preparation process will be improved by the composite enzymatic hydrolysis method, and it is hoped that the utilization efficiency of the eggshell inner membrane material can be improved.

2 Experimental materials and instruments

(1) Selection of experimental materials

| Main experimental material | other material | other material |
|---|---------------------|----------------|
| Egg shells (Beijing Deqingyuan Agricultural Technology Co., Ltd.) | Trypsin | Diethyl ether |
| | Papain | Acetic acid |
| | Alkaline proteinase | Copper sulfate |

(2) Selection of experimental instruments

| Instrument names | Model | Country of origin | COHR |
|-------------------------------|--------|-------------------|-------------------------------|
| Thermostatic magnetic stirrer | 85-2 | Shanghai | Siyue instrument |
| Universal crusher | FW100 | Tianjin | Teste instrument |
| PH meter | pHS-3C | Shanghai | Ray magnetic instruments |
| High speed centrifuge | H1850 | Hunan province | Xiangyi centrifuge instrument |
| Digestion furnace | QSL | Shanghai | Ailang instrument |
| Automatic nitrogen analyzer | QSY | Shanghai | Ailang instrument |

3 Experimental methods and procedures

3.1 Extracting egg membrane protein

(1) Protein extraction

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In the first step, weigh 10 grams of egg film, put it in 500ml of acetic acid and mercaptopropionic acid solution, and put it in a constant temperature stirrer at 90 degrees Celsius for 8 hours, and finally take it out;

In the second step, the operation is carried out in a centrifuge of 8000 r/min for 10 minutes, and a clear solution is obtained;

In the third step, the target solution is adjusted to the isoelectric point by using a sodium hydroxide solution, and then at a standstill for half an hour, and simultaneously subjected to centrifugation to obtain a milky white protein precipitate therefrom;

In the fourth step, the protein is precipitated in a 10% acetic acid solution and simultaneously subjected to dialysis treatment to obtain a soluble protease [1].

(2) Technological process

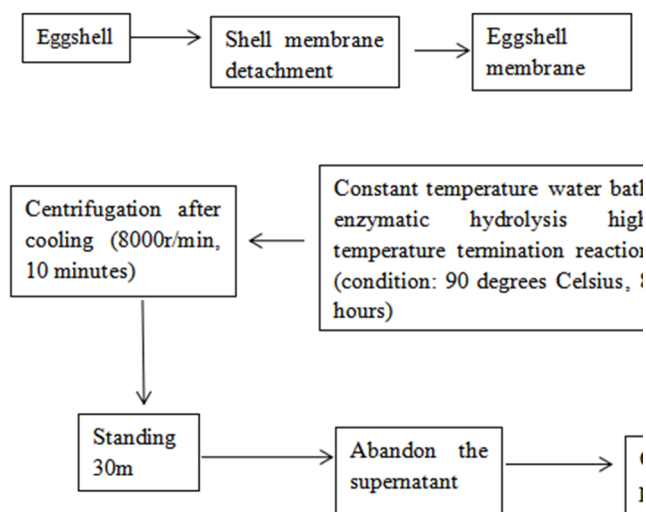


Fig. 1 Process flow of composite enzymatic hydrolysis of soluble protease preparation

3.2 Study on the optimum enzymatic hydrolysis conditions of composite enzyme

(1) Single enzyme screening

Take 50ml of egg membrane protein solution with protein quality of 1mg / ml, determine the total enzyme amount as 150000u / g substrate, and add the experimental materials according to the different cutting points of enzyme, and let them drop the substrate at the appropriate temperature, PH value and other conditions for hydrolysis, so as to determine the degree of

hydrolysis and nitrogen yield of different enzymes[2].

(2) Determination of the optimum conditions of composite enzymatic hydrolysis

In order to improve the utilization of egg membrane protein, it's necessary to determine the ratio of composite enzyme, time and temperature of enzymolysis.

A. Carrying out composite enzymolysis on the enzyme preparation with good effect, and then set the enzymolysis ratio, total enzyme dosage and enzymolysis time of 1:1, 1.5 * 104u / g, 120 minutes;

B. To observe and analyze the effect of adding enzyme in different order at the same time and at different time.

C. After the total amount is fixed, determine the ratio of enzymatic hydrolysis.

(3) Determination of total enzyme addition

On the premise that the total PH value of all kinds of protease is fixed, the amount of hydrolase is determined, the hydrolysis rate and nitrogen yield are determined, and finally the total enzyme amount is determined[3].

(4) Determination of PH value of enzymolysis

When all conditions are unchanged, papain is set at the PH value of 5.0-8.0 at the interval of 0.5, and alkaline protease is set at the PH value of 7.0-10.0 at the interval of 0.5. Then the hydrolysis rate and nitrogen yield are observed to obtain the optimal PH value of the composite enzymatic hydrolysis.

(5) Determination of enzymolysis time

Under the condition of no change in any conditions, eight time points are taken from papain hydrolysis time of 0.25-2.0, and seven time points are taken from alkaline protease time of 0.5-3.5. The hydrolysis rate, nitrogen yield and optimum time of egg membrane protein of two different enzymes are measured.

(6) Determination of enzymolysis temperature

Based on the same conditions, the hydrolysis degree and nitrogen yield of the enzyme at different temperatures are set at 40 °C, 50 °C and 60 °C, and the optimal hydrolysis temperature is finally determined.

Table 1 Horizontal coding of design factors of quadratic regression orthogonal rotation combined test

| Code | Factors | | | |
|------|---------------------------------|-----------------------|-------------------|---|
| | X ₁ temperature / °C | X ₂ time/h | X ₃ PH | X ₄ Total enzyme dosage/ (U/g) |
| -2 | 40 | 0.5 | 8.0 | 13000 |
| -1 | 45 | 1 | 8.5 | 14000 |
| 0 | 50 | 1.5 | 9.0 | 15000 |
| 1 | 55 | 2 | 9.5 | 16000 |
| 2 | 60 | 2.5 | 10.0 | 17000 |

3.3 Determination of the optimum conditions of enzymatic hydrolysis of composite enzyme

(1) Process optimization and composition determination

Through the above experimental methods and processes, it can be concluded that the four factors in Table 1, such as x₁, X₂, X₃, x₄, will directly affect the

enzymatic hydrolysis effect, so the best enzymatic hydrolysis process conditions can be determined by using the four factors and five levels regression orthogonal rotation combination (see Table 1 for details).

(2) Determination of basic components

The determination of basic components includes the determination of water, protein, fat, ash, protease activity and total nitrogen content. For the determination of the above components, a professional test formula should be

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used [4]. For example, the constant temperature drying method is used to determine the water content, and the ashing method is used to determine the ash content.

(3) Determination of degree of hydrolysis

Degree of hydrolysis /100%=

$$\frac{\text{Protein content in hydrolysate}}{\text{Protein content in raw materials}} \times 100$$

Protein content in raw materials

(4) Determination of nitrogen yield

Nitrogen yield/100%=

$$\frac{\text{Protein content in hydrolysate}}{\text{Protein content in raw materials}} \times 100$$

Protein content in raw materials

4 Results and discussion

4.1 Basic components of egg membrane

Table 2. Basic components and contents of egg membrane samples

| Items | Protein | Moisture | Aliphatics | Ash | Others |
|-----------|---------|----------|------------|-----|--------|
| Percent/% | 76.48 | 14.64 | 4.37 | 0.5 | 4.01 |

It can be seen from the above table that the egg membrane contains very high content of protein and collagen, so it is a good protein resource, which is very effective for improving the utilization rate of egg membrane.

4.2 The effect of various factors on enzymatic hydrolysis

(1) Effect of enzymatic hydrolysis time on enzymatic hydrolysis effect

The final experiment shows that the time of enzymatic hydrolysis is positively related to the degree of hydrolysis and the yield of nitrogen (as shown in Figure 1). It can be seen from Fig.2 that the optimal enzymatic time of alkaline protease and papain is 1.5h and 1h respectively, while the optimal total enzymatic time is 2.5h.

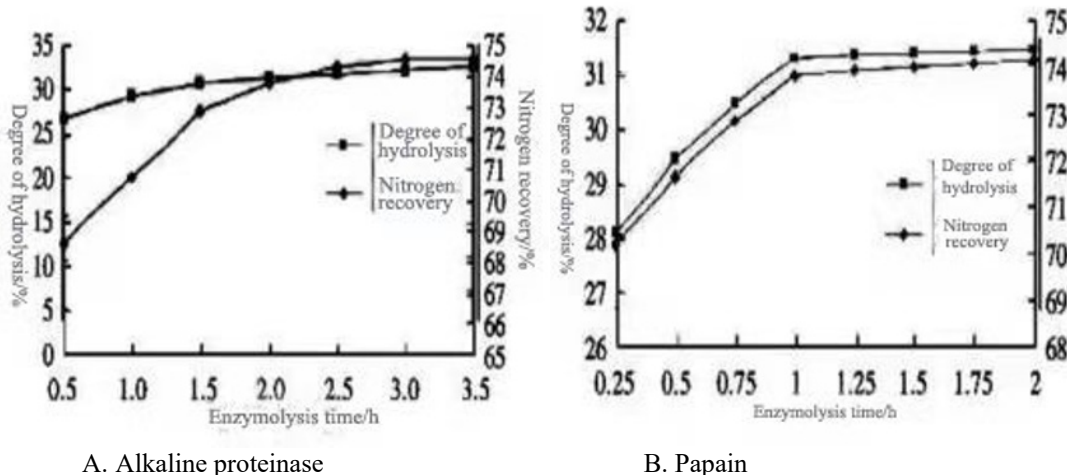


Fig. 2 Effect of alkaline protease and papain hydrolysis time on proteolysis of egg membrane

(2) Effect of enzymatic hydrolysis temperature on enzymatic hydrolysis effect

According to the above experiments, it can be observed that the degree of hydrolysis and the yield of nitrogen increase rapidly between 40 °C and 50 °C, and the degree of hydrolysis and the yield of nitrogen decrease when the temperature is over 50 °C. Therefore, the optimal temperature of proteolysis is 50 °C [5].

(3) Effect of total enzyme amount and PH value on enzymatic hydrolysis

There is a positive correlation between the degree of

hydrolysis and the nitrogen yield and the total amount of enzyme added and the PH value. After the total enzyme amount or PH value increases, the total amount of both will increase, and when it rises to a certain value. It will slow down the upward trend. Therefore, both the degree of hydrolysis and the nitrogen yield are affected by the total amount of enzyme added and the PH (see Fig. 3). Further, as can be seen from Fig.3, the optimum total enzyme amount is 1.5 x 10⁴ U/g, and the optimum PH values of alkaline protease and papain are 9.0 and 5.5, respectively.

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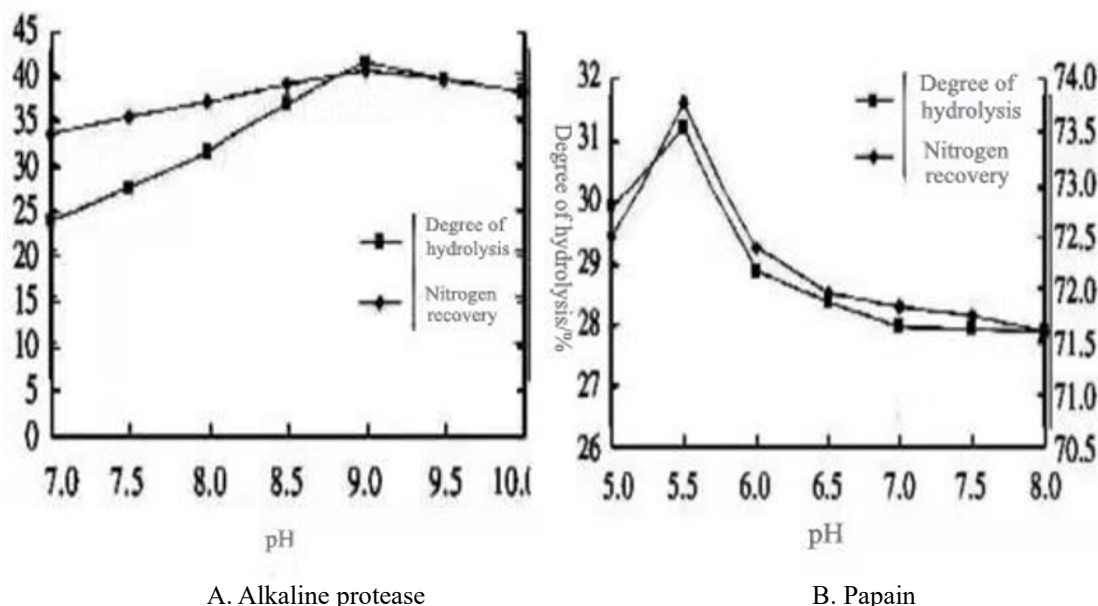


Fig. 3 Effect of PH on enzymatic hydrolysis

4.3 Analysis results of hydrolysis degree and nitrogen yield

(1) Analysis result of degree of hydrolysis

Using the statistical calculation method, the degree of hydrolysis is taken as the basic index, and the regression coefficients of various factors are calculated to obtain the following equation. The variance method is used to illustrate the fitting of the regression equation to the experiment.^[6]

$$Y=40.75667+1.48208X_1+1.78292X_2-0.14542X_3+5.56042X_4-1.98990X_1^2-1.18865X_2^2-2.54865X_3^2-1.81365X_4^2-0.01212X_1X_2-0.00688X_1X_3-0.01562X_1X_4-0.00813X_2X_3-0.00438X_2X_4-0.00562X_3X_4$$

(2) Nitrogen yield analysis results

Using the statistical calculation method, the nitrogen yield is used as the index, and the regression coefficients of various factors are calculated, and the validity of the regression equation is to test its significance.

$$Y=82.72333+177458X_1+1.77458X_1+1.49708X_2+0.39625X_3+4.64208X_4-2.60115X_1^2-1.49115X_2^2-2.54865X_3^2-1.81365X_4^2-0.01312X_1X_2-0.00688X_1X_3-0.01562X_1X_4-0.00813X_2X_3-0.00438X_2X_4-0.00562X_3X_4$$

5 Conclusion

In conclusion, to optimize the process of protease preparation, it needs to improve the utilization of protease. First of all, select two or more kinds of enzymes with the best and suitable composite enzymolysis to determine various conditions of the optimal composite enzymolysis, such as temperature, enzymolysis time, total enzyme amount, and PH value, etc., so as to obtain the optimal media effect and improve the utilization rate of soluble proteinase.

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