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Research Article

Study on Optimal Conditions of Alcalase Enzymatic Hydrolysis of Soybean Protein Isolate

[1]Yongsheng Ma, [2]Xianhui Sun and [1]Lintong Wang
[1]Chemistry and Chemical Engineering and Environment Engineering College, Weifang University, Weifang, Shandong 261061, P.R. China
[2]Agricultural Engineering College, Weifang Vocation College, Weifang, Shandong 261041, P.R. China

Abstract: Soybean protein isolate was hydrolyzed to obtain soybean polypeptide solution using Alcalase as hydrolase. Degree of hydrolysis and the recovery rate of protein were used to characterize the soybean protein hydrolysis reaction result. Influence factors of soybean protein hydrolysis reaction including the substrate concentration, temperature, pH, enzyme concentration characterized by E/S (ratio of Enzyme and Substrate) and hydrolysis time were systematically studied with single factor and multi-level experimental. The optimal reaction conditions of soybean protein isolate hydrolyzed by Alcalase were that the substrate concentration was 8%; E/S was 3.6 AU/100 g substrate; pH was 8.0; temperature was 60°C and hydrolysis time was 2 h. The study also showed that the hydrolytic activity of the Alcalase to soybean protein isolate was stronger in the front 1 h; the hydrolytic activity of Alcalase weakened gradually with time when reaction time was longer than 2 h.

Keywords: Alcalase, optimal hydrolysis conditions, soybean polypeptide, soybean protein isolate

INTRODUCTION

Hydrolysis methods of soybean protein can be divided into a number of ways including acid hydrolysis, alkaline hydrolysis and enzymatic hydrolysis method. Enzymatic hydrolysis has characteristics that reaction conditions are mild; the reaction is easy to control and damage to nutrients in the reaction process was less (Feng and Xiong, 2003). So, enzymatic hydrolysis which has become an important research direction of soybean polypeptides preparation methods produced from soybean protein isolate (Kim and Kim, 2010) received extensive attention from domestic and foreign researchers (Liu *et al*., 2002; Wu and Zhao, 2003; Li *et al*., 2010).

Trypsin, pepsin, neutral protease, papain, complex protease and microbial protease were the common enzyme used in soybean protein hydrolysis (Qian *et al*., 2002; Li and Luo, 2011). Action sites of different enzymes on the protein were different. So, the product obtained was different. Different enzymes may also bring a different flavor to the product.

In this study, subtilisin Alcalase was used to hydrolyze soybean protein isolate to obtain soybean polypeptides solution.

Enzymatic hydrolysis process is often influenced by conditions including the substrate of hydrolysis, temperature, pH and enzyme concentration.

In this study, different reaction conditions were studied and discussed to determine the optimal conditions of soybean protein isolate hydrolysis with Alcalase as hydrolase.

METHODOLOGY

Materials: Soybean protein isolate powder that protein content was 91% was provided by Jinan Guanli science and trading company.

Subtilisin alkaline protease Alcalase 2.4 L which labeling enzyme activity was 2.4 Au/g was purchased from Jinan BaiTai biological enzyme agent Co. LTD.

Main equipment: The Kjeldahl apparatus was customized. HH-6 digital constant temperature water bath was manufactured by Changzhou Guohua Electric Co., Ltd. PHS-2 type pH m was manufactured by Shanghai Leici Instrument Factory. Seven hundred and twenty one type spectrophotometer was manufactured by Shanghai Third Analytical Instrument Factory. LXJ-Ⅱ type centrifugal sedimentation machine was produced by Fuzhou Medical Instrument Factory.

Enzymatic hydrolysis process of soybean protein isolate: A certain amount of soybean protein isolate→ dissolved to a certain concentration→pretreatment at 90°C →cooled to a enzyme suitable temperature→ adjust enzyme suitable pH with 5 mol/L NaOH solution →placed in preheated constant temperature water bath→stirring→adding a certain amount of enzyme and maintaining the pH constant with 5 mol/L NaOH

Corresponding Author: Lintong Wang, Chemistry and Chemical Engineering and Environment Engineering College, Weifang University, Weifang, Shandong 261061, P.R. China, Tel.: 086-536-8785286

solution→the reaction was completed, inactivate enzymes for 15 min at 85°C →cooled to below 50°C, adjust pH to 4.2 with 6 mol/L HCl solution→the supernatant that was soybean polypeptide solution was obtained from centrifugal separation.

In the hydrolysis process hydrolysis temperature and pH should be strictly controlled to make enzyme hydrolysis carried out under optimum conditions.

Determination of protein content: Protein content was measured using semi-micro kjeldahl method. GB/T5511-85 was complied.

Determination of the degree of hydrolysis: Degree of hydrolysis was abbreviated as DH in full text. Ninhydrin chromotest method (Wang, 2000) was adopted to measure the DH of the soybean protein isolate Alcalase hydrolysates.

Calculation formula of DH was that DH = [NH_2 content in Alcalase hydrolysates (umol/mL) ÷ 6.25N (mg/mL) - 0.33 (umol/g)] ÷7.8 (umol/g) ×100%.

The 0.33 umol/g in the calculation formula was NH_2 content in raw soybean protein powder content. The 7.8 umol/g in the calculation formula was peptide bonds mmol number in per gram of raw soybean protein.

Determination of recovery rate of protein after hydrolysis: Calculation formula (Guo, 2000) of protein recovery rate was that protein recovery rate after hydrolysis % = Total Nitrogen in hydrolysates ÷ Total Nitrogen in raw protein×100.

RESULTS AND DISCUSSION

Effect of substrate concentration on hydrolysis of soybean protein: A certain amount of soybean protein isolate powder was formulated into 4, 6, 8 and 10% solution, respectively. Four solutions were heated at 90°C thermostatic bath for 15 min, then were cooled to 55°C. The pH of 4 solutions was all adjusted to 7.5. Then 2.4 Au/100 g substrate (Enzyme/Substrate) of the enzyme agent was added. Hydrolysis reaction was carried out for 2 h with stirring. The pH was maintained constant in the reaction process. After reaction, inactivating enzyme was done for 15 min in 85°C water bath. The supernatant that was hydrolysates was obtained from centrifugal separation. Effect of Substrate Concentration on DH of hydrolysates was shown in Fig. 1. Effect of Substrate Concentration on recovery rate of protein was shown in Fig. 2.

It can be seen from Fig. 1 that DH increased with the reducing of the substrate concentration. This change was not too significant. The substrate concentration had little effect on the reaction results. Taking into account its effect on the production efficiency, a large substrate concentration may be employed in the reaction process.

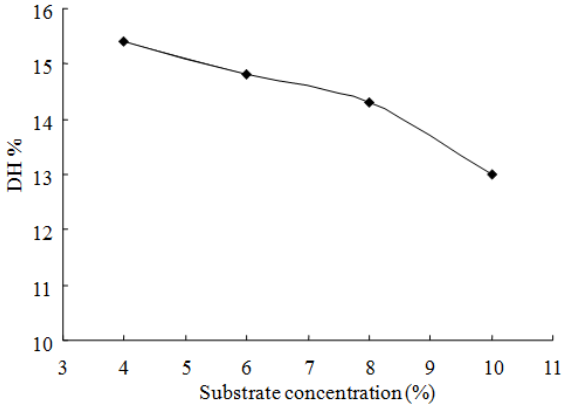

Fig. 1: Effect of substrate concentration on DH

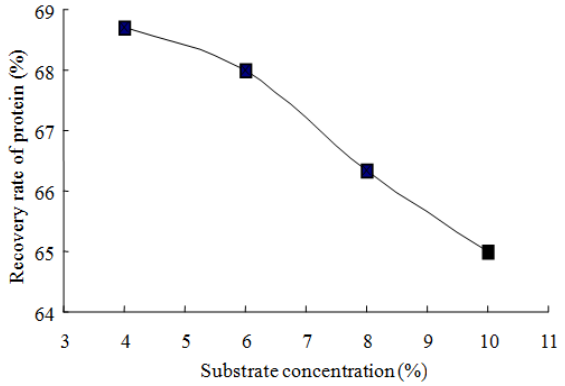

Fig. 2: Effect of substrate concentration on rate of recovery of protein

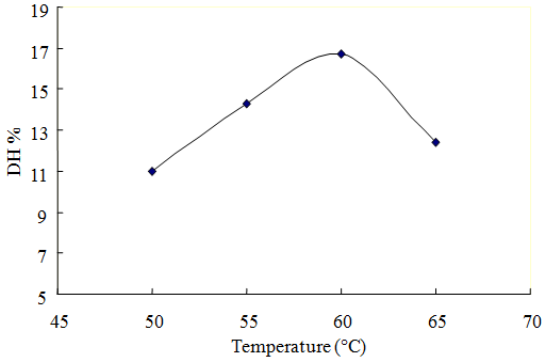

Fig. 3: Effect of temperature on degree of hydrolysis

It can be seen from Fig. 2 that effect of substrate concentration on rate of recovery of protein was not evident too. However, when the substrate concentration was 10%, soybean protein isolate powder solution was too thick to influence reaction uniformity. Therefore, 8% was determined as the proper substrate concentration.

Effect of temperature on hydrolysis of soybean protein: According enzymatic reaction dynamics

principle, as the temperature rise, the hydrolysis reaction become faster; but when the temperature exceeds the optimal temperature of the enzyme, the enzyme is easily fatigued even inactivation.

The pretreated 8% soybean protein isolate solutions were placed in 50, 55, 60 and 65°C thermostatic bath, respectively. The pH was adjusted to 7.5. Then 2.4 Au/100 g substrate (Enzyme/Substrate) of the enzyme agent was added. Hydrolysis reaction was carried out for 2 h. DH and recovery rate of protein of hydrolysates were determined. Effect of temperature on DH of hydrolysates was shown in Fig. 3. Effect of temperature on recovery rate of protein was shown in Fig. 4.

It can be seen from Fig. 3 and 4 that when temperature was 60°C, both the DH and recovery rate of protein of the hydrolysate were the highest value. When the temperature exceeded 60°C the DH decreased rapidly. The reason is that when temperature was too high, the enzyme activity decreased. Therefore, 60°C was determined as the optimal temperature.

Effect of enzyme concentration on hydrolysis of soybean protein: Enzyme concentration was characterized by E/S (ratio of enzyme and substrate) in this study. E/S was taken 1.2, 2.4, 3.6 and 4.8 Au/100 g substrate, respectively. Hydrolysis was carried out for 2 h under the other reaction conditions that the substrate concentration was 8%; reaction temperature was 60°C and pH was 7.5. After reaction DH and recovery rate of protein of hydrolysates were measured. Effect of E/S on DH of hydrolysates was shown in Fig. 5. Effect of E/S on recovery rate of protein was shown in Fig. 6.

It can be seen from Fig. 5 and 6 that under the same other reaction conditions, both the DH and recovery rate of protein of the hydrolysate increased with the increasing of E/S. When E/S was bigger than 3.6 Au/100 g substrate, increase of the DH and recovery rate of protein became very slow. Considering the cost of enzyme, 3.6 Au/100 g substrate was determined as the optimal enzyme concentration.

Effect of pH on hydrolysis of soybean protein: In the hydrolysis process of soybean protein using protease as hydrolase, the peptide bond opened can cause pH drop of hydrolysate. So, additional alkali is needed to maintain a constant pH of the hydrolysate in usually hydrolysis process. Constant pH may ensure optimal activity of the enzyme to enhance the rate of protein hydrolysis and to minimize the molecular weight of the peptide.

The pH of pretreated 8% soybean protein isolate solutions was adjusted to 7.0, 7.5, 8.0 and 8.5, respectively. Then 3.6 Au/100 g substrate (Enzyme/Substrate) of the enzyme agent was added. Hydrolysis reaction was carried out for 2 h at 60°C. In the course of the reaction, the pH of each reaction

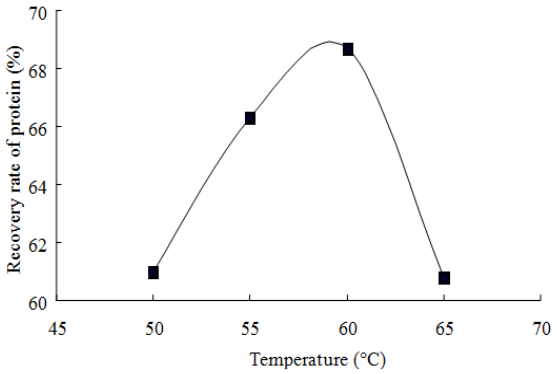

Fig. 4: Effect of temperature on rate of recovery of protein

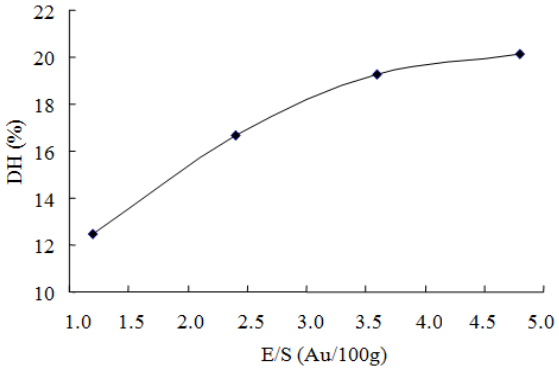

Fig. 5: Effect of E/S on DH

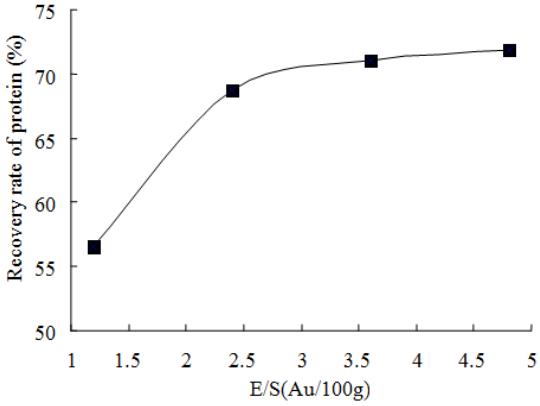

Fig. 6: Effect of E/S on recovery rate of protein

solution was maintained constant. DH and recovery rate of protein of hydrolysates were determined after enzyme inactivation and separation. Effect of pH on DH of hydrolysates was shown in Fig. 7. Effect of pH on recovery rate of protein was shown in Fig. 8.

It can be seen from Fig. 7 that the DH of soybean protein hydrolysate was gradually increased with increasing of pH; the DH reached maximum when the pH was 8.0; when the pH was 8.5 the DH had a slight decrease. It can be seen from Fig. 8 that recovery rate of protein of the hydrolysate increased with the increasing of the pH, the recovery rate of protein

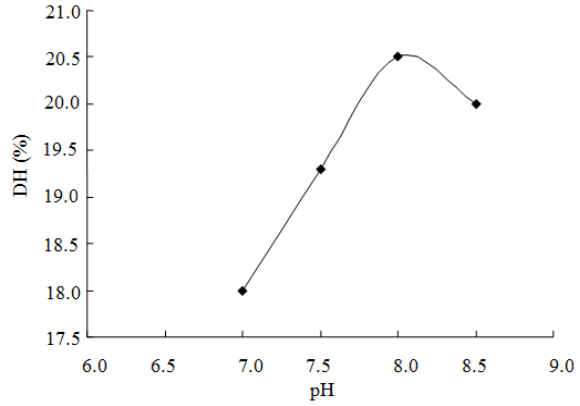

Fig. 7: Effect of pH on DH

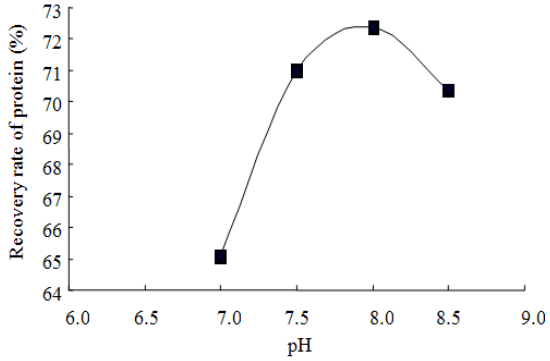

Fig. 8: Effect of pH on recovery rate of protein

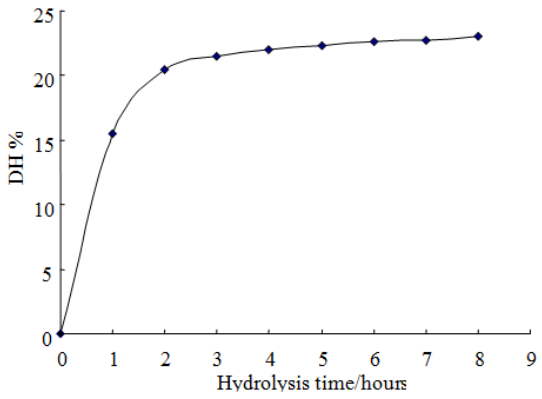

Fig. 9: Effect of hydrolysis time on DH

reached maximum when the pH was 8.0; change range of recovery rate of protein was not big. Considering the DH and recovery rate of protein, 8.0 was chosen as the optimal pH.

Effect of hydrolysis time on hydrolysis of soybean protein: Soybean protein hydrolysis reaction under conditions that the substrate concentration was 8%; enzyme concentration (E/S) was 3.6 Au/100 g substrate; the reaction temperature was 60°C and pH was 8.0. Eight parallel experiments which hydrolysis

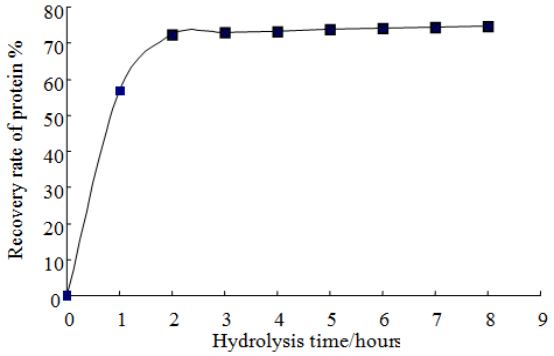

Fig. 10: Effect of hydrolysis time on rate of recovery of protein

time was 1, 2, 3, 4, 5, 6, 7 and 8 h were carried out, respectively. The results were shown in Fig. 9 and 10.

It can be seen from Fig. 9 and 10 that both the DH and recovery rate of protein of the hydrolysate increased rapidly with the increasing of hydrolysis time in the front 2 h. The hydrolytic activity of the enzyme to protein was stronger in the front 2 h especially the front 1 h. The hydrolytic activity of Alcalase weakened gradually with time when the reaction was longer than 2 h. Therefore, 2 h was determined as the optimal hydrolysis time.

CONCLUSION

Soybean protein isolate was hydrolyzed to obtain soybean polypeptide with Alcalase as protein hydrolase. Degree of hydrolysis abbreviated as DH and recovery rate of protein were used to characterize the soybean protein hydrolysis reaction result.

Influence factors of soybean protein hydrolysis reaction including the substrate concentration, reaction temperature, pH, enzyme (Alcalase) concentration characterized by E/S (weight ratio of Enzyme and Substrate) and hydrolysis reaction time were systematically studied with single factor and multi-level experimental to determine the optimal reaction condition of Alcalase hydrolysis soybean protein.

The optimal hydrolysis condition of soybean protein was that the substrate concentration was 8%, Alcalase concentration (E/S) was 3.6 AU/100 g substrate, pH was 8.0, temperature was 60°C and hydrolysis time was 2 h.

The study also showed that the hydrolytic activity of the enzyme (Alcalase) to soybean protein was stronger in the front 2 h especially in the front 1 h; the hydrolytic activity of Alcalase to soybean protein weakened gradually with time after 2 h.

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