Enzymatic Hydrolysis Conditions for Egg White Proteins

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Abstract

The enzymatic hydrolysis of proteins in egg white by Alcalase was systematically studied through dual quadratic rotary, orthogonal and regressive design. The optimum conditions of hydrolysis were determined. The results showed that the optimum temperature was 68.5℃, pH 8.21 at the substrate concentration of 5.5%. The regression equation, Y=42.6994 + 0.3344X_1 + 7.53X_2 −0.0086X_1X_2 −0.001X_1^2 −0.4726X_2^2 (Y-nitrogen recovery rate, NR; X_1-enzyme concentration /substrate concentration, E/S; X_2-hydrolytic time), was established to reveal the relationship between enzyme concentration and hydrolytic time with respect to the same NR. All these were fit to pilotscale experiment.

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Key words : enzymatic hydrolysis, egg white, Alcalase,

Introduction

Protein in egg white is the best among all kinds of protein for its amino acid composition resembles human bodies'. But its application has been limited for it's special properties such as heat instability and high viscosity. The properties of protein in egg white can be improved by enzymatic hydrolysis and thus its application could be widen, for example, the decline of viscosity is beneficial to process of fluid and stirring (Bo, 2001). Comparing with original protein, the hydrolysate is more digestible for its low molecular weight, its biological utilization rate is increased, its allergy is decreased, and it may have potential biological functions (Benheng and Baohua, 1996; Freitas and Padovan, 1993; William and Steven, 1994; Mahmond, 1994).

In this study, protein of egg white was hydrolysed by Alcalase and the hydrolysis conditions were optimised such as temperature and pH through dual quadratic rotary, orthogonal and regressive design (Zhongru, 1988; Xinhuai and Honghua, 1996). A regression equation was set up according to the design to reveal the relationship between enzyme concentration and hydrolytic time with respect to the same nitrogen recovery rate (NR). The equation was also developed to optimise hydrolysis condition in pilotscale experiment.

Materials and methods

1.1 Materials

Eggs were purchased from the Animal Nutrition Institute of Northeast Agricultural University. Alcalase, enzyme activity 2.4 AU/G, was supplied by Novo Nordisk Biotechnology Co., Ltd, China.

1.2 Egg White Protein Preparation

Eggs washed and broken → egg white separated from egg yolk → protein in egg white diluted → concentration of protein regulated to 5.5% → denaturalization by heating.

1.3 Technology of Hydrolysis

The pretreated protein solution 150g was added as substrate into a 250 ml three-necked bottle and then stirred it uniformly before protease was added. Temperature and pH were adjusted according to the dual quadratic rotary, orthogonal and regressive design. NaOH solution was added to maintain pH required and pH was permitted varying within ±0.05 during hydrolysis. Until the designed incubation time, 6 mol/L HCl was added to regulate pH to 4.5. The hydrolysate was heated until its center temperature up to 90℃ and maintained 10 min to inactivate the enzyme. Then it was cooled down to room temperature, centrifuged for 30 min at 4000 rpm before the supernatant was collected and its volume (ml) was determined.

1.4 Measurement of Nitrogen

The content of nitrogen in the hydrolysate was measured according to Kjeldahl method (National standard of China).

1.5 Determination of Denaturization Time

Egg white was diluted by water to its protein concentration 5.5% and heated at different time from 0 to 30 min and hydrolysed under the same condition, then each NR was measured.

1.6 Determination of Substrate Concentration

The substrate was diluted to its protein concentration at 2, 3, 4, 5, 5.5, 6% and 7%, respectively and hydrolysed under the same condition, then NRs were measured.

$$NR = \frac{\text{nitrogen in supernate}}{\text{nitrogen in substrate}} \times 100\%$$

1.7 Determination of Optimum Temperature and pH

In order to optimize the temperature and pH, dual quadratic rotary, orthogonal and regressive design was

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Table 1 Code of factor and level

levels	factors	
	X_1 Temperature (℃)	X_2 pH
−1.414	47.0	7.40
−1	50.5	7.78
0	55.0	8.70
1	67.5	9.62
1.414	71.0	10.00

adopted. Under the conditions of substrate concentration 5.0%, E/S 4%, and hydrolytic time 1h, temperature was assumed as variable X_1, pH as variable X_2, and NR as function Y so as to set up a model. The factors and levels were shown in Table 1.

1.8 Relationship between The Hydrolytic Time and Enzyme Concentration

Under the conditions of substrate concentration 5.5%, temperature and pH at their optimum levels, enzyme concentration was assumed as variable X_1, hydrolytic time as X_2, and NR as function to set up a pattern to show their relationship.

Results and Discussion

2.1 Determination of Degree of Denaturization

Protein can be denatured by physicochemical methods such as heating so that its configuration is loosed and peptide bonds are exposed, thus it is easily hydrolysed by proteases. So heat treatment can accelerate hydrolysis proceeding. But in order to get better hydrolytic effect, heating time should be limited to avoid aggregation of subunits through disulfide bonding.

The results (Figure 1) showed that NR increased in company with heating time in 10 min, and fluctuated to less extent after 10 min. The NRs did not have remarkable difference at time from 10 to 30 min when test error was taken into account, so 15 min was the optimum heating time for denaturing protein.

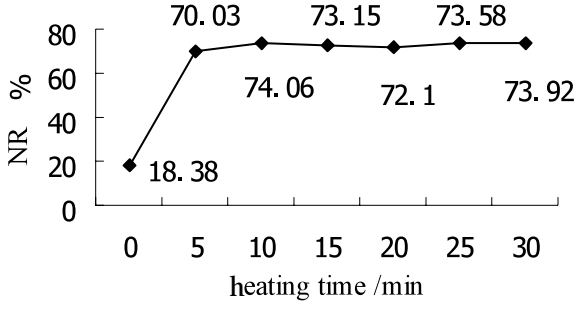

Figure 1. Effect of heating time on NR

Table 2 Effect of substrate concentration on NR

Substrate Concentration (%)	2	3	4	5	5.5
NR (%)	76.02	76.15	75.78	74.52	73.59

2.2 Determination of Substrate Concentration

Hydrolytic substrate was not stirred easily and had disadvantage of protease hydrolysis because gel was formed when protein concentration at heating treatment was high to 6% or 7%. But heat treatment could accelerate hydrolysis proceeding, so substrate should be diluted properly. The NRs of substrates at low protein concentrations were given in Table 2.

NR descended in company with ascending of the substrate concentration, but the changing range was small. The results showed 5.5% was the optimum if production efficiency was taken into account (Table 2).

2.3 Determination of optimum temperature and pH

The results of the experiments to optimize temperature and pH were given in Table 3.

The special software of dual quadratic rotary, orthogonal and regressive design was used to analyze the data. The pattern was as below, $Y=74.28+9.55X_1+0.21X_2-3.57X_1X_2-5.09X_1^2-3.59X_2^2$.

F-test was available to test the pattern. The results were given in Table 4.

The results of F_1-test indicated that the regression

Table 3 Experimental design and results

order	X_1	X_2	Temperature (℃)	pH	NR(%)
1	1	1	67.5	9.62	69.18
2	1	−1	67.5	7.78	77.72
3	−1	1	50.5	9.62	58.26
4	−1	−1	50.5	7.78	52.54
5	1.414	0	71.0	8.70	79.53
6	−1.414	0	47.0	8.70	51.05
7	0	1.414	59.0	10.00	69.89
8	0	−1.414	59.0	7.40	66.68
9	0	0	59.0	8.70	74.16
10	0	0	59.0	8.70	75.30
11	0	0	59.0	8.70	73.50
12	0	0	59.0	8.70	75.56
13	0	0	59.0	8.70	74.61
14	0	0	59.0	8.70	72.70
15	0	0	59.0	8.70	75.79
16	0	0	59.0	8.70	72.65

Table 4 Results of F-test

origin	quadratic sum	degree of freedom	ratio of F	critical value
regression	1090.550	5	F_2=70.4618	$F_{0.01}(5,10)$ =6.64
residual	30.954	10		
fitting	20.112	3	F_1=4.3283	$F_{0.05}(3,7)$= 4.35
error	10.842	7		
summation	1121.504	15		

equation fitted well, and there were no other factors affecting the experiment. The results of F_2--test indicated that the equation tallied with the actual experimental results.

In this study, contribution rates of temperature and pH are 2.45 and 1.44, respectively. The result indicated that the effect of temperature on NR was stronger than that of pH. So temperature should be considered at first in practice.

By the derivation of the extrema, we got the maximum value, F_{max} (1.12-0.53) =79.59, thus the true value was F_{max} (68.5 8.21) =79.59. So the optimum temperature of hydrolysis was 68.5℃ at pH 8.21.

2.4 Relationship between enzyme concentration and hydrolytic time and their effect on NR

2.4.1 Experimental design and regression analysis

To a hydrolytic technology, the enzyme concentration and hydrolytic time should be set in accordance with the investment and return, as well as reaction speed comprehensively. Under the conditions of substrate concentration 5.5%, temperature 68.5℃, pH 8.21, 16 groups of hydrolysates were prepared. A regression equation was set up and then the relationship between the enzyme concentration and hydrolytic time as well as their effect on NR were analyzed.

The special software of dual quadratic rotary, orthogonal and regressive design was used to analyze the data. The pattern built was $Y=84.33+4.37X_1+5.26X_2-0.54X_1X_2-1.22X_1^2-1.48X_2^2$. The code values were replaced by the true values, so corresponding regression equation was $Y=42.6994+0.3344X_1+7.53X_2-0.0086X_1X_2-0.001X_1^2-0.4726X_2^2$. F-test was available to test the pattern in table 6.

The results of F-test indicted that the pattern tallied with the actual experimental results. The significance of the coefficients of the equation was tested by t-test. The results were given in Table 7.

The values above were greater than 2.228 of $t_{0.05}$ (10) except for t_{12}. The result of t-test indicted the relationship between the enzyme concentration and hydrolytic time was trivial, and can be neglected. The equation was modified as

Table 5 Experimental Design and Results

order	X_1	X_2	hydrolytic time/min	E/S/%	Y- NR/%
1	1	1	125.4	5.77	90.27
2	1	−1	125.4	2.23	80.66
3	−1	1	54.6	5.77	82.51
4	−1	−1	54.6	2.23	70.75
5	1.414	0	140.0	4.00	88.58
6	−1.414	0	40.0	4.00	76.33
7	0	1.414	90.0	6.50	89.27
8	0	−1.414	90.0	1.50	74.63
9	0	0	90.0	4.00	85.05
10	0	0	90.0	4.00	84.61
11	0	0	90.0	4.00	83.53
12	0	0	90.0	4.00	84.32
13	0	0	90.0	4.00	84.69
14	0	0	90.0	4.00	83.98
15	0	0	90.0	4.00	84.57
16	0	0	90.0	4.00	83.86

Table 6 Results of F-test

origin	quadratic sum	degrees of freedom	ratio of F	critical value
regression	4049.49	5	F_2=179.8909	$F_{0.01}(5,10)$ =6.64
residual	4.502	10		
fitting	2.730	3	F_1=3.5933	$F_{0.05}(3,7)$ =4.35
error	1.773	7		
summation	409.452	15		

Table 7 Results of t-test

Coefficient	t_0	t_1	t_2	t_{11}	t_{12}	t_{22}
t-test value	355.47	18.44	22.17	5.16	1.60	6.23

$Y= 42.6994 + 0.3344X_1 + 7.53X_2 - 0.001X_1^2 - 0.4726X_2^2$

In this experiment, the contribution factor of hydrolytic time was 2.26 while E/S's was 2.28.This indicated the effects of the hydrolytic time and E/S were similar. So in practice, we should take them into account equally.

2.4.2 The effects of hydrolytic time (X_1) and enzyme concentration (X_2) on NR.

With assuming the values of E/S (X_2) at different levels

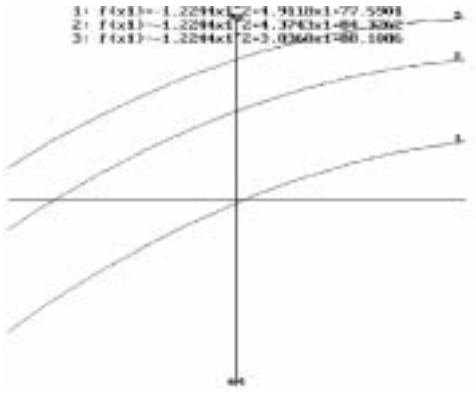

Figure 2. Effect of hydrolytic time on NR

(−1,0 1), three curves (curve 1, 2, 3) were gotten to reveal the effect of hydrolytic time on NR. (Figure 2). The figure showed that whatever values E/S was, NR increased continuously until balance when the hydrolytic time increased. NR increased sharply when E/S was low but NR increased slowly when E/S was high.

With assuming the values of hydrolytic time (X_1) at different levels (−1, 0, 1), three curves (curve 1, 2, 3) about the effect of E/S on NR were gotten (Figure 2). Figure 2 showed whatever values the hydrolytic time was, NR increased continuously until balance when E/S increased. NR increased sharply when hydrolytic time was short but NR increased slowly when the hydrolytic time was prolonged.

Conclusions

The optimum temperature was 68.5℃ at pH 8.21 and the substrate concentration at 5.5% for the enzymic hydrolysis of egg white proteins by Alcalase. The regression equation $Y= 42.6994 + 0.3344X_1 + 7.53X_2 - 0.001X_1^2 - 0.4726X_2^2$, which revealed the relationship between the enzyme concentration and hydrolytic time, could be applied to optimize hydrolysis condition. In the equation, when the function Y is confined, once the value of the enzyme concentration is changed, a corresponding hydrolytic time can be calculated. The reverse is true. NR can be gotten largely under the condition of more enzyme being added and longer hydrolytic time being prolonged. But it does not mean that it is better to use more enzyme and longer hydrolytic time. To get high efficiency, the best technique is to invest low cost and to get high output. This equation may be useful to select enzyme concentration and hydrolytic time for this reaction in scale-up studies.

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