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STUDY ON THE OPTIMIZATION OF HYDROLYSIS CONDITIONS FOR POULTRY BONE

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Introduction

China leads the world for the production of poultry and livestock. Although the resource of bones is abundant, the utilization of this high quality and nutritional substance is less emphasized . Every year, tons of bones from livestock and poultry are treated as industry materials and animal food with low value. The protein and other nutritional elements of the bones are not be fully utilized. Enzymatic hydrolysis is an effective way to utilize the bones properly with a procession that will change collagen protein of bones into peptide and L-amino acid in normal temperature and a short time. Enzymatic hydrolysis will improves the nutritional value and functional characteristic of bones, which facilitates the effective utilization of bones rather than treating as wastes. Better and more complete utilization of bones from these animals can perhaps reduce the economic loss of enterprise and bring more dollars back to the producers. Up to now, various bones had been hydrolyzed with enzymes. Surowka and Fik (1994) optimized the conditions of nitrogen recovery from chicken heads using pepsin and analyzed the amino acid composition of protein hydrolysis. Linder et al (1995) used enzymatic hydrolysis to recover protein from veal bones. Soottawat Benjakul (1997) used enzyme to hydrolyze the protein from *Pacific Whiting* solid wastes. Wang et al (2001)reported the hydrolysis of fresh swine bones with trypsin to study the optimal conditions.

The objective of the present study was to optimized the hydrolysis conditions for protein of poultry bones with enzyme Alcalase using a quadratic rotation perpendicular regressive design with three factors and five levels.

Materials And Methods

Poultry bones were ground, dried and defatted before being used.

The enzyme that was used was Alcalase 2.4 L, a food grade preparation of Novozymes, Beijing, China.

All chemicals / reagents used in this work were food-grade of reagent or reagent-grade.

Enzymatic hydrolysis

The defatted bone sample was mixed with water that made the substrate concentration was 8%. The hydrolysis was performed under different conditions with respect to temperature, pH and E/S. The pH of the mixture was kept constant by continuous addition of a 6M NaOH solution to the reaction mixture. The reaction was stopped by lowering the pH to 3-4 and temperature was 90 , waiting 10 min in order to deactivate the enzyme.

Nitrogen recovery (NR)

The nitrogen recovery (NR) was calculated according to the method of Linder (1995):

NR%=total nitrogen in supernatant (mg) / total nitrogen in substrate (mg) ×100%

Degree of hydrolysis (DH)

The degree of hydrolysis (DH) was calculated according to the method of Adler-Nissen (1986):

$$DH = \frac{h}{h_{tot}} \times 100 = \frac{B \times N_b \times 100}{\alpha \times M_P \times h_{tot}}$$

Optimized Designing

The data were analyzed according to the model of quadratic rotation perpendicular regressive design that was described by Xu (1988)

Results And Discussions

Modeling

Total of twenty-three experiments were carried out with three times according to the three factors and five levels quadratic rotation perpendicular regressive design, in order to estimate residual variance for the condition of hydrolysis duration. Table 1 described that the optimal time for hydrolysis was 60 min.

The rotation design was used with three independent variables (E/S, pH, temperature), The explanatory model equation for NR% was as follows:

$$y=71.1718+2.8639x_1+1.7778x_2+2.3704x_3+0.4962x_1x_2+0.0512x_1x_3-1.6938x_2x_3-1.1869x_1^2-1.7172x_2^2-2.267x_3^2 \quad (a)$$

Table 1 Responses of Dependent Variables to the Hydrolysis Conditions with Alcalase

Test number	X_1	X_2	X_3	E / S %	pH	T	Y NR%
1	1	1	1	3	9	65	71.78
2	1	1	-1	3	9	45	70.06
3	1	-1	1	3	7	65	71.39
4	1	-1	-1	3	7	45	62.74
5	-1	1	1	1	9	65	66.32
6	-1	1	-1	1	9	45	64.65
7	-1	-1	1	1	7	65	67.76
8	-1	-1	-1	1	7	45	59.47
9	1.682	0	0	3.682	8	55	73.07
10	-1.682	0	0	0.318	8	55	60.38
11	0	1.682	0	2	9.68	55	69.04
12	0	-1.682	0	2	6.32	55	61.41
13	0	0	1.682	2	8	72	67.25
14	0	0	-1.682	2	8	38	60.09
15	0	0	0	2	8	55	72.83
16	0	0	0	2	8	55	71.09
17	0	0	0	2	8	55	72.45
18	0	0	0	2	8	55	70.30
19	0	0	0	2	8	55	71.56
20	0	0	0	2	8	55	71.90
21	0	0	0	2	8	55	70.72
22	0	0	0	2	8	55	69.24
23	0	0	0	2	8	55	70.83

F-test

The model adequacy for the equation was tested by F-test. The result was showed in Table 2.

Table 2 Result of F-test of Nitrogen Recovery

Source	square sum	free degree	F-ratio	critical value
Regress	405.892	9	F_2=17.9765	$F_{0.01}(9,13)$=4.19
Residual	32.614	13		
Imitate	22.743	5	F_1=2.6866	$F_{0.05}(5,8)$=3.69
Error	9.871	8		
Summation	438.506	22		

F_1-test verified that three factors namely E/S, pH-value and temperature were the main factors that affected the result of Alcalase for hydrolyzing proteins of bones. F_2-test indicated that the model was adequate.

T-test

T-test was referred by the regression coefficients of the model. The result was as follows:

t_0=134.8905, t_1=6.6819, t_2=4.1484, t_3=5.5304, t_{11}=2.9870, t_{12}=0.8862, t_{13}=0.0915, t_{22}=4.3216, t_{23}=3.0246, t_{33}=5.7052

Coefficients except of t_{12}=0.8862 and t_{13}=0.0915 were bigger than $t_{0..05}(13)$=2.160. This result indicated that b_{12} and b_{13} were not prominence. The best explanatory model equation for NR% was:

$$y=71.1718+2.8639x_1+1.7778x_2+2.3704x_3-1.6938x_2x_3-1.1869x_1^2-1.7172x_2^2-2.267x_3^2$$

b

The model was verified to reflecte theoretically intrinsic rule on protein hydrolysis of bone according to the F-test and T-test results.

The evaluation of the effects of contribution ratio of x_1, x_2 and x_3 was 1.8655 2.3337 and 2.3819 respectively. This indicated that temperature x_3 was the most important variable from the three codes, which affected NR and had the highest regression coefficient, followed by pH x_2 and E/S x_1 .

Interaction effects on Nitrogen Recovery(y) were observed between E/S x_1 and pH x_2 , E/S x_1 and T x_3 and pH x_2 and T x_3 .

Effect of technologic conditions on NR(y)

Effects of E/S x_1 and pH x_2 interaction on NR y

When the temperature (x_3)of model b was fixed at 0 coded level, we could get the model of E/S x_1 and pH x_2 on NR y .

$$y=71.1718+2.8639x_1+1.7778x_2-1.1869x_1^2-1.7172x_2^2$$

A three-dimensional response surfaces and contour plots were protracted based on this model (Fig.1).

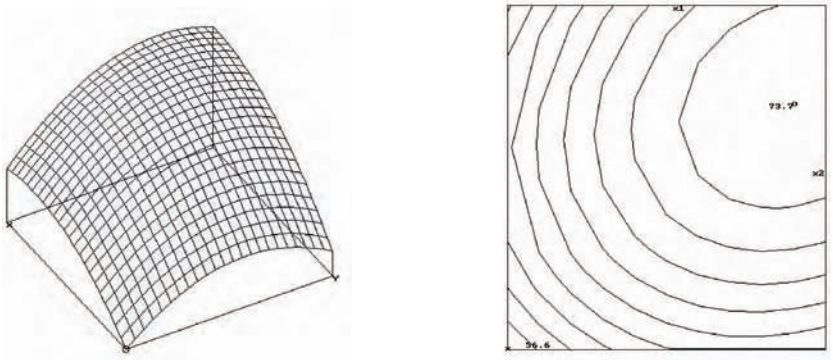

Figure 1 Effects of E/S and pH Interaction on the Amount of NR

In Figure 1 when E/S x_1 and pH x_2 were moderate, NR got maximum. The contour plot indicated that interaction effects of x_1 and x_2 were mutual. The NR was same with high E/S and low pH-value or low E/S and high pH-value. It was useful for optimizing the resultant in practice.

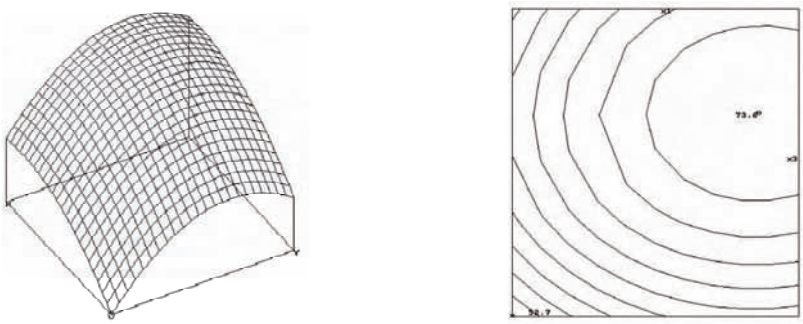

Figure 2 Effects of E/S and T Interaction on the Amount of NR

Effects of E/S x_1 and T x_3 interaction on NR y

When pH x_2 of model b was fixed at 0 coded level, we got the model of E/S x_1 and T x_3 on NR y .

$$y=71.1718+2.8639x_1+2.3704x_3-1.1869x_1^2-2.267x_3^2$$

Three-dimensional response surfaces and contour plots were protracted based on this model Fig.2 .

In Figure 2 when E/S x_1 and T x_3 were moderate, NR got maximum. The contour plot indicated that interaction effect of x_1 and x_3 were mutual.

Effects of pH x_2 and T x_3 interaction on NR y

When E/S x_1 of model b was fixed at 0 coded level, we could get the model of pH x_2 and T x_3 on NR y .

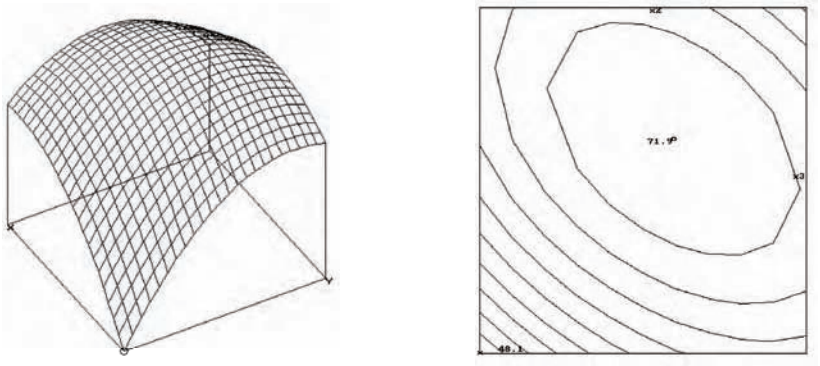

Figure 3 Effects of pH and T Interaction on the Amount of NR

Three-dimensional response surfaces and contour plots were protracted based on this model Fig.3 .

In Figure 3 when E/S x_1 was fixed at 0 coded level, the three-dimensional response surfaces of x_2 and x_3 interaction effects were presented as a parabola. At optimum pH x_2 and T x_3 , the amount of NR increased from contour plot: NR reached the maximum when pH x_2 and T x_3 were moderate. We could get a definite NR with low x_2 and high x_3 or high x_2 and low x_3 .

Optimization of hydrolysis time

According to the optimized hydrolysis condition, when the condition was E/S=3.33%, pH=8.54, temperature=58 , nitrogen recoveries of hydrolyzed protein in different periods of hydrolysis were showed in Figure 4.

From the curve, during the optimum reaction time (0-60 min), the amount of nitrogen recovery increased as reaction time increased, and remained constant after 90 min.

Therefore, the optimal hydrolysis time was 90 min.

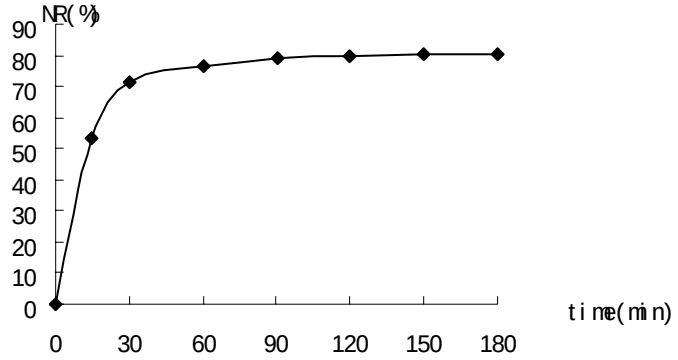

Figure 4 Dependence of Nitrogen Recovery on the Length of Hydrolysis Period

Conclusions

The regression model of quadratic rotation perpendicular regressive design with three factors and five levels was used in this study, when concentration of substrate was 8%. According to the F-test and T-test, the model reflected theoretically intrinsic law on protein hydrolysis of poultry bone. The results obtained from this pattern coincided in the actual experiment results and reflected the intrinsic relationship of hydrolysis process. With the design of rotation regression, three factors, namely E/S, pH-value and temperature were confirmed to be the main factors that affected the hydrolysis of the protein of poultry bone with Alcalase. The contributions of the three factors to nitrogen recovery ratio were: temperature > pH-value > E/S. The optimal hydrolysis conditions of hydrolyzing the protein of poultry bone with Alcalase were: concentration of enzyme (E/S)=3.33%, pH=8.54, hydrolyzing temperature=58℃, hydrolysis time=90 min and NR>75%.

References

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