Journal of Computational Science & Engineering 27 (2016) 779-782

Available at http://www.asocse.org ISSN 1710-4068

RSM-BASED Optimization of Enzymatic Hydrolysis Conditions for Chicken Bones

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Abstract: **The response surface methodology was used to study the enzymolysis technology of chicken bone and the enzymolysis technology model was established so as to obtain the optimum enzymatic hydrolysis technology conditions. Five factors, namely the size of bone, enzymolysis temperature, time of enzymolysis, solid-liquid ratio and quantity of enzyme were investigated using one way ANOVA. Then the model was established via response surface optimization. The results showed that the optimum enzymolysis conditions were: bone size: 1.9cm, the enzymolysis time 2.5h, and enzymolysis temperature 63℃， the obtained chicken bone enzymolysis production has an DEH of 0.921.**

Keywords: Response surface methodology; Enzyme hydrolysis; Chicken bones

Received 14 October 2016, Revised 23 November 2016, Accepted 25 November 2016

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1. Introduction

Chicken bones provide a complete spectrum of nutritional ingredients like water, protein, fat and minerals, and contain many kinds of nutrients beneficial for the health of man [1]. For example, the bones are particularly rich in minerals like phosphorus, calcium, and iron, vitamin A, vitamin B, as well as phospholipids and phosphoprotein which are indispensable for brain development [2]. Nevertheless, as the proteins in chicken bones are not easily digested and absorbed by the human body, a huge amount of chicken bones are either wasted or processed into traditional raw materials and animal feeds, resulting in a great loss of high quality nutrients [3].

Known as an effective way to improve the utilization of chicken bone protein, enzymolysis can hydrolyze the bone collagen, which is hard to digest and absorb by the human body, into polypeptides and L-amino acids, thereby improving the nutritive value and functional performance of the protein. [4]The enzymolysis of protein mainly produces polypeptides and free amino acids. The process that the enzyme cleaves the peptide bonds of the protein and produces small molecule peptides is described by the degree of hydrolysis (DEH) [5]. Chicken bones provide a complete spectrum of nutritional ingredients like water, protein, fat and minerals, and contain many kinds of nutrients beneficial for the health of man. For example, the bones are particularly rich in minerals like phosphorus, calcium, and iron, vitamin A, vitamin B, as well as phospholipids and phosphoprotein which are indispensable for brain development. Nevertheless, as the proteins in chicken bones are not easily digested and absorbed by the human body, a huge amount of chicken bones are either wasted or processed into traditional raw materials and animal feeds, resulting in a great loss of high quality nutrients. Known as an effective way to improve the utilization of chicken bone protein, enzymolysis can hydrolyze the bone collagen, which is hard to digest and absorb by the human body, into polypeptides and L-amino acids, thereby improving the nutritive value and functional performance of the protein [6]. The enzymolysis of protein mainly produces polypeptides and free amino acids. The process that the enzyme cleaves the peptide bonds of the protein and produces small molecule peptides is described by the degree of enzyme hydrolysis (DEH). The higher the DEH, the more the peptide bonds cleaved, and the more free amino acids and low-molecular-weight peptides produced. Protein hydrolysis also produces bitter substances. After enzymolysis, the protein has such three characteristics as decreased molecular weight, fewer numbers of ionic groups, and exposed hydrophobic groups. These characteristics, which ultimately determine the product properties, can be controlled by enzymolysis conditions. In recent years, many scholars use the amino acids and peptides in enzymatic hydrolysate as the precursors of meat-flavor essence to prepare essences of various flavors by Maillard reaction [7]. As important taste compounds, peptides in the enzymatic hydrolyzed are polymers of amino acids. With a complex taste-producing mechanism, peptides improve the flavor and texture of food by participating in and affect the formation of aroma and taste of seasonings so that the food has a coordinated, delicate, mellow and rich taste. The taste varies with changes in the length of peptide chain, the composition and sequence of amino acids. Generally speaking, researchers basically agree with each other on sweet, sour and bitter peptides, but differ greatly on umami and salty peptides [8].

Taking the response surface methodology, the parameters are optimized in enzymolysis of chicken bones, ranging from temperature, duration, solid-to-liquid ratio to enzyme dosage, establish a forecast model of enzymolysis rate, and obtains the optimal enzymolysis conditions, thus providing

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substrate for the formation of taste compounds in the Maillard reaction.

2. Materials and Methods

2.1. Pre-treatment of raw materials

Chicken bones → cutting into different lengths → rising→ adding fat, enzyme, and proper amount of water → temperature setting, boiling and filtering → isochoric process → detecting amino acid content.

2.2. Measurement of amino acid content

The amino acid content is measured by ninhydrin colorimetric method [9]. Figure1 is shown for the standard curve.

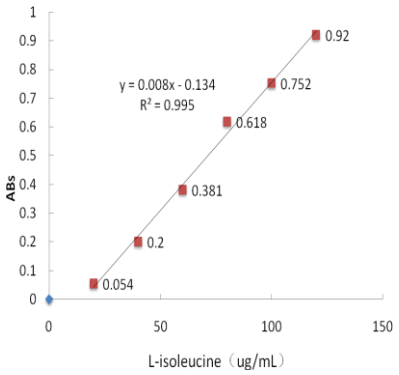

Figure 1. The standard curve of L−isoleucine.

2.3. Degree of enzyme hydrolysis(DEH)

DEH =(AN1/ AN0)－1

AN0 : Amino acid content in the initial solution

AN1 : amino acids content after reaction

2.4. Single factor test

Without changing other conditions, observe how chicken bone ezymolysis rate changes respectively with bone size, temperature, duration, solid-to-liquid ratio and enzyme dosage, choose the top 3 influencing factors, and design response surface optimization test for these 3 factors.

2.5. Design of response surface test

On the basis of the single factor test, use the software Design Expert 9.0 to design a three-factor three-level response surface test, with DEH as the response value, according to the design principle of Box-Behnken Design. [10]The 17 group tests include 12 factorial tests, and 5 repeated tests at the central point. Determine the optimal conditions for chicken bone proteins hydrolysis with compound protease. After establishing the forecast model, verify the forecast technology.

3. Results and Discussion

3.1. Effect of bone size on DEH

Without changing other conditions, observe DEH of chicken bones of different lengths. See Figure 2 for the results. When the bone size is ≤1cm, DEH changes very little. When the bone size is around 2cm, the degree is relatively high.

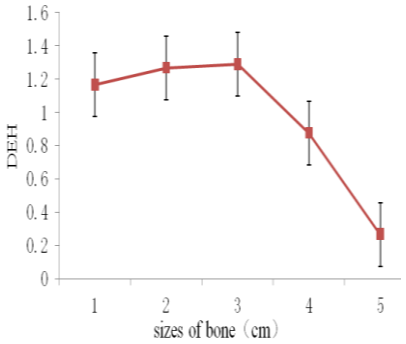

Figure 2. Effect of sizes of bone on DEH.

3.2. Effect of temperature on DEH

See Figure 3 for the effect of different temperatures on DEH of chicken bones. When the temperature is lower than 60 ℃, DEH has no obvious changes. When the temperature is about 65 ℃, the rate is relatively high. At above 65 ℃, the higher the temperature, the lower the rate is.

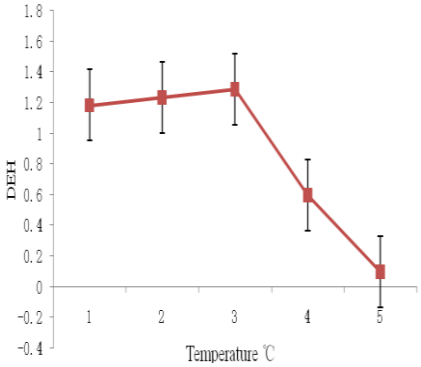

Figure 3. Effect of enzymatic hydrolysis temperature on DEH.

3.3. Effect of duration on DEH

See Figure 4 for the effect of different durations on the DEH. When the duration is about 2.5h, DEH is very satisfactory.

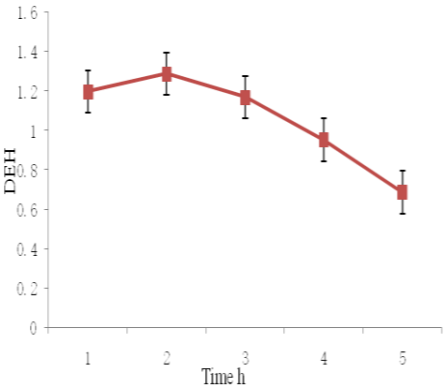

Figure 4. Effect of duration on DEH.

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3.4. Effect of solid-liquid ratio on DEH

Solid-liquid ratio has an obvious influence on DEH of chicken bones. When the ratio stands at 2:3, DEH is relatively high (Figure 5).

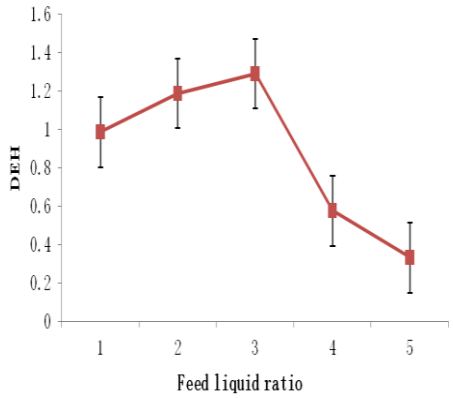

Figure 5. Effect of feed liquid ratio on DEH.

3.5. Effect of enzyme dosage on DEH

Without changing other conditions, add different amounts of papain into the chicken bones, and observe the impact on DEH. As shown in Figure 6, when the enzyme dosage is increased to 0.25%, DEH is relatively high. At above 0.25%, the higher the dosage, the lower the rate is.

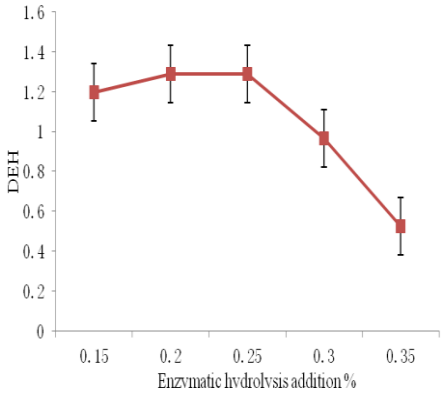

Figure 6. Effect of enzymatic hydrolysis addition on DEH.

3.6. Design of the response surface test of enzymolysis conditions

On the basis of the single factor test, design a three-factor three-level response surface optimization plan according to the design principle of Box-Behnken Design. The three influencing factors are bone size (A), duration (B) and temperature (C). See Table 1 for the plan, and Tab. 2 for variance analysis.

Table 1 The design of Box - Behnken and the response values

Number	A Size	B Duration	C Temperature	R1 DEH
1	1.5	2.5	55	0.85
2	2	3	65	0.54
3	1.5	3	60	0.16
4	1.5	2.5	65	0.80
5	1	3	65	0.28
6	1.5	3.5	65	0.06
7	2	2.5	60	0.93
8	1.5	3	60	0.20
9	1.5	3	60	0.11
10	1	3	55	0.53
11	1.5	3	60	0.12
12	2	3	55	0.37
13	2	3.5	60	0.06
14	1	3.5	60	0.07
15	1.5	3	60	0.20
16	1.5	3.5	55	0.39
17	1	2.5	60	0.71

Use software to fit the test results to a multivariate quadratic regression equation. After variance analysis of the data in Tab. 1, the multivariate quadratic regression equation of the model is obtained as follows:

$R1=+33.61588-2.86475*A-3.18150*B-0.84103*C-0.23900*A*B+0.042000*A*C-0.028100*B*C+0.37900*A^2+0.75800*B^2+7.09000E-003*C^2$

Table 2 The variance Analysis

Source	F value	P-value Prob＞F	significant
Model	33.92	＜0.0001	significant
A-A	2.49	0.1589	
B-B	201.79	＜0.0001	significant
C-C	5.83	0.0465	significant
AB	3.13	0.12	
AC	9.68	0.0171	significant
BC	4.33	0.759	
A^2	8.3	0.0236	significant
B^2	33.19	0.0007	significant
C^2	29.03	0.001	significant
Residual			
Lack of Fit	4.51	0.0899	not significant

Note: $R^2 = 0.9776$， $R^2_{Adj} = 0.9488$

According to Tab.2, the model has a high goodness of fit, small test error and high significance. As the hydrolysis rate has a significant variance under different conditions, there is no significant lack-of-fit, indicating that the model is very reliable. Specifically, the models

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of B, C, A*C, A2, B2, and C2 have high significance. Therefore, time, temperature, and the interaction between bones and temperature have a significant impact on the response value. As for the influence of each factor, the importance of each factor on test indices is reflected by the F value. The greater F is, the greater the impact on test indices, and the greater the importance of the corresponding factor. As shown in the table of variance analysis, FA = 2.49, F B= 201.79 and FC=5.83. Hence, the influences of the factors are ranked in the following order: duration> temperature> bone size.

See Figure 7 for the response surface of the AC interaction term in the manually optimized regression equation. When the temperature remains constant, the DEH increases and then decreases as either bone size or temperature increases.

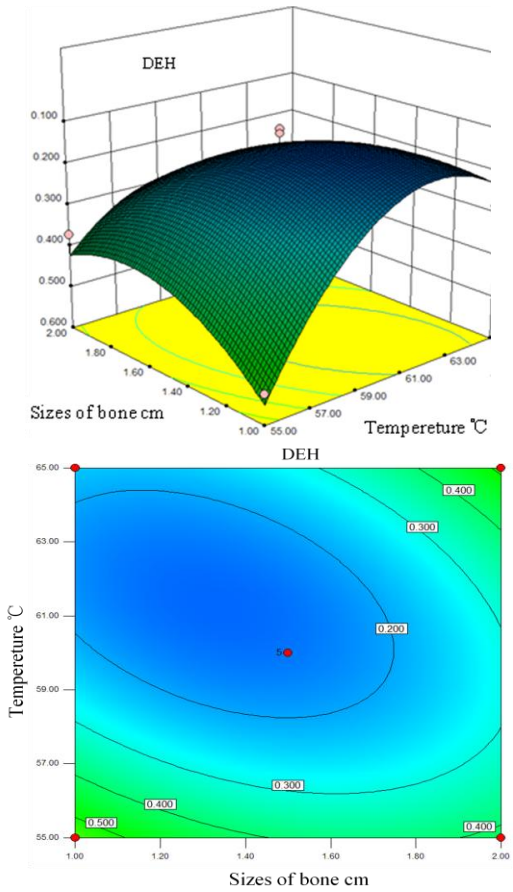

Figure 7 The size of bone and effect of enzymatic hydrolysis temperature on the rate of enzymatic hydrolysis.

3.7. Forecast and verification of optimal enzymolysis conditions

Solve the manually optimized regression equation with the software Design Expert9.0. Within the range of test factors, it is forecasted that the optimal enzymolysis conditions of chicken bones are: bone size 1.89cm, duration 2.51h, and temperature 63.22 ℃. Considering the feasibility of test operation, the optimal enzymolysis conditions of chicken bones are revised into: bone size 1.9cm, duration 2.5h, and temperature 63 ℃. Three tests are carried out under the revised conditions for verification. On average, the measured enzymolysis rate of chicken bones is 0.921. The average rate is very close to the theoretical forecast rate 0.934, with a relative error of only 1.39%, indicating that it is very reliable to conduct analysis and forecast of chicken bone enzymolysis rate with the manually optimized regression equation.

4. Conclusion

To sum up, this paper optimizes the chicken bone enzymolysis conditions through single factor test, Box-Behnken test design and response surface analysis. The influences of the factors on enzymolysis rate are ranked in the following order: duration> temperature> bone size. The optimal conditions are determined as: bone size 1.9cm, duration 2.5h, and temperature 63 ℃. With high regression significance, insignificant lack-of-fit, high goodness of fit and results close to the forecast value, the model has a certain application value.

$$R1=+33.61588-2.86475*A-3.18150*B-0.84103*C-0.23900*A*B+0.042000*A*C-0.028100*B*C+0.37900*A^2+0.75800*B^2+7.09000E-003*C^2$$

Acknowledgments

The author wishes to thank the Doctoral Scientific Research Foundation of Liaoning Province of China for contract 201501200 and Doctoral Scientific Research Foundation of Anshan normal university of china for 2014.

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