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Analytical Methods

Response surface methodology for autolysis parameters optimization of shrimp head and amino acids released during autolysis

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Abstract

Protein hydrolysates were prepared from the head waste of *Penaens vannamei*, a China seawater major shrimp by autolysis method. Autolysis conditions (viz., temperature, pH and substrate concentration) for preparing protein hydrolysates from the head waste proteins were optimized by response surface methodology (RSM) using a central composite design. Model equation was proposed with regard to the effect of temperature, pH and substrate concentration. Substrate concentration at 23% (w/v), pH at 7.85 and temperature at 50 °C were found to be the optimal conditions to obtain a higher degree of hydrolysis close to 45%. The autolysis reaction was nearly finished in the initial 3 h. The amino acid compositions of the autolysis hydrolysates prepared using the optimized conditions in different time revealed that the hydrolysates can be used as a functional food ingredient or flavor enhancer. Endogenous enzymes in the shrimp heads had a strong autolysis capacity (AC) for releasing threonine, serine, valine, isoleucine, tyrosine, histidine and tryptophan. Endogenous enzymes had a relatively lower AC for releasing cystine and glycine.

Keywords: Autolysis; Endogenous enzymes; *Penaens vannamei*; Response surface methodology; Amino acid composition; Autolysis capacity

1. Introduction

Shrimp has been used as one of the most popular and important raw materials for many Chinese and international dishes. In China, shrimp industry has undergone a dramatic increase in recent years. It is estimated that the output of shrimp in China was up to 500,000 tonnes in 2004, 63% of which was *Penaens vannamei* (Cui, 2006). Processing of shrimp has increased. Generally, the head portion of shrimp is removed during processing and it accounts for approximately 35–45% of the total shrimp population (Meyers, 1986). Continued production of the shrimp head waste without corresponding development of utilizing technology has resulted in waste collection, disposal and pollution problems. As regulations have become stricter, there is now a need to treat and utilize the waste in the most efficient manner.

Recycling of shrimp wastes can provide an answer. A better economic use of the shrimp head would minimize the pollution problem and at the same time maximize the profits of the processor. Shrimp head is basically the dried waste of the shrimp industry, contain large amounts of nutritive components, extractives and enzymes (Fanimo, Oduguwa, Onifade, & Olutunde, 2000; Heu, Kim, Shahidi, Jeong, & Jeon, 2003). It can be utilized by extracting useful components and incorporating them into desirable seafood products. Studies on shrimp waste focused on chemical composition and proteases (Heu et al., 2003; Shahidi & Synowiecki, 1991), and natural antioxidants (Pasqual & Babbitt, 1991; Seymour, Li, & Morrissey, 1996). Efforts have been underway to utilize shrimp by-products for the extraction of carotenoids (Sachindra, Bhaskar, & Mahen-

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drakar, 2006; Sachindra, Bhaskar, Siddegowda, Sathisha, & Suresh, 2007; Sachindra & Mahendrakar, 2005), chitin (Cira, Huerta, Hall, & Shirai, 2002; Daum, Helen, Veltrup, Meinhardt, & Bisping, 2007), chitosan (Benjakul & Sophanodora, 1993; Weska, Moura, Batista, Rizzi, & Pinto, 2007) and their application in food processing (Jeon, Shahidi, & Kim, 2000; Shahidi, Arachchi, & Jeon, 1999). Shrimp head waste has also been used for inclusion into feeds (Cavalheiro, Souza, & Bora, 2007; Cobos, Pérez, Piloni, González, & Barcena, 2007; Guillermo, Agbogbo, & Holtzapple, 2006). The use of shrimp waste to obtain protein hydrolysates has recently been reviewed by several investigators (Ferrer, Paez, Marmol, Ramones, & Garcia, 1996; Gildberg & Stenberg, 2001; Synowiecki & Al-Khateeb, 2000). However, there are few studies on the recovery of protein, peptides and amino acids from shrimp by-products by autolysis method.

Many endogenous hydrolytic enzymes such as proteases, lipases, etc. are found in food biological materials. Under certain conditions they will hydrolyze the tissues spontaneously. The phenomenon is called "autolysis" (Zhang, Deng, & Hong, 1999). The viscera in shrimp head contain most of the endogenous enzymes (Gu & Wang, 1991). The autolysis of shrimp head depends on several physicochemical factors. The temperature of incubation, pH, and substrate concentration influence the rate of reaction to a large extent. It is necessary to study their performance during autolysis.

Proximate analysis of the shrimp head of *P. vannamei* indicated the presence of 60.3% protein, ash 19.9%, and 7.9% fat on dry weight basis. The autolysis phenomenon was observed in our preliminary study under certain conditions. The objective of the present investigation was to determine the influence of pH, reaction temperature and substrate concentration on the reaction rate as well as to identify the optimum autolysis conditions. Also, a trial was made to investigate the amino acids released during autolysis under optimized conditions. Moreover, the autolysis capacity was assessed.

2. Materials and methods

2.1. Sample

Shrimp heads of *P. vannamei*, used as raw materials, were obtained from Zhanjiang Guolian Fisheries Ltd., Guangdong Province, China. Clean sanitized containers were provided to pickers for the packages of shrimp heads, which were placed on ice and immediately transported to the laboratory of Marine Products Institute of the Department of Food Science and Technology, Guangdong Ocean University.

2.2. Autolysis of shrimp head

Shrimp heads were ground in a blender (DS-1, Exemplar and Mould Instruments Co., Shanghai, China) with distilled water added. The homogenized plasma was pour into a reaction vessel with distilled water added till the final substrate to the desired concentration; the pH was adjusted according to the trails design with 5 M NaOH and 5 M HCl. The system was pre-incubated for 10 min to the desired temperature. The system was vigorously stirred during reaction using a magnetic stirrer (SG-5404, Shuoguang Electron Technology Co., Shanghai, China). The reaction was terminated after 3 h and the reaction system was heated in a boiled water bath for 10 min to inactive the enzymes, then cooled down to room temperature. The resulting solution was filtered with a piece of gauze and then centrifuged (TD-5, Yingtai Instruments Co., Changsha, China) at 4000*g* for 15 min. Supernatant was combined for later analysis.

2.3. Degree of hydrolysis

Degree of hydrolysis (DH) is defined as the percentage of free amino groups cleaved from protein, which was calculated from the ratio of α-amino nitrogen (AN) and the total protein nitrogen (TPN). The AN was determined by a formol titration method (Chen, Tao, & Li, 2003). TPN was determined by Kjeldahl method (AOAC, 1990).

2.4. Experimental design

The reaction parameters were optimized using RSM. The central composite design (CCD) was employed in this regard. The range and center point values of three independent variables presented in Table 1 were based on the results of preliminary experiments. CCD in the experimental design consists of eight factorial points, six axial points and six replicates of the central point (Table 2). pH (x_1), reaction temperature (x_2) and substrate concentration (x_3) were chosen for independent variables. Degree of hydrolysis was selected as the response for the combination of the independent variables given in Table 2. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. The behavior of the system was explained by the following quadratic equation:

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \quad (1)$$

where y is the dependent variable (degree of hydrolysis in real value), β_0 is constant, and β_i, β_{ii} and β_{ij} are coefficients estimated by the model. x_i, x_j are levels of the independent

Table 1
Independent variable values of the autolysis and their corresponding levels

Independent variables	Symbol	Coded levels				
		−1.682	−1	0	+1	+1.682
pH	x_1	5.32	6.0	7.0	8.0	8.68
Temperature	x_2	46.6	50	55	60	63.4
Substrate concentration (%)	x_3	11.6	15	20	25	28.4

Table 2
Experimental design used in RSM studies by using three independent variables with six center points showing observed DH

Run no.	Coded levels of variable			DH[a] (%)
	x_1	x_2	x_3	
1	−1	−1	−1	32.67 ± 1.04
2	−1	−1	1	39.20 ± 0.96
3	−1	1	−1	36.44 ± 1.31
4	−1	1	1	37.33 ± 1.12
5	1	−1	−1	35.42 ± 0.85
6	1	−1	1	42.86 ± 1.22
7	1	1	−1	36.05 ± 0.98
8	1	1	1	40.28 ± 1.22
9	−1.682	0	0	32.52 ± 1.31
10	1.682	0	0	38.02 ± 1.07
11	0	−1.682	0	40.30 ± 0.73
12	0	1.682	0	38.83 ± 1.28
13	0	0	−1.682	33.38 ± 1.19
14	0	0	1.682	38.43 ± 0.89
15	0	0	0	42.38 ± 1.30
16	0	0	0	40.23 ± 1.09
17	0	0	0	42.44 ± 0.93
18	0	0	0	41.33 ± 0.77
19	0	0	0	41.36 ± 1.53
20	0	0	0	42.06 ± 1.32

[a] Values are shown as mean ± SD of three analyses.

variables. They represent the linear, quadratic and cross-product effects of the x_1, x_2 and x_3 factors on the response, respectively. The model evaluated the effect of each independent variable to a response. Analysis of the experimental design and calculation of predicted data were carried out using Design Expert Software (version 7.0, trial Stat-ease Inc., Silicon Valley, CA, USA) to estimate the response of the independent variables. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

2.5. Amino acid composition

The amino acid composition for both essential and non-essential amino acids was estimated by using a high speed amino acid analyzer (L-8500A, Hitachi Co., Tokyo, Japan) according to the method modified by Rubin, Schoonouer, and Bossard (1975). In a test tube 0.1 g sample was taken and 10 ml of 6 M HCl were added. The test tube was placed under vacuum and filled with nitrogen, subsequently sealed and placed in oven at 110 °C for 22 h. The test tube was allowed to cool at room temperature. The hydrolysate so formed was evaporated under vacuum at 60 °C to dryness to remove HCl. The hydrolysates was dissolved in 5 ml 0.02 M HCl, centrifuged (MiniSpin, Eppendorf Co., Hamburg, Germany) at 1000*g* and filtered to remove the visible sediments. A known volume (20 μl) of the supernatant was injected into an amino acid analyzer to estimate the amino acid profile. For determination of tryptophan, the sample (weight equivalent to about 2 mg tryptophan) was treated with 4.2 M NaOH (100 ml) and 0.3 ml triglycerine and placed in the oven at 110 °C for 24 h. Seven milliliter of 6 M HCl were then added to the mixture and the pH adjusted to 4.5 using pH 4.2 citric acid buffer solution and the mixture was made to a certain volume. Tryptophan content was determined by colorimetric analysis (UV-1700, Shimadzu Co., Kyoto, Japan) at 400 nm under the condition of pH 5.0–5.5, column oven temperature 55 °C, reactor temperature 100 °C, and reaction time 10–15 min (Deng, Peng, Yang, & Xia, 2002).

3. Results and discussion

3.1. Preliminary results

Preliminary experiments were performed to determine the effect of pH on the autolysis of shrimp head (at constant temperature 50 °C and time 3 h) (data not shown). The results showed clearly that maximum amino acids were produced between pH 6.5 and 7.5. Hence pH 7.0 was chosen as center point with 0.5 as step change (Table 1). Similarly, experiments were performed to study the effects of temperature on shrimp head autolysis (at constant pH 7.5 and time 3 h). The results clearly indicated that the maximum DH was produced in the temperature range of 50–60 °C. Hence 55 °C was chosen as center point with 5 °C as step change (Table 1). The substrate concentration experiments were performed and DH reached a plateau then dropped. The hydrolysates showed a higher DH when the substrate concentration was between 15% and 25% as compared to other concentrations. Hence the center point for substrate concentration was 20% with a step change of 5%.

3.2. Model fitting

Eq. (2) shows the dependence of DH on pH, temperature and substrate concentration. The parameters of the equation were obtained by multiple regression analysis of the experimental data. The following quadratic model explains the experimental data:

$$y = 41.64 + 1.33x_1 - 0.18x_2 + 2.02x_3 - 0.48x_1x_2 + 0.53x_1x_3 - 1.11x_2x_3 - 2.07x_1^2 - 0.55x_2^2 - 1.85x_3^2 \quad (2)$$

where y is the predicted response in real value, x_1 the coded value of variable pH; x_2 the coded value of variable temperature and x_3 the coded value of variable substrate concentration.

The closer the value of R^2 to unity, the better the empirical models fits the actual data. On the other hand, the smaller the value of R^2, the lesser will be the relevance the dependent variables in the model have in explaining the behavior of variations (Little & Hills, 1978; Mendenhall, 1975). The value of R^2 (0.9522) suggests that there is a good agreement between the experimental values presented in Table 2 and predicted values obtained from the model.

3.3. Effects of autolysis parameters

Statistical testing of the regression model has been done by the Fisher's statistical test for ANOVA – the analysis of variance (Table 3). The F value is the ratio of the mean square due to regression to the mean square due to residual. The F value is 22.11. In general, the calculated F value should be several times greater than the tabulated value for a good model. If the F value is greater than tabulated $F_{0.05\ (9,10)}$ (3.02), then the null hypothesis is rejected at the a level of significance and implies that the variation accounted for by the model is significantly greater than the unexplained variation.

The probability (P) value of the regression model was less than 0.0001, with no significant lack-of fit ($P = 0.2631$). The determination coefficient ($R^2 = 0.9522$) was satisfactory, having a low experimental error according to ANOVA (Table 3). The pH (x_1) and substrate concentration (x_3) had a highly significant effect ($P < 0.001$) at the maximum DH. The pH-temperature interaction term (x_1x_2) had a significant effect ($P < 0.05$), and the quadratic terms (x_1^2 and x_3^2) also had a highly significant effect ($P < 0.001$).

3.4. Optimization of autolysis

Response surface methodology (RSM) has been used successfully to optimize the parameters affecting the protein hydrolysis (Cheison, Wang, & Xu, 2007; Nilsang, Lertsiri, Suphantharika, & Assavanig, 2005). It is a statistical technique for designing experiments, building models, evaluating the effects of several factors, and searching optimum conditions for desirable responses. The 3D response surfaces and the 2D contour plots of the response using Eq. (2) when one of the variables is fixed at the central point and the other two are allowed to vary are shown in Fig. 1. The plots depicted interaction of pH (x_1) and temperature (x_2), pH (x_1) and substrate concentration (x_3), temperature (x_2) and substrate concentration (x_3), respectively. The maximum predicted value is indicated by the surface confined in the smallest ellipse in the contour diagram. Elliptical contours are obtained when there is a perfect interaction between the independent variables (Muralidhar, Chirumamila, Marchant, & Nigam, 2001). The contour plots in Fig. 1 (A_2) are relatively perfect elliptical. It indicates that there is relatively significant interaction between pH and temperature corresponding to the response surface, which is consistent with the results of the ANOVA for quadratic model.

The protuberant shapes of the 3D response surfaces (Fig. 1) show that there is a maximum value for this model. The optimal conditions were extracted by Design Expert Software with its optimization menus: $x_1 = 0.85$, $x_2 = -1$, $x_3 = 0.60$. The real values were – pH at 7.85, temperature at 50 °C, and substrate concentration at 23%. The maximum DH obtained by using the above optimized concentrations of the variables is 43.13%. The maximum DH obtained experimentally was found to be 45.06. This is obviously in close agreement with the model prediction.

Table 3
ANOVA for quadratic model

Source	SS	DF	MS	F-Value	P-Value
Model	195.79	9	21.75	22.11	<0.0001
x_1	58.81	1	58.81	59.77	<0.0001
x_2	0.47	1	0.47	0.47	0.5071
x_3	22.33	1	22.33	22.70	0.0008
x_1x_2	9.79	1	9.79	9.96	0.0103
x_1x_3	2.26	1	2.26	2.29	0.1608
x_2x_3	1.85	1	1.85	1.88	0.2000
x_1^2	61.09	1	61.09	62.09	<0.0001
x_2^2	4.21	1	4.21	4.28	0.0655
x_3^2	48.50	1	48.50	49.29	<0.0001
Residual	9.84	10	0.98		
Pure error	3.53	5	0.71		
Lack of fit	6.31	5	1.26	1.79	0.2691
Total	205.63	19			

$R^2 = 0.9522$; SS, sum of square; DF, degree of freedom; MS, mean square; adjust $R^2 = 0.9091$.

3.5. Confirmation experiments and adequacy of the regression models

To confirm the validity of the statistical experimental strategies and to gain a better understanding of DH, three additional confirmation experiments were conducted. The chosen conditions for pH, temperature and substrate concentration, determined with the statistical model, and the experimental results are listed in Table 4. The measured DHs were close to the predicted values for DH using RSM. These results confirm the predictability of the model for the autolysis of the shrimp head in the experimental condition used.

3.6. Amino acid composition of hydrolysates during autolysis

The observed responses in Table 2 were all obtained at 3 h for autolysis reaction. To check whether 3 h was really optimal, time course experiments were performed at optimal pH, temperature and substrate concentration (Fig. 2). The results clearly showed that hydrolysis increases nonlinearly with time and reached a plateau after 3 h. It indicated that the autolysis reaction was nearly finished in the initial 3 h.

The results for the amino acid composition of the hydrolysates is presented in Table 5, which reveals that, a total of 18 amino acids were recorded in the hydrolysates during autolysis investigated in this study. During the autolysis evaluated in this study, most amino acids exhibited great change in the initial 3 h, apart from the amino acid cystine, all of which appeared to exhibit a tendency toward an increase in concentration with the passage of time as autolysis continued. Amongst the amino acids analyzed herein, the content of aspartic acid (36.61–62.72 mg/g), glutamic acid (50.89–82.81 mg/g) and arginine (32.81–50.67 mg/g) tended to predominate in the hydrolysates

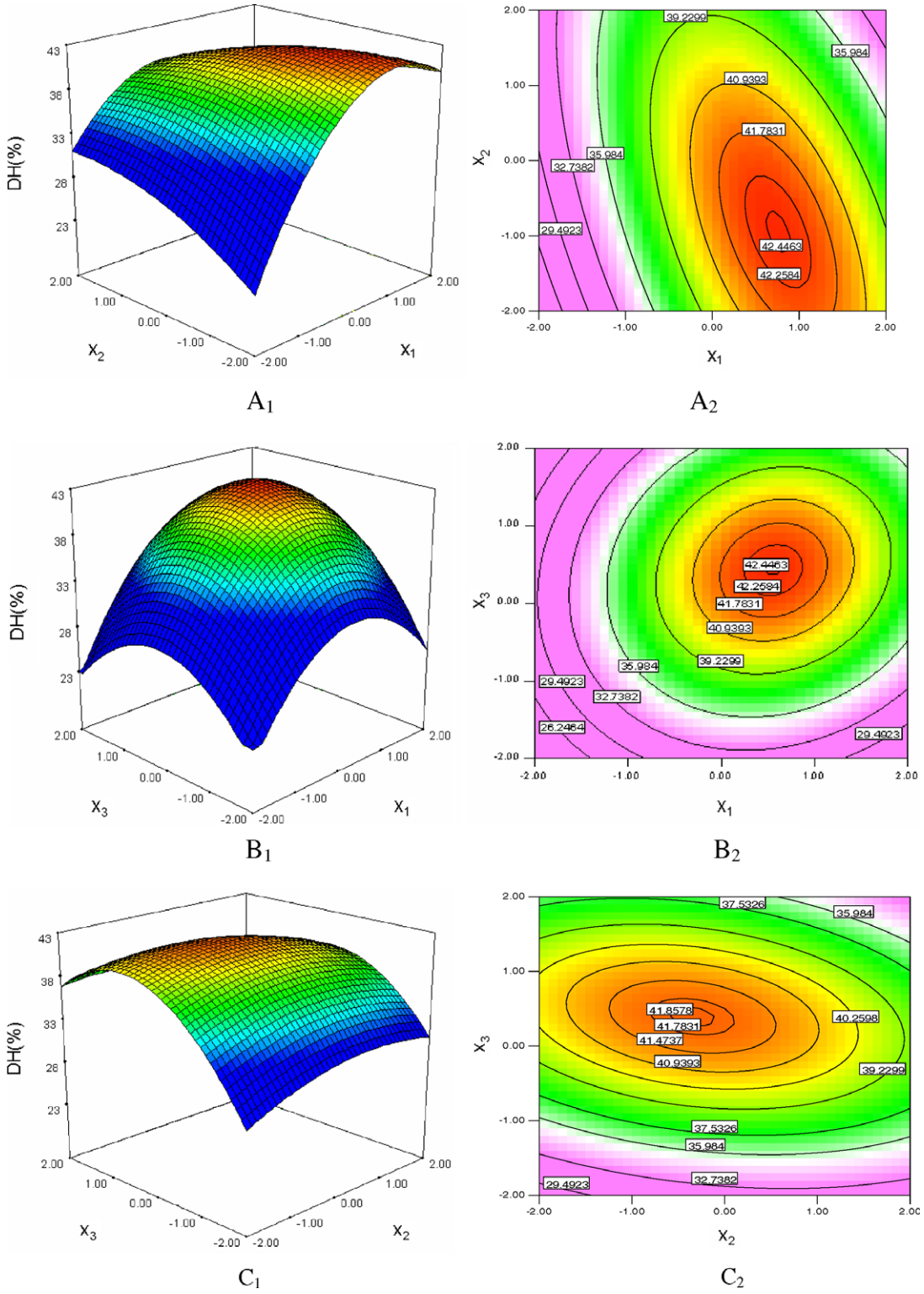

Fig. 1. Response surfaces and contour plots for the effects of variables on DH: (A) pH (x_1) and temperature (x_2); (B) pH (x_1) and substrate concentration (x_3); and (C) temperature (x_2) and substrate concentration (x_3).

during autolysis. The hydrolysates of the shrimp head autolysis in different time revealed to be a high quality food for human being. Firstly, the essential amino acids make up 47.15–48.92% of all amino acids, and the ratio of essential amino acids (EAA) to non-essential amino acids (NEAA) is 0.892–0.957. Both these values exceed the reference values of 40% and 0.6 recommended by World Health Organization (WHO)/Food and Agriculture Organization (FAO) (FAO/WHO, 1991). Secondly, the hydrolysates has an extremely high content of the flavor enhancers, glu-

Table 4
Observed and predicted values of the confirmation experiments

Trial	Conditions	Observed DH[a]	Predicted DH
1	$x_1 = -0.5$, $x_2 = -1$, $x_3 = 0.5$	38.99 ± 1.32	39.43
2	$x_1 = 0$, $x_2 = 1$, $x_3 = 0$	41.60 ± 1.52	40.88
3	$x_1 = 0$, $x_2 = 0.5$, $x_3 = 1$	41.22 ± 1.24	40.58

[a] Mean ± S.D. ($n = 4$).

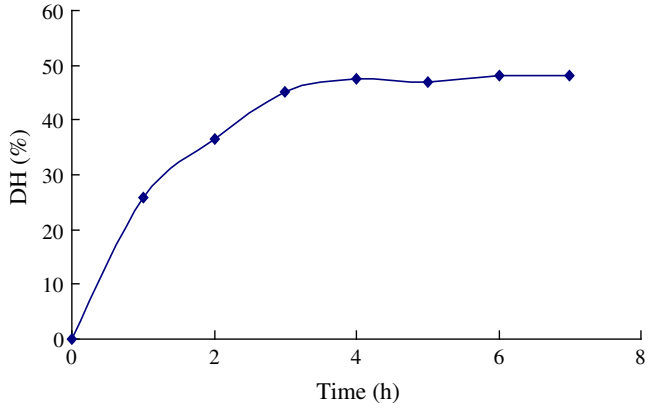

Fig. 2. Degree of hydrolysis (DH) at various reaction time.

tamic acid, aspartic acid, glycine and alanine (37.12–39.18% of the total amino acids), which may account for the good taste. Thirdly, the hydrolysates contain very high levels of both lysine (30.80–46.21 mg/g and about 7–8% of the total amino acids) and arginine (32.81–50.67 mg/g and about 8–9% of the total amino acids). Lysine, which is extremely rare in cereals, is an essential amino acid required for proper development and is also a precursor in the production of carnitine, a nutrient with roles in converting fatty acids into energy and in regulating cholesterol levels. Arginine is classified as a semi-essential or conditionally essential amino acid and it participates in protein synthesis and other physiological functions such as detoxification and energy conversion (Morris, 2005). Consequently, the nutritional value the hydrolysates of the shrimp head is high. It could be expected to be excellent functional food stuff or a good taste enhancer.

To explain distinctly the fact that peptide linkages supplied by each of amino acid were broken by the endogenous hydrolytic enzymes, a concept, namely autolysis capacity (AC), which was defined as a percentage of content of a amino acid released from proteins of the shrimp head at any time to total content of this amino acid of the proteins, was introduced. So AC reveals the ability of endogenous enzymes in shrimp heads releasing amino acids (in the form of protein, peptides and free amino acids). Table 6 shows that there was a significantly difference among amino acids in AC, although endogenous enzymes can break the peptide linkages supplied by all amino acids. Apparently, the endogenous enzymes had a strong AC for

Table 5
Amino acid composition of the hydrolysates in different time

Amino acids	Amount[a] (mg/g shrimp head) on dry basis						
	0 h	0.5 h	1 h	2 h	3 h	5 h	7 h
Asparagine[b]	36.61	38.39	50.22	52.46	60.27	62.05	62.72
Threonine[c]	15.18	16.29	22.1	23.21	26.78	27.23	27.46
Serine	11.61	11.83	18.3	19.2	22.32	22.54	22.54
Glutamic acid[b]	50.89	58.71	67.41	68.3	77.68	80.36	82.81
Proline	23.66	25.89	29.91	31.25	35.27	36.16	36.38
Glycine[b]	32.59	35.49	40.18	41.29	47.54	49.11	49.78
Alanine[b]	27.01	31.03	35.71	36.84	42.41	43.75	43.75
Cystine	2.23	1.79	2.23	2.86	3.57	3.79	3.79
Valine[c]	18.53	22.32	27.23	28.79	34.82	35.71	36.16
Methionine[c]	8.93	10.27	12.95	12.95	15.4	16.29	16.74
Isoleucine[c]	14.51	18.53	22.54	24.11	29.2	29.69	29.91
Leucine[c]	27.90	33.71	38.84	40.4	46.65	47.77	48.66
Tyrosine	13.84	17.64	20.54	21.21	24.55	25.45	25.89
Phenylalanine[c]	15.40	19.42	23.44	25.22	30.13	30.80	31.03
Lysine[c]	30.80	34.82	39.29	39.96	45.08	45.98	46.21
Histidine[c]	8.93	10.71	12.95	14.06	16.29	16.96	16.96
Arginine[c]	32.81	35.27	41.52	42.63	47.77	49.78	50.67
Tryptophan[c]	4.02	5.13	6.47	7.14	8.26	8.48	8.48
ΣAA	375.45	427.24	511.83	531.88	613.99	631.9	639.94
ΣEAA	375.45	427.24	511.83	258.47	613.99	631.9	639.94
ΣFAA	177.01	206.47	247.33	198.89	300.38	308.69	312.28
ΣEAA/ΣAA	0.4715	0.4833	0.4832	0.4860	0.4892	0.4885	0.4880
ΣFAA/ΣAA	0.3918	0.3830	0.3781	0.3739	0.3712	0.3723	0.3736
ΣEAA/ΣNEAA	0.8920	0.9352	0.9351	0.9454	0.9578	0.9551	0.9531

[a] Values shown are the average from three replicates.
[b] Flavor amino acids, in terms of FAA (the same in following).
[c] Essential amino acid, in terms of EAA.

Table 6
AC of endogenous hydrolytic enzymes for releasing amino acids of shrimp head proteins (%)[a]

Amino acids	0.5 h	1 h	2 h	3 h	5 h	7 h
Asparagine	3.51	26.76	31.14	46.49	50.00	51.32
Threonine	5.81	36.04	41.85	60.45	62.78	63.94
Serine	1.42	42.73	48.43	68.37	69.80	69.80
Glutamic acid	11.59	24.50	25.83	39.74	43.71	47.35
Proline	7.69	21.54	26.15	40.00	43.07	43.84
Glycine	6.70	17.53	20.10	34.54	38.15	39.69
Alanine	12.36	26.79	30.22	47.39	51.51	51.51
Cystine	−7.82	0	9.38	23.46	27.36	27.36
Valine	13.20	30.28	35.71	56.68	59.78	61.34
Methionine	9.87	29.61	29.61	47.70	54.28	57.57
Isoleucine	17.05	34.09	40.72	62.50	64.40	65.34
Leucine	15.48	29.17	33.33	0.50	52.98	55.36
Tyrosine	20.10	35.47	39.01	56.75	61.48	63.84
Phenylalanine	15.36	30.72	37.54	56.32	58.88	59.73
Lysine	11.75	24.80	26.76	41.77	44.38	45.03
Histidine	15.21	34.22	43.73	62.74	68.45	68.45
Arginine	7.81	27.70	31.25	47.58	53.97	56.82
Tryptophan	19.82	43.61	55.51	75.33	79.29	79.29

[a] Values shown are the average from three replicates.

releasing threonine, serine, valine, isoleucine, tyrosine, histidine and tryptophan due to their maximum AC exceeding 60%, especially the maximum AC of cystine was up to 79.29%. However, they had a relatively lower AC for releasing cystine and glycine due to their maximum AC being below 40%, especially the maximum AC of cystine was only 27.36%. In addition, in the period of autolysis, the AC of almost all the amino acids arrived at a plateau at 3 h. It indicated that the endogenous enzymes in shrimp head were compounded by several or dozens of enzymes due to the broad specificity. However, further research should be carried out to isolate and characterize the enzymes that take the main effects in the autolysis procedure, from which the mechanism of the autolysis action could be fully understood and more measures could be taken to make the autolysis process more controllable.

4. Conclusions

The autolysis DH of WSH was significantly influenced by the autolysis conditions that included temperature, pH of the substrate and the substrate concentration. The conditions were optimized by RSM. Autolysis of WSH under optimized conditions resulted in a DH about 45% Time course experiments revealed the autolysis reaction was nearly finished in the initial 3 h. The autolysis conditions for obtaining the maximum DH were – temperature at 50 °C, time at 3 h, pH at 7.85, and a substrate concentration at 23%.

The hydrolysates in different autolysis time showed a high ratio of both Σ EAA/Σ AA and Σ EAA/Σ NEAA, which exceed the recommendations of WHO and FAO for an "ideal" food protein. The hydrolysates also had an extremely high content of flavor amino acids, which make up 37.12–39.18% of the total amino acids. The hydrolysates contained very high levels of both lysine and arginine, which may account for the healthy function for the hydrolysates. The endogenous enzymes in the shrimp heads have a strong AC for releasing threonine, serine, valine, isoleucine, tyrosine, histidine and tryptophan. While it showed a relatively lower AC for releasing cystine and glycine. Almost all the amino acids finished their release at about 3 h. The hydrolysates prepared from shrimp head by autolysis method have potential for applications in aquaculture/animal feeds and has a potential to be an effective flavor enhancer. It will take more profits for shrimp processors.

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