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Optimization of enzymatic hydrolysis of Salmon *(Salmo salar)* skin by Alcalase

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Abstract: Fish protein hydrolysate was produced from Salmon *(Salmo salar)* skin using Alcalase® 2.4 L. Hydrolysis conditions were optimized by using a response surface methodology (RSM). The model equation was proposed with regard to the effects of enzyme to substrate level, temperature and pH on the degree of hydrolysis. An enzyme to substrate level of 2.50% (v/w), temperature of 55.30°C and pH of 8.39 and were found to be the optimum conditions to obtain the highest degree of hydrolysis (77.03%) using Alcalase. The freeze dried protein hydrolysate was characterized with respect to chemical composition and amino acid composition. The protein hydrolysate produced contained high protein (89.53%) and higher level of indispensable amino acids. Protein hydrolysate from salmon skin may potentially serve as a good source of desirable peptide and amino acids.

Keywords: Alcalase, enzymatic hydrolysis, optimization, protein hydrolysate, salmon fish skin

Introduction

Fish constitutes 60-70% of the national animal protein intake. In Malaysia, fish is an important component diet with per capita consumption at 55 kg, which is the highest among ASEAN countries (Pawiro, 2008). Salmon fish is one of the most popular fish sources in Malaysia. It is usually regarded as a high quality protein source for human consumption and high in essential amino acids and high Omega-3 fatty acids. According to Department of Fisheries Malaysia (2007), more than 2000 tonnes of salmon fish was imported to Malaysia annually. Salmon is mostly imported in the form of fresh, chilled or frozen of whole fish and then processed into the fillet and surimi product by local salmon fish processing industry. As the result, a large amount of by-product materials including skins, bones, head and frames were disposed annually from salmon processing industry. Most of these sources are utilized as animal feed ingredient. These by-products are important protein sources. Parts of these by-products should therefore be used for purposes other than the feed industry. From an economical point of view, these underutilized wastes could be converted into more marketable and acceptable forms to achieve a better utilization.

One of the methods for effective protein recovery from this protein rich by-product is production of protein hydrolysate through enzymatic hydrolysis. It is widely employed to improve and upgrade the functional and nutritional properties of the fish proteins. During enzymatic hydrolysis, soluble protein compounds are separated from insoluble particles and fish oil, thus offer good predictability of the products. In the effort of attempting to hydrolyze fish protein by using controlled enzymatic hydrolysis process, many factors have to be considered. One of the important factors is the choice of enzymes since different enzymes have different specific activities and optimal working parameters. Therefore, it gives products with different chemical and functional properties. There were many commercial proteases have been reported to be used for the hydrolysis of fish protein. They included those from plant sources such as bromelain (Aspmo *et al*., 2005) and papain (Hoyle and Merritt, 1994) or those from animal origin likes chymotrypsin and trypsin (Simpson *et al*., 1998) and pepsin (Viera *et al*., 1995). Proteolytic enzymes of microbial origin like Alcalase, Neutrase, Protamex and Protease N (Kristinsson and Rasco, 2000a; Guèrard *et al*., 2001; Liaset *et al*., 2002) have been also applied to the hydrolysis of fish protein. Proteases from microbial origin were found to be more suitable to produce fish protein hydrolysate. This was because they offer a wide variety of available catalytic activities and greater pH and temperature stabilities (Diniz and Martin, 1997).

Alcalase is an alkaline enzyme produced from *Bacillus licheniformis*. It has been reported to be one of the highly efficient bacterial protease used to prepare functional fish and other protein hydrolysates (Adler-Nissen, 1986; Benjakul and Morrisey, 1997; Diniz and Martin, 1997; Kristinsson and Rasco, 2000a, b).

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Alcalase has great ability to solublize fish protein and is nonspecific, with an optimum temperature that ranged from 50 to 70°C. It has optimal pH range at the value of 8 to 10 that could reduce the risk of microbial contaminations (Chabeaud *et al*., 2009). According to Adler-Nissen (1986), hydrolysates prepared by using Alcalase had the highest protein recovery and the lowest lipid content than those made using Papain and Neutrase. Moreover, it has been reported that Alcalase treated fish protein hydrolysates had less bitter principles compared to those prepared with papain (Hoyle and Merritt, 1994). Furthermore Alcalase has been documented to be a better candidate for hydrolyzing fish proteins based on enzyme cost per activity (Kristinsson and Rasco, 2000b).

There were several studies was conducted to on hydrolysis of salmon muscle, frame and skin. Kristinsson and Rasco (2000a and 2000b) focused on the enzymatic hydrolysis of muscle proteins from Atlantic salmon using one enzyme mixture extracted from pyloric ceca of salmon and four commercial proteases, Alcalase, Flavourzyme, Corolase PN-L and Corolase 7089. Liaset *et al*. (2000) used cod and salmon frames from the filleting industry in enzymatic hydrolysis with the commercial enzymes Alcalase, Neutrase, pepsin and a combination of Alcalase and Kojizyme™. Liaset *et al*. (2002) studied the optimum hydrolyzing process conditions (enzyme to substrate ratio, frames–water ratio, temperature, starting pH and hydrolysis time) on nitrogen recovery in the enzymatic hydrolysis of Atlantic salmon frames without head using the commercial protease, Protamex™. Kołodziejska *et al*. (2008) investigated the effect of temperature and time on the yield of gelatin extraction from fresh salmon skins or smoked salmon skins by using partially thermal hydrolysis method without using enzyme. However, there was no study was carried out on optimization of hydrolysis process conditions on the degree of hydrolysis (DH) of protein hydrolysate in the enzymatic hydrolysis of salmon skin.

In the process of enzymatic hydrolysis, factors such as pH, time, enzyme to substrate level and temperature will influence enzymatic activity. In this context, the main goal of enzymatic hydrolysis of salmon fish skin was to obtain the maximum possible degree of hydrolysis (DH). Studying the effect of these hydrolysis parameters on DH contribute to the understanding of the protein hydrolysate released. Optimization is one of the methods to find a best alternative from a specified set of alternatives. It is the modern statistically derived experimental designs that are viewed as a way to achieve this purpose at the lowest possible overall cost (Arteaga *et al*., 1996). Response surface methodology (RSM) has been effectiveness in the optimization and monitoring of food processes (Wangtueai and Noomhorm, 2009). It is a collection of mathematical and statistical modeling technique that relates product treatment to the outputs and establishes a regression equation to describe inter-relations between input parameters and product properties (Cho *et al*., 2004). The objective of this study was to determine optimal enzymatic hydrolysis parameter (i.e., enzyme to substrate level, temperature and pH) of salmon skin to obtain the maximum DH by using RSM. Additionally, the chemical composition and amino acid composition of the protein hydrolysate prepared using the optimized hydrolysis conditions were determined.

Materials and Methods

Chemicals and raw materials

Salmon *(Salmo salar)* skin, provided by a salmon fish processing industry located in Penang, Malaysia, was used as a substrate. Alcalase® 2.4 L (declared activity of 2.4 AU/kg, density of 1.18 g/ml), an endoproteinase from *Bacillus licheniformis*, was purchased from Sigma-Aldrich. All reagents used were of analytical grade.

Enzymatic hydrolysis

After thawing overnight in cold room (4°C), the salmon fish scales were removed and the salmon skins were cut into pieces (about 30 mm x 30 mm). The fish skins were first washed under running tap water and minced in a Waring blender. Then, the minced skin substrates were mixed with 10 mM sodium phosphate in a ratio of 1:10 (w/v). The pH of the mixture was adjusted to desired value using 2N NaOH or 2N HCl. The mixtures were then incubated at the reaction temperature (38-72°C) for 10 min before the reaction was initiated by adding the enzyme of Alcalase 2.4 L at desired concentration (0.2 to 1.8%). The hydrolysis was conducted for 120 min. At the end of the reaction, the enzyme was inactivated at the temperature of 90°C for 10 min. The samples were then centrifuged at 10,000 g for 20 min.

Degree of hydrolysis

Degree of hydrolysis (DH) was calculated according to percent of trichloroacetic acid (TCA) ratio method as described by Hoyle and Merritt (1994). After hydrolysis, 20 ml of protein hydrolysate was added to 20 ml of 20% (w/v) TCA to produce 10% TCA soluble material. The mixtures were left to stand for 30 min to allow precipitation, followed by

centrifugation (7800 g for 15 min). The supernatant was analyzed for protein content by using Kjeldahl method (AOAC, 2000). The degree of hydrolysis (DH) was computed as the formula below:

$$DH\ (\%) = \frac{\text{10\% TCA soluble nitrogen in the sample}}{\text{Total nitrogen in the sample}} \times 100\%$$

Proximate composition

The moisture, protein, fat and ash of the salmon skin and its protein hydrolysate samples were determined according to AOAC (2000) methods. The moisture content was determined according to oven method (AOAC, 2000). The total crude protein content was determined using Kjeldahl method (AOAC, 2000). A nitrogen conversion factor of 6.25 was used for calculation of crude protein content of samples. Total lipid content of samples was evaluated by Soxhlet extraction (AOAC, 2000). Ash content was determined by charring the predried sample in crucible at 600°C until a white ash was formed (AOAC, 2000).

Amino acid composition

The sample of salmon skin protein hydrolysate was digested for 16 h in 15 mL of 6N HCl at 110ºC. The hydrolyzate was dissolved in deionized water and filtered. The amino acid composition was examined by a high performance liquid chromatography (HPLC), equipped with a Waters 410 Scanning Flourescence and AccQ Tag column (3.9 x 150 mm). AccQ Tag Eluent A and AccQ Tag Eluent B or 60% acetonitrile acid was used as the mobile phase (flow rate=1 ml/min).

Chemical score

The chemical score of the protein hydrolysate was computed according to Ovissipour *et al*. (2009). It was calculated relative to the indispensable amino acid (IAA) profile in a standard reference protein as described by FAO/WHO (1990) or NRC (1993). Briefly, the chemical score was calculated based on the following equation:

$$\text{Chemical score} = \frac{\text{IAA in sample protein (g/100 g)}}{\text{IAA in standard reference protein (g/100 g)}} \times 100\%$$

Experimental design for optimization

The optimum enzymatic hydrolysis conditions of salmon skin were determined by response surface methodology. The three independent variables chosen were enzyme to substrate level (%v/w, X_1), temperature (C°, X_2) and pH (X_3) and the levels used for each independent variable were showed in Table 1. The range of each independent variable was determined according to the results of preliminary study (data not shown). Degree of hydrolysis (DH) was selected as dependent variables. The experiment was optimized by using three-factors, five levels central composite design. The central composite design composed of 20 treatments including 2^3 factorial points, six axial points (α=1.68) and six replicates of the central point. The design of experiments and dependent variable values was presented in Table 2. Randomized experimental runs were carried out with the purpose of minimizing the effect of unexpected variability in the observed responses (Wangtueai and Noomhorm, 2009).

Table 1. Independent variables and their level in the 3-factors, 5-levels central composite design for optimizing the enzymatic hydrolysis condition of salmon skin

Independent Variables	Symbol	Coded level				
		-1.68(-α)	-1	0	1	1.68(α)
Enzyme to substrate level (% v/w)	X_1	0	0.50	1.50	2.50	3.18
Temperature (°C)	X_2	29.77	40	55	70	80.23
pH	X_3	6.23	7.00	8.00	9.00	9.68

Table 2. Predicted and experimental results of the central composite design for optimization of enzymatic hydrolysis of salmon skin

Std. order	Code level of variable			Degree of Hydrolysis (%)	
	X_1	X_2	X_3	Predicted	Experimental
1	-1	-1	-1	61.39	59.52
2	1	-1	-1	64.95	64.38
3	-1	1	-1	50.46	48.73
4	1	1	-1	57.35	55.04
5	-1	-1	1	56.33	56.92
6	1	-1	1	62.87	62.89
7	-1	1	1	61.90	60.76
8	1	1	1	68.03	68.19
9	-1.682	0	0	65.46	67.40
10	1.682	0	0	75.64	76.51
11	0	-1.682	0	48.34	48.60
12	0	1.682	0	41.83	43.98
13	0	0	-1.682	56.14	59.16
14	0	0	1.682	68.63	68.02
15	0	0	0	77.59	74.54
16	0	0	0	72.22	74.54
17	0	0	0	73.58	74.54
18	0	0	0	74.22	74.54
19	0	0	0	75.61	74.54
20	0	0	0	74.82	74.54

X_1: Enzyme to substrate level (% v/w), X_2: temperature (°C)., X_3: pH

Statistical analysis

The response surface methodology (RSM) was statistically analyzed by Design-Expert, Version 6.0.11 software (Stat-ease Inc., Minneapolis, Minn., U.S.A.). The multiple regressions analysis was performed by the taking into account the main, quadratic and interaction effects to develop a quadratic polynomial equation. As three parameters were varied, 10 β-coefficients had to be estimated which included coefficients for the three main effects, three quadratic effects, three interactions and one constant. It is assumed that the estimated behavioral model of both dependent variables was described by a second-order polynomial equation:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$$

Where Y represented the estimated dependent variable (nitrogen recovery or antioxidant activity); β_0

represented the constant term; β_i, β_{ii} and β_{ij} represented the linear, quadratic and interaction terms (i=1-4; and j=1-4) respectively.

The R^2 value and the lack of fit value were determined. After the multifactor analysis of variance and the second-order model prediction determinations, the optimal enzymatic hydrolysis conditions were obtained by the desirability function approach. The response surface plots were developed to represent a function of two independent variables while keeping the other independent variable at the optimal value.

Table 3. Analysis of variance for the second order response surface model for degree of hydrolysis

Source	Sum of squares	DF	Mean square	F value	P value
Model	1922.06	9	213.56	43.17	< 0.0001
X_1	121.92	1	121.92	24.65	0.0006
X_2	25.75	1	25.75	5.21	0.0457
X_3	94.89	1	94.89	19.18	0.0014
X_1^2	15.57	1	15.57	3.15	0.1065
X_{12}	1443.38	1	1443.38	291.80	< 0.0001
X_{22}	216.78	1	216.78	43.83	< 0.0001
$X_1 X_2$	1.06	1	1.06	0.22	0.6527
$X_1 X_2$	0.62	1	0.62	0.12	0.7311
$X_2 X_3$	107.06	1	107.06	21.64	0.0009
Residual	49.46	10	4.95		
Lack of Fit	32.63	5	6.53	1.94	0.2425
Pure Error	16.83	5	3.37		
Cor Total	1971.529	19			

X_1: Enzyme to substrate level (% v/w), X_2: temperature (°C)., X_3: pH

Results and Discussion

Optimization of enzymatic hydrolysis of salmon skin

A central composite design was performed to study the combined effect of three factors (enzyme to substrate level, temperature and pH) on the responses, degree of hydrolysis (DH). The predicted and experimental results of the 3-factors, 5-levels central composite design was presented in Table 2.

A multiple regressions analysis technique that is included in the RSM was performed to determine all the coefficients of linear (X_1, X_2, X_3), quadratic (X_1^2, X_2^2, X_3^2) and interaction (X_1X_2, X_1X_3, X_2X_3) terms to fit a full response surface model for the responses. In this experiment, the quadratic model was suitable to the response of Y. The determinant coefficient (R^2) was 0.9699 (data not shown), which suggested that this quadratic model was appropriately to represent the real relationships among the chosen hydrolysis parameter. When fitting the model, various statistical analysis techniques were used to judge the experimental error, the suitability of the model, and the statistical significance of the terms in the model.

In the present study, the adequacy of the model is justified through analysis of variance (ANOVA), as shown in Table 3. By using ANOVA, the statistical significance of the quadratic polynomial model equation was evaluated. The result demonstrated that the model was significant at a 95% confidence level ($p<0.05$). This indicated that the quadratic model can explain a high percentage of the variability in the observed data. In this case, all the linear model terms (X_1, X_2, X_3) were significant ($P<0.05$). Among the quadratic coefficients, X_2^2 and X_3^2 had significant effects ($P<0.05$). However, X_1^2 was not significant ($P>0.05$). For the interaction coefficients (X_1X_2, X_1X_3, X_2X_3), only X_2X_3 were significant ($P<0.05$). X_1X_2 and X_1X_3 did not have significant effects ($P>0.05$).

The lack of fit test was used to evaluate the fitness of the model. The P-values for the lack of fit test was large and not significant ($P>0.05$), which indicated that the model was adequate for predicting enzymatic hydrolysis condition of salmon skin. Overall, the analysis of variance suggested that the predicted quadratic model for enzymatic hydrolysis conditions of salmon skin was statistically valid. The final response surface regression equation obtained by RSM was as follows:

$$Y = -217.97 + 2.89\ X_1 + 2.80\ X_2 + 50.73\ X_3 - 1.13\ X_1X_1 - 3.87\ X_2X_2 - 0.04\ X_3X_3 + 0.02\ X_1X_2 + 0.28\ X_1X_3 + 0.24X_2X_3$$

Where Y, X_1, X_2 and X_3 are DH, enzyme to substrate level (% v/w), temperature (°C) and pH respectively. The examination of fitted models was done to ensure that it gave an adequate approximation to the true system and to verify that they were no least squares regression assumption is violated (Zhou and Regenstein, 2004).

Analysis of variance

Conditions for optimum responses

The effect of enzyme to substrate level (X_1), temperature (X_2) and pH of (X_3) on the degree of hydrolysis (DH) was determined using response surface methodology (RSM) as mentioned in the previous section. Desirability profile was employed to establish the optimum level of each condition. Enzyme to substrate level (X_1), temperature (X_2) and pH of (X_3) were set in arranged ranges, whereas dependent variable, DH was expected to respond maximally.

The optimum conditions of enzymatic hydrolysis of salmon skin were 2.5% for enzyme to substrate level (X_1), 55.30°C for temperature (X_2) and 8.39 for pH (X_3). The predicted value of response, DH was 77.03% obtained from calculation by using the model equation with desirability of 0.984. The actual experimental results repeated three times under optimal hydrolysis conditions was 76.43±0.98%, which was close to and agreed well with the predicted value.

The influences of the independent variables on the DH can be judged through three-dimensional views of response surface plots and respective contour plots (Figure 1 and 2). The plots are represented as a function of two factors at a time, holding other factors at a fixed level (optimal level).

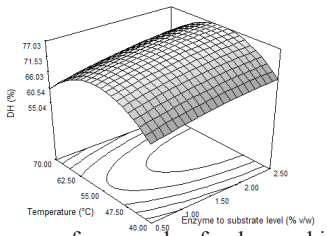

Figure 1. Response surface graph of salmon skin enzymatic hydrolysis with Alcalase for degree of hydrolysis as a function of enzyme to substrate level (% v/w) and temperature (°C)

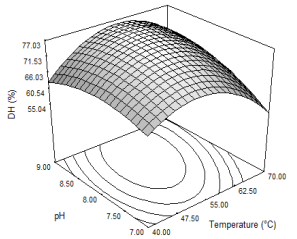

Figure 2. Response surface graph of salmon skin enzymatic hydrolysis with Alcalase for degree of hydrolysis as a function of temperature (°C) and pH

Figure 1 showed the three dimensions plot for DH as a function of enzyme to substrate level and temperature, where the pH was constant at 8.39. The result showed that DH increased as the enzyme to substrate level increased. The result may be caused by greater hydrolysis of protein when higher level of enzyme was added. Figure 2 showed the three dimensions plot for DH as a function of temperature and pH, where the enzyme to substrate level was constant at 2.50°C. DH was raised with an increasing temperature. However, DH exhibited a decreasing trend from the temperature of 55.3°C to 70°C. This was consistent with the optimal temperature of Alcalase enzyme, which is 55°C. The DH increased with an increase in substrate pH up to pH 8.39, beyond which, the yield of DH decreased. This was agreed with the working pH range of Alcalase since it is an alkaline enzyme with optimal pH of 8 – 8.5.

Proximate composition

Chemical composition of salmon skin and protein hydrolysate were shown in Table 4. The raw material of salmon skin was a rich source of protein. According to Muyonga *et al*. (2004), the crude protein content of the proteinous materials was the maximum possible yield of protein hydrolysate that can be isolated. The crude protein of salmon skin was high, which was 35.28%. Furthermore, the salmon skin composed of low fat and ash content, which was 1.65% and 0.43% respectively.

Table 4. Proximate composition of salmon skin and protein hydrolysate

	Salmon skin	Protein hydrolysate
Moisture (%)	58.53 ± 0.18	3.21 ± 0.11
Crude protein (%)	35.28 ± 0.46	89.53 ± 0.51
Fat (%)	1.65 ± 0.25	0.72 ± 0.04
Ash (%)	0.43 ± 0.04	3.58 ± 0.06

The crude protein content can be used as an indicator of the purity of the protein hydrolysate. It was found that the crude protein was the major component of the protein hydrolysate. The crude protein of the protein hydrolysate from salmon skin was 89.53%. This indicated high purity of the protein hydrolysate. The protein hydrolysate consisted of low fat content, which was only 0.72%. The low content of fat can ensure the stability of sample during storage. The ash content of the protein hydrolysate was quite high, which was 3.58%. The ash may partially consist of sodium phosphate buffer used during enzymatic hydrolysis.

Amino acid composition

Table 5 indicated the amino acid composition and chemical score of protein hydrolysate from salmon skin. The protein hydrolysate from salmon skin resembled the composition of interstitial collagen, presenting 19.45 g glycine/100 g sample and 11.42 g imino acid (proline and hydroxyproline)/100 g sample of the sum of the dispensable amino acids glycine, hydroxyproline and proline are abundantly present in connective tissues containing collagens.

Table 5. Amino acid composition of salmon skin and protein hydrolysate

Amino Acid	g/ 100g	Chemical score			
	Protein hydrolysate	Reference protein 1[a]	Reference protein 2[a]	RP 1[a]	RP 2[a]
Histidine	4.95	1.6	2.1	3.09	2.36
Isoleucine	11.18	1.3	2.5	8.60	4.47
Leucine	2.05	1.9	3.3	1.08	0.62
Lysine	0.52	1.6	5.7	0.33	0.09
Methionine	4.89	1.7[c]	3.1	-	1.58
Phenyl alanine	4.87	-	6.5	-	0.75
Tyrosine	1.91	-	-	-	-
Threonine	3.27	0.9	3.9	3.63	0.84
Tryptophan	0.01	-	0.8	-	0.01
Arginine	11.91	-	1.31	-	9.09
Valine	1.52	1.3	3.6	1.17	0.42
Total indispensable amino acid (IAA)	47.08				
Aspargine	1.87				
Glutamine	3.02				
Serine	3.26				
Glycine	19.45				
Alanine	4.81				
Proline	6.21				
Hydroxyproline	5.21				
Cystine	-				
Total amino acid (DAA)	43.83				
Ratio IAA:DAA	1.07				

RP1: Chemical score of salmon skin protein hydrolysate computed based on FAO/ WHO reference protein (1990)
RP2: Chemical score of salmon skin protein hydrolysate computed based on amino acid requirement as per NRC (1993)
[a] Suggested profile of essential amino acid requirements for adults (FAO/ WHO,1990).
[b] Essential amino acid requirements of common carp according to NRC (1993).
[c] Methionine + Cystine

From a nutritional point of view, the ratio of indispensable (IAA) to dispensable amino acids (DAA) of the protein hydrolysate was more than one, suggested that it was suitable as supplier of dietary amino acid. The nutritional value of a food ingredient

can also be judged from its protein capacity to fulfill the nutritional requirement with respect to essential amino acid. One of the methods to assess the dietary protein quality of a food ingredient is by determining its chemical score. Chemical score is used to estimate the nutritional value of a protein. It measures the ratio of the level of an indispensable amino acid to the corresponding amino acid in a standard protein. In this study, the amino acid requirements for adults as listed by FAO/WHO (1990) and amino acid requirements of juvenile common carp, as listed by NRC (1993) were used as the standard were used as reference protein to compute the chemical score of the protein hydrolysate from salmon skin.

Based on the amino acid requirements for adults provided by FAO/WHO (1990), it was found that the salmon skin protein hydrolysate generally had higher level in indispensable amino acids except lysine. Furthermore, the results of chemical score calculated based on amino acid requirement of juvenile common carp as listed by NRC (1993) revealed that tryptophan and lysine were the most limiting amino acids. Tryptophan content of salmon skin protein hydrolysate was very low, only 0.01 g/100 g. The protein hydrolysate was deficient in leucine, valine and phenyl alanine, which were present in inadequate quantities. All other indispensable amino acids (except threonine, which was in almost similar quantity as that of the requirement of juvenile common carp) were in adequate or excess quantities.

Conclusion

Salmon skin can be used as the material of protein hydrolysate due to its high content of protein. The degree of hydrolysis of protein hydrolysate from salmon skin was significantly affected by the hydrolysis conditions including enzyme to substrace level, temperature and pH. Accodring to the model, the optimum hydrolysis parameters were 2.50% (v/w) of enzyme to substrate level, pH 8.39 and the temperature of 55.3°C. The corresponding response was 77.03% of the DH. With these optimum hydrolysis conditions, the protein hydrolysate obtained composed of high percentage of protein. It also composed of higher level of indispensable amino acid. Protein hydrolysate from salmon skin may potentially serve as a good source of desirable peptide and amino acids.

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