Asian Fisheries Science **31** (2018):230–244

ISSN 0116-6514
E-ISSN 2073-3720
https://doi.org/10.33997/j.afs.2018.31.3.005

Asian Fisheries Science
The Journal of the Asian Fisheries Society

Optimisation of the Enzymatic Hydrolysis of Oneknife Unicornfish, *Naso thynnoides* (Cuvier 1829) Skin Gelatin

GARNER ALGO LANGOTE ALOLOD[1,2] and SHARON NONATO NUNAL[1*]

[1]Institute of Fish Processing Technology, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao 5023, Iloilo, Philippines
[2]University of Antique-Tario Lim Memorial Campus, Tibiao 5707, Antique, Philippines

Abstract

Protein hydrolysates are products of splitting protein into peptides, which has been shown to provide superior nutritive benefits than the parent material. Hydrolysis can be carried out enzymatically or by using acid or alkali treatments. In this study, conditions for enzymatic hydrolysis of unicornfish *Naso thynnoides* (Cuvier 1829) skin gelatin was optimised through response surface methodology (RSM) to yield the highest degree of hydrolysis. Extracted skin gelatin was hydrolysed using a crude preparation of neutral protease from *Bacillus* sp. The considered optimum hydrolysis conditions were: enzyme concentration of 2.11 % (v/v), the temperature of 47.60 °C and hydrolysis time of 137.45 min resulting in 29.12 % degree of hydrolysis (DH) point prediction value. Foaming capacity expressed as foaming expansion percentage (FE) was also considered as a surface model response. Response surface methodology predicted an optimum FE value of 11.14 % with optimal conditions as follows: enzyme concentration of 2.13 % (v/v), the temperature of 54.16 °C and hydrolysis time of 147.73 min.

Keywords: unicornfish, gelatin, gelatin hydrolysates, response surface methodology, degree of hydrolysis, foaming expansion

Introduction

Processing of fish and other marine organisms to produce desired food product involves removal of unneeded parts such as skin, bones, viscera and scales (Amiza et al. 2011). Efforts are made to utilise these discards as a source of functional compounds like collagen and gelatin.

*Corresponding author. E-mail: snnunal@up.edu.ph

Approximately 30 % of skin and bones from fish filleting process is composed of collagen which can be further processed into gelatin, a collagen derivative extensively utilised as an ingredient in the improvement of elasticity, consistency, and stability of food (Binsi et al. 2009; Gómez-Guillén et al. 2010). Studies on gelatin production from fish sources have focused on its potential to substitute bovine and porcine counterparts. However, developments on gelatin production from fish is considered minimal comprising only 1.5 % of total annual world gelatin produced due to its poor rheological properties such as low melting point and low gel strength limit (Gómez-Guillén et al. 2011).

Protein hydrolysates are valuable products which may be derived from fish skin gelatin through controlled enzymatic hydrolysis, cleaving protein peptide chains into smaller fragments (Chalamaiah et al. 2012). In the food industry, enzymatic hydrolysis of protein is widely applied as compared to other methods because of the ease of controlling the reaction whereby it forms only minimal by-products and the milder processing conditions improve the nutritional and functional properties of proteins and peptides (Kristinsson and Rasco 2000; See et al. 2011; Mohammad et al. 2015; Jamil et al. 2016). The efficiency of the hydrolysis process is significantly dependent on the type of enzyme to be used since various enzymes have respective specific activities and optimal working parameters (Ji et al. 2016). For practicality, production of gelatin hydrolysates is widely favoured over collagen due to the high cost of the enzyme collagenase (Mohammad et al. 2015).

One of the desirable properties of proteins needed in industrial applications is good foaming capacity which relates to the protein's ability to be adsorbed quickly at the air-water interface resulting in a lowered surface tension (Medina et al. 2011; Razali et al. 2015). In the production of protein hydrolysates, foaming capacity determines the functionality of the protein fragments. Optimising the conditions to obtain desired enzymatic hydrolysis and foaming capacity of the gelatin hydrolysates can be done by response surface methodology (RSM). This multivariate tool creates a mathematical model estimating the effect of variables and its interactions to response, resulting in the generation of optimum conditions and response values (Bezerra et al. 2008; Amiza et al. 2011).

Acanthurids such as unicornfish *Naso thynnoides* (Cuvier 1829) is usually processed for human consumption as fillets, generating skin as a by-product which can be a viable source of gelatin (Sweetser 2009). In this study, gelatin was extracted from the skin of unicornfish and was hydrolysed using a crude bacterial protease. The objective of this study was to optimise the hydrolysis conditions in relation to enzyme concentration, temperature and reaction time using RSM to obtain the highest degree of hydrolysis and the highest foaming capacity of the hydrolysed protein.

Materials and Methods

Raw material

Skin of oneknife unicornfish *Naso thynnoides* was collected from a dried fish processor in Culasi, Antique, Philippines. The sample was frozen and transported to the laboratory for washing and cleaning. Skin samples were cut into small pieces (approximately 0.5 × 0.5 cm), packed in polyethylene bags and stored in a freezer at –20 °C until use.

Extraction of gelatin from fish skin

Gelatin was extracted from the cleaned fish skin following the method of Razali et al. (2015) with slight modification. Approximately 30 g of fish skin was mixed with 120 mL of 0.15 M acetic acid (Macron Fine Chemicals, Thailand) for 1 h at 4 °C. The mixture was swirled every 10 min to promote better absorption of acid to the skin. The acetic acid was drained and skin was rinsed twice with 150 mL distilled water and later mixed with distilled water at a ratio of 1:6 (skin/water). The mixture was stirred for 3 h in a water bath (Kottermann, Germany) at 60 °C with the flask swirled every 5 min. The extract was filtered with two layers of cheese cloth and the liquid fraction was collected and freeze dried. The yield was calculated following the equation below:

$$\text{Yield (\%)} = \frac{\text{Dry weight of gelatin (g)}}{\text{Wet weight of skins (g)}} \text{ x } 100$$

Bacterial protease

A crude enzyme preparation from *Bacillus* sp. was used in this study due to its desirable hydrolytic capacity at a wide range of pH. The bacterial protease was obtained from the Enzyme Laboratory of University of the Philippines Los Baños (UPLB), Los Baños, Laguna, Philippines and was determined to have a specific activity of 0.3 $U.mg^{-1}$ (U defined as μmol tyrosine liberated min^{-1} mL^{-1} enzyme preparation) and a neutral pH optimum. The solution was stored at 4 °C until use.

Response surface model experimental design

Optimisation was done by the use of response surface methodology (RSM) using Design Expert version 7 software (Stat-Ease, Inc., USA) by central composite design using three independent factors at five levels including 2 axial points, 2 factorial points and 1 centre point. Hydrolysate production conditions identified as independent variables are enzyme concentration (% v/v, X_1), hydrolysis time (min, X_2) and hydrolysis temperature (°C, X_3) with range of values shown in Table 1. A pH of 7 in the treatments was maintained during the whole enzymatic hydrolysis process by adding 1N NaOH (Scharlau Chemie, Spain). Responses monitored for the experimental model were degree of hydrolysis (DH) and foaming expansion (FE).

Table 1. Independent variables and corresponding coded and actual value levels used in the optimisation of enzymatic hydrolysis of Oneknife unicornfish *Naso thynnoides* skin gelatin.

Variable	Code	Actual value level				
		–1.68 (-α)	–1	0	1	1.68 (α)
Enzyme concentration (% v/v)	X_1	1	1.41	2	2.59	3
Hydrolysis time (min)	X_2	60	84.32	120	155.68	180
Hydrolysis temperature (°C)	X_3	40	44.05	50	55.95	60

The suggested number of runs by the software were experimentally done and the values for degree of hydrolysis and foaming expansion were used as data for RSM. The centre point was carried out six times to obtain precise estimation of the experimental error. For this study, experimental runs were done in random order to minimise the effects of unanticipated variability in the observed values of the response. Each combination run was done in triplicate.

Model fitting

After inputting experimental values to the software, analysis of the model was done by identifying the significant ($P < 0.05$) and non-aliased highest polynomial order among the linear, quadratic and cubic sources. In this study, analysis of variance (ANOVA) suggested the quadratic response surface as the chosen model for both degree of hydrolysis and foaming expansion. The independent and interaction coded terms which had significant effects on the model were identified. The non-significant lack of fit value ($P > 0.05$) was considered since it indicates the model's fitness.

Predicted values of the response were calculated using the equation generated by the software. It consisted of 10 β-coefficients including three linear effects, three quadratic effects, three interaction effects and one constant. The equation follows the format given below:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} V_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

The relationship between independent variables by holding the third variable as constant was illustrated by 3D response surface plots generated by the software. Effect of the variables with respect to the response in observations was also shown by the plot.

Preparation of fish skin gelatin hydrolysates

Unicornfish skin gelatin hydrolysates were prepared following the method of Lassoued et al. (2015) with slight modification. Gelatin powder of 2.5 g was dissolved in 100 mL of distilled water and hydrolysed using the crude bacterial protease at varying concentrations, reaction times, and temperatures that the RSM software identified.

After every run, the tubes were exposed to 80 °C for 10 min to deactivate the enzyme. The solution was centrifuged at 3500 rpm for 30 min. The soluble fractions were stored in the freezer at –20 °C until analysis.

Degree of hydrolysis determination

The degree of hydrolysis of the fish skin gelatin was determined by the trichloroacetic acid (TCA) (Sigma-Aldrich, Singapore) ratio method following the process of Haslaniza et al. (2010). In this method, 20 mL of the hydrolysates was added with 20 mL of 20 % (w/v) TCA to produce 10 % TCA soluble matter. The mixture was left to stand for 30 min to allow precipitation and centrifuged at 3500 ×*g* for 30 min. The soluble fraction was analysed for protein content determination by the Kjeldahl method (AOAC, 2005). The hydrolysate samples were also subjected to the same test and degree of hydrolysis was calculated using the formula below:

$$\text{DH \%} = \frac{\text{Souble N in TCA 10 \% } (\frac{\text{W}}{\text{V}})}{\text{Total N in the hydrolysate sample}} \text{ x } 100$$

Foaming capacity of hydrolysates

Foaming capacity of the hydrolysates was determined according to the method of Giménez et al. (2009). The sample solution (0.5 % v/v) at 50 mL volume was homogenised for 1 min using a homogeniser (approximately 8000 rpm) (WiseTis, Humanilab Instrument Corp., Germany) to incorporate air at room temperature. The whipped sample was immediately transferred into a 100 mL graduated cylinder and total volume was measured and recorded. Foaming expansion percentage was calculated using the following equation where V_t is the total volume after whipping (mL) and V_o is the volume (mL) before whipping. The analysis was done in triplicate.

$$\text{FE} = \frac{\text{Vt}-\text{Vo}}{\text{Vo}} \text{ x } 100$$

Results

Prior to optimisation, the yield of the gelatin powder from unicornfish skin was determined at 10.65 % (10.65 g.100 g^{-1} of wet skin). Response surface modelling with use of 3 factors-five level central composite design and the detailed experimental and predicted values for both of the responses are shown in Table 2. Experimental values for DH ranged from 27.71–49.69 % with maximum to minimum ratio of 1.79 and for FE, 4.27 % to 18.81 % with maximum to minimum ratio of 4.41. Model generation with basis on the sum of sequential squares (Table 3) selected quadratic model as the significantly highest order polynomial criterion ($P < 0.05$) among the sources presented.

Table 2: Central composite design of experimental and predicted results for the optimisation of enzymatic hydrolysis and foaming expansion of Oneknife unicornfish *Naso thynnoides* skin gelatin.

				Degree of hydrolysis (%)		Foaming expansion (%)	
Standard order	X_1	X_2	X_3	Experimental value	Predicted value	Experimental value	Predicted value
1	1.41	84.32	44.05	49.69	46.21	10.89	12.21
2	2.59	84.32	44.05	32.52	35.47	9.57	8.17
3	1.41	155.68	44.05	38.28	38.15	6.93	6.05
4	2.59	155.68	44.05	29.81	27.41	4.29	3.49
5	1.41	84.32	55.95	37.29	35.53	12.87	12.89
6	2.59	84.32	55.95	32.35	34.83	6.6	6.69
7	1.41	155.68	55.95	36.1	35.51	16.83	17.45
8	2.59	155.68	55.95	35.47	34.81	14.85	12.73
9	1	120	50	38.41	41.57	18.81	17.77
10	3	120	50	33.78	31.95	8.25	10.40
11	2	60	50	39.14	38.60	6.27	5.87
12	2	180	50	29.96	31.80	4.27	5.78
13	2	120	40	34.99	36.38	4.95	5.61
14	2	120	60	33.72	33.62	13.53	13.96
15	2	120	50	29.72	30.08	10.23	8.88
16	2	120	50	27.71	30.08	8.25	8.88
17	2	120	50	29.87	30.08	8.91	8.88
18	2	120	50	33.42	30.08	9.24	8.88
19	2	120	50	30.53	30.08	7.26	8.88
20	2	120	50	29.43	30.08	9.57	8.88

Analysis of variance (ANOVA) of the quadratic response surface model for DH was significantly different ($P < 0.05$) suggesting that it has sufficiently represented the true relationship among the chosen independent variables. Parameters with code X_1, X_2, X_1X_3, X_2X_3, X_1^2, X_2^2, X_3^2 were identified to contribute to the significance of the quadratic model ($P < 0.05$). For FE, parameter codes such as X_1, X_3, X_2X_3, X_1^2, X_3^2 were significant model terms.

For both of the responses, the non-significant ($P > 0.05$) relationship of lack of fit to the pure error suggests that the experimental data fits well in the model. Response model equations used for the calculation of the predicted responses' value (Table 2) are shown in Table 4.

Table 3. Response surface model fitting through sum of sequential squares suggesting quadratic source for both degree of hydrolysis and foaming expansion.

Response	Source	Sum of squares	DF	Mean square	F value	P-value	
Degree of hydrolysis (%)	Mean vs Total	23269.15981	1	23269.15981			
	Linear vs Mean	176.4767161	3	58.82557204	3.1463858	0.0542	
	2FI vs Linear	103.7084375	3	34.56947917	2.299545334	0.1254	
	Quadratic vs 2FI	143.4448599	3	47.81495332	9.197574443	0.0032	Suggested
	Cubic vs Quadratic	30.25443389	4	7.563608473	2.088236312	0.2006	Aliased
	Residual	21.73204755	6	3.622007926			
	Total	23744.7763	20	1187.238815			
Foaming expansion (%)	Mean vs Total	1850.310845	1	1850.310845			
	Linear vs Mean	149.9272804	3	49.97576012	5.057713021	0.0118	
	2FI vs Linear	60.9159375	3	20.3053125	2.716244244	0.0876	
	Quadratic vs 2FI	72.97068092	3	24.32356031	10.04650956	0.0023	Suggested
	Cubic vs Quadratic	15.69496965	4	3.923742412	2.764501124	0.1282	Aliased
	Residual	8.515986579	6	1.419331096			
	Total	2158.3357	20	107.916785			

Table 4. Analysis of variance (ANOVA), coefficient of determination (R^2) and second order polynomial equation of the quadratic model for degree of hydrolysis and foaming expansion.

Response	Source	SS	D	MS	F	P-	R^2	Model equation
Degree of hydrolysis (%)	Model	423.63	9	47.07	9.05	0.0010	0.8907	$Y=30.00-2.86X_1-2.02X_2-0.82X_3+1.63X_1X_2+2.51X_1X_3+2.01X_2X_3+2.36X_1^2+1.81X_2^2+1.74X_3^2$
	Residual Error	51.99	10	5.20				
	Lack of Fit	34.42	5	6.88	1.96	0.2390		
	Pure Error	17.56	5	3.51				
	Total	475.62	19					
Foaming expansion (%)	Model	283.81	9	31.53	13.03	0.0002	0.9214	$Y=8.88-2.19\ X_1-0.029\ X_2+2.48\ X_3+0.37\ X_1\ X_2-0.54\ X_1\ X_3+2.68\ X_2\ X_3+1.84\ X_1^2-1.08\ X_2^2+0.32\ X_3^2$
	Residual Error	24.21	10	2.42				
	Lack of Fit	18.77	5	3.75	3.45	0.1003		
	Pure Error	5.45	5	1.09				
	Total	308.02	19					

The effect of the hydrolysis parameter to the response is graphically illustrated in Figure 1. Figure 1a shows the effect to the response of the relationship between enzyme concentration (% v/v) and hydrolysis time (min) by maintaining the hydrolysis temperature at 50 °C. Degree of hydrolysis decreased from 1–2 h peaking at an enzyme concentration of 1.67 %. Also, when hydrolysis time was held at a mean level of 120 min, degree of hydrolysis increased while enzyme concentration and temperature decreased with highest range values at 1.79 % and 53.56 °C (Fig. 1b).

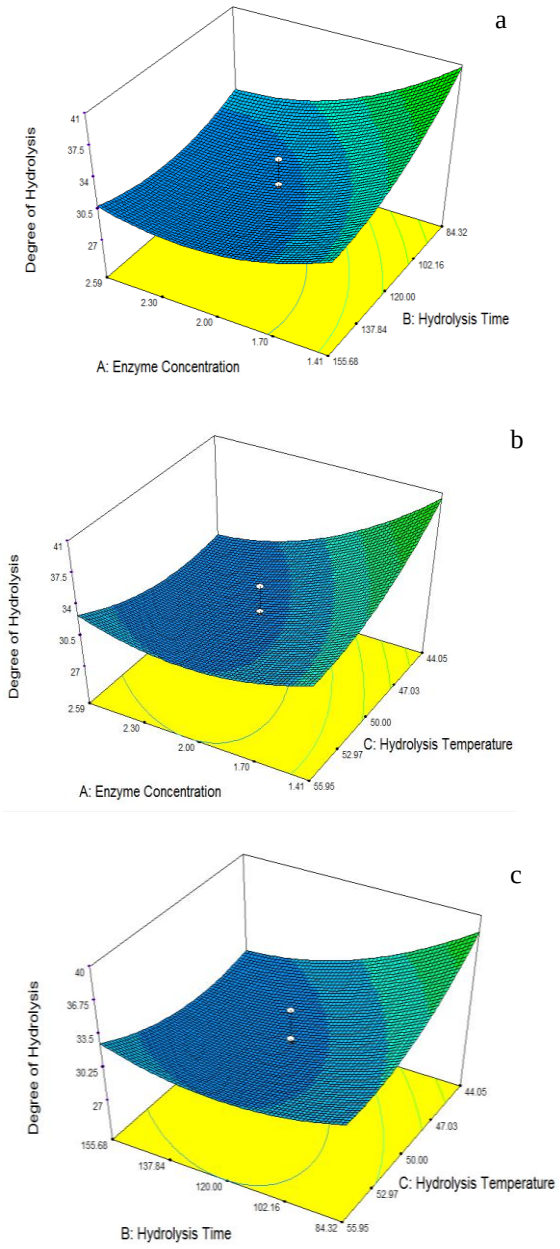

Fig. 1. 3D response surface plot showing the effect of independent variables to degree of hydrolysis: a – enzyme concentration and hydrolysis time; b – enzyme concentration and hydrolysis temperature; c – hydrolysis time and hydrolysis temperature.

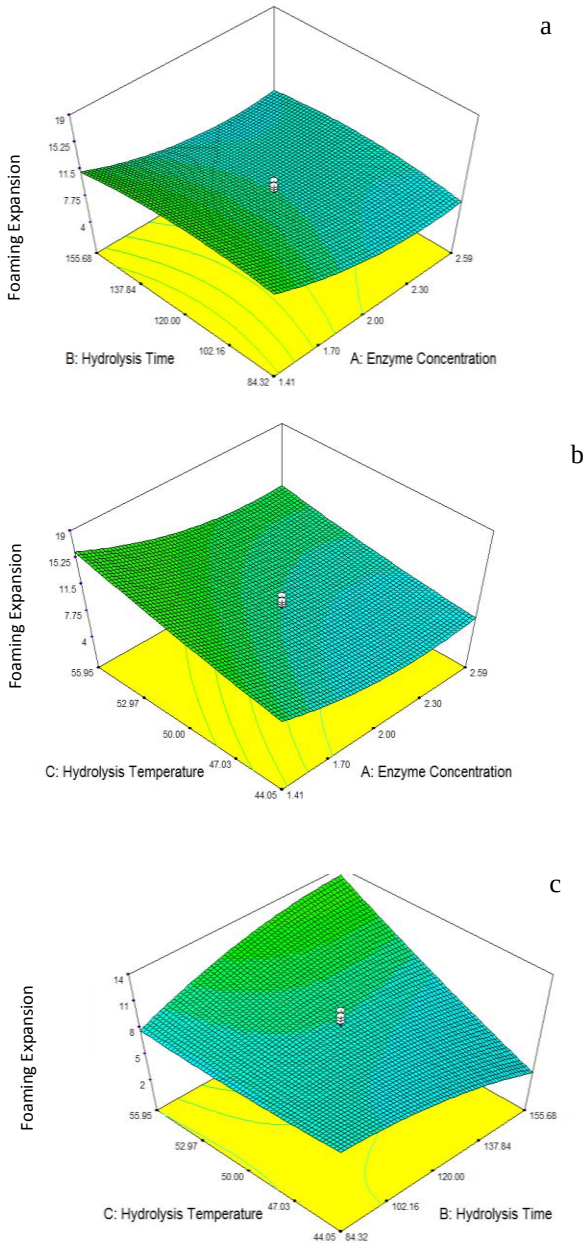

Fig. 2. 3D response surface plot showing the effect of independent variables on foam expansion: a – hydrolysis time and enzyme concentration; b – hydrolysis temperature and enzyme concentration; c – hydrolysis temperature and hydrolysis time.

This observation was also true for the relationship between hydrolysis time and temperature when enzyme concentration was fixed at 2 % with the lowest decrease in DH observed at 103.08 min hydrolysis time and 53.70 °C hydrolysis temperature (Fig. 1c). The effect of the independent variables to FE is shown in Figure 2. As illustrated by the 3D response surface plots, FE decreased from 1–2 h as it approached enzyme concentration of 1.62 % and hydrolysis temperature at 50 °C (Fig. 2a). At 120 min hydrolysis time, FE also decreased peaking at 1.93 % enzyme concentration (Fig. 2b). When enzyme concentration was at central value of 2 %, FE decreased as temperature and time increased up to 53.31 °C and 111.61 min respectively (Fig. 2c).

For DH, optimisation by response surface modelling yielded the following optimum conditions: 2.11 % enzyme concentration, 137. 45 min hydrolysis time and 47.60 °C hydrolysis temperature suggesting a point predicted value of 29.11 %. For FE, optimum conditions were 2.13 % enzyme concentration, 147.73 min hydrolysis time and 54.16 °C hydrolysis temperature. The predicted value of FE for this combination of conditions was 11.14 %.

Table 5. Optimised variable conditions and point prediction for degree of hydrolysis and foaming expansion responses.

Response	Variables			Predicted value
	Enzyme concentration (% v/v)	Hydrolysis time (min)	Hydrolysis temperature (°C)	
Degree of hydrolysis (%)	2.11	137.45	47.60	29.12
Foaming expansion (%)	2.13	147.73	54.16	11.14

Discussion

In the present study, the 10.65 % gelatin powder yield was within the reported range of fish gelatin extraction percentages of 6–19 % from different aquatic organisms as indicated in Table 6. It is much higher than the gelatin extracted from bigeye snapper, giant squid, dover sole, megrim, cod, hake, rainbow trout, nile perch, bigeye snapper, brownstripe red snapper and shortfin scad. However, this result is lower than those obtained from walking catfish, striped catfish, grass carp and catfish.

The observed variation in the recovery of gelatin from different species can be attributed to the intrinsic properties of fish skin since physical and structural characteristics of gelatin vary among fish species (Gómez-Guillén et al. 2001; Razali et al. 2015). Furthermore, the lower content of inter- and intra-chain non-reducible crosslinks in the fish skin collagenous material leads to high susceptibility to degradation. This is evident with the possible leaching out of extracted collagen after a series of washings and incomplete collagen hydrolysis which can result to lower yields (Jamilah and Harvinder 2002; Karim and Bhat 2009; Tabarestani et al. 2010).

Table 6. Gelatin yield from skins and bones of different aquatic organisms.

Species	Gelatin yield (%)	Reference
Oneknife unicornfish *Naso thynnoides* (Cuvier 1829)	10.65	Present study
Bigeye snapper *Priacanthus macracanthus* Cuvier 1829	4	Jongjereonrak et al. 2006
Giant squid *Dosidicus gigas* (D'Orbigny 1835)	7.5	Gómez-Guillén et al. 2002
Dover sole *Solea vulgaris* Quensel 1806	7.3	Gómez-Guillén et al. 2002
Megrim *Lepidorhombus boscii* (Risso 1810)	7.4	Gómez-Guillén et al. 2001
Cod *Gadus morrhua* Linnaeus 1758	7.2	Gómez-Guillén et al. 2002
Hake *Merluccius merluccius* (Linnaeus 1758)	6.5	Gómez-Guillén et al. 2002
Rainbow trout *Onchorynchus mykiss* (Walbaum 1792)	9.36	Tabarestani et al. 2010
Nile perch *Lates niloticus* (Linnaeus 1758)	3.4	Muyonga et al. 2004
Bigeye snapper *Priacanthus hamrur* (Forsskål 1775)	6.5	Binsi et al. 2009
Brownstripe red snapper *Lutjanus vitta* (Quoy and Gaimard 1824)	9.4	Jongjereonrak et al. 2006
Shortfin scad *Decapterus macrosoma* Bleeker 1851	7.3	Cheow et al. 2007
Walking catfish *Clarias batrachus* (Linnaeus 1758)	13.06	Jamilah et al. 2011
Striped catfish *Pangasius sutchi* Fowler 1937	11.97	Jamilah et al. 2011
Grass carp *Ctenopharyngodon idella* (Valenciennes 1844)	19.83	Kasankala et al. 2007
Catfish *Clarias gariepinus* (Burchell 1822)	17.57	Sanaei et al. 2013

The peak maximum on the 3D response surface plots for degree of hydrolysis and foaming expansion were used to find the optimal values of the respective independent variables. The inverse relationship of the independent variables to DH is mainly due to rapid gelatin hydrolysis kinetics during the first few minutes of enzymatic hydrolysis of the fish skin gelatin sample. This finding is comparable to the results of tuna *Thunnus* sp. and giant squid *D. gigas* skin hydrolysate production in which proteolysis was increased at 5–30 min and reached the maximum value roughly after 2 h (Gomez–Guillen et al. 2010). Sai–Ut et al. (2014) also reported that proteolysis of unicorn leatherjacket *Aluterus monoceros* (Linnaeus 1758) skin gelatin using extracellular protease from *Bacillus amyloliquefaciens* H11 was rapid at the primary phase of 30 min.

The observed high initial rate of protein hydrolysis in the present study suggested that the maximum peptide cleavage is within 2 h (You et al. 2010). The fast hydrolysis of unicornfish skin gelatin can be attributed to its composition of peptides mainly with molecular weight of <100 kDa facilitating a more efficient access of the enzyme to the substrate (Gomez–Guillen et al. 2010). The further increase in enzyme concentration resulting in lower DH may be attributed to decreasing substrate amount, enzyme autodigestion and product inhibition (Khantaphant and Benjakul 2008). Further, the efficiency of enzymatic hydrolysis decreased at higher hydrolysis temperature which may have led to deactivation of the enzyme (Mohammad et al. 2015).

The degree of hydrolysis predicted value (29.12 %) obtained in the study is comparable to the optimum DH of cuttlefish *Sepia officianalis* (Linnaeus 1758) gelatin using crude enzymes from *Bacillus mojavensis* A21 and *Bacillus licheniformis* NH1 with values at 26.9 % and 24.1 % respectively (Jridi et al. 2014). This is higher than tilapia *Orechromis* spp. scale gelatin hydrolysates at 10.91 % (Mohammad et al. 2015) but lower than the salmon *Salmo salar* Linnaeus 1758 skin hydrolysates at 77.03 % (See et al. 2011) which both utilised the commercial Alcalase enzyme. Comparing the obtained optimal value, results of the present study showed higher DH than published works that used *Bacillus subtilis* A26 proteases on thornback ray's *Raja clavata* Linnaeus 1758 skin gelatin hydrolysates (11.35 %) and cuttlefish *S. officianalis* gelatin hydrolysates (12.7 %) (Jridi et al. 2014; Lassoued et al. 2015).

Optimum values of the independent variables for the degree of hydrolysis using a crude protease from *Bacillus* sp.in the present study was within the range of optimised conditions for fish protein hydrolysis by Alcalase, an endoproteinase obtained from *Bacillus licheniformis* with 35–64 °C hydrolysis temperature and 0.20 % – 2.50 % enzyme concentration (Jamil et al. 2016). Further, the optimum hydrolysis time of 137.45 min in the present study is close to the optimal hydrolysis time of 136 min for protein hydrolysis of tuna dark muscle (Saidi et al. 2013) and Catla *Catla catla* (Hamilton 1822) fish visceral waste proteins (Bhaskar et al. 2008).

The 3D response surface plots for FE which shows that the decrease in the response is due to the increase of independent variables with maximum values is presented in Figure 2. Foaming capacity as expressed by foaming expansion has a direct relationship with the degree of hydrolysis wherein at higher hydrolysis percentage, foaming expansion increased. This could be due to the presence of smaller peptides which migrate faster at the air-water interface (Intarasirisawat et al. 2012). Hydrolysing fish proteins can increase the foaming capacity of the polypeptides, but it often lacks stability due to its inability to stabilise the air cells in the foam (Kristinsson and Rasco 2000).

The 11.14 % point prediction for FE in the present study is higher than the value reported by Giménez et al. (2009) at 6–7 % using the same concentration. This can be attributed to the assumption that the unicornfish skin gelatin hydrolysates have small peptide fractions that can easily be adsorbed in the air-water interface resulting in the lowering of surface tension.

Variation in the FE maybe due to the interface adsorption efficiency of the peptide affected by the molecular size and structure of the parent protein, the hydrophobicity of the hydrolysates and the employed hydrolysis procedure (Martin et al. 2002). The results of the present study are comparable to the foaming expansion percentage of cobia *Rachycentron canadum* (Linnaeus 1766) skin gelatin hydrolysate of 9.33 % at 5 kDa molecular weight (Razali et al. 2015).

Conclusion

Optimisation by response surface methodology of the production of unicornfish skin gelatin hydrolysates showed that degree of hydrolysis is affected by enzyme concentration, hydrolysis temperature and interaction of hydrolysis time with hydrolysis temperature. Also, foaming capacity is mainly influenced by enzyme concentration, hydrolysis time and hydrolysis time-hydrolysis temperature interaction. Characterisation of the hydrolysates in terms of its molecular profile, amino acid profile, other functional properties and bioactivity is however recommended.

Acknowledgements

The authors would like to acknowledge the support of the Philippines' Department of Science and Technology-Accelerated Science and Technology Human Resource Development Program and Institute of Fish Processing Technology of the College of Fisheries and Ocean Sciences of the University of the Phillipines Visayas, Miagao, Iloilo, Philippines. The authors also wish to thank Ms. Edna Monreal, Ms. Nida Momblan and Ms. Rosanna Alama for the technical assistance.

References

Amiza, M.A., S. Nurul Ashikin and A.L. Faazaz. 2011. Optimization of enzymatic protein hydrolysis from Silver Catfish (*Pangasius* sp.) frame. International Food Research Journal 18:775–781.

AOAC. 2005. Fish and other marine products. In: Official methods of analysis of AOAC International (ed. P.A. Cunniff), pp. 16–17. AOAC International, Virginia.

Bezerra, M.A., R.E. Santelli, E.P. Oliveira, L.S. Villar and L.A. Escaleira. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. International Journal of Pure and Applied Analytical Chemistry 76:965–977.

Bhaskar, N., T. Benila, C. Radha and R.G. Lalitha. 2008. Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease. Bioresource Technology 99:335–343.

Binsi, P.K., B.A. Shamasundar, A.O. Dileep, F. Badii and N.K. Howel. 2009. Rheological and functional properties of gelatin from the skin of bigeye snapper (*Priacanthus hamrur*) fish: influence of gelatin on the gel-forming ability of fish mince. Food Hydrocolloids 23:132–145.

Chalamaiah, M., B. Dinesh Kumar, R. Hemalatha and T. Jyothirmayi. 2012. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chemistry 135:3020–3038.

Cheow, C.S., M.S. Norizah, Z.Y. Kyaw and N.K. Howell. 2007. Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). Food Chemistry 101:386–391.

Giménez, B., A. Alemán, P. Montero and M.C. Gómez-Guillén. 2009. Antioxidant and functional properties of gelatin hydrolysates obtained from skin of sole and squid. Food Chemistry 114:1322–1327.

Gómez-Guillén, M.C. and P. Montero. 2001. Extraction of gelatin from megrim (*Lepidorhombus boscii*) skins with several organic acids. Journal of Food Science 66:213–216.

Gómez-Guillén, M.C., J. Turnay, M.D. Fernández-Diaz, N. Ulmo, M.A. Lizarbe and P. Montero. 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocolloids 16:25–34.

Gómez-Guillén, M.C., M.E. Lopez-Caballero, A. Aleman, A. Lopez de Lacey, B. Gimenez and P. Montero. 2010. Antioxidant and antimicrobial peptide fractions from squid and tuna skin gelatin. In: Sea by-products as real material: new ways of application (ed. E. Le Bihan), pp. 89–115. Transworld Research Network, India.

Gómez-Guillén, M.C., B. Gimenez, M.E. Lopez-Caballero and P. Montero. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocolloids 25:1813–1827.

Haslaniza, H., M.Y. Maskat, W.M. Aida and S. Mamot. 2010. The effects of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipitate from cockle (*Anadara granosa*) meat wash water. International Food Research Journal 17:142–152.

Intarasirisawat, R., S. Benjakul and W. Visessanguan. 2012. Antioxidative and functional properties of protein hydrolysate from defatted skipjack (*Katsuwonous pelamis*) roe. Food Chemistry 135:3039–3048.

Jamil, N.H., N.R.A. Halim and N.M. Sarbon. 2016. Optimization of enzymatic hydrolysis condition and functional properties of eel (*Monopterus* sp.) protein using response surface methodology (RSM). International Food Research Journal 23:1–9.

Jamilah, B. and K.G. Harvinder. 2002. Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). Food Chemistry 77: 81–84.

Jamilah, B., K.W. Tan, M.R. Umi Hartina and A. Azizah. 2011. Gelatins from three cultured freshwater fish skins obtained by liming process. Food Hydrocolloids 25:1256–1260.

Ji, Y., G. Zhang and Y. Zhang. 2016. Optimization of enzymatic hydrolysis of protein from small yellow croaker (*Psendosciaena polyactis*) using response surface methodology. Advance Journal of Food Science and Technology 11:1–6.

Jongjareonrak, A., S. Benjakul, W. Visessanguan, T. Prodpran and M. Tanaka. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloids 20:492–501.

Jridi, M., I., R. Lassoued, M. Nasri, M. Ayadi, N. Nasri and N. Soussi. 2014. Characterization and potential use of cuttlefish skin gelatin hydrolysates prepared by different microbial proteases. Biomed Research International 2014:461728. doi.org/10.1155/2014/461728.

Karim, A.A. and R. Bhat. 2009. Fish gelatin: Properties, challenges and prospects as an alternative to mammalian gelatin. Food Hydrocolloids 23:563–576.

Kasankala, L.M., Y. Xue, Y. Weilong, S.D. Hong and Q. He. 2007. Optimization of gelatin extraction from grass carp (*Ctenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technology 98:3338–3343.

Khantaphant, S. and S. Benjakul. 2008. Comparative study on the proteases from fish pyloric caeca and the use for production of gelatin hydrolysate with antioxidative activity. Comparative Biochemistry and Physiology 151:410–419.

Kristinsson, H.G. and B.A. Rasco. 2000. Fish protein hydrolysates: production, biochemical, and functional properties. Critical Reviews in Food Science and Nutrition 40:43–81.

Lassoued, I., L. Mora, A. Barkia, M. Conception-Aristoy, M. Nasri and F. Toldra. 2015. Bioactive peptides identified in thornback ray skin's gelatin hydrolysates by proteases from *Bacillus subtillis* and *Bacillus amyloliquefaciens*. Journal of Proteomics 128:8–17.

Martin, A.H., K. Grolle, M.A. Bos, M.A.C. Stuart and T. Van Vliet. 2002. Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability. Journal of Colloid and Interface Science 254:175–183.

Medina, A.C., H. Jimenez-Islas, L. Dendooven, R. Patiño-Herrera, G. Gonzalez-Alatorre and E.M. Escamilla-Silva. 2011. Emulsifying and foaming capacity and emulsion and foam stability of sesame protein concentrates. Food Research Journal 44:684–692.

Mohammad, A.W., A.G. Kumar and R.K. Basha. 2015. Optimization of enzymatic hydrolysis of tilapia (*Oreochromis* spp.) scale gelatine. International Aquatic Research 7:27–39.

Muyonga, J.H., C.G.B Cole and K.G. Duodu. 2004. Extraction and physico-chemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocolloids 18:581–592.

Razali, A.N., A.M. Amin and N.M. Sarbon. 2015. Antioxidant activity and functional properties of fractionated cobia skin gelatin hydrolysate at different molecular weight. International Food Research Journal 22 (2):651–660.

Saidi, S., M. Belleville, A. Deratani and R. Amar. 2013. Optimization of peptide production by enzymatic hydrolysis of tuna dark muscle by-product using commercial proteases. African Journal of Biotechnology 12:651–660.

Sai-Ut, S., S. Benjakul, P. Sumpavapol and H. Kishimura. 2014. Antioxidant activity of gelatin hydrolysate produced from fish skin gelatin using extracellular protease from *Bacillus amyloliquefaciens* H11. Journal of Food Processing and Preservation 39:1–10.

Sanaei, A.V., F. Mahmoodani, S.F. See, S.M. Yusop and A.S. Babji. 2013. Optimization of gelatin extraction and physico-chemical properties of catfish (*Clarias gariepinus*) bone gelatin. International Food Research Journal 20:423–430.

See, S.F., L.L. Hoo and A.S. Babji. 2011. Optimization of enzymatic hydrolysis of salmon (*Salmo salar*) skin by alcalase. International Food Research Journal 18:1359–1365.

Sweetser, W. 2009. The connoisseur's guide to fish and seafood. Sterling Publishing Company, Inc. 50 pp.

Tabarestani, H.S., Y. Maghsoudlou, A. Motamedzadegan and A.R.S. Mahoonak. 2010. Optimization of physico-chemical properties of gelatin extracted from fish skin of rainbow trout (*Onchorhynchus mykiss*). Bioresource Technology 101:6207–6214.

Uriarte-Montoya, M.H., H. Santacruz-Ortega, J. Cinco-Moroyoqui-F, O. Rouzaud-Sandez, M. Plascencia-Jatomea and J.M. Ezquerra-Brauer. 2011. Giant squid skin gelatin: chemical composition and biophysical characterization. Food Research International 44:3243–3249.

You, L., J.M. Regenstein and R.H. Liu. 2010. Optimization of hydrolysis conditions for the production of antioxidant peptides from fish gelatin using response surface methodology. Journal of Food Science 75:582–587.

Received: 24/10/2017; Accepted: 20/09/2018; (AFSJ-2017-0079)