AJAB

Asian J Agric & Biol. 2019; Special Issue:92-99.

Original Article

Optimization and characterization of enzymatic protein hydrolysis of Asiatic hard clam (*Meretrix meretrix*) compared with two other bivalve species

Amir Izzwan Zamri*, Farhana Sukor, Fisal Ahmad, Tuan Zainazor Tuan Chilek, Shamsul Bahri Abd Razak, Qamarul Haziman Abdullah, Najma Farhaten Latiff

Faculty of Fisheries and Food Science,University Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

Received:
March 10, 2019
Accepted:
November 06, 2019
Published:
December 05, 2019

**Corresponding author email:*
amir@umt.edu.my

Abstract

In this study, hydrolysate was produced from Asiatic hard clam (*Meretrix meretrix*) meat by enzymatic hydrolysis using Alcalase® 2.4 L and the optimum hydrolysis condition was determined. Optimization was carried out with face centred central composite design in response surface methodology. The optimum condition of hydrolysis conditions was determined by three levels and four independent variables, which were temperature °C (45, 55, 65°C), enzyme to substrate level, % v/w, (1, 1.5, 2%) time, (60, 120,180 mins) and pH of the substrate (7.5, 8.5, 9.5). Degree of hydrolysis (%) (DH) was chosen as the dependent variable. The optimum conditions for enzymatic hydrolysis of Asiatic hard clam meat were obtained at temperature ºC, enzyme to substrate concentration (ES), hydrolysis time and pH of 65°C, 1%, of 60 minutes and pH 7.5 respectively. In this condition, the predicted and actual value of degree of hydrolysis (DH) obtained were 25.79% and 26.32 ± 0.35% respectively. Suggested model for the enzymatic protein hydrolysis of Asiatic hard clam meat was a two-factor interaction (2FI) model. Asiatic hard clam hydrolysate powder contained of moisture, crude fat, ash, crude protein of 60.09 ± 0.88%, 7.36 ± 0.10%, 2.18 ± 0.29%, and 8.12 ± 0.02% respectively. DH (%) and proximate analysis of hydrolysate from Asiatic Hard clam was compared with hydrolysate of two different species of mollusk which was green mussel (*Perna viridis*) and blood cockle (*Anadara granosa*) from previous studies.

Keywords: Protein hydrolysate, Meretrix meretrix, Hydrolysis

How to cite this:

Zamri AI, Sukor F, Ahmad F, Chilek TZT, Razak SBA, Abdullah QH and Latiff NF, 2019. Optimization and characterization of enzymatic protein hydrolysis of Asiatic hard clam (*Meretrix meretrix*) compared with two others bivalve species. Asian J. Agric. Biol. Special Issue: 92-99.

Introduction

Protein is one of the most essential components for both humans and animals. Protein is affected by its surface structure (Toro and Garcia-Carreno, 2002). Particular functional and organoleptic properties of protein can be obtained by modifying the structure of protein, where hydrolysis is one of the most important protein structure modification processes in the food industry (Toro and Garcia-Carreno, 2002). Protein hydrolysate is the product obtained after the hydrolysis of proteins is achieved by acid, alkali, enzymes and

fermentation methods. Among them, enzymatic hydrolysis method is more preferred because enzymes remain active under mild pH (6-8) and temperature conditions (40-60°C), avoiding the extremes usually required for chemical and physical treatments thus minimizing side reaction (Boscoe and Listow, 2008). Besides, the advantages of the process are more specific, faster rate of reaction and are capable of yields approaching 100%. In protein hydrolysis, the key parameter for monitoring the reaction is the degree of hydrolysis (DH) (Nielsen et al., 2001) and associated with bitterness as a result from the presence of high peptide content. Protein hydrolysate from shellfish is not really commercialized even though the shellfish enzymes have emerged to accelerate seafood industry processes or produce other food products, like fish and shellfish protein hydrolysates and seafood flavourings (Flick Jr, 2009). There are only a few studies of protein from shellfish as most of the previous studies were done on protein from fish resources, probably due to the abundance of protein content in fish resources. Asiatic hard clam (*Meretrix meretrix*) is a bivalve mollusc and found in freshwater, seawater, on intertidal beaches and in very deep water. In Malaysia, this clam can be found at Besut and Setiu in Terengganu. This study aimed to optimize the enzymatic protein hydrolysis conditions of Asiatic hard clam (*Meretrix meretrix*), to determine the proximate composition of Asiatic hard clam meat and hydrolysate and to compare the degree of hydrolysis (%) and proximate value of Asiatic hard clam hydrolysate with hydrolysate from two different species of molluscs which were green mussel (*Perna viridis)* and blood cockle (*Anadara granosa*) from previous studies done by Normah and Nordallila Diyana (2018) and Amiza et al. (2011).

Material and Methods

Material

The Asiatic hard clams (*Meretrix meretrix*) were purchased from Kampung Pengkalan Gelap, Setiu at Terengganu, Malaysia. Alcalase® 2.4 L was purchased from Novozymes (Denmark), is a liquid food grade preparation and has a declared activity of 2.4 Anson U/g and a density of 1.18 g/ml. Alcalase® is an endoproteinase produced by a selected *Bacillus licheniformis*, whose main component is subtilisin Calsberg with a molecular mass of 27.3 kDa. The enzyme was stored at 4°C until they were used for hydrolysis experiment. All reagents used in this work were of analytical grade chemicals were purchased from the local suppliers.

Preparation of Asiatic hard clam (AHC)

The Asiatic hard clam was removed by using a knife to get the clam meat. The clam meat then was rinsed with tap water several times to remove any dirt from it. Then, the clam meat was packed and sealed in polyethylene bags and frozen at -40°C for further analysis.

Preparation of Asiatic hard clam hydrolysate (AHCH) powder

First, the protein content of clam meat was determined by Kjeldahl method (AOAC, 2002). The calculation is necessary because the mass of raw materials and enzyme depend on the protein content of clam meat. The calculation was carried out according to the previous study by Kristinsson and Rasco (2000) as shown below:

a) Mass of protein per batch

$MP = M \times (S\% / 100)$

Where,

MP = the mass of protein

M = the mass of the hydrolysis mixture (in g or kg)

S = substrate concentration (protein) in % w/w of protein

Thus, protein in Asiatic hard clam meat is 10.56%

$S\% = (10.56/100 \times 75) / 150 \times 100 = 5.28\%$

$MP = 150 \times 5.28\% = 7.92$

(b) The mass of raw material will be weighed and mix with a mass of water

$MR = 1.1 \times MP \times (100 / PR\%)$

Where,

MR = Raw material (Asiatic hard clam meat)

PR = Protein contains in Asiatic hard clam meat = 10.56%

Thus,

$MR = 1.1 \times 7.92g \times (100 / 10.56)$

$= 82.5g$

$MW = 1.1 \times (M\text{-}M_{enz}) – MR$

Where,

MW = Mass of water

M_{enz} = The mass of enzyme solution (Alcalase)

Thus,

$MW = 1.1 \times (M – M_{enz}) - MR$

$MW = 1.1 \times (150 – 20g) – 82.5g$

$= 60.5g$

(c) Mass of Alcalase® solution will be needed per batch

ME = ES% / 100 x MP

Where,

ME = Mass of enzyme (g)

ES% = Enzyme to substrate ratio

Thus, If ES% = 1

ME = 1 / 100 x 7.92g = 0.0792g

If ES% = 1.5

ME = 1.5 / 100 x 7.92g =0.1188g

If ES% = 2

ME = 2 / 100 x 7.92g

= 0.1884g

Preparation of Asiatic hard clam hydrolysate (AHCH) was carried out according to Amiza et al. (2011) with some modification. From the calculation, for one run, 82.5g of clam meat was mixed with 60.5g of distilled water and was homogenized by mincing it using a Waring commercial blender at low speed until a viscous type mixture was obtained. The mixture was heated at 85°C for 20 minutes in order to inactivate the endogenous enzymes. At the same time, the desired pH of the mixture was adjusted with addition 1N sodium hydroxide (NaOH) solution and 0.1N (hydrochloric acid) HCl solution. Then, 20g of the Alcalase® enzyme solution was added into the slurry and enzymatic hydrolysis process was started immediately. After the hydrolysis was completed, the sample was heated at temperature 85°C for 20 minutes to inactivate the Alcalase® enzyme activity. Next, the mixture was centrifuged at 4000 rpm for 20 minutes and was freeze dried to obtain the hydrolysate powder.

Optimization

Four independent variables with three levels (temperature (°C, A), enzyme to substrate concentration (%v/w, B), time (minutes, C), and pH (D)) was shown in Table 1 with degree of hydrolysis (DH) was selected as the dependent variable. The experiment was optimized by using central composite design (CCD) with four independent variables at three levels (+1, 0, -1) was performed by applying the Design expert 6.0.10, Stat-Ease Inc. software. It was assumed that the estimated behavioural model of both dependent variables was described by a second-order polynomial equation (1):

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{44} D^2 + \beta_{12} AB + \beta_{13} AC + \beta_{14} AD + \beta 23BC + \beta_{24} BD + \beta_{34} CD \quad (1)$$

The analysis of variance (ANOVA) methods was applied to evaluate the adequacy of the developed mathematical model (by applying the lack-of-fit test) and to evaluate the statistical significance of the factors in the model. In order to examine the goodness and evaluate the adequacy of a fitted model, the coefficient of the determination (R^2) was calculated.

Table-1: Range and level of a parameter that was used in the RSM design

Independent Variable Symbol		Range and Level		
		-1	0	+1
Temperature (°C)	A	45	55	65
Enzyme concentration (E/S, %v/w)	B	1	1.5	2
Hydrolysis time (min)	C	60	120	180
pH	D	7.5	8.5	9.5

Determination of degree of hydrolysis (DH)

Degree of hydrolysis was calculated according to the 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 mixture was homogenized and allowed to stand for 30 minutes to allow precipitation, followed by centrifugation (7800 rcf for 15 min). The supernatant and sample from the hydrolysate were analysed for protein content by Kjeldahl method (AOAC, 2002). Degree of hydrolysis (DH) was calculated using the formula below:

$$\%DH = \frac{\text{Soluble in TCA 10\% w/v}}{\text{Total N in the sample}} \times 100$$

Where,

DH = Degree of hydrolysis;

TCA = Trichloroacetic acid;

N = Nitrogen

2.2.5 Proximate analysis

The proximate analysis was carried out on Asiatic hard clam meat and on the Asiatic hard clam hydrolysate powder prepared using optimum condition. The method was carried out by using AOAC method (AOAC, 2002). The analysis was carried out are moisture analysis, ash analysis, crude protein analysis and crude fat analysis.

Results and Discussion

Table-2: Analysis of variance (ANOVA) for different models

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Model Summary Statistics						
Linear	12.83	0.18	0.05	-0.27	6364.85	
2FI	12.43	0.41	0.10	-1.34	11715.46	Suggested
Quadratic	12.85	0.51	0.04	-1.77	13886.33	
Cubic	13.51	0.74	-0.06	-25.02	130258.11	Aliased

Optimization of enzymatic protein hydrolysis

Overall 30 runs with six replicates at the centre point of design space were carried out in order to find the best hydrolysis condition for the hydrolysate from Asiatic hard clam (*Meretrix meretrix*). Four independent variables with three levels each were chosen, which were temperature °C (45, 55, 65°C), enzyme to substrate level, % v/w, (1, 1.5, 2%) time, min (60, 120,180 mins) and pH of the substrate (7.5, 8.5, 9.5).

Based on Table 2, model 2- factor interaction (2FI) was suggested by the software for the optimization of the hydrolysis condition. According to Liu et al. (2010), the larger the value of F-value and the smaller the value of Prob > F (p-value), the more statistically significant is the corresponding coefficient term. The model with p-value is lower than 0.05 was statistically significant. The result obtained in Table 2 revealed that the 2FI model was suggested to be the most suitable model as the F-value and p-value was 2.56 and 0.048 respectively. Lack of fit is the variation of the data around the fitted model. If the model fit the actual response behavior well, this value was not significant. P-value of lack of fit was 0.6271 which is higher than 0.05, thus indicated it was not significant. The R^2 value of 0.4 indicates that 40% of the variability in the dependent variable could be explained by the model. The adjusted R^2 was 0.1. The closer the adjusted R^2 value to the R^2 advocates for a high significant of the model (Ng et al., 2014).

The final equation which can show the relationship between factors in term of coded was shown in Eq. (2):

$$Y = 82.08 - 1.21A - 4.60C - 5.12D - 6.22AC + 3.62AD + 4.31CD \quad (2)$$

Analysis of response surface

The optimum conditions of enzymatic hydrolysis of AHC meat were at temperature, E/S, time and pH of 65°C, 1.0%, 60 minutes and 7.5 for pH. The predicted value of response DH (%) was 25.79% obtained from the calculation by using the model equation with the highest desirability, which is 0.988. The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value. The value obtained was closed to 1 indicated that the response was close to the ideal value.

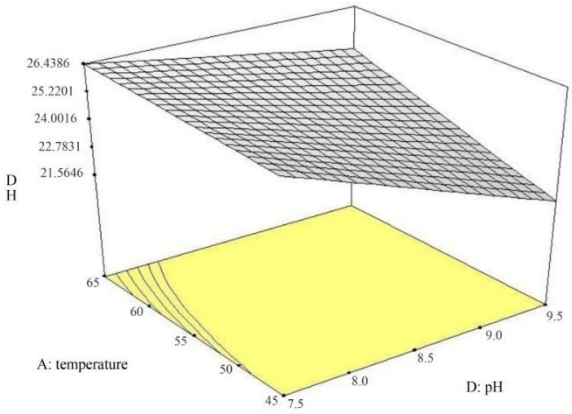

Figure-1: DH as function of temperature and pH during protein hydrolysis of Asiatic hard clam with Alcalase®

As it can be observed from the data presented in Figure 1, an increase of hydrolysis temperature (ºC) led to the increment in the degree of hydrolysis (%). However, DH (%) was decreased as the pH increased. Microbial enzymes such as Alcalase® operating at alkaline pH, have been reported to be the most efficient in the seafood protein hydrolysis (Amiza et al., 2011). From Figure 1, it shows that the reaction started to decrease when the pH was higher than pH 7.5. The enzyme might be denatured and was inactivated at higher pH as reported by Clemente (2000). Besides, it is due to the reduction in peptide bonds capable of being cleaved, competition between the substrate and the hydrolysis products and enzyme denaturation that

decreases its activity. The optimal pH may vary according to the substrate and enzyme concentration used in the hydrolysis (Salwanee et al., 2013). Figure 1 also showed that DH (%) reached its maximum level when the temperature at 65°C. The rate of chemical reaction being catalysed increased as the temperature was increased (Illanes, 2008). This is in agreement with Mukhin and Novikov (2000) as they reported that the exposures of peptide bonds during the enzymatic hydrolysis and in the presence of heat, lead to the increased of the DH (%). Previous study reported the optimum temperature for silver catfish (*Pangasius* sp.) was 55 ºC (Amiza et al., 2011), *Catla viscera* waste was 55°C (Bashkar et al., 2008), and for salmon skin was 55.3°C (See et al., 2011). It shows that the Alcalase® reacts at higher temperature for AHC compared to previous studies (Amiza et al., 2011; Bashkar et al., 2008; See et al., 2011).

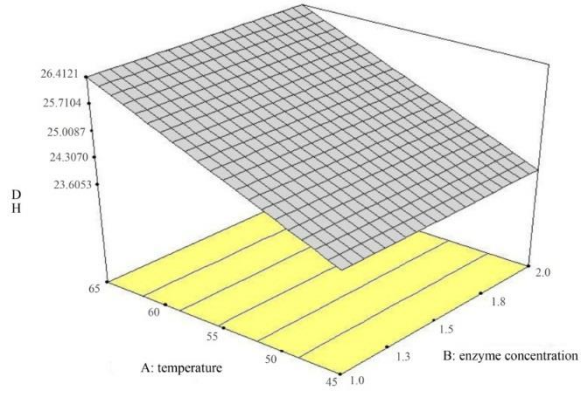

Figure-2: DH as function of temperature and enzyme concentration during protein hydrolysis of Asiatic hard clam with Alcalase®

Figure 2 shows the effect of temperature and enzyme concentration on the DH (%) of AHC hydrolysis. From the results presented, the DH (%) increased as the temperature and enzyme concentration increased. As stated by Amiza et al. (2011), the chances for the hydrolysis to occur are higher as enzymes molecule present in enzyme to substrate ratio increased in higher concentration of enzyme. The result obtained might be due to the increased of smaller peptides and amino acids present in the hydrolysate. Figure 3 shows the response surfaces and their corresponding contours of the combined effect of hydrolysis time and pH on DH using Alcalase®. As it can be seen, DH (%) started to decrease as the time and pH were increased. The decreasing of DH (%) might be due to the decrease in enzyme activity, substrate saturation, product inhibition or extreme pH concentration that might cause the denaturation of enzyme.

In order to verify the optimized model, validation test was performed to determine the adequacy of the suggested models. According to the 2FI model suggested, the predicted value for DH (%) was 25.79% and the optimum conditions were at 65°C, 1% E/S, 60 min and pH 7.5. The hydrolysis was repeated in 5 replicates following the same conditions suggested in optimization. The DH (%) obtained was 26.32 ± 0.35 %.

Independent sample T-test was conducted to verify the optimized result. P-value obtained was 0.234, indicated the null hypothesis (H_0: μ = 25.79%) were accepted at the 0.05 α-level. The mean degree of hydrolysis (%) of the experimented value showed no significant difference to the predicted value. Hence, the optimum condition of hydrolysis from Asiatic hard clam suggested by RSM software was verified and could be used for further research.

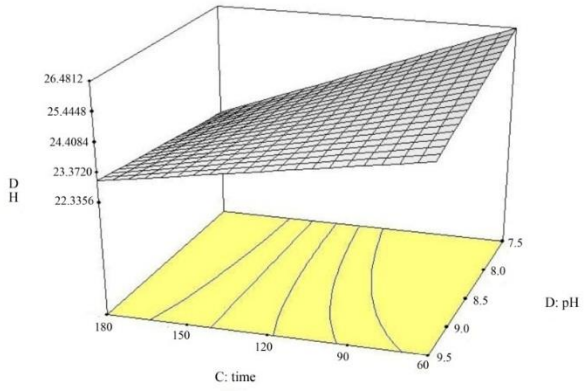

Figure-3: DH as function of time and pH during protein hydrolysis of Asiatic hard clam with Alcalase®

Comparisons on the degree of hydrolysis (%) between Asiatic hard clam meat hydrolysate with the different species of mollusk hydrolysate.

Hydrolysate was extracted from Asiatic hard clam (*Meretrix meretrix*) meat at the optimum hydrolysis condition (65°C, 1% E/S, 60 min and pH 7.5) obtained from the optimization step by response surface methodology (RSM). The degree of hydrolysis DH (%) was calculated and compared with other two different species from the previous studies (green mussel and cockle meat). DH (%) obtained in this study was 26.43%. Normah and Nurdalila Diyana (2018) hydrolyze protein from green mussel (*Perna*

viridis) by using flavorzyme at pH 8, E/S 3%, time 120 mins and temperature 50ºC. The study aimed to evaluate the effect of using flavorzyme and the addition of sodium tripolyphosphate (STPP) and sodium chloride (NaCl) on the development of umami flavor in green mussel hydrolysate. Degree of hydrolysis (%) for green mussel hydrolysate was done by pH-stat method. DH (%) value obtained for the hydrolysate hydrolyzed by using enzyme flavorzyme was lower than the hydrolysate hydrolyzed by the addition of STPP and NaCl (23.18%). The DH (%) obtained for Asiatic hard clam hydrolysate hydrolyzed by Alcalase was higher than those obtained by Normah and Nurdalila Diyana (2018). This study was in agreement with Sbroggio et al. (2016) where it showed hydrolysate hydrolyzed by Alcalase and Flavorzyme presented different DH (%) values of 33.6% and 5.8%, respectively. Type of enzyme used as enzyme type used during the hydrolysis process affects the DH (%) (Liu et al., 2010). This is because alcalase is an endopeptidase enzyme and has a range of specificity of peptide bonds for hydrolysis, where flavorzyme is a mixture of an endoprotease and exopeptidase, produced by *Aspergillus oryzae*, giving it a broader range of action and thus a higher DH is expected when compared with hydrolysis by alcalase (Pedroche et al., 2002).

On the other hand, DH (%) of hydrolysate from Asiatic hard clam meat was lower compared to the hydrolysate from cockle (*Anadara granosa*) meat (Amiza and Masitah, 2012). Study from Amiza and Masitah (2012) aimed to optimize the enzymatic protein hydrolysis from blood cockle in terms of hydrolysis time, hydrolysis temperature, hydrolysis pH and concentration of enzyme to achieve the maximum degree of hydrolysis (DH). Degree of hydrolysis was determined by pH-stat method and the enzyme used in the hydrolysis was Alcalase®. The statistical results suggested that quadratic model was the most suitable model for DH. The optimum condition of the enzymatic hydrolysis of blood cockle were found to be at 65ºC, pH 9.5, enzyme concentration at 2% and hydrolysis time of 180 minutes. DH (%) of hydrolysate from blood cockle obtained was 37.27%. DH (%) of hydrolysate from Asiatic hard clam meat was lower compared to the hydrolysate from cockle (*Anadara granosa*) meat which was 26.43% and 37.27% respectively. DH (%) of hydrolysate depends on the reaction time and the type of enzyme. DH (%) of hydrolysate from blood cockle (time: 180 mins) is higher compared to hydrolysate from Asiatic hard clam (60 mins) as the hydrolysis time is longer a there are higher peptide fragments are released during protein hydrolysis (Sbroggio et al., 2016)

Proximate composition

The proximate composition of Asiatic hard clam (AHC) meat and Asiatic hard clam hydrolysate (AHCH) were shown in the Table 3 (based on dry matter). Protein content of AHC meat and AHCH were 10.53 ± 0.04% and 60.09 ± 0.88%, respectively. The higher protein content in AHCH compared with the AHC meat was probably due to solubilization of proteins during hydrolysis and removal of insoluble solid matter by centrifugation (Liceaga- Gesualdo and Li-Chan, 1999; Chalamaiah et al., 2010).

According to See et al. (2011), the crude protein content can be used as an indicator of the purity of the protein hydrolysate. The ash content of the AHC meat and AHCH were 1.98 ± 0.82% and 7.36 ± 0.1%, respectively. The high ash content might be caused by the addition of NaOH and HCl during enzymatic hydrolysis. Fat content for AHC meat and AHCH were 8.23 ± 0.09% and 2.18 ± 0.29% respectively. The low-fat content of Asiatic hard clam hydrolysates is due to the removal of lipids and insoluble protein fractions by centrifugation (Chalamaiah et al., 2012). Low fat content in protein hydrolysate is desirable as it contributes to lipid oxidation stability (Motamedzadegan et al., 2010), thus can ensure the stability of sample during storage (See et al., 2011). Moisture content (%) of AHC meat and AHCH were 71.92 ± 1.76% and 9.12 ± 0.02% respectively. The low moisture content of protein hydrolysates is depending on the type of sample and the effect of freeze drying. During the process of freeze drying, the sample loses most of its moisture (Bueno-Solano et al., 2009).

Table-3: Proximate composition of the Asiatic hard clam meat and Asiatic hard clam hydrolysate

	Asiatic hard clam meat (%)	Asiatic hard clam hydrolysate (%)
Protein	10.53 ± 0.04*	60.09 ± 0.88
Ash	1.98 ±0.82	7.36 ± 0.1
Fat	8.23 ± 0.89*	2.18 ± 0.29
Moisture	71.92 ± 1.76	8.12 ± 0.02

Comparison of proximate profiles between the hydrolysate from Asiatic hard clam to the two different mollusk

The proximate analysis data for the hydrolysate from the three different species of mollusc were presented in Table 4. Protein content (%) of hydrolysates from cockle was the highest compared the other two species. This is probably due to the hydrolysis reaction time. As hydrolysis time for hydrolysates from cockle was the highest (180 mins) compared with Asiatic hard clam (60 mins) and Green mussel (120mins), higher amount of protein was solubilized during hydrolysis. Ash content (%) for cockle hydrolysates was the highest, followed by green mussel hydrolysate and Asiatic hard clam hydrolysates.

Table-4: Proximate composition of the hydrolysate from three different species

	Type of species		
Analysis (%)	**Asiatic Hard Clam**	**Green mussel**	**Cockle**
Protein content	60.09± 0.88	64.52± 0.24	74.00 + 0.57
Ash content	7.36± 0.1	9.42± 075	10.22+ 0.68
Fat content	2.18± 0.29	3.79± 0.69	5.80 + 0.91
Moisture content	8.12± 0.02	8.97± 0.12	8.59 + 0.08

The enzymatic hydrolysis and freeze-drying process had increased the protein, ash and fat content. One of the main factors affects the ash content is the usage of added acid or base for adjustment of pH of medium. Higher amount of acid/base used leads to higher ash content (%). Amiza and Masitah (2012) stated that NaOH was added during enzymatic hydrolysis to maintain the specified pH throughout the hydrolysis. It is assumable that the NaOH used in hydrolysis of cockle was higher compared with hydrolysates from the other two species. Fat content (%) of Asiatic hard clam hydrolysate was the lowest as compared with the other two species. Protein hydrolysate from Asiatic hard clam was the most desirable among the three as it contributes to lipid oxidation stability (Motamedzadegan et al., 2010), thus ensuring the stability of sample during storage. The moisture content (%) for the hydrolysates from the three species were closed to each other (<10%) which were suitable for the storage.

Conclusion

The hydrolysis condition of Asiatic hard clam meat by Alcalase® was optimized by using response surface methodology (RSM). According to the two-factor interaction (2FI) model suggested, the optimum conditions suggested were temperature, enzyme to substrate concentration, hydrolysis time and pH, at 65°C, 1%, 60 minutes and 7.5, respectively. The actual value of degree of hydrolysis obtained under these optimum conditions was 26.32 ± 0.35% which was closed to the predicted value of 25.79%. With these optimum hydrolysis conditions, the protein hydrolysate obtained composed of protein, ash content, fat and moisture of 60.09 ± 0.88%, 7.36 ± 0.10%, 2.18 ± 0.29% and 8.12 ± 0.02%, respectively. The proximate composition of the hydrolysate from Asiatic hard clam was compared with the two species.

Contribution of Authors

Zamri AI: Literature search, Statistical analysis
Sukor F: Data collection and manuscript writing, Data interpretation and mathematical calculations
Ahmad F: Statistical analysis
Chilek TZT: Conceived idea and manuscript final approval
Razak SBA: Manuscript final reading and approval, Data interpretation
Abdullah QH: Manuscript writing, Designed research methodology
Latiff NF:

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: None.

References

Amiza MA and Masitah M, 2012. Optimization of enzymatic hydrolysis of blood cockle (*Anadara granosa*) using Alcalase®. Borneo Sci. 31. 1-10.

Amiza M, Nurul Ashikin S and Faazaz A, 2011. Optimization of enzymatic protein hydrolysis from silver catfish (*Pangasius sp.*) frame. Int. Food Res. J. 18: 775-781.

AOAC, 2002. Official Method of Analysis. 16th Edition. Association of Official Analytical, Washington DC, USA.

Bashkar N, Benilla T, Radha C and Lathila R, 2008. Optimization of enzymatic hydrolysis of visceral waste protein of Catla (*Catla catla*) for preparing protein hydrolysate using commercial protease. Bioresour. Technol. 99 (2): 335-343.

Boscoe AB and Listow CR, 2008. Protein Research Progress. New York: Nova Science Publishers, Inc. p. 257.

Bueno-Solano C, López-Cervantes J, Campas-Baypoli ON Lauterio-García R, Adan-Bante NP and Sánchez-Machado DI, 2009. Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. Food Chem. 112(3): 671-675.

Chalamaiah M, Rao GN, Rao DG and Jyothirmayi T, 2010. Protein hydrolysates from meriga (*Cirrhinus mrigala*) egg and evaluation of their functional properties. Food Chem. 120(3): 652-657.

Chalamaiah M, Hemalatha R and Jyothirmayi T, 2012. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chem. 135(4): 3020-3038.

Clemente A, 2000. Enzymatic Protein Hydrolysates in Human Nutrition. *Trends Food* Sci. *Tech*. 11: 254-262.

Flick Jr GJ, 2009. Enzymes in seafood. Part III. New applications lead to new products. Glob. Aquacult. Allian. 12(5): 47-49.

Hoyle NT and Merrit JH, 1994. Quality of fish protein hydrolysate from Herring (*Clupea harengus*). J. Food Sci. 59: 76-79.

Illanes A, 2008. Enzyme biocatalysis. Principles and Applications. Editorial Springer-Verlag New York Inc., United States. pp. 1-56.

Kristinsson HG and Rasco BA, 2000. Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle proteins hydrolysed with various alkaline proteases. J. Agric. Food Chem. 48: 657-666.

Liceaga-Gesualdo AM and Li-Chan ECY, 1999. Functional properties of fish protein hydrolysate from herring (*Clupea harengus*). J. Food Sci. 64(6): 1000-1004.

Liu Y, Zheng Y and Wang A, 2010. Response Surface Methodology for Optimizing Adsorption Process Parameters for Methylene Blue Removal by a Hydrogel Composite. Adsorpt Sci Technol. 28. 10: 913-922.

Motamedzadegan A, Davarniam B, AsadiG, Abedian AM and Ovissipour M, 2010. Optimization of enzymatic hydrolysis of Yellowfin Tuna (*Thunnus albacares*) viscera using Neutrase. Int. Aquatic Res. 2: 173-181.

Mukhin VA and Novikov VY, 2001. Enzymatic hydrolysis of proteins from Crustaceans of the Barents Sea. Prikl. Biokhim. Mikrobiol. 37(5): 633-638.

Nielsen P, Petersen D and Dambmann C, 2001. Improved method for determining food protein degree of hydrolysis. J. Food Sci. 66(5): 642-646.

Normah I and Nurdalila Diyana MR, 2018. Evaluation of umaminess in green mussel hydrolysate (Perna viridis) produced in the presence of sodium tripolyphosphate and NaCl. Int. Food Res. J. 25(6): 2524-2530.

Pedroche J, Yust MM, Giron-Calle J, Alaiz M, Millán F and Vioque J, 2002. Utilisation of chickpea protein isolate for production of peptides with angiotensin I-converting enzyme (ACE)-inhibitory activity. J. Sci. Food Agric. 82(9): 960- 965.

Salwanee S, Wan Aida WM, Mamot S, Maskat MY and Ibrahim S, 2013. Effects of Enzyme Concentration, Temperature, pH and Time on the Degree of Hydrolysis of Protein Extract from Viscera of Tuna (*Euthynnus affinis*) by Using Alcalase. Sains Malays. 42 (3): 279–287.

See S, Hoo L and Babji A, 2011. Optimization of enzymatic hydrolysis of Salmon (*Salmo salar*) skin by Alcalase. Int. Food Res. J. 18 (4): 1359-1365.

Sbroggio MF, Montilha MS, Figueiredo VRGD, Georgetti SR and Kurozawa LE, 2016. Influence of the degree of hydrolysis and type of enzyme on antioxidant activity of okara protein hydrolysates. Food Sci. Technol. 36(2): 375-381.

Toro MA and Garcia-Carreno FL, 2002. Current Protocols in Food Analytical Chemistry. La Paz: John Wiley & Sons, Inc. pp. B2.2.1-B2.2.14.