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BIOCHEMICAL PROPERTIES AND PROXIMATE COMPOSITION OF CATFISH ENZYMATIC PROTEIN HYDROLYSATES MADE USING SUBTILISIN

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Abstract—**This study was designed to facilitate enzymatic protein hydrolysis of catfish (*Clarias batrachus*) using subtilisin. The effect of incubation time (30, 60, 120, 180 min) with enzyme concentration of 1% (v/w substrate); pH = 8.0; 55°C was studied to determine the degree of hydrolysis (DH), peptide content, amino acid profile and proximate composition of catfish protein hydrolysate (CPH). Results showed that CPH obtained with maximum incubation time had the highest DH; 59% and peptide content; 7200 µg/ml. The DH and peptide content of CPH significantly increased ($p < 0.05$) as hydrolysis time increases from 0 min to 180 min. The major amino acids of CPH were Glu, Lys and Asp. CPH showed significantly higher ($p < 0.05$) crude protein content and lower fat, moisture and ash content compared to raw catfish. From the findings, *C.batrachus* protein hydrolysates may contribute as an excellent source of amino acids, bioactive peptides and proteins.**

Key words— **Enzymatic hydrolysis, substilisin, catfish, biochemical properties, proximate composition.**

I. INTRODUCTION

Clarias batrachus or commonly known in Malaysia as *Keli* is one of the high demand freshwater fishes contributing by its unique taste and cheaper price as compared to other freshwater fishes. According to Department of Fisheries Malaysia (2012), the total amount of catfish production in year 2012 was 46,523 metric tons. The sale of catfish produced in Malaysia in year 2012 earned RM 189 million out of the total aquaculture fish production of RM 992 million [1]. Though catfish is on a high demand in Malaysia, yet its commercial value is low. This is due to the utilization of cultured catfish is still limited to local consumption. Hence, an alternative to enhance the commercial value of catfish is by generating catfish protein hydrolysates. These protein hydrolysates can be a source of raw material for various industries.

Protein hydrolysis refers to all possible process in which the protein is broken down to produce lower molecular weight peptides [2]. Hydrolysis decreases the peptide size, making hydrolysates the most available amino acid source for protein biosynthesis [3]. The use of protein hydrolysate as ingredients for animal feed or human consumption dates back to the 1960–1970s [4,5,6]. Nowadays the applications of protein hydrolysates have expanded in a variety of industries, including pharmaceuticals, nutraceuticals, cosmetics or animal nutrition. Protein hydrolysates are also useful for maintaining the growth of different microorganisms [7]. Recently, fish protein hydrolysates have become popular in the food industry due to the high protein content. Because some proteins possess a range of dynamic functional properties, fish protein hydrolysates exhibit a wide spectrum of physicochemical behavior such as emulsifying activity, foamability and moisture sorption [8]. With the increasing knowledge regarding the functional properties of fish protein hydrolysates, there are many researchers conducting studies on the developments and applications of fish-derived bioactive peptides as functional foods and nutraceuticals [9]. During the last decade, several studies have shown that fish protein hydrolysates or peptides possess biological properties such as inhibition of angiotensin-I-converting enzyme, antioxidant, immunomodulatory and antimicrobial activities [10,11,12,13,14].

The use of commercial enzyme is preferred in protein hydrolysis since the hydrolysis process and the properties of resulting products such as the peptide chain length of the hydrolysates can be controlled [15]. A number of different enzymes have been used for hydrolysis of fish [16]. There are plant-derived enzymes such as papain [13] and animal-originated enzymes for example trypsin [17]. Enzymes of microbial source have been also applied. Subtilisin is an alkaline proteinase originating from *Bacillus subtillis* [18]. From a technical and economic point of view, enzymes from microbial sources operating at alkaline pH were shown to be one of the most efficient in the hydrolysis of fish proteins [19].

This study was designed to facilitate enzymatic protein hydrolysis of catfish (*Clarias batrachus*) muscle using subtilisin in order to characterise its biochemical properties and proximate composition as affected by hydrolysis time.

II. MATERIALS AND METHODS

A. Materials

Clarias batrachus, sized 0.3 - 0.5 kg were bought from Bangi Wet Market, Selangor. AccQ Fluor reagent kit and AccQ Tag eluent were obtained from Sigma (Sigma-Aldrich Chemical Co., USA). Amino acid standards were purchased from Pierce (Rockford, IL, USA). Subtilisin and other chemicals of analytical grade were purchased from Sigma (Sigma–Aldrich).

B. Protein Hydrolysis

For enzymatic hydrolysis, catfish muscle was mixed with distilled water (2% w/v). The mixture was adjusted to the enzyme pH of 8 and temperature of 55°C as suggested by manufacturer. The sample was incubated for 30, 60, 120 and 180 min with subtilisin ratio of 1% (w/w).). Each solution was heated at 90°C for 10 min to inactivate the protease and centrifuged at 3000 g for 20 min at 4°C to remove the insoluble materials. The supernatant was lyophilized and stored at -80°C for further experiments.

C. Degree of Hydrolysis

Degree of hydrolysis (DH) was calculated as described by Hoyle & Merit [20]. After hydrolysis, 20 ml of FPH 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 the Kjeldahl method [21]. The degree of hydrolysis (DH) was computed as the formula below:

DH (%) = (10% TCA soluble nitrogen in the sample /Total nitrogen in the sample) x 100%

D. Peptide Content

The peptide contents of catfish protein hydrolysates (CPH) were measured by the method of Church et al. [22] using o-phthaldialdehyde (OPA) spectrophotometric assay. 50 ml of fresh OPA reagent was prepared by mixing 25 ml of 100 mM sodium tetra hydroborate, 2.5 ml of 20% (w/w) sodium diodecyl sulphate, 40 mg of OPA solution (dissolved in 1 ml of methanol) and 100 ml of b-mercaptoethanol and then adjusted the volume to 50 ml with deionized water. 50 µl of CPH, containing 5–100 µg protein, was mixed with 2 ml of OPA reagent and incubated for 2 min at room temperature. The absorbance at 340 nm was measured with a spectrophotometer (Model UV-160A, Shimadzu, Kyoto, Japan). Leucine was used as a standard to quantify the peptide content.

E. Amino Acid Profile

Amino acid composition of purified hydrolysate was measured according to Alaiz et al. [23]. Samples were hydrolysed with 5 ml 6 N hydrochloric acid (HCl) in a closed test tube, shaken for 20 min and then kept in oven for 24 h at 110°C. Internal standard a-aminobutyric acid was added to the hydrolysed samples and filtered through a nylon 0.2 mm cellulose acetate membrane filter (Whatman No. 1). Derivatization of amino acid was done at 55°C in a heating block for 10 min. Separations of amino acid was carried out on a C18 AccQ-Tag amino acid analysis column (150 x 3.9 mm, Waters, USA). The column temperature was set at 37°C with a flow rate set at 1 ml/min. AccQ Tag Eluent A and AccQ Tag Eluent B or 60% acetonitrile acid was used as the mobile phase. The UV detector was operated at 248 nm (for peak identification), and the fluorescence detector was with a 250 nm excitation and a 395 nm emission wavelength (for amino acid quantification). The hydrolysed sample was then analysed using an automatic amino acid analyser (Waters Corporation, USA) equipped with Waters 717 Autosampler. Alkaline hydrolysis was also done for determination of tryptophan level.

F. Proximate Composition

Crude protein content was determined using Kjedahl method, AOAC 981.10 [21]. One gram of sample, one Kjeltec catalyst tablet and 10mL H_2SO_4 were put into Kjedahl tube and digested for 2 hr at 420°C. The product was then made basic with 30% (w/v) NaOH before distillation into 0.1M HCl and titration against 0.25M NaOH. The factor used to convert nitrogen into crude protein was 6.25. Moisture content was determined with a modified version of the AOAC 925.04 [21]. 10g of sample was oven-dried at 105oC for 24 hr and the water content of the sample was gravimetrically determined. Fat content was determined by using Soxhlet extractor (Behrotest, Behr Labor Technik Gro bH, Dusseldorf, Germany). The dried sample was inserted into a Soxhlet tube and petroleum ether was recycled though the sample for 2 hr. Remaining ether was evaporated and the sample was dried at 105°C overnight. Fat content was then calculated gravimetrically. The ash content was analyzed using a modified version of AOAC 938.08 [21]. The water and fat free sample was combusted in the furnace at 550°C for 12 hr and the ash content was determined gravimetrically.

G. Statistics

Each treatment was conducted in triplicate. The results were presented as mean ± standard deviation and subjected to an analysis of variance (SPSS 14, SPSS Inc., 2005) in order to determine significant statistical differences among samples. Data analysis was carried out using One-way Analysis of Variance (ANOVA) and the mean comparisons were run by Duncan's multiple range test. The differences in means between the samples were determined at the 5% confidence level ($P < 0.05$).

III. RESULTS AND DISCUSSION

Compared to animal or plant derived enzymes, microbial enzymes offer several advantages, including a wide variety of available catalytic activities, and higher pH and stability of temperature [24]. Subtilisin or its commercial name; Alcalase®, is an alkaline bacterial protease which has proven to be one of the best enzymes used for producing fish protein hydrolysates [13,16,20,24]. Fish protein hydrolysates produced by subtilisin tend to have less bitter components compared to those made with papain [20]. The cost of the enzyme may influence the economy and commercial viability of the process [13], and subtilisin may exhibit a lower cost per unit of enzyme activity compared to other enzymes that could be utilized [24].

Properties of protein hydrolysates are mainly dependent on the primary structure of the peptide residuals as a consequence of the substrate protein, the substrate pretreatment, the proteolytic enzymes, and the hydrolysis conditions. A careful choice of process parameters is essential to successful hydrolysis [25]. The extent of hydrolysis is monitored using degree of hydrolysis. DH is defined as the percentage of the total number of peptide bonds in a protein which have been cleaved during hydrolysis [26]. In current study, the degree of hydrolysis of *C.batrachus* depends on the reaction time (Fig. 1). The highest DH value of 59% was obtained at maximum incubation time (180 min) for subtilisin-assisted hydrolysis. The DH values were significantly increased ($p < 0.05$) with increasing hydrolysis time. The result was in agreement with previous studies. Study by Herpandi et al. reported the degree of hydrolysis of skipjack flesh treated with Alcalase increased linearly with the increment of hydrolysis time [27]. The DH of yellow stripe trevally was found to increase with longer the incubation time and higher enzyme concentration [28]. Norma et al. reported the same conclusion on the hydolysis of threadfin bream by Alcalase [29].

The increasing DH value resulted from dynamic hydrolysis kinetics. Subtilisin-assisted hydrolytic curve of *C.batrachus* (Fig. 1) exhibited an initial fast reaction rate. Thereafter, the rate of enzymatic hydrolysis decreased and reached a stationary phase. Such shapes of hydrolysis curves were similar to those previously published for salmon muscle [30] and cod muscle [31]. In the initial period of hydrolysis, a large number of peptide bonds were cleaved, leading to an increase of soluble peptides in the reaction mixture. Meanwhile in the second stage, the reaction speed decreases. This may due to some enzyme partial inactivation [32]. In addition, an increase of peptides may also act as effective substrate competitors to the undigested or partially digested proteins [33]. Quantitatively the peptide content of CPH was affected by

hydrolysis time (Fig. 2). At maximum incubation time (180 min), the peptide content of subtilisin-treated CPH was 7200 µg/ml. The result also indicated that increasing hydrolysis time from 0 min to 180 min increased the peptide content of

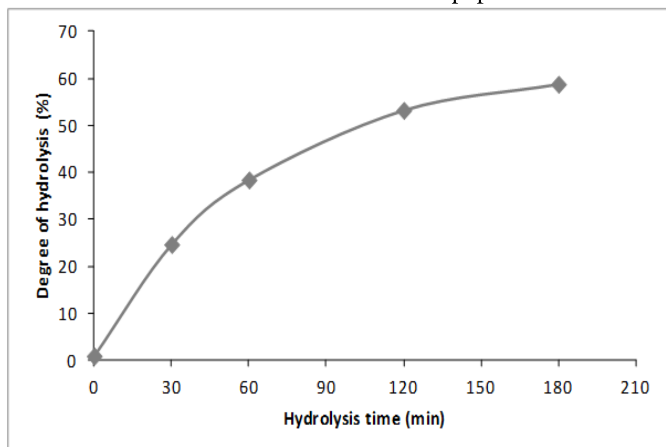

FIGURE 1. Effect of hydrolysis time on degree of hydrolysis of *C.batrachus* protein.

CPH. From these results, we infer that the peptide content increased with increasing hydrolysis time as more peptides are released as a result of proteolytic enzymes activity.

Table 1 showed the amino acid composition of CPH. The hydrolysates were rich in glutamic acid, lysine and aspartic acid. Studies by Ghassem et al. and Hou et al. found glutamic acid and aspartic acid to be higher compared to other amino acids in the reported fish protein hydrolysates [34,35]. CPH samples contain the essential and nonessential amino acids. Fish muscle hydrolysates produced by other researchers are reported to have all the essential and nonessential amino acids [34,35,36]. Based on the total amino acids, the composition of essential amino acids are 42.85%, 43.61%, 43.92% and 44.05% of the CPH incubated for 30 min, 60 min, 120 min and 180 min respectively. Several studies have reported high essential amino acids composition of the fish protein hydrolysate including those of herring [8] and round scad muscle hydrolysate [12].

Table 2 presented the proximate composition of raw catfish and CPH at different hydrolysis time. The protein content of CPH was significantly higher ($p < 0.05$) than that of the raw catfish sample. The high protein content reported for fish protein hydrolysates is due to solubilization of proteins during hydrolysis and removal of insoluble solid matter by centrifugation [8,37]. There was no significant difference in protein content between all CPH samples. Similar trend was reported in the sardinella byproducts hydrolysate [38] as well as Pacific whiting muscle hydrolysate [39]. The amount of protein content in spray-dried sardinella byproducts hydrolysate and freeze-dried Pacific whiting muscles hydrolysate were within the range of 72 – 75% and 85 – 88% respectively. The fat, moisture and ash content of CPH was significantly lower ($p < 0.05$) than raw *C.batrachus*. Other studies also reported low fat, moisture and ash contents in fish hydrolysates [12,39,40]. The low fat content of fish protein hydrolysates is due to the removal of lipids and insoluble protein fractions by centrifugation [37]. The low moisture content of protein hydrolysates is related to the type of sample and the effect of freeze drying. During the process, the sample

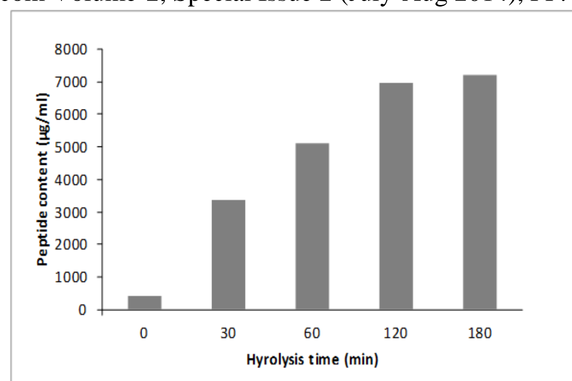

FIGURE 2. Effect of hydrolysis time on peptide content of *C.batrachus* protein.

Amino acid (%)	CPH30	CPH60	CPH120	CPH180
Aspartic acid	9.06 ± 0.01	9.17 ± 0.05	9.41 ± 0.25	9.86 ± 0.01
Serine	5.50 ± 0.05	5.45 ± 0.04	5.51 ± 0.04	4.15 ± 0.10
Glutamic acid	13.66 ± 0.22	13.86 ± 0.22	14.40 ± 0.20	15.66 ± 0.22
Glycine	7.66 ± 0.02	7.65 ± 0.01	6.96 ± 0.11	5.66 ± 0.04
Histidine	2.21 ± 0.06	2.21 ± 0.04	2.21 ± 0.19	2.21 ± 0.01
Arginine	6.42 ± 0.14	6.50 ± 0.05	6.52 ± 0.15	6.61 ± 0.14
Threonine	4.76 ± 0.03	4.71 ± 0.04	4.75 ± 0.04	4.60 ± 0.07
Alanine	6.02 ± 0.15	6.52 ± 0.13	5.62 ± 0.63	5.29 ± 0.05
Proline	5.53 ± 0.07	4.60 ± 0.08	4.12 ± 0.04	4.53 ± 0.97
Tyrosine	3.04 ± 0.00	3.03 ± 0.04	3.53 ± 0.04	4.04 ± 0.14
Valine	6.00 ± 0.08	5.98 ± 0.10	5.77 ± 0.13	5.48 ± 0.08
Methionine	3.03 ± 0.05	3.03 ± 0.07	3.08 ± 0.13	3.20 ± 0.06
Lysine	10.05 ± 0.04	10.06 ± 0.03	10.26 ± 0.39	10.35 ± 0.09
Isoleucine	4.23 ± 0.14	4.37 ± 0.08	4.47 ± 0.08	4.86 ± 0.23
Leucine	7.08 ± 0.05	7.35 ± 0.23	7.75 ± 0.12	8.08 ± 0.46
Phenylalanine	4.33 ± 0.00	4.25 ± 0.03	4.20 ± 0.01	4.13 ± 0.00
Cysteine	0.37 ± 0.01	0.35 ± 0.00	0.32 ± 0.00	0.17 ± 0.05
Tryptophan	1.05 ± 0.07	1.10 ± 0.09	1.12 ± 0.09	1.11 ± 0.18
TAA	100	100	100	100
TEAA	42.85	43.61	43.92	44.05

TABLE 1. Amino acid profile of *C.batrachus* protein hydrolysates.

TAA, total amino acids; TEAA, total essential amino acids
CPH30, 30min incubated CPH; CPH60, 60min incubated CPH; CPH120, 120min incubated CPH; CPH180, 180min incubated CPH

loses most of its moisture [41]. In general, there were no significant differences in fat, moisture and ash content between all CPH samples with different hydrolysis time. CPH samples contained 0.95 – 1.06% fat content. This result was in agreement to the study of Pacific whiting muscles and

sardinella byproducts hydrolysates, which reported no significant different in fat content between different DH [38,39]. CPH possessed low ash content, which is in the range of 0.70-0.72%. The low ash content of samples might due to the minimal addition of alkali required for pH adjustment and its control during the hydrolytic process.

Hydrolysis time (min)	Proximate composition (%)			
	Crude Protein	Fat	Moisture	Ash
Control	20.32 ± 1.42[b]	4.75 ± 0.61[a]	76.85 ± 0.17[a]	1.09 ± 0.04[a]
30	83.94 ± 0.52[a]	0.95 ± 0.06[b]	3.02 ± 0.12[b]	0.70 ± 0.05[b]
60	84.69 ± 0.55[a]	0.97 ± 0.05[b]	3.09 ± 0.46[b]	0.71 ± 0.08[b]
120	85.02 ± 0.24[a]	1.00 ± 0.08[b]	3.13 ± 0.12[b]	0.72 ± 0.06[b]
180	85.40 ± 0.62[a]	1.06 ± 0.08[b]	3.18 ± 0.12[b]	0.71 ± 0.05[b]

TABLE 2. Proximate compositions of raw *C.batrachus* and catfish protein hydrolysates.

Control, raw catfish.
[a b] Means with different superscripts within a column are significantly different (P<0.05)

IV. Conclusion

Degree of hydrolysis and peptide content of CPH were significantly affected by hydrolysis time. In contrast, the amino acids profile and proximate composition of CPH samples were not significantly affected by incubation time. Nevertheless *C.batrachus* protein hydrolysates prepared by subtilisin demonstrated a high protein content as well as high peptide and amino acid content. Therefore, the CPH may potentially serve as a good source of useful nutrients and bioactive peptides for application in health, food and other industries.

References

[1] Department of Fisheries Malaysia. "Estimated aquaculture production from freshwater culture system by species 2012," 2012, *http://www.dof.gov.my/web/guest/perangkaan-perikanan-tahunan-2012*
[2] B. Lieske and G. Konrad, "Protein hydrolysis – the key to meat flavoring systems," Food Rev. Int., vol. 10, pp. 287-312, 1994.
[3] A. Gildberg and E. Stenberg, "A new process for advanced utilization of shrimp waste," Process Biochem., vol. 36, pp. 809–812, 2001.
[4] C. W. Keyes and W. W. Meinke, Method of processing fish, US Patent 3 249 442, 1966.
[5] U. H. Bertullo and C. R. Pereira, Protein hydrolysis, US Patent 3 516 349, 1970.
[6] M. Rutman, Process for preparing high energy fish protein concentrate, US Patent 3 561 973, 1971.
[7] H. Duarte de Holanda and F. M. Netto, F. M., "Recovery of components from shrimp (Xiphopenaeus kroyeri) processing waste by enzymatic hydrolysis," J. Food Sci., vol. 71, pp. 298–303, 2006.
[8] A. M. Liceaga-Gesualdo and E. C. Y. Li-Chan, "Functional properties of fish protein hydrolysate from herring (*Clupea harengus*). J. Food Sci., vol. 64, pp. 1000–1004, 1999.
[9] L. Najafian and A. S. Babji, "A review of fish-derived antioxidant and antimicrobial peptides: their production, assessment, and applications," Peptides, vol. 33, pp. 178-185, 2012.
[10] K. Arihara, "Functional Foods" in Encyclopedia of Meat Sciences, edited by W.K. Jensen, C. Devine and M. Dikeman, Oxford: Elsevier, 2004, pp. 492–499.
[11] S. Raghavan and H. G. Kristinsson, "ACE-inhibitory activity of tilapia protein hydrolysates," Food Chem., vol. 117, pp. 582–588, 2009.
[12] Y. Thiansilakul, S. Benjakul and F. Shahidi, "Antioxidative activity of protein hydrolysate from round scad muscle using Alcalase and Flavourzyme," Food Biochem., vol 31, pp. 266–287, 2007.
[13] F. Shahidi, X. Q. Han and J. Synowiecki, "Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*)," Food Chem., vol. 53, pp. 285-293, 1995.
[14] G. Bergsson, B. Agerberth, H. Jornvall and G. H. Gudmundsson, "Isolation and identification of antimicrobial components from the epidermal mucus of Atlantic cod (Gadus morhua)," J. FEBS, vol. 272, pp. 4960–4969, 2005.
[15] S. Benkajul and M. T. Morrissey, "Protein hydrolysate from pacific whiting solid waste," J. Agric. Food Chem., vol. 45, pp. 3423-3430, 1997.
[16] S. I. Aspmo, S. J. Horn and V. G. H. Eijsink, "Enzymatic hydrolysis of Atlantic cod (*Gadus morhua* L.) viscera," Process Biochem., vol. 40, pp. 1957–1966, 2005.
[17] B. K. Simpson, G. Nayeri, V. Yaylayan and N. A. Ashie, "Enzymatic hydrolysis of shrimp meat," Food Chem., vol. 61, pp. 131–138, 1998.
[18] A. N. Glazer, "Esteratic reactions catalyzed by subtilisins," J. Bio. Chem., vol. 242, pp. 433-436, 1967.
[19] L. Dufosse´, D. De La Broise and F. Gue´rard, "Fish protein hydrolysates as nitrogen sources for microbial growth and metabolite production," Recent Res. Dev. Microbiol., vol. 1, pp. 365-381, 1997.
[20] N. T. Hoyle and J. H. Merritt, "Quality of fish protein hydrolysate from herring (Clupea harengus)," J. Food Sci., vol. 59, pp. 76–79, 1994.
[21] AOAC, Official Methods of Analysis of the Association Analytical Chemistry, 18th Edn., AOAC International, Washington, USA., 2005, pp. 1141.
[22] F. C. Church, H. E. Swaisgood, D. H. Porter and G. L. Catignani, "Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins," J. Dairy Sci., vol. 66, pp. 1219–1227, 1983.
[23] M. Alaiz, J. L. Navarro, J. Girón, and E. Vioque, "Amino acid analysis by highperformance liquid chromatography after derivatization with diethyl ethoxymethylenemalonate," J. Chromatography, vol. 591, pp. 181–186, 1992.
[24] H. G. Kristinsson and B. A. Rasco, "Fish protein hydrolysates: production, biochemical and functional properties," Crit. Rev. Food Sci. Nutr., vol. 40, pp. 43–81, 2000.
[25] S. M. Kamau and R. R. Lu, "The effect of enzymes and hydrolysis conditions on degree of hydrolysis and DPPH radical scavenging activity of whey protein hydrolysates," Curr. Res. Dairy Sci., vol. 3, pp. 25-35, 2011.
[26] J. Adler-Nissen, "Enzymatic hydrolysis of proteins for increased solubility," J. Agric. Food Chem., vol. 24, pp. 1090-1093, 1976.
[27] H. Herpandi, A. Rosma and W. A. Wan Nadiah, "Degree of hydrolysis and free tryptophan content of skipjack tuna (Katsuwonus pelamis) protein hydrolysates produced with different type of industrial proteases," Int. Food Res. J., vol. 19, pp. 863-867, 2012.
[28] V. Klompong, S. Benjakul, D. Kantachote and F. Shahidi, "Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type," Food Chem., vol.102, pp.1317–1327, 2007.
[29] I. Norma, B. Jamilah, N. Saari, and B. C. M. Yaakob, "Optimization of hydrolysis conditions for the production of threadfin bream (*Nemipterus japonicus*) hydrolysate by alcalase," J. Muscle Foods, vol. 16, pp. 87-102, 2005.
[30] H. G. Kristinsson and B. A. Rasco, "Kinetics of the hydrolysis of Atlantic salmon (*Salmo salar*) muscle proteins by alkaline proteases and a visceral serine protease mixture," Process Biochem., vol. 36, pp. 131–139, 2000.

[31] R. Ravallec-Plé, L. Gilmartin, A. Van-Wormhoudt and Y. Le Gal, "Influence of the hydrolysis process on the biological activities of protein hydrolysates from cod (*Gadus morhua*) muscle," J. Sci. Food Agric., vol. 80, pp. 2176–2180, 2000.

[32] F. Guérard, L. Guimas and A. Binet, "Production of tuna waste hydrolysates by a commercial neutral protease preparation," J. Mol. Catal. B-Enzym., vol. 19, pp. 489–498, 2002.

[33] H. T. M. Nguyen, K. S. B. Sylla, Z. Randriamahatody, C. Donnay-Moreno, J. Moreau, L. T. Tran and J. P. Bergé, "Proteolysis of tuna by-products," Food Technol. Biotechnol., vol. 49, pp. 48–55, 2011.

[34] M. Ghassem, S. S. Fern, M. Said, Z. M. Ali, S. Ibrahim and A. S. Babji, "Kinetic characterization of Channa striatus muscle sarcoplasmic and myofibrillar protein hydrolysates," J. Food Sci. Tech., DOI: 10.1007/s13197-011-0526-6, 2011.

[35] H. Hou, B. Li and X. Zhao, "Enzymatic hydrolysis of defatted mackerel protein with low bitter taste," J. Ocean Uni. China, vol. 10, pp. 85–92, 2011.

[36] S. Khantaphant, S. Benjakul, and H. Kishimura, "Antioxidative and ACE inhibitory activities of protein hydrolysates from the muscle of brownstripe red snapper prepared using pyloric caeca and commercial proteases," Process Biochem., vol. 46, pp. 318–327, 2011.

[37] M. Chalamaiah, G. N. Rao, D. G. Rao, and T. Jyothirmayi, "Fish protein hydrolysates: proximate composition, amino acid composition,antioxidant activities and applications: a review," Food Chem., vol. 120, pp. 652–657, 2010.

[38] N. Souissi, A. Bougatef, Y. Triki-Ellouz and M. Nasri, "Biochemical and functional properties of sardinella (*Sardinella aurita*) byproduct hydrolysates," Food Tech and Biotechnology 45(2): 187-194, 2007.

[39] R. Pacheco-Aguilar, M. A. Mazorra-Manzano and J. C. Ramirez-Suarez, "Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease," Food Chem., vol. 109, pp. 782-789, 2008.

[40] S. Nilsang, S. Lertsiri, M. Suphantharika and A. Assavanig, "Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases," J. Food Eng., vol. 70, pp. 571–578, 2005.

[41] *C.* Bueno-Solano, J. Lopez-Cervantes, O. N. Campas-Baypoli, R. Lauterio-Garcia, N. P. Adan-Bante, and D. I. Sanchez-Machado, "Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products," Food Chem., vol. 112, pp. 671–675, 2009.