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The properties, compositions and qualities of Yellowstripe scad *(Selaroides leptolepis)* and its liquid protein hydrolysate based on different enzyme concentrations, hydrolysis time and choice of buffer

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Abstract

This study aimed to determine the best parameters (types of buffer, hydrolysis time and enzyme concentration) used to produce good quality of liquid protein hydrolysate from Yellowstripe scad in terms of high yield, protein content and concentration. The choice of buffer (sodium or potassium buffer), hydrolysis time (1 h, 2 h, 3 h or 4 h) and enzymes concentrations (0.5%, 1.0%, 1.5% and 2.0%) were investigated. The results obtained from two way ANOVA showed that these parameters had significant difference ($p<0.05$) indicating these three parameters had interaction among each other. The optimum conditions found were sodium buffer with 1h and 2 h of hydrolysis time, in terms of have higher percentage of yield (70 – 80%), protein content (4.2 – 5.8%) and concentration of protein hydrolysate (68 – 92 mg/ml).

Introduction

Yellowstripe scad belongs to the small pelagic group which is categorised as low value fishes, is one of the plentiful marine source in South China Sea (Vietnam sea area) (Bui Tran Nu Thanh Viet and Toshiaki, 2014). This species is distinguished by its prominent lateral yellow band and smaller eye, differing from scads of Selar. In order to increase the value and utilization of low value proteinacious fish, processes such as protein hydrolysis via enzymatic hydrolysis is used to produce a more marketable and functional protein hydrolysate (Aspmo *et al*., 2005).

Unutilized fish or underutilized fish or fish waste can be used to produce fish protein concentrate or hydrolysate since they contain so much amino acids and functional protein (Ramakrishnan *et al*., 2013). Fish protein hydrolysate produced by controlled enzymatic hydrolysis, is considered to be the best fish protein hydrolysate due to its nutritional properties of well balanced amino acids composition and these hydrolysate is highly digestible by consumers (Kristinsson and Rasco, 2000). Protein hydrolysate with different degree of hydrolysis and different functional properties could be produced by proper control during hydrolysis process. Physicochemical properties of protein hydrolysate are greatly affected by the degree of hydrolysis, type of substrate and protease enzyme used.

There are many different types of proteolytic enzymes that can be used to produce protein hydrolysate (Liceaga–Gesualdo and Li–Chan, 1999). The most common source of proteolytic enzymes are found to be either plant or microorganisms, which are suitable for the production of fish protein hydrolysate (Bhaskar *et al*., 2008). Alcalase is a commercially obtainable enzyme which is widely used in protein hydrolysis because of its thermostability (50°C) and high optimal pH (pH8.5) where it can minimise the growth of microorganisms along hydrolysis process (Salwanee *et al.*, 2013). Alcalase is originated from a strain of *Bacillus licheniformis,* subtilisin A (Subtilisin Carlsberg) which act as the main enzyme component. This enzyme is an endopeptidase, also available in food grade form that complies with FAO/WHO (Novo Nordisk, 1995).

Yellowstripe scad is often regarded as waste or used as animal feed since the usage for consumption is limited to traditional cooking and simple processing although the yield of capture and protein content are high. Besides, limited studies regarding the proper extraction techniques and parameters for Yellowstripe scad, which contain high amount of protein, is prone to degradation, oxidation and other undesirable

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processes. Hence, there is a need to widen the use of this fish in producing fish protein hydrolysate rather than only domestically used or processed into feeds. Proper handling technique and parameters are needed in order to obtain optimum protein from this fish and this can serve as a reference for further study. Thus, this study aimed to determine the best parameters (types of buffer, hydrolysis time and enzyme concentration) used to produce good quality of liquid protein hydrolysate from Yellowstripe scad in terms of high yield, protein content and concentration.

Materials and Methods

The raw material used in this study was fresh Yellowstripe scad obtained from fish market in Pulau Kambing, Kuala Terengganu, East Coast of Peninsular Malaysia. The fish had approximately 5 – 10 cm long and weighed in the range of 35 – 45 g. They had a variety of maturity since these fishes were caught randomly from South China Sea within May to June. Raw fish was divided into 2 categories; whole fish (whole fish was ground) and edible parts (degutted with fish head, tail and fins were removed). The ground edible portion was used to produce protein hydrolysate. Moisture, protein, fat and ash content were determined using AOAC (2000) methods. Generally in moisture analysis, the weight before and after drying according to the gravimetric method, while fat was determined according to Soxhlet method with fat extracted using petroleum ether.

Determination of micronutrients (macro-minerals and micro-minerals)

Micronutrients of fish was determined using Inductive Coupled Plasma Mass Spectrophotometry (ICP) (Perkin Elmer, USA). The elements analyzed were Zinc (Zn), Iron (Fe), Calcium(Ca), Potassium (K), Magnesium (Mg), Boron (B), Manganese (Mn) and Copper (Cu). Approximately two grams of sample was ashed and left to cool before the addition of 2 ml of concentrated HCl. The sample was evaporated until dry, then 10 ml of 20% nitric acid was added. The sample was made up to 100 ml using distilled water and analyzed using ICP.

Properties of raw fish (Characterization of functional group)

The properties of raw fish were determined using transmission technique of Fourier Transform Infra Red (FTIR) (Thermo Scientific Nicolet iS10 – Transmission, USA). Fish sample was dried in oven for overnight at 55°C before ground into powder form. Approximately 25 mg of sample was added with potassium bromide (KBr) with a ratio of 1:100. The sample mixture was pressed into a pellet form using a presser with pressure of 10000 to 15000 psi.

Protein extraction

Protein was extracted from the edible portion of fish (without the head, viscera, tails and fins). The fish was ground using a blender (7011S, Waring, USA). Fifty grams of fish meat was deactivated by immersing into water bath at 90°C for 10 min. It was centrifuged at 3500 rpm for 20 min for oil separation. Then it was mixed with 100 ml of buffer (either sodium phosphate buffer or potassium phosphate buffer) and adjusted to pH 8 using 2.0 M sodium hydroxide. Four different concentrations of enzymes were added; 0.5%, 1.0%, 1.5% and 2.0%. The hydrolysis was conducted for several hours; 1 h, 2 h, 3 h and 4 h. The solutions were centrifuged at 10000 rpm for 20 min and filtered. The liquid hydrolysate was frozen (−28°C) prior to further analysis.

Yield of liquid protein hydrolysate

Yield analysis for liquid hydrolysate was determined by recording the amount of supernatant obtained from centrifugation of solution after protein hydrolysis. The solution from centrifugation was filtered using filter paper and the amount was recorded as (a). The percentage of yield from liquid hydrolysate was calculated as shown in [1].

Yield of liquid hydrolysate= (liquid protein hydrolysate (a))/(total solution) ×100% [1]

Protein content of liquid protein hydrolysate

Protein content was measured using Kjeldahl method to determine the ammonium compound present in the solution (AOAC, 2000). Briefly, one gram of sample (whole fish and edible portion) was weighed and placed into the digestion tube of the instrument, while powdered protein hydrolysate used was only approximately 0.5 g. Two tablets of Kjeltabs catalyst, Cu 3.5 and 12 ml of the concentrated sulphuric acid was added consecutively. After that, the tubes were connected to the digester (2006 Digester, FOSS, Sweden, 1998). This process of digestion was continued until green or light blue solution was formed. Then distillation was continued using distillation unit (2100 Kjeltec Distillation Unit, FOSS, Sweden, 2002). The values from the titration was calculated using formula [2] and [3] given below.

$$\text{Percentage of nitrogen (\%)} = \frac{(T-B)\times N\times 14.007\times 100}{\text{weight of the sample (mg)}} \quad [2]$$

$$\text{Percentage of protein (\%)} = \text{percentage of nitrogen} \times F \quad [3]$$

Where,

T = Titration volume for the sample (ml)
B = Titration volume for the control (ml)
N = Concentration of hydrochloric acid (HCl)
F = Protein factor (6.25)

Concentration of liquid protein hydrolysate

Concentration of liquid protein hydrolysate was obtained by substituting the absorbance in the standard curve prepared. 10 ml of sample was prepared and analyzed using UV Spectrophotometer at 280 nm.

Statistical analysis

Statistical analyses were performed using SPSS 16 on the percentage of yield, protein and concentration of liquid protein hydrolysate. Samples were replicated thrice. Both analysis of variance (ANOVA) and Tukey's grouping were carried out. Data transformation was conducted on percentage of yield using $\log_{10}$ (x+1).

Results and Discussion

Chemical analysis (whole and edible portion of fish)

The results depict that moisture content was found to be slightly higher in edible portion as compared to whole fish with 77.58%±0.06 and 77.42%±0.07, respectively. Previous study by Nurnadia *et al*. (2011) had recorded 79.48%, slightly higher moisture content in Yellowstripe scad. This might be due to different form of samples used, where in this study ground sample used but fish fillet was used in previous study. Analysis on protein content showed 19.73% and 26.04% for whole fish and edible portion respectively. Edible parts had higher protein content than those reported in previous study mostly due to different raw materials obtained. Different geographical area and maturity of fish are the most influential factors (Nurnadia *et al*., 2011).

Whole fish recorded 1.06% while edible portion had higher percentage of fat which is 1.10%. Wan Rosli *et al.* (2012) reported that this fish has less than 4% of fat. Previous study by Nurnadia *et al.*, (2013) also stated that Yellowstripe scad was categorized in low fat fish that contained 2 – 4% of fat. The lower values obtained in this study were probably due to different geographical area and season of harvest. Furthermore, Piggot and Tucker reported that age variation and maturity could cause significant differences in total lipid content within the same fish species (Piggot and Tucker, 1990). Ash content was slightly higher in whole fish (3.18%) compared to that of edible portion (2.71%), which is probably due to higher amount of bones and cartilage found in fish head, tail and fins.

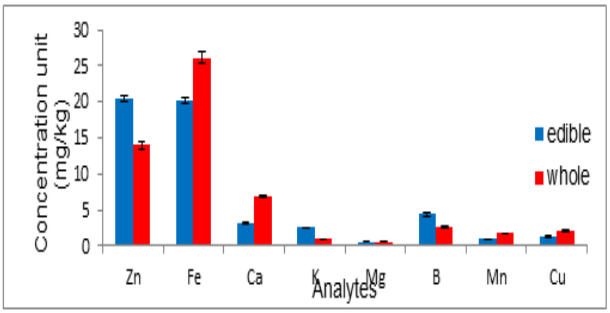

Figure 1. Concentration of micro and macro–minerals for whole fish and edible portion of Yellowstripe scad

Micronutrients of whole and edible portion

Minerals in food can be essential, non essential or toxic for human consumption. Micro–minerals and macro–minerals of Yellowstripe scad obtained from whole fish and edible parts had only slight differences. Generally, edible parts had higher Zinc (Zn), Potassium (K), Boron (B) and Magnesium (Mg), while whole fish had higher Iron (Fe), Calcium(Ca), Manganese (Mn) and Copper (Cu). Based on Figure 1, whole fish was observed to have higher amount of iron (26.17 mg/kg) and calcium (6896.10 mg/kg), probably contributed by internal organs and fish head, respectively. Internal organs had more blood content which is richer in iron, while fish head, tail and fins contribute higher amount of calcium as they are made up of bones.

Potassium was higher in edible parts than whole fish with 259 mg/kg and 104 mg/kg, respectively. Iron, potassium and calcium were seen to be significantly higher than previous study by Nurnadia (2013) with 4.54 mg/kg, 10.98mg/kg and 83.29mg/kg, respectively. This could be due to species, individuals and sampling period (Yilmaz *et al.*, 2010). Zinc was found to be higher in edible portion (20.40 mg/kg) compared to whole fish (13.97 mg/kg). The amount of zinc reported slightly higher than previous studies but they were still in the range permitted by FAO/WHO (1984), 150 mg/kg.

Figure 1 illustrates that magnesium content in both whole fish and edible portion were almost at similar readings, while boron content was seen to be higher in edible portion than whole fish. Manganese and copper content were seen to be higher in whole fish, 1.78 mg/kg and 2.08 mg/kg than edible portion, 0.99 mg/kg and 1.36 mg/kg, respectively. The content of manganese and copper were found to be lower than

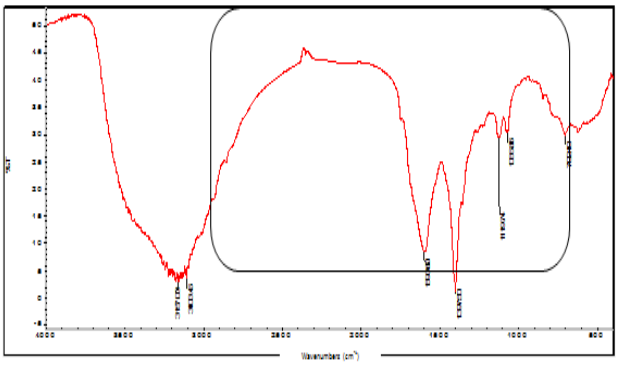

Figure 2a. FTIR spectrum for whole fish of Yellowstripe scad

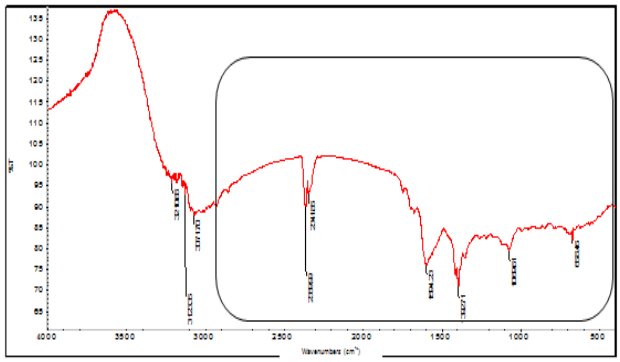

Figure 2b. FTIR spectrum for edible portion of Yellowstripe scad

the permissible limit set by FAO/WHO (1984) with 5.4 mg/kg and 10 mg/kg respectively. Malaysian standards had no record for manganese while permissible limit for copper was 30 mg/kg (Nurnadia *et al*., 2013). Variation in results obtained in this study for micro and macro–minerals as compared to previous studies could be due to different parts of fish used as sample, species, area sampling, season, maturity and other environmental difference (Erkan and Ozden, 2007).

Properties of whole and edible portion (FTIR)

The overall spectral profile for both samples (Figures 2a and 2b) were similar except for variation of intensities of certain bands. Whole fish had sharper and clearer FTIR spectrum as compared to edible parts especially from 3000 to 600 cm^{-1}. Figures 2a and 2b showed functional groups from whole fish and edible portion from Yellowstripe scad, mainly found was primary amines.

The N–H stretching of primary amines usually found to occur in two bands in the region of 3400 to 3300 cm^{-1}. Referring to Figures 2a and 2b, the primary amines found in these two samples were also deduced by the broad band for N–H bending (amide II) at 1640–1560 cm^{-1}. Whole fish showed N–H band at 1593.60 cm^{-1} while edible portion showed

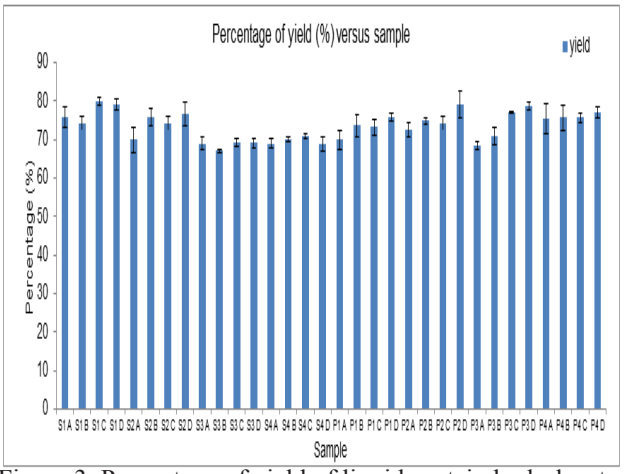

Figure 3. Percentage of yield of liquid protein hydrolysate
Note: S= sodium buffer, P= potassium buffer [Buffer type]
1= 1 hr, 2=2 hrs, 3=3 hrs, 4=4 hrs [Hydrolysis time]
A= 0.5%, B=1.0%, C=1.5%, D=2.0% [Enzyme concentration]

strong N–H bond at 1594.23 cm^{-1} which was found to be amide bending vibration. Similar to previous findings, sharp bands observed at this region are assigned to C=O stretching vibration (amide) and C–N stretching or N–H bending vibrations (amide II) of protein (Sorensen, 1991; Parveez *et al*., 1999).

The band at 1397.50 cm^{-1} and 1392.71 cm^{-1} in whole fish and edible portion respectively are mainly due to asymmetric and symmetric CH_3 bending of methyl group of protein (Venkataramana *et al*., 2010). The low intensity bands observed at 1066.86 cm^{-1} and 1069.61 cm^{-1} from Figures 2a and 2b respectively, mainly contributed by symmetric stretching of PO_2 group in nucleic acid and phospholipid (Senthamilselvan and Chezhian, 2014). Dovbeshko *et al*. (2000) reported at band observed around 669 cm^{-1} may be due to CH_2. Primary amines had the highest intensity band in the whole spectrum. Based on the transmission spectrum, there is only primary amines and no sign of secondary amines which usually occurred in amide I region (1700 – 1600 cm^{-1}).

Yield for liquid protein hydrolysate

Yield of liquid protein hydrolysate was conducted to determine the efficiency of hydrolysis conducted. Figure 3 shows that the yield of liquid hydrolysate from sodium and potassium buffers with different hydrolysis times and enzymes concentrations were seen to be in the range of 65% to 80%. Highest yield were produced using sodium buffer with 1 and 2 hrs of hydrolysis.

Statistic analysis (ANOVA) showed significant interaction between the types of buffer, hydrolysis time and concentration of enzymes used. Generally, the data showed that the yield of liquid hydrolysate

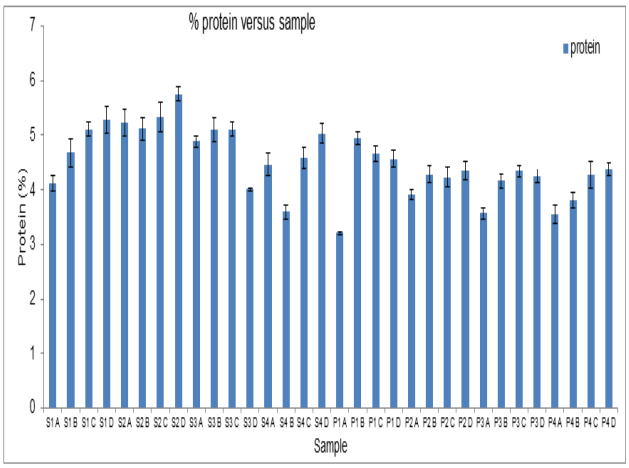

Figure 4. Percentage of protein content of liquid protein hydrolysate

Note: S= sodium buffer, P= potassium buffer [Buffer type]
1= 1 hr, 2=2 hrs, 3=3 hrs, 4=4 hrs [Hydrolysis time]
A= 0.5%, B=1.0%, C=1.5%, D=2.0% [Enzyme concentration]

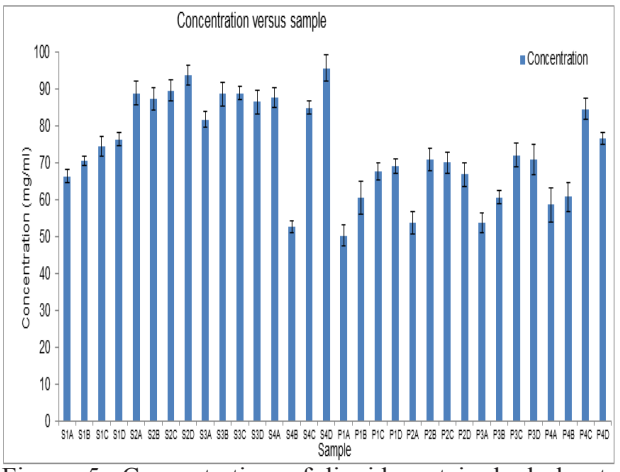

Figure 5. Concentration of liquid protein hydrolysate viewed on 280 nm

Note: S= sodium buffer, P= potassium buffer [Buffer type]
1= 1 hr, 2=2 hrs, 3=3 hrs, 4=4 hrs [Hydrolysis time]
A= 0.5%, B=1.0%, C=1.5%, D=2.0% [Enzyme concentration]

in every hour increased as the concentration of enzyme used increase, but sodium buffer had higher extraction yield as compared to potassium buffer. The finding from Figure 3 showed that sodium buffer with 1 h and 2 h of hydrolysis time and increasing enzyme concentration, were better than the others, aligned with previous study by Ramakrishnan *et al.* (2013) also suggested that shorter hydrolysis time (1h) for protein extraction. In addition, Kristinsson and Rasco (2000) had reported that Alcalase enzyme was used in fish hydrolysis due to its high degree of hydrolysis in relatively short time (1 h and 2 h) increasing concentration of enzyme used. Sodium is attracted to the protein surface more strongly than potassium due to stronger interaction of sodium with carboxylate group in aspartate and glutamate side chains (Heyda *et al.*, 2009).

Protein content for liquid protein hydrolysate

Generally, the liquid hydrolysate produced using sodium phosphate buffer had higher percentage of protein and the results were more consistent as compared to the result shown by hydrolysate obtained from potassium phosphate buffer treatment. The results shown in Figure 4, were significantly difference ($p \leq 0.05$) among each other, indicating that there were interaction between types of buffer, hydrolysis time and enzyme concentration.

Based on Figure 4, the increment of enzyme concentration from 0.5 to 2% had resulted in the increase in percentage of protein, similarly reported by previous study done by Ramakrishnan *et al.* (2013) and some other studies had disclosed that more enzymes molecules were associated with fish particles, releasing more protein molecules during hydrolysis (Shahidi *et al.*, 1995; Kristinsson and Rasco, 2000). However, there were inconsistent results obtained (seen in Figure 4) such in sodium buffer with 3 h and 4 h of hydrolysis and potassium 1hr of hydrolysis. Gildberg (1992) reported that an increase in enzyme concentration increased the rate of reaction but fish tissue is a very complex substrates that contains large amount of proteinase inhibitors which make it difficult to explain protein hydrolysis.

Concentration for liquid protein hydrolysate

Concentration obtained from absorbance reading using spectrophotometer at 280 nm (near UV absorbance) is basically contributed by tyrosine and tryptophan (also a small amount of phenylalanine and disulfide bonds) (Alastair and Michele, 2009). Generally Figure 5 showed that sodium buffer had high concentration of protein compared to potassium buffer. The finding from two way ANOVA showed that the three parameters; buffer type, hydrolysis time and concentration of enzymes used, showed that there were significant interaction ($P<0.05$) between these three parameters. Increasing hydrolysis time and protein concentration were supposed to increase concentration of amino acid. However, the results obtained from Figure 5 showed increment for the first two hours, gradually decreased for the third and fourth hour. This might be due to decrease in enzyme activity, denaturation of enzyme or product inhibition (Liaset *et al.*, 2000).

Conclusion

The study clearly denotes the best choice of parameters suitable to produce protein hydrolysate

from Yellowstripe scad. It is observed that liquid hydrolysate extracted using sodium buffer are significantly better than potassium buffer in terms of having higher percentage of yield (70 – 80%), protein content (4.2 – 5.8%) and concentration of protein hydrolysate (68 – 92 mg/ml). Thus, it could be concluded conclude that sodium buffer with 2 h of hydrolysis have higher advantage to be used to extract protein from Yellowstripe scad. However, enzyme concentrations are subjected to further study since results obtained were not consistent.

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References

Alastair, A. and Michele, P.L. 2009. Protein determination by UV absorption. The Protein Protocols Handbook. 2nd ed, p.1–5. USA: Humana Press Inc.

Anthony, J.A., Roby, D.D. and Turco, K.R. 2000. Lipid content and energy density of forage fishes from the Northern Gulf of Alaska. Journal of Experimental Marine Biology and Ecology 248: 53–78

Association of Official Analytical Chemist (AOAC). 2000. Official Method of Analysis. 17th ed. Washington D.C., USA: AOAC

Aspmo, S.I., Horm, S.J. and Eisiak, G.H. 2005. Hydrolysate from Atlantic cod viscera as components of microbial growth media. Process Biochemistry 40: 3714–3722

Bhaskar, N., Benila, T., Radha, C. and Lalitha, R.G. 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.

Bui Tran Nu Thanh Viet and Toshiaki, O. 2014. The stability of bioactive compounds in Yellowstripe scad *(Selaroides leptolepis)* under subatmospheric pressure storage. International Journal of Research In Agriculture and Food Science 2(2): 23–28

Dovbeshko, G.I., Gridina, N.Y., Kruglova, E.B. and Pashchuk.O.P. 2000. FTIR spectroscopy studies of nucleic acid damage. Talenta 53:233–246

Erkan, N. and Ozden, O. 2007. Proximate composition and mineral contents in aqua cultured sea bass *(Dicentrarchuslabrax),* sea bream *(Sparusaurata)* analyzed by ICP–MS. Food Chemistry 102(3): 721–725.

FAO/WHO. 1984. List of macimum levels recommended for contaminants by the Joint FAO/WHO Codex Alimentarius Commission. Second Series. Rome: FAO/WHO.

Gildberg, A. 1992. Recovery of proteinases and protein hydrolysates from fish viscera. Bioresource Technology 39: 271–276.

Heyda, J., Pokorna, J., Vrbka, L., Jagoda−Cwiklik, B., Konvalinka, J., Jungwirth, P. and Vondrasek, J. 2009. Ion specific effects of sodium and potassium on the catalytic activity of HIV–1 protease. Journal of Physical Chemistry 11(35): 7599–7604

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

Liaset, B., Lied, E. and Espe, M. 2000. Enzymatic hydrolysis of by–products from the fish–filleting industry, chemical characterization and nutritional evaluation. Journal of Science and Food Agriculture 80: 581–589.

Liceaga-Gesualdo, A.M. and Li-Chan, E.C.Y. 1999. Functional properties of fish protein hydrolysate from Herring *(Clupea harengus)*. Journal of Food Science 64(6): 1000–1004

Novo Nordisk. 1995. Alcalase® Food Grade: Enzyme Process Division. Novo Nordisk Technical Bulletin.

Nurnadia, A. A., Azrina, A. and Amin, I. 2011. Proximate composition and energetic value of selected marine fish and shellfish from the West Coast of Peninsular Malaysia. International Food Research Journal 18(1): 137–148

Nurnadia, A. A., Azrina, A., Amin, I., Mohd Yunus, A. S. and Mohd Izuan, E. H. 2013. Minerals content of selected marine fish and shellfish from the west coast of Peninsular Malaysia. International Food Research Journal 20(1): 431–437

Parveez, I., Haris, F. and Servercan. 1999. FTIR Spectroscopic characterization of protein structure in aqueous and non aqueous media, Journal of Molecular Catalysis B: Enzymatic 7: 207–221

Payne, S.A., Johnson, B.A. and Otto, R.S. 1999. Proximate composition of some north–eastern Pacific forage fish species. Fish Oceanography 8(3): 159–177.

Piggot, G.M. and Tucker, B.W. 1990. Seafood: Effects of Technology on Nutrition. New York, USA: Marcel Dekker, Inc.

Ramakrishnan, V.V., Ghaly, A.E., Brooks, M.S. and Budge, S.M. 2013. Extraction of Protein Mackerel Fish Processing Waste Using Alcalase Enzyme. Journal Bioprocess Biotechniques 3(2):1–9

Salwanee, S., Wan Aida, W.M., Mamot, S., Maskat, M.Y. and Ibrahim, S. 2013. Effects of Enzyme Concentration, Temperature, pH and Time on the Degree of Hydrolysis of Protein Extract from Viscera of Tuna. Sains Malaysian 42(3): 279–287

Senthamilselvan, D and Chezian, A. 2014. Ammonia induced biochemical changes on the muscle tissues of the fish *Cyprinus carpio* FTIR study. Research Journal of Environmental Toxicology 8(3): 117–123

Shahidi, F., Han, X.Q. and Synowiecki, J. 1995. Production and characteristics of protein hydrolysates from capelin. Food Chemistry 53: 285–293

Sorensen, E.M. 1991. Metal poisoning in fish. CRC press: Boca Raton 1–374.

Venkataramana, G.V., Komal Kumar, J., Devi Prasad, A.G. and Karimi, P. 2010. Fourier transform infrared

spectroscopic study on liver of freshwater fish *(Oreochromis mossambicus)*. Romanian Journal of Biophysic 20(4): 315–322

Wan Rosli W. I., Rohana, A.J., Gan, S.H., Noor Fadzlina, H., Roliza, H., Helmy, H., Mohd Nazri, S., Mohd Ismail, I., Shaiful Bahri, I., Wan Mohamad, W.B. and Kamarul Iman, M. 2012. Fat content and EPA and DHA levels of selected marine, freshwater fish and shellfish species from the east coast of Peninsular Malaysia. International Food Research Journal 19(3): 815–821.

Yilmaz, A.B., Sangun, M.K., Yanghoglu, D. and Turan, C. 2010. Metals (major, essential to non–essential) composition of the different tissue of 3 demersal fish species from Iskenderun Bay, Turkey. Food Chemistry 123(2): 410–415.