PH levels effect on functional properties of different molecular weight eel (*Monopterus* sp.) protein hydrolysate

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Abstract The aim of this study is to investigate the effect of pH levels on functional properties of various molecular weights of eel (*Monopterus* sp.) protein hydrolysate (EPH). The eel was enzymatically hydrolyzed and fractionated through membranes filter (10 kDa, 5 kDa and 3 kDa). The foaming capacity and stability, emulsifying capacity and stability index, water holding capacity and fat binding capacity between pH 2 and 10 were determined. The 5 kDa EPH was found to have the highest foaming capacity at pH 2, pH 4 and pH 6, and foaming stability and emulsifying activity index at all pH levels, except pH 8 and fat binding capacity at pH 2, as compared to 10 kDa and 3 kDa EPH fractions. The 10 kDa EPH had the highest emulsifying stability index and water holding capacity at all pH levels. This study shows that the EPH fractions at low pH level had high foaming and oil binding capacity, while at neutral pH, the fractions had high foaming stability and water holding capacity. These properties are important in making whipped cream, mousse and meringue. In contrast, EPH fractions demonstrated strong emulsifying properties at high pH levels and show potential as an emulsifier for breads, biscuits and frozen desserts.

Keywords Eel protein hydrolysate · Molecular weight · pH · Functional properties

Introduction

Protein hydrolysates are produced from the hydrolysis of animal and plant protein sources (Halim and Sarbon 2017). Since the 1990's, protein hydrolysates have been widely used as milk replacers, flavor enhancers, protein supplements, surimi production and beverage stabilizers (dos Santos et al. 2011). The food industry typically employs proteases to produce protein hydrolysates (Halim et al. 2016). Several types of commonly used enzyme to meet this purpose are pepsin, trypsin, flavourzyme, alcalase and papain (Chalamaiah et al. 2012). The functional properties of protein can be improved by enzymatic hydrolysis and made more suitable to serve as high quality food ingredients for industrial food products (Chi et al. 2014).

Recently, fish protein hydrolysis has been examined by researches around the world. Fish protein is used in producing fish protein hydrolysate (FPH) due to large amount of fish sources and wastes in the world market. This waste offers strong potential to be utilised as functional food products. There are numerous studies on the extraction of freshwater and seawater fish in producing FPH. Examples include fish hydrolysates from herring (Prabha et al. 2013), daggaa (Chi et al. 2014) and shortfin scad (Ishak and Sarbon 2017). In addition, eel is also one of the potential sources to be enzymatically hydrolyzed. Studies on eel protein hydrolysate have previously been conducted (Jamil et al. 2016; Baharuddin et al. 2016; Halim and Sarbon 2017). The chemical composition of the eel flesh was 83.87% of moisture (wet based), 16.88% of protein, 3.41% of fat and 0.89% of ash. After hydrolysis, the freeze-dried eel protein hydrolysate (EPH) had 2.64% of moisture (wet based), 98.53% of protein, 0.12% of fat and 17.28% of ash (Halim and Sarbon, 2017). The high amount of protein showed the potential to release more amino acids during hydrolysis

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which will improve the functional properties of EPH such as water holding capacity, emulsifying properties and foaming properties. However, the functional characteristics of eel protein hydrolysate (EPH) at molecular level have not been explored.

Additionally, Chi et al. (2014) also mentioned that one of the key factors affecting the biological and functional properties of hydrolysates is its average molecular weight (AMW). Research has revealed correlations between AMW and functional properties, which are important for the utilization of hydrolysates. A study conducted by Razali et al. (2015) found that lower levels of MW peptides led to higher functional properties, including emulsifying and foaming properties, water holding and oil binding capacity. The fractionation technique is a part of ultrafiltration (UF) membrane technology to enrich peptides for various food products (Halim et al. 2016). Several researchers have examined the fractionation of fish protein hydrolysates from *Channa striatus*, *Sphyrna lewini*, *Dasyatis akjei* and *Raja porosa* (Razali et al. 2015; Li et al. 2013).

The pH level of hydrolysates is a primary factor affecting the functional properties of hydrolyzed proteins, including their foaming and emulsifying capacity, water holding and fat binding capacity. According to Taheri et al. (2013), the environmental pH may significantly affect the emulsifying properties of hydrolysate by changing the surface hydrophobicity and charge of the protective layer surrounding the lipid globules (Taheri et al. 2013). Research by Taheri et al. (2013) found that the foaming capacity of rainbow trout hydrolysate was low at pH 4 and stable at pH 6–10. In addition, the emulsifying activity index (EAI) of this hydrolysate was also the highest at pH 6–10. Meanwhile, the determination of pH level's effect on water holding and fat binding capacity of the fish hydrolysates is also of interest. Therefore, the aim of this study was to prepare eel protein hydrolysate (EPH) enzymatically using alcalase and fractionate into 3, 5 and 10 kDa molecular weight to determine the effect of pH on functional properties of these fractionated EPHs.

Materials and methods

Eels (*Monopterus albus*) were purchased in Kota Bharu, Malaysia and brought alive to laboratory. The eels were then eviscerated, filleted, and beheaded in order to obtain eel flesh. The flesh was homogenized and frozen at -80°C prior to use. The commercial protease used in the hydrolysis process was liquid Alcalase[®] 2.4 L (2.4 AU/g) purchased from Novo Industry (Denmark). All other chemicals used were of analytical grade.

Preparation of eel protein hydrolysate (EPH)

The hydrolysis of eel protein hydrolysate (EPH) was conducted following Jamil et al. (2016). The hydrolysis conditions were time of 2 h, temperature of 56°C , and pH of 9. The frozen eel flesh was firstly thawed in chiller (4°C) for overnight prior to use. Approximately 176 g of eel was added to 242 ml of distilled water. The endogenous enzyme of eel flesh was inactivated by heating the mixture (85°C) for 20 min before cooled to a specified temperature (56°C). Hydrolysis process was initiated immediately after adding 20 g of alcalase solution into the mixture. 1 N NaOH was used to maintain the required pH value (pH 9) during the process. Alcalase activity was inactivated after 2 h by heating the mixture (85°C) for 20 min, which ended the hydrolysis process. The mixture was then cooled and centrifuged (6000 rpm, 4°C) (Gyrozen 158R, Korea) for 30 min.

In order to gain the EPH at different molecular weight, the supernatants were collected and fractionated using a Vivaspin ultrafilter membrane (Sartorius, Germany) with 3 different molecular weight cut-off (MWCO) membranes. The hydrolysate was first fractionated through 10 kDa MWCO membrane with centrifuge (3500 rpm, 30 min) and the resultant fraction was then fractionated through 5 kDa MWCO membrane, then, further fractionation through 3 kDa molecular weight cut-off (MWCO) membrane. The fractionated eel protein hydrolysates were freeze-dried, weighed, and kept at -80°C for analysis (Razali et al. 2015).

Functional properties of fractionated eel protein hydrolysate (EPH)

Foaming properties

The foaming capacity and stability of eel protein hydrolysates were determined according to the method of Jamil et al. (2016). Approximately 20 ml of 0.5% sample at different MW (3, 5 and 10 kDa) were adjusted to pH 2, 4, 6, 8 and 10 followed by homogenization at a speed of 16,000 rpm to incorporate the air for 2 min at room temperature. The whipped sample was immediately transferred into a 25 ml cylinder and the total volume was read after 30 s. The foaming capacity was calculated according to the following equation:

$$\text{Foaming capacity (\%)} = [(A_w - B_w)/B_w] \times 100$$

where A_w = volume after whipping (ml), B_w = volume before whipping (ml).

Next, the whipped sample was allowed to stand at 20 °C for 3 min and the volume of whipped sample was then recorded. Foaming stability was calculated as follows:

$$\text{Foaming stability (\%)} = [(A_s - B_s)/B_s] \times 100$$

where A_s = volume after standing (ml), B_s = volume before standing (ml).

Emulsifying properties

The emulsifying properties of fractionated eel protein hydrolysates were determined according to the method of Elavarasan et al. (2014). Approximately, 10 ml of corn oil and 30 ml of 1% (1 g EPH diluted in 100 ml of distilled water) sample at different MWs (3, 5 and 10 kDa) were mixed and the pH of the resulting solution of oil-protein was adjusted to 2, 4, 6, 8 and 10. The mixture was then homogenized (IKA T25 Ultra-Turrax, Malaysia) (20,000 rpm) for 1 min. 50 µl aliquot of the emulsion at the bottom was pipetted at 0 and 10 min after homogenization and mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. Absorbance was measured with a spectrophotometer (500 nm) (Cary WinUV software, United States) immediately at 0 min (A_0) and 10 min (A_{10}) after emulsion and the values were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as follows:

$$\text{ESI (min)} = A_0 \times A_{10}/\Delta A$$

where A_0 = ESI at 0 min, A_{10} = ESI at 10 min.

Water holding capacity

The water holding capacity (WHC) of fractionated eel protein hydrolysates was determined according to the method of Razali et al. (2015). Approximately 0.5 g of sample (3, 5 and 10 kDa) were dispersed in 50 ml distilled water and mixed for 2 min. The mixture was then kept at room temperature for 30 min. Then, the mixture was centrifuged (Gyrozen 158R, Deejan, Korea) at 5000 rpm for 30 min. The supernatant was filtered by using Whatman filter paper and the volume was measured. Water holding capacity was calculated as follows:

$$\text{Water holding capacity (ml/g)} = \frac{\text{Volume of water added (ml)} - \text{Volume of supernatant (ml)}}{\text{Mass of hydrolysate (g)}}$$

Fat binding capacity

Approximately 0.5 g of sample at different MWs (3, 5 and 10 kDa) were placed in a centrifuge tube and 10 ml of corn oil was added. The solutions were mixed for 30 s followed

by the centrifugation of the mixture (Gyrozen 158R, Deejan, Korea) at 2800 rpm for 25 min (Razali et al. 2015). The fat binding capacity was calculated as follows:

$$\text{Fat binding capacity (ml/g)} = \frac{\text{Volume of fat added (ml)} - \text{Volume of fat decanted (ml)}}{\text{Mass of hydrolysate (g)}}$$

Statistical analysis

The EPH was prepared in triplicates. Both one-way and two-way analyses of variance (ANOVA) were conducted to analyze all data. The results were stated as mean $\pm$ standard deviation. MINITAB Statistical Software version 14.0 and Microsoft Excel were also used for all statistical analysis. All results were recorded in triplicate.

Results and discussion

Fractionation of eel protein hydrolysate (EPH)

After fractionation, the yields obtained for 10 kDa, 5 kDa and 3 kDa of EPH fraction were 59.0%, 24.5% and 16.5%, respectively. The results showed that the crude EPH consisted of higher amount of larger molecular weight peptide than lower molecular weight peptide. In addition, Razali et al. (2015) stated that yield decreased with decrease in molecular weight because the smallest peptides are lost during filtration. The high amount of protein after hydrolysis showed the potential to release more amino acids during hydrolysis which will improve the functional properties of EPH such as water holding capacity, emulsifying properties and foaming properties. Halim and Sarbon (2017), reported that the hydrolysis of eel protein significantly increased the amount of amino acids such as serine, threonine, valine, isoleucine, phenylalanine and methionine in hydrolysates. During the hydrolysis process, the muscle cell membranes was rounded up, forming insoluble vesicles which to the structured lipids membrane's removal (Thiansilakul et al. 2007). Thus, the alcalase enzyme rapidly interacted with the compact, insoluble protein particles and cleaved the polypeptides chains (Benjakul and Morrissey 1997).

A study on cobia skin gelatin hydrolysate (CSGH) by Razali et al. (2015) reported that the yields for 10 kDa, 5 kDa and 3 kDa CSGH as 21.21%, 13.82%, and 5.94%, respectively. This finding was lower than the yield of fractionated EPH obtained in this current study. In contrary with the yield of fish hydrolysates, the rapeseed protein hydrolysate fractions were increased with the decrease of molecular weight (5–10 kDa, 3–5 kDa and 1–3 kDa with

yield 10.46%, 14.42% and 21.81%, respectively) (McCarthy et al. 2013). The level of hydrolysates produced depends on the number of cleaved peptides and hydrolysis parameters, including time, temperature, enzyme concentration and pH level.

Functional properties of fractionated eel protein hydrolysate (EPH)

Foaming properties

Figure 1 shows that the foaming capacity of fractionated (3 kDa, 5 kDa, 10 kDa) EPH which decreased with the increase of pH level (pH 2–pH 10). The two-way ANOVA found an interaction between different molecular weight of EPH and pH level ranging from pH 2–pH 10. Based on Fig. 1, there was a significant difference ($p < 0.05$) between all EPH fractions at different pH levels. However, 5 kDa and 10 kDa EPH at pH 2, pH 4 and pH 10 and also 3 kDa and 5 kDa EPH at pH 8 were not significantly different ($p > 0.05$). In short, the 5 kDa and 10 kDa EPH fractions had similar foaming capacity in acidic and highly alkaline conditions. During fractionation of EPH, the hydrolysate was collected according to its specific molecular weight, as the 5 kDa and 10 kDa EPH had the same capacity to be absorbed at the air–water interface and foaming. According to Razali et al. (2015), the ability of a protein to be quickly absorbed at the air–water interface is required for foam formation. Moreover, the ionic repulsion of peptides at the air–water interface in highly alkaline condition reduced foam capacity (Klompong et al. 2007). This decrease in foam capacity with the increase of pH has also been reported by Naqash and Nazeer (2013) on pink perch hydrolysate.

Fig. 1 Foaming capacity (%) of fractionated eel protein hydrolysates at different pH (2, 4, 6, 8 and 10). Results are presented as mean $\pm$ SD. Different superscript indicates significant difference ($p < 0.05$) with the increase of pH value

Meanwhile, foam stability is mainly depending on the protein flexibility and structure together with the protein–protein interactions within the matrix of films surrounding air bubbles (Razali et al. 2015). Based on Fig. 2, the foaming stability of fractionated EPH (3 kDa, 5 kDa, 10 kDa) was the highest at pH 6 (24.26%, 27.10%, 21.30%, respectively) as compared to other pH levels. The results showed that the hydrolysate had a well-ordered orientation under neutral conditions. However, there was no significant difference ($p > 0.05$) between 5 kDa and 10 kDa EPH fraction at pH 2 and pH 8. The result showed that 5 kDa EPH fraction has the highest foam stability at all pH values (pH 2–pH 10) as compared to 3 kDa and 10 kDa EPH fractions. These findings agree with a study by Razali et al. (2015) on fractionated cobia skin gelatin hydrolysate (CSGH), in which 5 kDa CSGH had the highest foaming stability, as compared to 3 kDa and 10 kDa CSGH.

Emulsifying properties

The emulsifying activity index of fractionated EPH (3 kDa, 5 kDa, 10 kDa) was observed increased with the increases of pH value (pH 2–pH 10) (Fig. 3). However, there was a significant decrease ($p < 0.05$) on EAI of 3 kDa EPH fraction from pH 2 to pH 4. The highest EAI was shown by 5 kDa EPH fraction at pH 10 (3.95 m^2/g). The results showed that the 5 kDa EPH fraction had the highest amount of hydrophobic amino acids as compared to 3 kDa and 10 kDa of EPH fractions. The N-terminals of hydrophobic amino acids bound with oil and form emulsion at the air–water interface. Hence, more hydrophobic amino acids resulted in a higher emulsifying activity index. The fractionation of EPH had affected the EAI of the hydrolysate, in which the EAI was increased as the molecular weight decreased. The results obtained in this study are in agreement with the EAI of fractionated cobia skin gelatin hydrolysate (CSGH) as reported by Razali

Fig. 2 Foaming stability (%) of fractionated eel protein hydrolysates at different pH (2, 4, 6, 8 and 10). Results are presented as mean $\pm$ SD. Different superscript indicates significant difference ($p < 0.05$) with the increase of pH value

Fig. 3 Emulsifying Activity Index (m^2/g) of fractionated eel protein hydrolysates at different pH (2, 4, 6, 8 and 10). Results are presented as mean $\pm$ SD. Different superscript indicates significant difference ($p < 0.05$) with the increase of pH value

et al. (2015). However, the finding on EAI of EPH was in contrast with the study conducted by dos Santos et al. (2011) using bluewing searobin hydrolysate in which the highest EAI was at pH 3. Theoretically, there were other factors that affect the EAI of the hydrolysate produced. For example, the bluewing searobin hydrolysate solubility and enzyme's specificity during hydrolysis process (dos Santos et al. 2011).

The same trend was found on emulsifying stability index of fractionated EPH (3 kDa, 5 kDa, 10 kDa) at pH value ranging from pH 2–pH 10 except at pH 4 where all EPH fractions were observed decreased in ESI (Fig. 4). In addition, there was a significant difference ($p < 0.05$) between all EPH fractions (3 kDa, 5 kDa, 10 kDa) at all pH levels (pH 2–pH 10) regardless 3 kDa and 5 kDa EPH at pH 2 ($p > 0.05$). The highest ESI was shown by 3 kDa EPH at pH 10 (69.67%). Taheri et al. (2013) mentioned that the presence of negative charge peptides in highly alkaline conditions leads to more polypeptides cleavage. As a result, higher hydrophilic and hydrophobic peptide residues were exposed during emulsion hence, promoting

Fig. 4 Emulsifying Stability Index (%) of fractionated eel protein hydrolysates at different pH (2, 4, 6, 8 and 10). Results are presented as mean $\pm$ SD. Different superscript indicates significant difference ($p < 0.05$) with the increase of pH value

significant interactions at the oil-water interface and stabilize the emulsion. Based on Fig. 3, the EPH fractions were unable to reduce the interfacial tension at the oil-water interface in an acidic environment (pH 2–pH 4). This finding was in contrast with the ESI of fractionated CSGH as the ESI of the hydrolysate decreased with the increase of CSGH molecular weight. Hence, it showed that the ionic condition of the fractionated EPH significantly influenced its ESI.

Water holding capacity

Figure 5 shows the water holding capacity of fractionated EPH (3 kDa, 5 kDa, 10 kDa) at different pH levels. The water holding capacity of fractionated EPH was decreased with the increase of pH level (pH 2–pH 10) except at pH 6 where all EPH fractions were increased significantly ($p < 0.05$). In addition, it can be seen that the water holding capacity was decreased with the decrease of molecular weight. The two-way ANOVA analysis found an interaction between different molecular weight of EPH and pH value ranging from pH 2–pH 10. The highest water holding capacity was shown by 10 kDa EPH fraction at pH 6 (22.80 ml/g) while the lowest water holding capacity was from 3 kDa EPH at pH 4 (0.57 ml/g). The larger peptides had less surface area than the smaller peptides. Hence, the larger peptides had a greater ability to bind with water and retain the gravitational force, as more area did not contact with water.

Water holding capacity refers to the ability of a protein to bind with water and retain it against a gravitational force within a protein matrix (Foh et al. 2010). Besides that, the presence of polar amino acids such as histamine, asparagine, histidine, threonine, tyrosine and tryptophan may be the reason for the increase of water absorption of fractionated EPH at pH 6. Halim and Sarbon (2017) reported

Fig. 5 The water holding capacity of fractionated eel protein hydrolysates at different pH (2, 4, 6, 8 and 10). Results are presented as mean $\pm$ SD. Different superscript indicates significant difference ($p < 0.05$) with the increase of pH value

that these amino acids were found in EPH. The results obtained for WHC of EPH at all fractions are in agreement with a study by Razali et al. (2015) on cobia skin gelatin hydrolysate, in which the water holding capacity increased with the increase of molecular weight of the hydrolysate.

Fat binding capacity

Figure 6 showed the fat binding capacity of fractionated EPH. The results decreased from pH 2 to pH 6 and increased from pH 6 to pH 10 for all EPH fractions, except for 10 kDa EPH fraction in which the fat binding capacity was decreased with the increase of pH value. The 5 kDa EPH fraction at pH 2 was found as the highest in the ability to bind oil (43.01 ml/g) as compared to other EPH fractions. There were significant difference ($p < 0.05$) on fat holding capacity of 3 kDa and 5 kDa EPH at all pH values (pH 2–pH 10). The two-way ANOVA analysis found an interaction between different molecular weight of EPH and pH value ranging from pH 2–pH 10.

According to dos Santos et al. (2011), the fat binding capacity was directly related to the surface hydrophobicity of the protein. The 5 kDa EPH at pH 2 showed the highest surface hydrophobicity as compared to other fractions at different pH values (Fig. 6). This finding was supported by the results obtained for emulsifying properties (Figs. 3, 4). The 5 kDa EPH in pH 2 was among the lowest in emulsifying properties. It is because the emulsifying properties of the hydrolysate were also depended on the surface hydrophobicity of the protein to maintain at the oil-water interface. Therefore, the results for the emulsifying properties of EPH fractions may be related to their fat binding capacity.

However, these results contrast with the study by Razali et al. (2015), which found that 10 kDa cobia skin gelatin hydrolysate had the highest fat binding capacity and it

Fig. 6 The fat binding capacity of fractionated eel protein hydrolysates at different pH (2, 4, 6, 8 and 10). Results are presented as mean $\pm$ SD. Different superscript indicates significant difference ($p < 0.05$) with the increase of pH value

increased with the increase of the hydrolysate molecular weight. Therefore, the study on EPH fractions found that the fat binding capacity was also affected by the difference in pH values of the peptides. The fat binding ability of hydrolysate is important in the production of food, because it not only influences the taste of the product, but also allows the prevention of phase separation, such as in the production of cake, mayonnaise, and salad dressings (dos Santos et al. 2011).

Conclusion

In conclusion, a novel eel protein hydrolysate (EPH) with good functional properties via fractionation technique was successfully produced. The ultrafiltration (UF) by fractionation method on the eel protein hydrolysate (EPH) affected the functional properties of the hydrolysate, including foaming properties, emulsifying properties, water holding capacity and fat binding capacity. Besides that, these functional properties were also affected by the pH values of the peptides. The 5 kDa EPH fraction was found to have the highest foaming capacity, foaming stability, emulsifying activity index and fat binding capacity. Meanwhile, the 10 kDa EPH fraction had the highest emulsifying stability index and water holding capacity. Hence, the study shows that the functional properties of fractionated EPH at various pH values have potential for improving the production of food in terms of the taste and physical characteristics.

References

- Baharuddin NA, Halim NRA, Sarbon NM (2016) Effect of degree of hydrolysis (DH) on the functional properties and angiotensin I-converting enzyme (ACE) inhibitory activity of eel (*Monoptychus* sp.) protein hydrolysate. *Intl Food Res J* 23(4):1424–1431
- Benjakul S, Morrissey MT (1997) Protein hydrolysate from Pacific whiting solid waste. *J Agric Food Chem* 45:3432–3440
- Chalamaiah M, Kumar BD, Hemalatha R, Jyothirmayi T (2012) Fish protein hydrolysate: proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chem* 135:3020–3038
- Chi C, Cao Z, Wang B, Hu F, Li Z, Zhang B (2014) Antioxidant and functional properties of collagen hydrolysates from Spanish mackerel skin as influenced by average molecular weight. *Molecules* 19:11211–11230
- dos Santos S, Martins VG, Salas-Mellado M, Prentice C (2011) Evaluation of functional properties in protein hydrolysates from bluewing searobin (*Prionotus punctatus*) obtained with different microbial enzymes. *Food Bioprocess Technol* 4:1399–1406
- Elavarasan K, Kumar NV, Shamasundar BA (2014) Antioxidant and functional properties of (FPH) from fresh water carp (*Catla catla*) as influenced by the nature of enzyme. *J Food Process Preservation* 38:1207–1214

- Foh MBK, Amadou I, Foh BM, Kamara MT, Xia WS (2010) Functionality and antioxidant properties of tilapia (*Oreochromis niloticus*) as influenced by the degree of hydrolysis. *Inter J Mol Sci* 11:1851–1869
- Halim NRA, Sarbon NM (2017) A response surface approach on hydrolysis condition of eel (*Monopterus* sp. protein hydrolysate with antioxidant activity. *Int Food Res J* 24(3):1081–1093
- Halim NRA, Yusof HM, Sarbon NM (2016) Functional and bioactive properties of fish protein hydrolysates and peptides: a comprehensive review. *Trends Food Sci Technol* 51:24–33
- Ishak NH, Sarbon NM (2017) Optimization of enzymatic hydrolysis condition of shortfin scad (*Decapterus Macrosoma*) waste for production of ACE inhibitory peptide by RSM. *Int Food Res J* 24(4):1735–1743
- Jamil NH, Halim NRA, Sarbon NM (2016) Optimization of enzymatic hydrolysis condition and functional properties of eel (*Monopterus* sp.) protein using response surface methodology (RSM). *Int Food Res J* 23(1):1–9
- Klompong V, Benjakul S, Kantachote D, Shahidi F (2007) 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* 102:1317–1327
- Li Z, Wang B, Chi C, Gong Y, Luo H, Ding G (2013) Influence of average molecular weight on antioxidant and functional properties of cartilage collagen hydrolysates from *Sphyrna lewini*, *Dasyatis akjei* and *Raja porosa*. *Food Res Int* 51:183–193
- McCarthy AL, O'Callaghan YC, O'Brien NM (2013) Protein hydrolysates from agricultural crops-bioactivity and potential for functional food development. *Agriculture* 3:112–130
- Naqash SY, Nazeer RA (2013) Antioxidant and functional properties of protein hydrolysates from pink perch (*Nemipterus japonicas*) muscle. *J Food Sci Technol* 50(5):972–978
- Prabha J, Narikimelli A, Sajini MI, Vincent S (2013) Optimization for autolysis assisted production of fish protein hydrolysate from underutilized fish *Pellona ditchela*. *Int J Sci Eng Res* 4(12):1863–1869
- Razali AN, Sarbon NM, Amin AM (2015) Antioxidant activity and functional properties of fractionated cobia skin gelatin hydrolysate at different molecular weight. *Int Food Res J* 22(2):651–660
- Taheri A, Anvar SAA, Ahari H, Fogliano V (2013) Comparison the functional properties of protein hydrolysates from poultry by-products and rainbow trout (*Onchorhynchus mykiss*) viscera. *Iran J Fish Sci* 12(1):154–169
- Thiansilakul Y, Benjakul S, Shahidi F (2007) Composition, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). *Food Chem* 103:1385–1394