INFLUENCE OF HYDROLYSIS TIME ON THE FUNCTIONAL PROPERTIES OF PROTEIN HYDROLYSATES FROM SAILFISH TRIMMINGS

Nguyen Thi My Huong[1, *]

ABSTRACT

Influence of hydrolysis time on the functional properties of protein hydrolysates from sailfish trimmings was studied. Four protein hydrolysates were obtained by hydrolysis of sailfish trimmings with enzyme Flavourzyme 0.5% at the temperature of 50°C and different hydrolysis times (2 h, 4 h, 6 h, 8 h). The study results showed that the degree of hydrolysis increased with the increase of hydrolysis time. The degrees of hydrolysis of the protein hydrolysates from sailfish trimmings after 2 h, 4 h, 6 h and 8 h of hydrolysis were 18.6%, 25.8%, 29.7% and 30.5%, respectively. The protein hydrolysate from sailfish trimmings after 8 h of hydrolysis had contents of moisture 7.2%, protein 73.4%, lipid 0.6% and ash 8.6%. The sailfish trimmings protein hydrolysate had a total amino acid content of 32.87 g/100 g of protein hydrolysate and essential amino acid content of 12.32 g/100 g of protein hydrolysate. The ratio of essential amino acids to total amino acids was 37.48%. The protein hydrolysate was rich in glycine, glutamic, aspartic, proline, leucine and alanine. The solubility of sailfish trimmings protein hydrolysates increased when hydrolysis time and degree of hydrolysis increased. The sailfish trimmings protein hydrolysates had solubility in the range of 88.3 - 95.2%. The study results showed that the foaming capacity of sailfish trimmings protein hydrolysate reached a maximum value (45.8%) at hydrolysis time of 2 h and degree of hydrolysis of 18.6%. The foaming capacity of protein hydrolysates from sailfish trimmings decreased when the hydrolysis time increased from 2h to 8h of hydrolysis. The emulsifying properties of protein hydrolysates obtained after 2 h, 4 h, 6 h and 8 h of hydrolysis of sailfish trimmings were 29.2 ml/g, 24.9 ml/g, 18.8 ml/g and 17.6 ml/g, respectively. The sailfish trimmings protein hydrolysates were found to have high nutritional value and good functional properties. The study results suggested that protein hydrolysates from sailfish trimmings could be used as a protein source in food systems and were a promising potential food ingredient.

Keywords: *Fish protein hydrolysate, functional property, nutritional property, sailfish trimmings.*

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1. INTRODUCTION

Seafood processing industry generates up to 60% by - products including head, frames, viscera, skin, trimmings, fins, roes, and only 40% fish products for human consumption [1]. The sailfish processing industry generates a large amount of trimmings, which are a rich source of protein. High protein content in fish trimmings make them more perishable, may bring undesirable effects to environment pollution. Production of protein hydrolysate from fish by - products by enzymatic hydrolysis is one way to add value to fish by - products. Enzymatic hydrolysis is the process of breaking down the peptide bonds of the parent protein using proteases, whereas the degree of hydrolysis is the ratio of the number of peptides cleaved to the number of peptide bonds contained in the protein mass [2]. Enzymatic hydrolysis is one of the most efficient methods to recover proteins from fish by - products. Moreover, the enzymatic hydrolysis of fish proteins improved its functional properties, including solubility, foaming capacity and emulsifying capacity.

Fish protein hydrolysates have a high content of amino acids and are used as available sources of protein for humans due to their good functional properties. However, there is a little information regarding protein hydrolysates from the sailfish trimmings. Therefore, the study on influence of hydrolysis time on the functional properties of protein hydrolysates from the sailfish trimmings was necessary. The fish protein hydrolysates could be used in foods for the production of nutritional drinks and milk substitutes. In addition, they could be used as ingredients to aid in foam formation and stability in some products such as beer, fresh cream, and to aid in emulsion formation and stability in some products such as margarine and sausages. Fish protein hydrolysates have been used for incorporation into different food systems such as cereal products, fish and meat products, desserts and crackers [3].

2. MATERIALS AND METHODS

2.1. Sailfish trimmings

Sailfish (*Istiophorus platypterus*) trimmings were provided by Hoang Hai, a seafood processing company in Nha Trang, Vietnam. The sailfish trimmings were frozen and transported to the laboratory. After their arrival, they were thawed and ground in a grinder through 3 mm plate. The minced materials were packed in plastic bags (0.5 kg per unit), frozen and stored at - 20°C until use.

2.2. Enzyme Flavourzyme

Flavourzyme is a fungal protease/peptidase complex produced by submerged fermentation of a selected strain of *Aspergillus oryzae* and it contains both endoprotease and exopeptidase activities. The declared activity was 500 LAPU/g (Leucine Amino Peptidase Units per gram). This commercial enzyme was produced by Novozymes A/S (Bagsvaerd, Denmark). The optimal working conditions provided by the manufacturer for Flavourzyme are at 50°C and pH 5 - 7.

[1] Faculty of Food Technology - Nha Trang University
* Email: huongntm@ntu.edu.vn

2.3. Preparation of fish protein hydrolysate from sailfish trimmings

The four samples of sailfish trimmings was hydrolyzed with the water/trimmings ratio of 1/1 using Flavourzyme with 0.5% of the weight of sailfish trimmings (w/w) at 50°C and natural pH of substract (pH = 6) for 2 h, 4 h, 6 h and 8 h. After hydrolysis, the enzyme was inactivated by heating in a water bath at 85°C for 15 min. The mixture was centrifuged at 10000 rpm for 20 min to separate the liquid protein hydrolysate and the sludge. The liquid protein hydrolysate was then spray - dried to obtain the protein hydrolysate powder which was stored in a sealed plastic bag at 4°C until analyses.

2.4. Analysis methods

The moisture content was determined by drying the samples in an oven at 105°C until constant weight. Ash content was measured by incinerating the samples in a furnace at 600°C. Total nitrogen content was determined by the Kjeldahl method. Crude protein content was determined by multiplying total nitrogen content by 6.25. Lipid content was determined according to Folch *et al.* (1957) [4]. Amino acid composition was determined according to Kechaou *et al.* (2009) [5]. Degree of hydrolysis was determined according to Nguyen *et al.* (2011) [6].

Solubility of fish protein hydrolysate was determined according to Klompong *et al.* (2007) [7]. Each 200 mg of protein hydrolysate sample was dispersed in 20 ml of deionized water. The mixture was stirred at the room temperature for 30 min and centrifuged at 7500 rpm for 15 min. Protein content in the supernatant was determined using the biuret method. The solubility of fish protein hydrolysate, defined as the amount of soluble protein from the total protein, was calculated as follows:

Solubility = (Protein in supernatant/Total protein in sample) x 100.

Foaming capacity (FC) was determined according to Amiza *et al.* (2012) [8]. Fish protein hydrolysate (3 g) was dispersed in 100 ml of distilled water and the mixture was homogenized for 1 min using a homogenizer at high speed. The mixture was then poured into a 250 ml graduated cylinder and the total volume was read. Foaming capability was expressed as percentage of volume increase upon whipping. Foaming capability was calculated as follows:

FC% = (Volume after homogenisation - Volume before homogenisation) x 100/Volume before homogenisation.

Emulsification capacity (EC) was determined according to Diniz and Martin (1997) [9]. Protein hydrolysate samples (0.5 g) and 30 ml of vegetable oil were added to 60 ml of NaCl solution (30 g/l) and mixed using a homogenizer at 9500 rpm for 30 min. Then, another 30 ml of oil were added over 1.5 min and mixed for a further 30 seconds. The mixture was transferred to centrifuge tubes, held in a water-bath at 85°C for 15 min, and then centrifuged at 3000 rpm for 30 min. Emulsification capacity was calculated by the following equation:

EC (ml/g) = $(V_A - V_R)/W_S$ where V_A was the volume of oil added to form an emulsion (ml), V_R was the volume of oil released after centrifugation (ml), W_S was the weight of sample (g).

2.5. Statistical analysis

The experiments were carried out in triplicates. The data obtained were subjected to one way analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine the significant difference between samples at $p<0.05$ level using the SPSS programme (SPSS Version 20.0).

3. RESULTS AND DISCUSSION

3.1. Proximate composition of the sailfish trimmings

The proximate composition of the sailfish trimmings is shown in table 1.

Table 1. Proximate composition of the sailfish trimmings

Proximate composition (%)	Sailfish trimmings
Moisture	75.2 ± 0.4
Protein	21.3 ± 0.2
Lipid	1.5 ± 0.1
Ash	1.2 ± 0.1

Data are expressed as mean ± standard deviation (n=3).

The study result showed that the moisture content of sailfish trimmings was 75.2%, which was higher than those of tilapia waste (66.29%) [10] and trout viscera (71.65%) [11]. The protein content of sailfish trimmings (21.3%) was far higher than those of carp meat (18.4%) [12] and eel meat (16.88%) [13]. Lipid content of sailfish trimmings (1.5%) was lower than those of carp meat (3.52%) [12] and eel meat (3.41%) [13]. Ash content (1.2%) of sailfish trimmings was higher than those of carp meat (1.07%) [12] and eel meat (0.89%) [13]. The study result showed that the sailfish trimmings was a good source of protein which can be used for production of protein hydrolysate.

3.2. Chemical composition of protein hydrolysate from sailfish trimmings

The chemical composition of the protein hydrolysate from sailfish trimmings after 8 h of hydrolysis is shown in table 2.

Table 2. Chemical composition of protein hydrolysate from sailfish trimmings

Proximate composition	Content (%)
Moisture	7.2 ± 0.1
Protein	73.4 ± 0.3
Lipid	0.6 ± 0.1
Ash	8.6 ± 0.2

Data are expressed as mean ± standard deviation (n=3).

The protein content of sailfish trimmings protein hydrolysate was 73.4%, which was higher than those of salmon head protein hydrolysates (62.3 - 64.8%) [14] but lower than those of tilapia waste protein hydrolysate

(82.19%) [10] and trout viscera protein hydrolysate (88.32%) [11]. The sailfish trimmings protein hydrolysate had protein content similar to thornback ray muscle hydrolysate (73.41%) [15]. The lipid content of protein hydrolysate from sailfish trimmings was 0.6%, which was lower than that of shortfin scad waste hydrolysate (7.55%) [16]. The low lipid content of sailfish trimmings protein hydrolysate might enhance stability of the hydrolysate towards lipid oxidation. The ash content of sailfish trimmings protein hydrolysate was 8.6%, which was lower than that of shortfin scad waste hydrolysate (10.4%) [16].

3.3. Amino acid composition of the protein hydrolysate from sailfish trimmings

The composition and content of amino acids indicated the nutritional quality of sailfish trimmings protein hydrolysate obtained after 8 h of hydrolysis.

Table 3. Amino acid composition of the protein hydrolysate from sailfish trimmings

Amino acids	Content (g/100 g protein hydrolysate)
Alanine	2.28 ± 0.14
Aspartic	3.58 ± 0.16
Glutamic	4.14 ± 0.19
Glycine	5.35 ± 0.21
Histidine	1.62 ± 0.08
Isoleucine	1.72 ± 0.07
Leucine	2.62 ± 0.12
Lysine	1.25 ± 0.07
Methionine	0.75 ± 0.02
Phenylalanine	1.27 ± 0.06
Proline	2.96 ± 0.11
Serine	1.27 ± 0.06
Threonine	1.33 ± 0.08
Tyrosine	0.97 ± 0.04
Valine	1.76 ± 0.07
Total amino acids	32.87 ± 0.35
Total essential amino acids	12.32 ± 0.28
Total essential amino acids/Total amino acids (%)	37.48 ± 0.32

Data are expressed as mean ± standard deviation (n=3).

Sailfish trimmings protein hydrolysate had a total amino acid content of 32.87 g/100 g of protein hydrolysate and total essential amino acid content of 12.32 g/100 g of protein hydrolysate. The ratio of essential amino acids to total amino acids was 37.48%. The content of essential amino acids showed the potential of hydrolysate to use as a nutrition source in food for human. Sailfish trimmings protein hydrolysate was rich in glycine, glutamic, aspartic, proline, leucine and alanine. However, the protein hydrolysate from sailfish trimmings contained low contents of methionine and tyrosin. According to Chalamaiah *et al.* (2012), aspartic acid and glutamic acid contents were found to be

higher than others amino acids in most fish protein hydrolysates [17]. The amino acid content of sailfish trimmings protein hydrolysate was higher than that of protein hydrolysate from sardine by-product (19.72 g of total amino acid /100 g and 8.2 g of essential amino acid/100 g) [18]. The present study indicated that sailfish trimmings protein hydrolysate was found to have high nutritional value and could be used in human diets.

3.4. Functional properties of the protein hydrolysate from sailfish trimmings

3.4.1. Solubility

The degree of hydrolysis and solubility of the protein hydrolysate from sailfish trimmings are displayed in figure 1. The solubility is one of the most important functional properties of fish protein hydrolysates. The high solubility of protein hydrolysates showed potential applications in food industry [8].

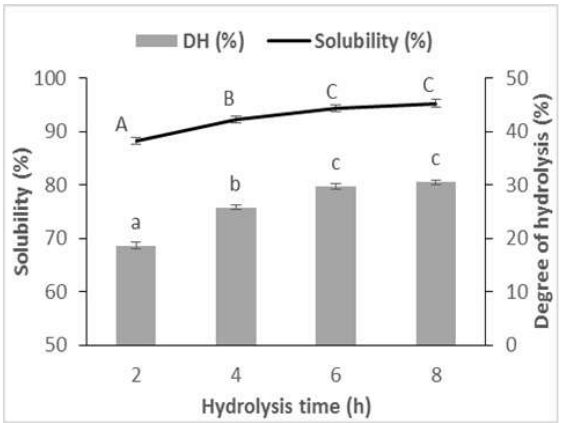

Figure 1. The degree of hydrolysis (DH) and sobubility of sailfish trimmings protein hydrolysate. Different letters show significant differences ($p < 0.05$)

The study results indicated that the degree of hydrolysis (DH) increased with increasing hydrolysis time. The degrees of hydrolysis of the protein hydrolysates from sailfish trimmings after 2 h, 4 h, 6 h and 8 h of hydrolysis were 18.6%, 25.8%, 29.7% and 30.5%, respectively. There was not a significant difference in degree of hydrolysis between hydrolysis time of 6 h and 8 h. The hydrolysis of the fish protein was characterized by an initial rapid phase, during which a large number of peptide bonds were hydrolyzed. Thereafter, the rate of enzymatic hydrolysis decreased. This result was similar to those previously reported for herring head [19] and salmon head [14].

According to results showed in figure 1, the solubility of protein hydrolysates from sailfish trimmings increased when hydrolysis time and degree of hydrolysis increased. Sailfish trimmings protein hydrolysates had solubility in the range of 88.3 - 95.2%. The fish protein hydrolysates with high degrees of hydrolysis gave higher solubility than those with low degrees of hydrolysis. The protein hydrolysate with the highest degree of hydrolysis of 30.5% at hydrolysis time of 8 h showed the highest solubility (95.2%). There was a significant difference ($p<0.05$) in solubility between hydrolysis time of 2 h and hydrolysis time of 4 h as well as between 4 h and 6 h. However, no significant difference in solubility was observed between hydrolysis

time of 6h and 8h. The similar result was also indicated in the study by Gbogouri *et al.* (2004) where the solubility of salmon by - products hydrolysates increased with the increase in degree of hydrolysis [20]. Gbogouri *et al.* (2004) showed that the solubility of salmon by - products hydrolysates was more than 90% [20] while the solubility of cobia frame hydrolysates was in the range of 85 - 86% [8].

In present study, the hydrolysis of sailfish trimmings formed a large number of hydrophilic polar group peptides when the hydrolysis time increased resulting in the high solubility of sailfish trimmings protein hydrolysate. Enzymatic breakdown of fish protein leaded to a major structural change in that the protein was gradually cleaved into smaller peptides. The increase in solubility of the hydrolysates with increased hydrolysis time may be due to the cleavage of proteins into smaller peptides that have more polar residues, with the ability to form hydrogen bonds with water and enhance solubility [2, 20].

3.4.2. Foaming capacity

The foaming capacity of protein hydrolysate from sailfish trimmings is indicated in figure 2.

When hydrolysis time increased, the sailfish trimmings protein hydrolysate displayed a lower foaming capacity. The study results showed that the foaming capacity of protein hydrolysates from sailfish trimmings decreased when the hydrolysis time increased from 2 h to 8 h. The foaming capacity of sailfish trimmings protein hydrolysate reached a maximum value (45.8%) at hydrolysis time of 2 h and degree of hydrolysis of 18.6%. The degree of hydrolysis obtained the highest value (30.5%) at hydrolysis time of 8 h but at that time the foaming capacity of sailfish trimmings protein hydrolysate was lowest (35.7%). The decrease in foaming capacity of protein hydrolysate with increased hydrolysis time may be due to the formation of smaller peptides with less surface activity and due to the reduction of hydrophobicity of protein hydrolysate during extensive hydrolysis [2].

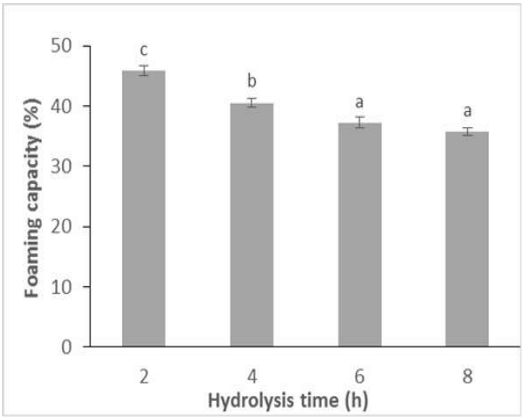

Figure 2. Foaming capacity of sailfish trimmings protein hydrolysate. Different letters show significant differences ($p < 0.05$)

Baharuddin *et al.* (2016) indicated that the foaming capacity of eel protein hydrolysate decreased significantly when the degree of hydrolysis increased from 36% to 69% [21].

The similar trend of foaming capacity was also observed for the protein hydrolysates from cobia frame [8], sardine by - product [2] and yellow stripe trevally muscle [7]. Cobia frame protein hydrolysate with the lowest degree of hydrolysis gave highest foaming capacity (122.7%) [8]. The capelin protein hydrolysate possessed the foaming capacity of 90% [22] while the protein hydrolysate from yellow stripe trevally muscle gave the foaming capacity up to 200% [7].

3.4.3. Emulsifying capacity

Emulsifying capacity of sailfish trimmings protein hydrolysate is showed in figure 3.

The study results showed that the emulsifying capacity of sailfish trimmings protein hydrolysates decreased with increasing hydrolysis time. Hydrolysates with low degree of hydrolysis had high emulsifying capacity and extensive hydrolysis resulted in a decrease in emulsifying capacity. After 2 h, 4 h, 6 h and 8 h of hydrolysis corresponding to degree of hydrolysis 18.6%, 25.8%, 29.7% and 30.5%, the emulsifying capacity of sailfish trimmings protein hydrolysates were 29.2 ml/g, 24.9 ml/g, 18.8 ml/g and 17.6 ml/g, respectively. The similar trend was also reported for protein hydrolysates from sardine by-products [2], cobia frame [8] and carp skin [23]. Souissi *et al.* (2007) indicated that the protein hydrolysates from sardine by - products with degrees of hydrolysis of 6.62%, 9.31% and 10.16% had emulsifying capacity of 20 ml/g, 15.2 ml/g and 10.8 ml/g, respectively [2]. The carp skin protein hydrolysates had emulsifying capacity in the range of 20 - 38 ml/g [23] while the cobia protein hydrolysates had emulsifying capacity in the range of 3 - 12 ml/g [8].

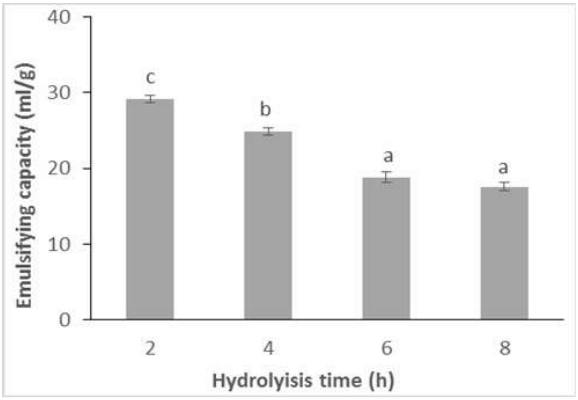

Figure 3. Emulsifying capacity of sailfish trimmings protein hydrolysate. Different letters show significant differences ($p < 0.05$)

Fish protein hydrolysates are surface active materials and promote oil - in water emulsions. The emulsifying properties of protein hydrolysis can be explained based on their surface properties. According to Foh *et al.* (2010), an increase in the number of peptide molecules and exposed hydrophobic amino acid residues due to hydrolysis of proteins contribute to an improvement in the formation of emulsions [24]. However, the hydrolysates with a higher degree of hydrolysis had poorer emulsifying capacity due to their small peptide size. Moreover, the diminishing in emulsifying capacity with an

extensive hydrolysis process is also due to the reduction of hydrophobicity of the hydrolysate during hydrolysis [2].

4. CONCLUSION

The sailfish trimmings were a rich source of protein and utilized for the production of fish protein hydrolysate. Enzymatic hydrolysis of sailfish trimmings with different hydrolysis times had a significant effect on the solubility, foaming capacity and emulsifying capacity of protein hydrolysates. The solubility of sailfish trimmings protein hydrolysates increased with increasing hydrolysis time. The sailfish trimmings protein hydrolysate at hydrolysis time of 8 h showed the highest solubility (95.2%). The protein hydrolysate after 8 h of hydrolysis had contents of moisture 7.2%, protein 73.4%, lipid 0.6% and ash 8.6%. The sailfish trimmings protein hydrolysate had a high nutritional value with total amino acid content of 32.87 g/100 g. On the contrary, the foaming capacity and emulsifying capacity decreased with increasing hydrolysis time from 2 h to 8 h. The sailfish trimmings protein hydrolysate at hydrolysis time of 2 h reached the highest foaming capacity (45.8%) and highest emulsifying capacity (29.2 ml/g). Research results suggested that sailfish trimmings protein hydrolysates had potential application as functional ingredients in different foods and could be used in food industry for human consumption.

REFERENCES

1. Dekkers E, Raghavan S, Kristinsson HG. Marshall MR. (2011). Oxidative stability of *Mahi mahi* red muscle dipped in tilapia protein hydrolysates. Food Chem. 124: 640 - 645.

2. Souissi N., Bougatef A., Triki-Ellouz Y., Nasri M. (2007). Biochemical and functional properties of Sardinella (*Sardinella aurita*) by-product hydrolysates. Food Techology Biotechnology, 45 (2): 187 - 194.

3. Kristinsson H. G., Rasco B. A. (2000). Fish protein hydrolysates: production, biochemical, and functional properties. Critical Review in Food Science and Nutrition, 40: 43 - 81.

4. Folch J., Lees N., Sloan-Stanley G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497 - 509.

5. Kechaou, E. S., Dumay, J., Donnay - Moreno, C., Jaouen, P., Gouygou, J. P., Bergé, J. P., Amar, R. B. (2009). Enzymatic hydrolysis of cuttlefish (*Sepia officinalis*) and sardine (*Sardina pilchardus*) viscera using commercial proteases: Effects on lipid distribution and amino acid composition. *Journal of Bioscience and Bioengineering*, 107, 158 - 164.

6. Nguyen T. M. H, Sylla K. S. B., Randriamahatody Z., Donnay - Moreno C., Moreau. J., Tran T, L, Bergé J. P. (2011). Enzymatic hydrolysis of yellowfin tuna (*Thunnus albacares*) by - products using

Protamex protease. *Food Technology and Biotechnology*, 49 (1): 48 - 55.

7. 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 Chemistry, 102: 1317 - 1327.

8. Amiza M. A., Kong Y. L., Faazaz A. L. (2012). Effects of degree of hydrolysis on physicochemical properties of Cobia *(Rachycentron canadum)* frame hydrolysate, *International Food Research Journal*, 19 (1): 199 - 206.

9. Diniz F. M., Martin A. M. (1997). Effects of the extent of enzymatic hydrolysis on functional properties of shark protein hydrolysate, Lebensm. - Wiss. U. - Technol., 30: 266 - 272.

10. Srikanya, A., Dhanapal, K., Sravani1, K., Madhavi K., and Kumar, P G. (2017). A study on optimization of fish protein hydrolysate preparation by enzymatic hydrolysis from tilapia fish waste mince. *International Journal of Current Microbiology and Applied Sciences*, 6 (12) pp. 3220 - 3229.

11. Taheri, A, Anvar, S. A. A, Ahari H , Fogliano, V. (2013). Comparison the functional properties of protein hydrolysates from poultry byproducts and rainbow trout (*Onchorhynchus mykiss*) viscera. *Iranian Journal of Fisheries Sciences*, 12 (1) 154 - 169.

12. Saputra, D., Nurhayati, T. (2016). Production of fish hydrolysate protein from waste of fish carp (*Cyprinus carpio*) by enzymatic hydrolysis. Com Tech, 7 (1): 11 - 18.

13. Halim, N. R. A., Sarbon, N. M. (2017). A response surface approach on hydrolysis condition of eel (*Monopterus* sp.) protein hydrolysate with antioxidant activity. *International Food Research Journal,* 24 (3) 1081 - 1093.

14. Sathivel S., Smiley S., Prinyawiwatkui W., Bechtel P. J. (2005). Functional and nutritional properties of red salmon (*Oncorhynchus nerka*) enzymatic hydrolysates. *Journal of Food Science*, 70 (6): 401 - 406.

15. Lassoued, I., Mora, L., Nasri, R., Aydi, M., Toldrá, F., Aristoy, M. C., Barkia, A. and Nasri, M. (2015). Characterization, antioxidative and ACE inhibitory properties of hydrolysates obtained from thornback ray *(Raja clavata)* muscle. *Journal of Proteomics,* 128: 458 - 468.

16. Ishak, N. H; *Sarbon, N. M. (2018). Physicochemical characterization of enzymatically prepared fish protein hydrolysate from waste of shortfin scad *(Decapterus macrosoma). International Food Research Journal*, 25 (6): 2593 - 2600.

17. Chalamaiah M., Dinesh kumar B., Hemalatha R., Jyothirmayi T. (2012). Fish protein hydrolysates: Proximate composition,

amino acid composition, antioxidant activities and applications: A review. Food Chemistry, 135: 3020 - 3038.

18. Batista I., Pires C. (2011). Feasibility study - Production of protein hydrolysates from sardine byproducts. Atlantic Area Programme.

19. Sathivel S., Bechtel P. J., Babbitt J., Smiley S., Crapo C., Reppond K. D., Prinyawiwatkul W. (2003). Biochemical and functional properties of herring (*Clupea harengus*) byproduct hydrolysates. Food Science, 68: 2196 - 2200.

20. Gbogouri G. A., Linder M., Fanni J., Parmentier M. (2004). Influence of hydrolysis degree on the functional properties of salmon byproducts hydrolysates. *Journal of Food Science*, 69 (8): 615 - 622.

21. Baharuddin, N. A., Halim, N. R. A., Sarbon, N. M. (2016). Effect of degree of hydrolysis (DH) on the functional properties and angiotensin I - converting enzyme (ACE) inhibitory activity of eel (*Monopterus* sp.) protein hydrolysate. *International Food Research Journal*, 23 (4), 1424 -1431.

22. Shahidi F., Han X. Q, Synowieck J. (1995). Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem, 53: 285 - 293.

23. Wasswa J., Tang J., Gu X., Yuan X. (2007). Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp (*Ctenopharyngodon idella*) skin. Food Chemistry, 104: 1698 - 1704.

24. Foh, M. B. K., Amadou I., Foh, B. M., Kamara, M. T., Xia, W. (2010). Functionality and antioxidant properties of Tilapia (*Oreochromis niloticus*) as influenced by the degree of hydrolysis. *International Journal of Molecular Sciences*., 11, 1851 - 1869.