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Molecular characterization and structural analysis of fish protein hydrolysate from bycatch *Upeneus teniopterus* (Cuvier, 1829)

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Abstract
Fish is usually regarded as a quality protein sources for human and animal consumption, rich in essential amino acids and good for digestibility. Fish by-product also contains the same valuable protein as the fish muscles. Recovery of proteins presents in the by-products and using these as functional ingredients, such as bioactive peptides is a very exciting and promising alternative. By using enzyme technology, it may be possible to produce a broad spectrum of food ingredients for wide range of applications. In the present study molecular characterization and structural analysis of fish protein hydrolysate from by catch fish *Upeneus teniopterus* were investigated. The electrophoretic techniques (SDS – PAGE) are promising tools for identifying protein profile in fish protein hydrolysate from by catch fishes. The samples *Upeneus teniopterus* control (UTC) and *Upeneus teniopterus* control - lyophilized (UTCL) had higher molecular weight bands; whereas in *Upeneus teniopterus* hydrolysate (UTH) and *Upeneus teniopterus* hydrolysate – lyophilized (UTHL) showed low molecular weight bands as acid aided in breakdown of protein into small peptides. The HR-SEM analysis was conducted to observe the microstructure of UTCL and UTHL. The microstructure of samples was obtained at different magnifications like 500x, 1000x and 1200x. HR-SEM analysis of UTCL and UTHL revealed different morphological structures due to degree of hydrolysis. The changes in functional properties of native proteins are related to produce by enzymatic hydrolysis, which are mainly characterized by a lower molecular mass, exposure of hydrophobic groups and by an increased number of ionic groups. Fish protein hydrolysate has a broad spectrum of applications ranging from high-value peptones to food ingredients with special functional properties.

Keywords: Fish protein hydrolysate, SDS-PAGE and HR –SEM

1. Introduction
Proteins are important in food processing and product development, as they are accountable for various functional properties that influence consumer acceptability. Fish by products are usually discarded and cause numerous environmental problems and contain considerable amounts of proteins that are known to possess high nutritional value (Arvanitoyannis and Kassaveti, 2008; Jung *et al*., 2006, Fahmi *et al*., 2004) [13]. It has been estimated that the value addition of human food developed from the by product will increase significantly in the future (Kristinsson and Rasco 2000; Tahergorabi *et al*. 2012) [33]. Recovery of proteins from these by-products and conversion to high value products, such as bioactive peptides is a very exciting and promising alternative (Sathivel *et al*. 2004) [31]. Thus, production of fish protein hydrolysate from fish waste is the most convenient method considered by researchers around the world for the utilization of fish waste (Chalamaiah *et al*., 2012; Olsen *et al*., 2014) [26]. Fish protein hydrolysate (FPH) is a breakdown product of fish proteins containing smaller peptides and amino acids. Biopeptides exhibits various biological activities such as antioxidant activity, antimicrobial activity, anticancer activity, antihypertensive activity and anti-inflammatory activity (Lordan *et al*., 2011).
Enzymatic hydrolysis is employed more frequently to produce fish protein hydrolysate. A variety of enzymes are employed in the preparation of protein hydrolysate includes alcalase, bromelain, flavourzyme, neutrase, pepsin, trypsin and papain (Razali *et al*., 2013). The enzymatic hydrolysis influences the protein functional properties by changing the peptides and amino acid composition of the proteins. Therefore, a controlled enzymatic process can result in hydrolysate products with improved functional properties such as improved pH functionality compared to the native proteins (De Castro *et al*., 2015) [12]. In particular, protease affects the Molecular weight and hydrophobic properties of proteins and consequently the protein

functional properties (Villamil *et al*., 2017) [35]. Therefore, with proper selection of the enzyme and substrates, it is possible to achieve peptides in the hydrolysate with a desired molecular weight range. In the present study fish protein hydrolysate from *Upeneus taeniopterus* were studied. The *Upeneus taeniopterus* commonly called goat fish belongs to the family Mullidae. *U. taeniopterus* considered as non-commercial species abundantly landing around coastal waters termed as bycatch and discarded as waste. This is because people are unaware of high protein profile present in the goat-fish which has to be exploited and discovered. Also, it is ecologically and commercially important inhabitants of sand-associated, shallow habitats are a clade that requires enhanced biodiversity-related research. *U. taeniopterus* are indicators in tropical and temperate coastal habitat monitoring and management (Uiblein and Gouws, 2014) [34]. Recently, Akhila Narikimilli *et al*. (2019) studied the production and physcio chemical parameters of Fish Protein hydrolyste from *U. taeniopterus*. Nowadays, much attention has been paid to unravelling the structural, compositional, and sequential properties of bioactive peptides. The electrophoresis of proteins is an effective technique for generating systematic data from macromolecules; since proteins are species-specific and electrophoretic separations are easily performed (Corzo *et al*., 1984; Jesslin *et al*., 2013) [10]. Many researchers have used the protein electrophoresis as a valid tool to determining intra and inter-specific variation among species, which may reflect the metabolic level of the organism and its adaptations to environmental fluctuations and the different nutritional status of the fish and feeding habits (McDonald and Milligan, 1992; Zowail *et al*. 1994; Navarro and Gutiérrez, 1995) [23, 38]. SDS-PAGE is an important molecular technique used for the identification at species level of whole cell proteins and it has the advantage of being fairly simple and rapid to perform (Leisner *et al*. 1994) [20]. The micro-structural analysis is generally used to establish the relationships between protein structure and functional property. Structure of dried protein hydrolysate plays a vital role in determining functional properties of protein hydrloysate. Scanning electron microscope (HR-SEM) was used to show microstructure of dried samples, treated with different conditions. However there is no reported research on molecular characterization and structural analysis of fish hydrolysate from *U. teniopterus*. Hence the present study was undertaken to elucidate the molecular characterization and structural analysis of fish protein hydrolysate from bycatch fish *U. teniopterus*.

2. Materials and methods

In the present study molecular characterization and structural analysis of fish protein hydrolysate from bycatch fish *Upeneus teniopterus* was studied.

2.1 Collection and preparation of Sample

The by catch fish *Upeneus teniopterus* (Fig.1) was collected from shores across Kasimedu shore, Chennai, Tamil Nadu. *U. teniopterus* was kept in ice-box in cold condition for further analysis. *U. teniopterus* was cut into small pieces and were ground in a conventional mixer separately. The homogenised fish were stored in deep freezer -20 ^{0}C for experimental use.

Fig 1: *Upeneus taeniopterus*

2.2 Preparation of fish protein hydrolysate (FPH)

The fish protein hydrolysate was produced under sterilized conditions. 50gm of homogenate is added to 500 ml sterilized water. The pH is adjusted to 4 using 3% formic acid. Hydrolysis process is continued for 90 minutes at 40 ^{0}C. After hydrolysis, it is kept in water bath at 90 ^{0}C for 15 min to terminate the hydrolysis. Homogenate was centrifuged at 10000 rpm for 15 minutes; collect the supernatant and discarded the pellets. The supernatant was pre freezed to -80 ^{0}C in round bottomed flask and were lyophilised in lyophilizer. The samples were lyophilized until it turned to powder. This is essential to carry out characterization and property analysis. Also, lyophilisation increases the storage capacity and stability of sample. The lyophilised samples are more applicable in Pharmaceutical field and Food industries as they exhibit increased shelf-life (Jeff, 2009).

2.3 Sds-Page Analysis

SDS-PAGE electrophoresis method was used to determine the protein composition and molecular mass. The SDS-PAGE was performed according to the method described by Laemmli (1970) [18]. First all the required solutions to run SDS-PAGE were prepared with pH adjusted. The stacking gel, separating gel, stock, sample buffer, Tank buffer (pH should be accurate & not adjusted) staining and destaining solutions were prepared. The fish protein hydrolysate sample was prepared by mixing equal ratios of protein and sample buffer. The four different samples UTC (*Upeneus teniopterus* – control), UTH (*Upeneus teniopterus* –Hydrolysate) UTCL (*Upeneus teniopterus* – control lyophilised) and UTHL (*Upeneus teniopterus* – Hydrolysate lyophilised) samples were loaded into wells in gel and tracked using tracking dyes bromo phenol blue. The electrophoresis is run by applying current. The SDS-PAGE is run till the dye reaches the bottom part of gel. The proteins were separated into bands that are stained. After this process the gel slab was removed carefully using forceps and washed with destaining solution. The gel was then preserved in 7% acetic acid. The gel containing protein bands can be viewed under UV-illuminator.

2.4 HR- Scanning Electron Microscopy

The microstructure of the fish protein hydrolysate produced was observed under the Scanning electron microscope at 100x, 500x, 600x, 1000x & 1200x magnification at WD 9.9mm. The HR-SEM analysis was conducted in IIT at SAIF (Modified method of Moreira *et al*., 1997) [24].

3. Results and discussion

The present study elucidated the molecular characterization and structural analysis of fish protein hydrolysate from by catch *Upeneus teniopterus*

3.1 SDS-PAGE analysis

The electrophoretic pattern profile of four different samples UTC, UTH, UTCL and UTHL were clearly observed in SDS-gel in Fig 2. In L1 and L3 the intensity of the molecular mass was equal or less than 66 - 97 KDa and 20 - 35 KDa. Similarly, Prabha *et al*. (2016) [27]. studied the SDS – PAGE analysis of fish protein hydrolysate from *Leiognathus bindus* showed the range of 14-20 kDa due to the extent of protein hydrolysis was supported by the disappearance of large proteins in the electrophoretic profile of the protein hydrolysates. Likewise, protein with a MW of 97 kDa might be a vitellin like protein, which was found in *salmon Oncorhynchus keta* and *sturgeon Acipenser transmontanus* roes was reported by Al-Holy and Rasco (2006) [2]. Also, Souissi *et al*. (2007) [32] observed that the fish protein hydrolysate showed 63 KDa, due to larger proteins contained in raw material which are not totally hydrolysed by the enzyme. The fish 35 kDa protein as fish tropomyosin based on its thermal stability, similarity of molecular weight, migration of molecular weight and matched biological characteristics. Fish tropomyosin is a ubiquitous protein uniformly distributed throughout the muscle of the fish, from head to caudal fin. Tropomyosin was deemed a suitable marker protein to represent the presence of fish in food (Yi-Tien Chen, 2012) [9].

In L2 and L4 the sample exhibits an intensive banding pattern ranging from 14.3 - 3.5 KDa. Likewise, our results were akin with Balaswamy *et al*. (2011) [4] who observed the high proportion of shorter peptides below 14.4 kDa for *Catla catla* roe hydrolysate with 1 % alcalase. According to Roslan *et al*. (2014) [30], alcalase has shown protein cleavage, leading to production of small peptides. The molecular weight derived by gel filtration technique relates to undissociated molecule, while by SDS-PAGE gives that under reduced conditions (Binsi *et al*. 2009) [7]. Several studies have shown alcalase's ability to produce low molecular weight peptides via a high degree of hydrolysis (Lalasidis *et al*., 1978; Benjakul and Morrissey, 1997 and Liaset *et al*., 2000) [19, 5, 21]. According to Bhaskar *et al*. (2008) [6], fish protein hydrolysate with high nutritional values should be rich in low molecular weight peptides, and the successful production of such desired peptides from *Tilapia* by product indicated its potential application in functional food products. The samples UTC and UTCL had higher molecular weight bands. This can be due to improper breaking down of certain peptides during hydrolysis process as hydrolysis is carried out without adding acid. Whereas the samples in UTH and UTHL the peptides show low molecular weight bands as acid aided in breakdown of protein into small peptides. Smaller peptides have higher solubility than intact proteins. Hence, the presence of low molecular weight bands in UTH and UTHL may result in the production of peptides with potent biological properties. Also Prabha *et al*. (2016) [27] opined that low molecular weight peptides are obtained in the hydrolyzed samples which indicate high protein solubility confirmed to have the potential application as an ingredient in the balanced human diet.

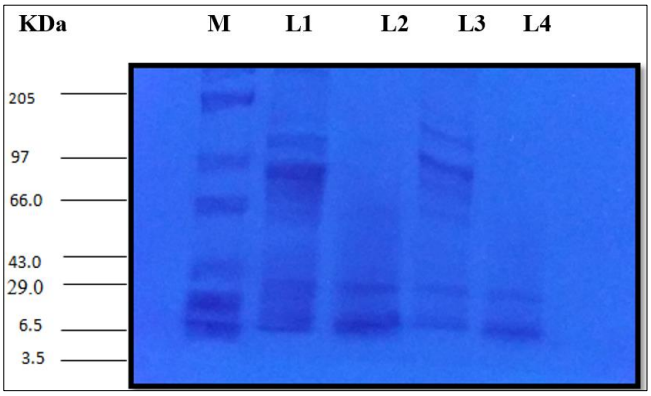

Fig 2: SDS-PAGE analysis of protein hydrosylates from *Upeneus taeniopterus* UTC, UTH, UTCL and UTHL

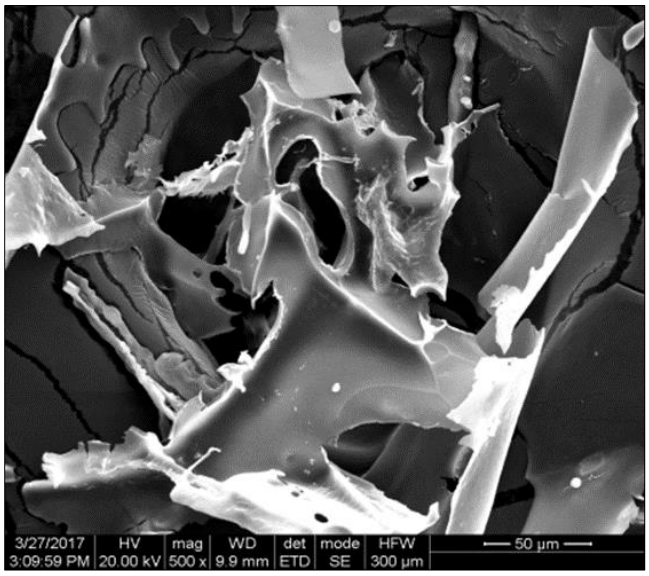

Fig 3: UTC sample examined at 500Mag, HV-20,00KV, WD-9.9mm and HFW-300µm showing flake-like structures

3.2 HR-SEM analysis

The HR-SEM analysis was conducted to observe the microstructure of the UTCL and UTHL. The micro-structural analysis is generally used to establish the relationships between protein structure and functional property. The microstructure of UTCL and UTHL were observed at different magnifications like 500x, 1000x and 1200x (Fig.4-9). The UTCL appeared as irregular broken micro flake-like structures at 500x Mag, W.D-9.9mm. When observed at 500 x Mag, W.D- 9.9mm appeared as smooth porous matte-like structures resembled honey-comb. When Mag was increased to 1200x at W.D-9.9mm they appeared as folded structures with flakes stacked. The UTHL were also observed under same wavelength like the UTCL. The UTHL appeared as smooth packed aggregates at 1000x Mag, W.D-9.6mm. When examined at 1000x Mag, W.D-9.6mm they appeared as smooth aggregates. When examined at same Mag with W.D-9.7mm appeared as rod-like porous structure.

Priyanka Dash and Goutam Ghosh (2017) observed that the protein hydrolysates from fish showed porous type morphological characters. Likewise, Wei *et al*. (2018) reported the micro structure analysis of fish protein

hydrolysate from Surimi exhibits smooth protein matrix structure. These changes in morphological characters may affect functional properties of protein hydrolysates (Zhao *et al*. 2015) [37]. The microstructure is dependent on the degree of hydrolysis, the irregular aggregates and smooth porous surfaces observed in UTHL is due to acid hydrolysis. In UTCL more flake-like structures was due to absence of hydrolysis. Similar results were concurrence with Rawdkuen *et al*. (2009) [28]. and Foh *et al*. (2011) [14]. However, drying of protein induce few stresses that can denature protein by modifying protein structures (Joshi *et al*. 2011).

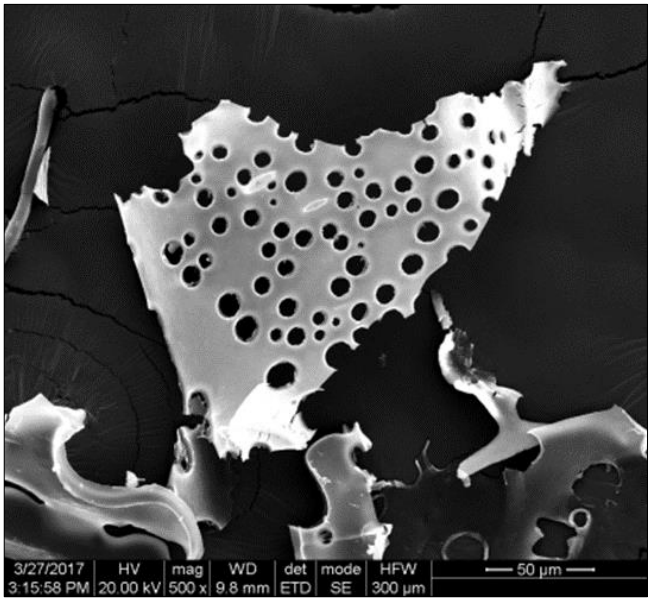

Fig 4: UTH sample examined at 500 Mag, HV-20,00KV, W.D-9.8 mm and HFW-300µm showing porous matrix

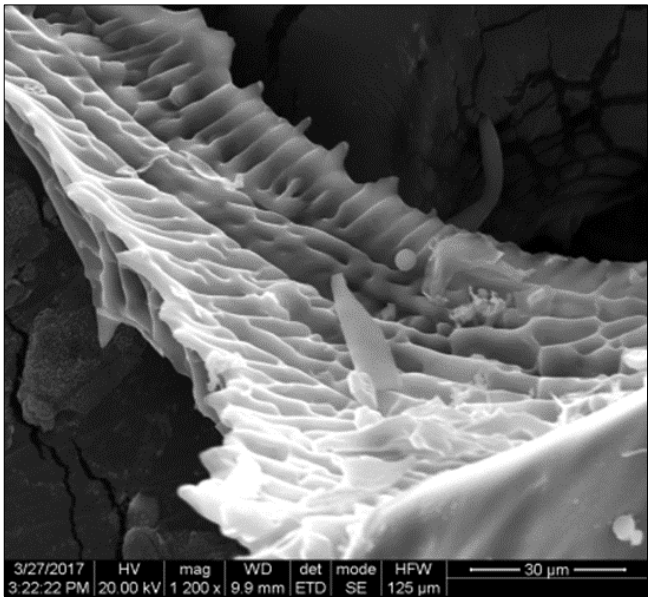

Fig 5: UTC sample examined at 1000 Mag, HV-20,00KV, W.D-9.9 and HFW-125µm showing folded structures

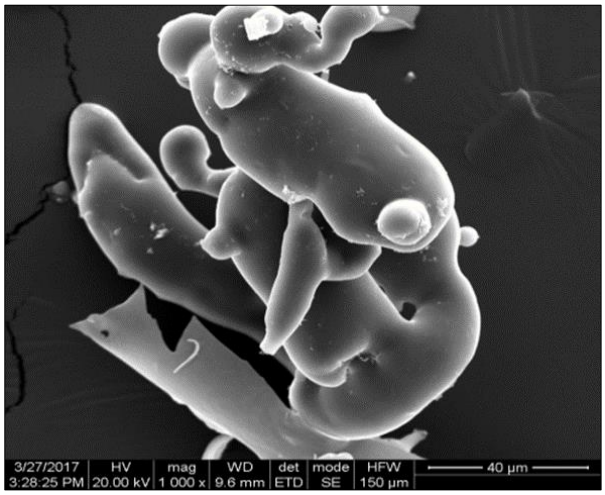

Fig 6: UTH sample examined at 1000Mag, HV-20.00KV, W.D-9.6mm and HFW-150µm showing smooth packed aggregates

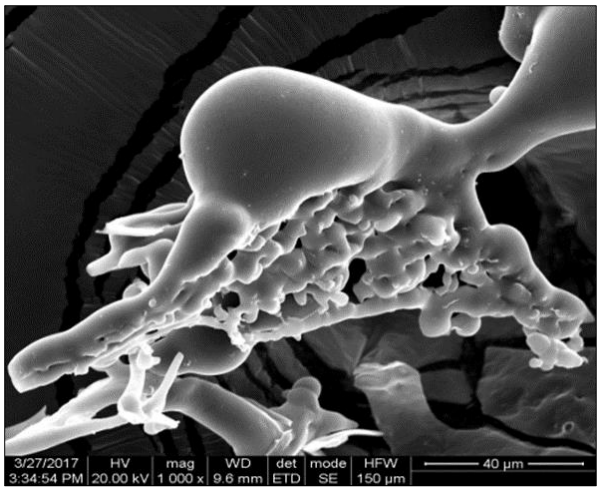

Fig 7: UTC sample examined at 1200Mag, HV-20.00KV, W.D-9.6mm and HFW-150µm showing smooth aggregates

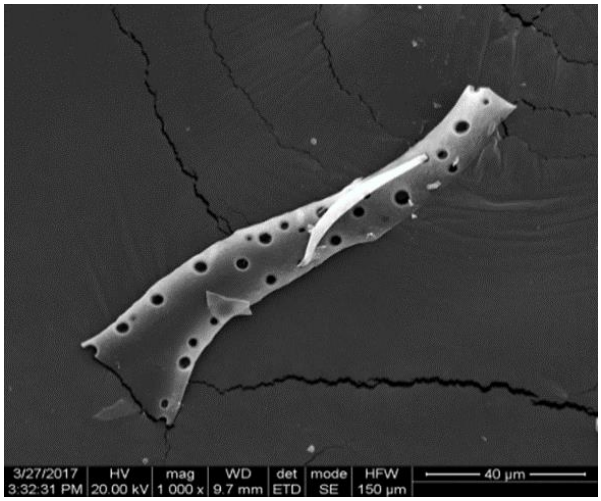

Fig 8: UTH sample examined at 1200Mag, HV-20.00KV, W.D-9.7mm and HFW-150µm showing smooth porous flake.

4. Conclusion

The outcome of this study shows the potential usage of wasted fish resource for the production of FPH through novel and efficient technology. Fish protein hydrolysate (FPH) normally has a high content of protein primarily due to the solubilization of the protein during the hydrolysis reaction and removal of non-protein compounds such as fat and other insoluble materials. Fish hydrolysates contain essential amino acids making a good nutritional product. The amino acid composition of the FPH product can affect its bioactive and functional properties. The enzymatic hydrolysis influences the protein functional properties by changing the peptides and amino acid composition of the proteins. Some hydrolytic enzymes affect the molecular weight and hydrophobic properties of proteins and consequently the protein functional properties. Therefore, with proper selection of the enzyme and substrates, it is possible to achieve peptides in the hydrolysate with a desired molecular weight range.

In our study, fish protein hydrolysate from *Upeneus teniopterus* possessed low molecular bands as acid aided in breakdown of protein into small peptides. Hydrolysates with greater solubility can be obtained by increasing the time of the hydrolysis reaction resulting in smaller peptides with lower molecular weight. Small peptides have consequently more ionizable polar groups on their surface, which become more able to form hydrogen bonds with water molecules. Hydrolysis of proteins can result in the exposure of hydrophobic groups, with the only increase in ionizable groups due to the generation of amino and carboxyl termini on the peptides. HR – SEM studies on UTCL and UTHL revealed that the micro structure and functional properties of the protein. Due to acid hydrolysis, UTHL exhibits the better morphology structure when compared to UTCL. These fish protein hydrolysates from *Upeneus teniopterus* could be utilized in food and pharmaceutical industries for the development of functional foods with potent biological activities.

5. References

1. Akhila Narikimelli S. Vincent, Prathapa varma Digala and K. Manoj Dhanraj. Physico - chemical parameters of fish protein hydrolysate from bycatch *Upeneus taeniopterus* (Cuvier, 1829). International Journal of Research and Analytical Reviews. 2019; 6(2).
2. Al-Holy MA, Rasco BA. Characterization of salmon (*Oncorhynchus keta*) and sturgeon (*Acipenser transmontanus*) caviar proteins. Journal of food biochemistry. 2006; 30(4):422-428.
3. Arvanitoyannis IS, Kassaveti A. Fish Industry Waste: Treatments, Environmental Impacts, Current and Potential Uses. International Journal of Food Science and Technology. 2008; 43:726-745.
4. Balaswamy K, Prabhakara Rao PG, Narsing Rao G, Jyothirmayi T. Functional properties of roe protein hydrolysates from *Catla catla*. Electronic Journal of Environmental Agricultural and Food Chemistry. 2011; 10(4):2139-2147.
5. Benjakul S, Morrissey MT. Protein Hydrolysates from Pacific Whiting Solid Wastes. Journal of Agricultural and Food Chemistry. 1997; 45:3423-30.
6. Bhaskar N, Benila T, Radha C, Lalitha RG. Optimization of Enzymatic Hydrolysis of Visceral Waste Proteins of Catla (*Catla catla*) for Preparing Protein Hydrolysate Using a Commercial Protease. Bioresource Technology. 2008; 99:335-343.
7. Binsi PK, Shamasundar BA, Dileep AO, Badii F, Howell NK. Rheological and functional properties of gelatin from the skin of *Bigeye snapper* (*Priacanthus hamrur*) fish: Influence of gelatin on the gel-forming ability of fish mince. Food Hydrocolloids. 2009; 23(1):132-145.
8. Chalamaiah M, Narsing RG, Rao DG, Jyothirmayi T. Protein Hydrolysates from Meriga (*Cirrhinus mrigala*) Egg and Evaluation of Their Functional Properties. Food Chemistry. 2010; 120:652-657.
9. Chen YT. Characterization of a 36 kDa fish protein and its application to the development of an immunoassay for the detection of fish muscle (Doctoral dissertation, The Florida State University), 2012.
10. Corzo JM, Riol-Cimas E. MelCndez-Hevia Fish species identification by electrophoresis of muscle proteins in sodium dodecyl sulfate-containing polyacrylamide slab gels Electrophoresis. 1984; 5:168-170.
11. Dash P, Ghosh G. Amino acid composition, antioxidant and functional properties of protein hydrolysates from Cucurbitaceae seeds. Journal of food science and technology. 2017; 54(13):4162-4172.
12. De Castro RJS, Bagagli MP, Sato HH. Improving the functional properties of milk proteins: focus on the specificities of proteolytic enzymes. Current Opinion in Food Science. 2015; 1:64-69.
13. Fahmi A, Morimura S, Guo HC, Shigematsu T, Kida K, Uemura Y *et al*. Production of Angiotensin I Converting Enzyme Inhibitory Peptides from Sea Bream Scales. Process Biochemistry. 2004; 39:1195-1200.
14. Foh MBK, Kamara MT, Amadou I, Foh BM, Wenshui X. Chemical and physicochemical properties of tilapia (*Oreochromis niloticus*) fish protein hydrolysate and concentrate. International journal of Biological chemistry. 2011; 5(1):21-36.
15. Jesslin A. Sabaridasan P, Edwinthangam R, Palanikani R. Soranam Electrophoretic studies on the muscle proteins of three species of Genus puntius (Osteichthyes – Cyprinidae) Int. J Res. Fish. Aquacult, 2011, 16-20.
16. Jung WK, Mendis E, Je JY, Park PJ, Son BW, Kim HC. Angiotensin I-Converting Enzyme Inhibitory Peptide from Yellowfin Sole (*Limanda aspera*) Frame Protein and Its Antihypertensive Effect in Spontaneously Hypertensive Rats. Food Chemistry. 2006; 94:26-32.
17. Kristinsson HG, Ingadottir B. Recovery and properties of muscle proteins extracted from tilapia (*Oreochromis niloticus*) light muscle by pH shift processing. J Food Sci. 2006; 71:132-4
18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. nature, 227(5259), 680, 1970.
19. Lalasidis G, Bostrom S, Sjoberg LB. Low Molecular Weight Enzymatic Fish Protein Hydrolysates: Chemical Composition and Nutritive Value. Journal of Agricultural and Food Chemistry. 1978; 26(3):751-756.
20. Leisner JJ, Millan JC, Huss HH, Larsen LM. Production of Histamine and tyramine by Lactic Acid bacteria isolated from Vacuum packed sugar-salted fish. J Appl. Bacteriol. 1994; 76:417-423.
21. Liaset B, Lied E, Espe M. Enzymatic Hydrolysis of by-Products from the Fish-Filleting Industry: Chemical Characterisation and Nutritional Evaluation. Journal of Science and Food Agricultural. 2000; 80(5):581-589.
22. Lordan RP, Ross C. Stanton, Marine bioactives as

functional food ingredients: potential to reduce the incidence of chronic diseases, Marine Drugs, 2000, 91056-1100,
23. McDonald CL, Milligan Chemical proper ties of the blood WS, Hoar, DJ Randall AP. Farrell (Eds.), Fish physiology, Academic Press, San Diego, CA, 1992, 56-133.
24. Moreira RG, Sun X, Chen Y. Factors affecting oil uptake in tortilla chips in deep-fat frying. Journal of Food Engineering. 1997; 3:485-498.
25. Navarro J. Gutiérre. Fasting, starvation PW, Hochachka TP. Mommsen (Eds.), Biochemistry and Molecular Biology of Fishes, Vol. 4, Metabolic Biochemistry, Elsevier, Amsterdam. 1997; 4:393-434
26. Olsen RL, Toppe J, Karunasagar I. Challenges and realistic opportunities in the use of byproducts from processing of fish and shellfish. Trends in Food Science and Technology. 2014; 36(2):144-151.
27. Prabha J, Vincent S, Joseph S, Magdalene J. Bioactive and functional properties of fish protein hydrolysate from *Leiognathus bindus*. Asian J Pharm Clin Res. 2016; 9(5):277-81.
28. Rawdkuen S, Sai-Ut S, Khamsorn S, Chaijan M, Benjakul S. Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. Food Chem. 2009; 112:112-119.
29. Razali AN, Amin AM, Sarbon NM. Antioxidant activity and functional properties of fractionated cobia skin gelatin hydrolysate at different molecular weight. International Food Research Journal. 2015; 22(2):651-660.
30. Roslan J, Yunos KFM, Abdullah N, Kamal SMM. Characterization of fish protein hydrolysate from Tilapia (*Oreochromis niloticus*) by- product. Agriculture and Agricultural Science Procedia. 2014; 2:312-319.
31. Sathivel S, Bechtel PJ, Babbitt J, Prinyawiwatkul W, Negulescu II, Reppond KD. Properties of Protein Powders from Arrowtooth Flounder (*Atheresthes stomias*) and Herring (*Clupea harengus*) Byproducts. Journal of Agricultural and Food Chemistry. 2004; 52(16):5040-5046.
32. Souissi N, Bougatef A, Triki-ellouz Y, Nasri M. Biochemical and Functional Properties of Sardinella (*Sardinella aurita*) By-Product Hydrolysates. Food Technology and Biotechnology. 2007; 45(2):187-194.
33. Tahergorabi R, Beamer SK, Matak KE, Jaczynski J. Isoelectric solubilization/ precipitation as a means to recover protein isolate from Striped Bass (*Morone saxatilis*) and Its physicochemical properties in a nutraceutical seafood product. J Agric Food Chem. 2012; 60:5979-87.
34. Uiblein F, Gouws G. A new goatfish species of the genus Upeneus (Mullidae) based on molecular and morphological screening and subsequent taxonomic analysis. Marine Biology Research. 2014; 10(7):655-681.
35. Villamil O, Váquiro H, Solanilla JF. Fish viscera protein hydrolysates: Production, potential applications and functional and bioactive properties. Food chemistry. 2017; 224:160-171.
36. Wei W, Hu W, Zhang XY, Zhang FP, Sun SQ, Liu Y. et al. Analysis of protein structure changes and quality regulation of surimi during gelation based on infrared spectroscopy and microscopic imaging. Scientific reports. 2018; *8*(1): 5566.
37. Zhao X, Zhu H, Zhang B *et al.* XRD, SEM, and XPS analysis of soybean protein powders obtained through extraction involving reverse micelles. J Am Oil Chem Soc. 2015;92:975–983
38. Zowail, El-Deeb SI, El-Serafy SS, Rizkalla EH, El-Saied H. Biochemical genetic studies of serum properties of family Mugillidae in two different habitats of Egyptian water. Bulletin National Institute Oceanography Fish Egypt, 1994; 20:175-190.