

PROTEIN AND LIPID RECOVERY FROM TUNA HEAD USING INDUSTRIAL PROTEASE

Nguyen Thi My Huong

Faculty of Food Technology - Nha Trang University

Email: huongdhts@yahoo.com

Received date: 28.09.2013

Accepted date: 22.12.2013

ABSTRACT

Protein and lipid recovery from yellowfin tuna heads by enzymatic hydrolysis was studied. Hydrolysis of tuna head was carried out using 0.5% Protamex at 45°C without pH control for 120 minutes with a water/material ratio of 1:1 (ml/g). Nitrogen recovery, amino acid composition of protein hydrolysate, lipid recovery and fatty acid composition of fish oil obtained from hydrolysis of tuna heads were determined. Results showed that after 120 minutes of hydrolysis, the nitrogen and lipid recoveries were 70.3% and 65.4% respectively. Protein hydrolysate from yellowfin tuna heads had of 80% protein, 1.3% lipid and 7.9% ash. Protein hydrolysate had high content of essential amino acids (33.67%). The fish oil obtained from hydrolysis of tuna heads was rich in omega-3 fatty acids (18.99%), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The content of omega-6 fatty acids was 4.37%. Fatty acids with high contents in tuna head oil were palmitic acid (29.75%), oleic acid (16.76%) and docosahexaenoic acid (14.56%).

Key words: Enzymatic hydrolysis, protein hydrolysate, protein and lipid recovery, tuna head.

Thu hồi protein và lipid từ đầu cá ngừ bằng enzyme protease công nghiệp

TÓM TẮT

Sự thu hồi protein và lipid từ đầu cá ngừ vây vàng theo phương pháp thủy phân bằng enzyme đã được nghiên cứu. Sự thủy phân đầu cá ngừ được thực hiện bằng Protamex 0,5% ở nhiệt độ 45°C, không điều chỉnh pH, trong thời gian 120 phút, tỉ lệ nước/nguyên liệu là 1/1 (ml/g). Hiệu suất thu hồi nitơ, thành phần axit amin của sản phẩm thủy phân protein, hiệu suất thu hồi lipid và thành phần axit béo của dầu cá thu được từ sự thủy phân đầu cá ngừ đã được xác định. Kết quả đã chỉ ra rằng sau 120 phút thủy phân, hiệu suất thu hồi nitơ và lipid lần lượt là 70,3% and 65,4%. Sản phẩm thủy phân protein từ đầu cá ngừ vây vàng có hàm lượng protein 80%, lipid 1,3% và tro 7,9%. Sản phẩm thủy phân protein có hàm lượng axit amin không thay thế cao (33,67%). Dầu cá thu được từ sự thủy phân đầu cá ngừ giàu axit béo omega 3 (18,99%), đặc biệt axit docosahexaenoic (DHA) và eicosapentaenoic (EPA). Hàm lượng axit béo omega 6 là 4,37%. Các axit béo có hàm lượng cao trong dầu đầu cá ngừ là palmitic (29,75%), oleic (16,76%) và docosahexaenoic (14,56%).

Từ khoá: Đầu cá ngừ, sản phẩm thủy phân protein, thu hồi protein và lipid, thủy phân bằng enzyme.

1. INTRODUCTION

Tuna is one of the most economically important pelagic species. The main products from tuna are frozen tuna, canned tuna, smoked tuna and tuna sashimi, etc. The fish processing industry generates more than 60% by-products, which includes head, viscera, trimmings,

frames, skin, liver and roes, etc (Chalamaiah et al., 2012). Tuna heads generated from tuna processing contain not only valuable proteins, lipids as well as vitamins and minerals, but are also an important source of environmental contamination. Today, they are seen as a potential resource instead of a waste. There is a great potential in marine bioprocessing industry

to convert and utilize these by-products to value-added products. Enzymatic hydrolysis is one of the most efficient methods to recover proteins and lipids from fish by-products and to produce the protein hydrolysates with a high content of amino acids. Enzymatic hydrolysis of fish by-products was studied by several scientists (Liaset et al., 2002; Aspino et al., 2005; Sathivel et al., 2005; Slizyté et al., 2005; Mbatia et al., 2010; Nguyen et al., 2011). The results showed that the protein hydrolysates produced from fish by-products had high content of protein, and were rich in essential amino acids. The fish oil obtained from hydrolysis of fish by-products was a good resource of omega-3 fatty acids. The potential use of fish protein hydrolysate and fish oil in food and feed was also reported by several authors (Yu and Tan, 1990; Berge and Storebakken, 1996; Refstie et al., 2004; Tang et al., 2008; Nguyen et al., 2012). The possibility of tuna head use for the production of fish protein hydrolysate and fish oil could potentially generate significant revenue for fish processing industry and environment.

The purpose of this work was to study protein and lipid recovery from yellowfin tuna head by enzymatic hydrolysis as well as to analyse the amino acid compositions in tuna head protein hydrolysate and the fatty acid compositions in tuna head oil.

2. MATERIALS AND METHODS

2.1. Yellowfin tuna heads

Yellowfin tuna (*Thunnus albacares*) caught in the Pacific Ocean was transported to processing plant (Hai Vuong company, Khanh Hoa, Vietnam). The heads were taken from the frozen fish and transported to the laboratory at Nha Trang University. Upon arrival, the frozen tuna heads were thawed, cut and ground in a grinder through 3mm plate. Ground tuna heads were frozen in the plastic bags and stored at - 20°C until use.

2.2. Enzyme

Protamex is a *Bacillus* protease complex developed for the hydrolysis of food proteins. Protamex was produced by Novozymes A/S, (Bagsvaerd Denmark) and complies with the recommended purity specifications for food-grade enzyme issued by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC). The pH of 5.5-7.5 and temperature range of 35-60°C are optimal working conditions for Protamex.. Protamex has a declared activity of 1.5 Anson Units/g.

2.3. Hydrolysis process of yellowfin tuna heads

Hydrolysis process of yellowfin tuna heads is shown in Figure 1.

The ground and frozen tuna heads were thawed overnight in the refrigerator at 4°C. Five hundred grams of ground tuna head were mixed with 500 mL of distilled water. The hydrolysis was performed in a glass vessel. The mixture was stirred at 300 rpm with an impeller. The enzymatic hydrolysis was started when the temperature of the mixture reached 45°C by adding 0.5% Protamex (by weight of tuna head). The hydrolysis was carried out for 30, 60, 90 and 120 minutes at 45°C without pH control (initial pH = 6.5). After each hydrolysis period, the enzyme was inactivated by heating at 95°C for 15 minutes in a water bath. Then, the mixture was filtered through a mesh to remove the bones. The filtrate was centrifuged at 10000 rpm at 4°C for 30 minutes. After centrifugation, the following three fractions were collected: the oil fraction on the top; the liquid protein hydrolysate (water-soluble compounds) in the middle; and the sludge (non-water-soluble part) at the bottom. The liquid protein hydrolysate was then freeze-dried to obtain fish protein hydrolysate. The experiments were carried out in triplicates.

2.4. Chemical analyses

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 estimated by multiplying total nitrogen content by 6.25. Lipid content was determined after extraction of lipids from the samples according to Folch et al. (1957). The nitrogen recovery (NR) in the hydrolysate was calculated according to Liaset et al. (2002) as follows:

$$\text{NR} = (\text{Total nitrogen in the hydrolysate} / \text{Total nitrogen in 500g of ground tuna head}) \times 100.$$

Amino acid composition was determined using the EZ: faast™ procedure described by Kechaou et al. (2009). The lipid recovery (LR) in the oil fraction was calculated as follows:

$$\text{LR} = (\text{Lipid in the oil fraction} / \text{Lipid in 500 g of ground tuna head}) \times 100.$$

Fatty acid composition in the tuna head oil was determined by gas chromatography according to Noriega-Rodríguez et al. (2009).

2.5. Statistical analyses

The statistical program SPSS (SPSS Inc., Chicago, IL, USA) was used for data processing and statistical analysis. Data were subjected to analysis of variance (ANOVA). Means were separated by using Duncan's multiple range test (Tang et al., 2008). Differences in treatment means were considered significant at $P < 0.05$.

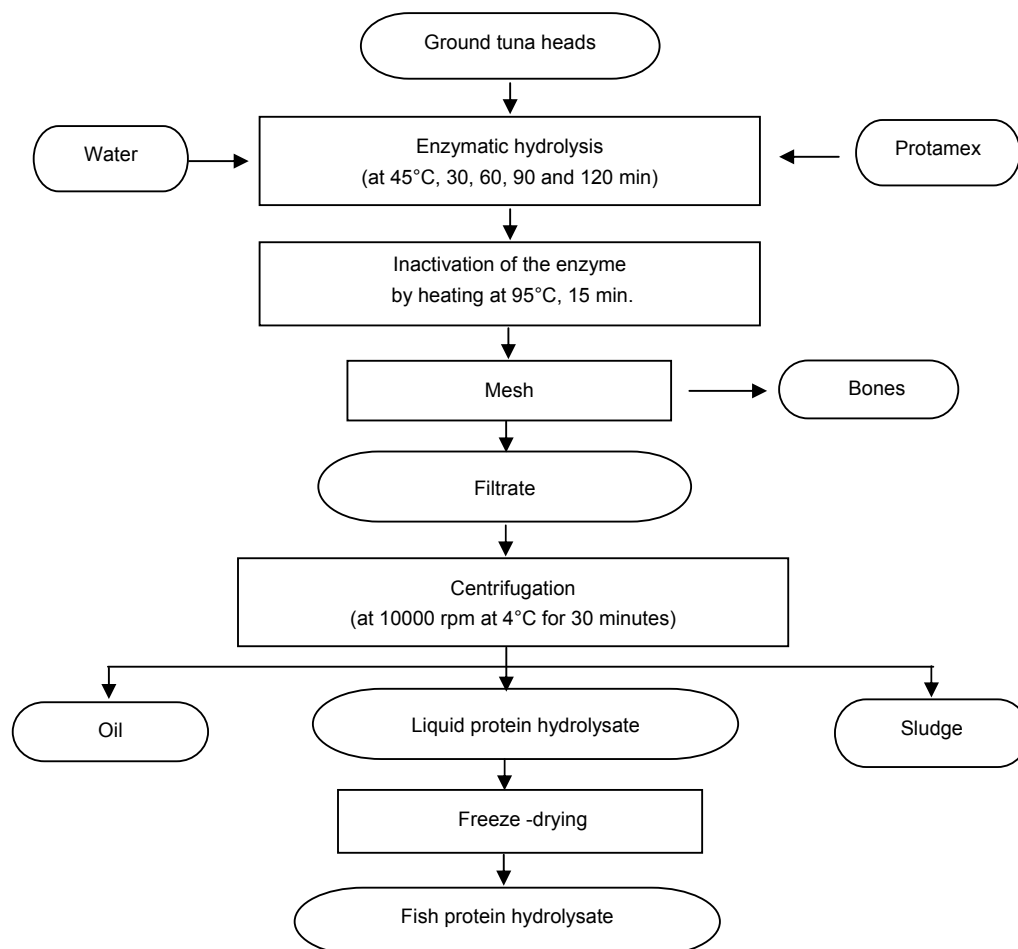


Figure 1. Scheme of the enzymatic hydrolysis of tuna heads

3. RESULTS AND DISCUSSION

3.1. Chemical compositions of yellowfin tuna heads and protein hydrolysate

The chemical compositions of yellowfin tuna heads and protein hydrolysate obtained from yellowfin tuna head after 120 minutes of hydrolysis are shown in Table 1.

The yellowfin tuna heads had 14.8% protein and 13.5% lipid, indicating that the yellowfin tuna heads are a good source of protein and lipid which can be recovered by enzymatic hydrolysis.

The protein content of tuna head protein hydrolysate was 80%, which was higher than those reported for salmon head protein hydrolysates (62.3% to 64.8%) by Sathivel et al. (2005) but lower than that reported for herring head protein hydrolysate (85.2%) by Sathivel et al (2003). The high protein content was a result of the solubilization of protein during hydrolysis, the removal of insoluble undigested non-protein substances and removal of lipid after hydrolysis (Benjakul and Morrissey, 1997). The lipid content of tuna head protein hydrolysate was low (1.3%). This result was similar to that reported for herring head protein hydrolysate (1.2%) (Sathivel et al., (2003). The removal of the oil layer after hydrolysis caused a low lipid content in the protein hydrolysate (Benjakul and Morrissey, 1997). The low lipid content might enhance stability of the hydrolysate towards lipid oxidation. The ash content of tuna head protein hydrolysate was 7.9%, which was higher than those reported for salmon head protein hydrolysates (6.9% to 7.7%) (Sathivel et al., (2005) but lower than that shown for herring head protein hydrolysate (10.1%) (Sathivel et al., (2003).

3.2. Nitrogen recovery

According to Benjakul and Morrissey (1997), the nitrogen recovery (protein recovery) reflects the yield that can be recovered in the protein hydrolysate from the hydrolysis process. Nitrogen recovery was used as an index of nitrogen solubilization to describe the hydrolysis yield (Guérard et al. 2002). Nitrogen recovery indicates the percentage of nitrogen solubilized into hydrolysate to total nitrogen in the raw material and is presented in Figure 2. The results indicated that the nitrogen recovery increased with increasing hydrolysis time. These results are in accordance with those reported in previous studies on fish by-products (Liaset et al., 2002; Aspino et al., 2005). The nitrogen recovery in protein hydrolysate after 30 minutes of hydrolysis of tuna head was 52.8% while nitrogen recovery of 70.3% was obtained after 120 minutes of hydrolysis. There were significant differences in nitrogen recovery among the samples with different hydrolysis time. The break of peptide bonds under the action of Proteome during hydrolysis resulted in the amount of soluble nitrogen in the protein hydrolysate. The hydrolysis of the fish protein was characterized by an initial rapid phase. Thereafter, the rate of enzymatic hydrolysis decreased. A reduction in the reaction rate might be due to limitation of the enzyme activity by formation of reaction products (Guérard et al., 2002). Shahidi et al. (1995) reported that considerable soluble protein was released during initial phase. The rate of hydrolysis and nitrogen recovery was reduced with high concentration of soluble peptides in the reaction mixture.

Table 1. Chemical compositions of yellowfin tuna head and protein hydrolysate

| | Moisture (%) | Crude protein (%) | Lipid (%) | Ash (%) |
|---------------------|--------------|-------------------|------------|------------|
| Yellowfin tuna head | 59.0 ± 1.1 | 14.8 ± 0.1 | 13.5 ± 0.1 | 11.8 ± 1.1 |
| Protein hydrolysate | 8.2 ± 0.4 | 80 ± 0.8 | 1.3 ± 0.3 | 7.9 ± 0.3 |

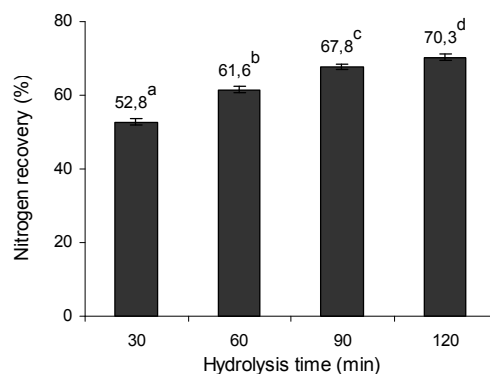


Figure 2. Nitrogen recovery in the tuna head protein hydrolysate. Values reported are means of three replicates. Mean values with different superscript letter are significantly different ($P < 0.05$)

3.3. Amino acid composition of the protein hydrolysate from yellowfin tuna heads

Table 2 shows the amino acid composition of protein hydrolysate from hydrolysis of yellowfin tuna heads for 120 minutes.

The protein hydrolysate from yellowfin tuna heads had a total amino acid content of 60.43% and total essential amino acid content of 33.67%. The tuna head protein hydrolysate was rich in aspartic acid, leucine, glycine, histidine,

Table 2. Amino acid composition of the protein hydrolysate from yellowfin tuna head

| Amino acids | Content (% of dry matter) |
|---|---------------------------|
| Arginine | 4.03 ± 0.08 |
| Histidine | 5.06 ± 0.06 |
| Isoleucine | 4.17 ± 0.12 |
| Leucine | 6.25 ± 0.11 |
| Lysine | 3.01 ± 0.18 |
| Methionine | 2.10 ± 0.07 |
| Phenylalanine | 2.42 ± 0.06 |
| Threonine | 3.15 ± 0.09 |
| Valine | 3.48 ± 0.11 |
| Alanine | 4.42 ± 0.16 |
| Aspartic | 7.53 ± 0.10 |
| Glutamic | 2.35 ± 0.08 |
| Glycine | 5.13 ± 0.13 |
| Hydroxyproline | 1.36 ± 0.06 |
| Proline | 1.45 ± 0.10 |
| Serine | 2.36 ± 0.07 |
| Tyrosine | 2.16 ± 0.10 |
| Total essential amino acids (TEAA) | 33.67 ± 0.35 |
| Total non-essential amino acids (TNEAA) | 26.76 ± 0.33 |
| Total amino acids (TAA) | 60.43 ± 0.30 |
| TEAA/TAA (%) | 55.72 ± 0.54 |
| TEAA/TNEAA | 1.26 ± 0.03 |

alanine, isoleucine and arginine. The ratio of essential amino acids to total amino acids was 55.72% and the ratio of essential amino acids to non-essential amino acids was 1.26. Both values exceeded the reference values of 40% and 0.6 for human, which are recommended by World Health Organization (WHO)/Food and Agriculture Organization (FAO) (FAO/WHO 1990). The results of the present study showed that the protein hydrolysate from yellowfin tuna heads was found to have high nutritional value and could be used in human and animal diets.

3.4. Lipid recovery

In addition to the protein hydrolysate obtained after the hydrolysis of tuna head with Protamex, the fish oil was also extracted during hydrolysis. According to Dumay et al. (2006), the disruption of tissues increased oil liberation during hydrolysis of fish by-product with enzyme. The lipid recovery in the oil fraction from hydrolysis of tuna head is shown in Figure 3.

The results indicated that the lipid recovery from tuna head increased within the first 90 minutes of hydrolysis. The prolonged hydrolysis (90 -120 minutes) did not increase the lipid recovery further. Decrease in oil yield after 90 minutes could be due to interaction of more lipids with the hydrolysed proteins. The reduced

release of lipid may result from the formation of lipid-protein aggregates as pointed out by Šližyte et al. (2005). These results are in accordance with those reported by Mbatia et al. (2010) who showed that the prolonged hydrolysis did not improve the oil yield further but resulted in a colour change of hydrolysate solution to brown.

After 120 minutes of hydrolysis of tuna head with 0.5% Protamex at 45°C, the lipid recovery in the oil fraction was 65.4%. Daukšas et al. (2005) showed that the lipid recovery in the oil fraction after hydrolysis of different by-products ranged from 36.4% to 82.8%. Batista et al. (2009) reported that the lipid recovery was 35% after 1h of hydrolysis of sardine by-product with Protamex and only a small increase of 5% was observed in the next 3 hours. The previous studies indicated that the oil liberation was dependent on the raw material and hydrolysis conditions such as the temperature, the pH, the hydrolysis time and the enzyme ratio (Daukšas et al., 2005; Batista et al., 2009; Mbatia et al., 2010).

3.5. Fatty acid composition in the oil recovered from yellowfin tuna head

Fatty acid composition in the oil recovered from hydrolysis of yellowfin tuna heads for 120 minutes is shown in Table 3.

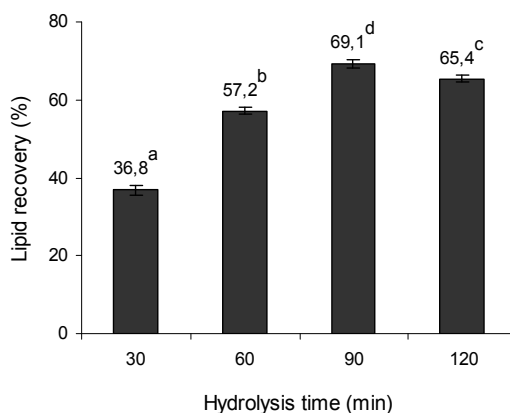


Figure 3. Lipid recovery in the oil fraction from hydrolysis of yellowfin tuna head. Values reported are means of three replicates. Mean values with different superscript letter are significantly different (P<0.05)

Table 3. Fatty acid composition in the oil recovered from yellowfin tuna head

| Fatty acids | | Content (% total fatty acids) |
|--|-------------------------|-------------------------------|
| C14:0 | (Myristic) | 3.52 ± 0.10 |
| C16:0 | (Palmitic) | 29.75 ± 0.35 |
| C18:0 | (Stearic) | 10.28 ± 0.10 |
| C24:0 | (Linoceric) | 1.35 ± 0.09 |
| C16:1 (ω-7) | (Palmitoleic) | 5.54 ± 0.13 |
| C18:1 (ω-9) | (Oleic) | 16.76 ± 0.16 |
| C18:1 (ω-7) | (Vaccenic) | 3.19 ± 0.06 |
| C20:1 (ω-9) | (Aldoleic) | 2.13 ± 0.09 |
| C22:1 (ω-9) | (Erucic) | 2.32 ± 0.07 |
| C24:1 (ω-9) | (Nervonic) | 1.80 ± 0.05 |
| C18:2 (ω-6) | (Linoleic) | 1.99 ± 0.04 |
| C18:3 (ω-3) | (Linolenic) | 0.75 ± 0.03 |
| C18:4 (ω-3) | (Stearidonic) | 0.94 ± 0.07 |
| C20:4 (ω-6) | (Arachidonic) | 1.52 ± 0.06 |
| C22:4 (ω-6) | (Docosatetraenoic) | 0.86 ± 0.07 |
| C20:5 (ω-3) | (Eicosapentaenoic, EPA) | 2.44 ± 0.06 |
| C22:5 (ω-3) | (Docosapentaenoic) | 0.30 ± 0.02 |
| C22:6 (ω-3) | (Docosahexaenoic, DHA) | 14.56 ± 0.14 |
| Total saturated fatty acids (SFA) | | 44.90 ± 0.33 |
| Total monounsaturated fatty acids (MUFA) | | 31.74 ± 0.16 |
| Total polyunsaturated fatty acids (PUFA) | | 23.36 ± 0.25 |
| ω-3 PUFA | | 18.99 ± 0.23 |
| ω-6 PUFA | | 4.37 ± 0.17 |

The results indicated that the content of saturated fatty acids in the oil recovered from yellowfin tuna heads was 44.9% of total fatty acids. Palmitic acid was the highest among the saturated fatty acids with the content of 29.75% followed by stearic acid with 10.28%. The content of monounsaturated fatty acids was 31.74%. The most abundant monounsaturated fatty acid was oleic acid with 16.76%. The content of polyunsaturated fatty acids was 23.36% of total fatty acids. Docosahexaenoic acid (DHA) was the highest among the polyunsaturated fatty acids with 14.56% followed by eicosapentaenoic acid (EPA) with 2.44%. DHA and EPA are known as essential fatty acids for human. DHA has an important

role in the development of the brain and the nervous system. EPA plays an important role in the prevention of cardiovascular diseases (Stansby et al., 1990). The tuna head oil had an omega-3 fatty acid content of 18.99% and omega-6 fatty acid content of 4.37%. The results in this study indicated that the fatty acids with high contents in tuna head oil were palmitic acid, oleic acid and docosahexaenoic acid. Previous studies have shown that the content of DHA in the tuna was higher than that in most other fish species and that the content of DHA was higher than the content of EPA (Stansby et al., 1990; Shimada et al., 1997).

4. CONCLUSIONS

The tuna head generated from tuna processing industry can be utilized as a good source for recovery of both proteins and lipids. Enzymatic hydrolysis of tuna head with Protamex for 120 minutes is suitable for production of high quality products that can be used for different applications. The tuna head protein hydrolysate produced was rich in essential amino acids and could be applied in food for human or feed for shrimp and fish. The produced tuna head oil was rich in DHA and EPA. Tuna head oil can be used in aquaculture feeds or could be used as an ingredient in food industries after when refined.

REFERENCES

- Aspmo, S. I., Horn, S. J., Eijsink, V. G. H. (2005). Enzymatic hydrolysis of Atlantic cod (*Gadus morhua* L.) viscera. *Process Biochemistry*, 40: 1957-1966.
- Batista, I., Ramos, C., Mendonca, R., Nunes, M. L., (2009). Enzymatic hydrolysis of sardine (*Sardina pilchardus*) by-products and lipid recovery. *Journal of Aquatic Food Product Technology*, 18: 120-134.
- Benjakul, S., Morrissey, M. T. (1997). Protein hydrolysates from Pacific whiting solid waste. *J Agric. Food Chemistry*, 45: 3423-30.
- Berge, G.M., Storebakken, T. (1996). Fish protein hydrolysate in starter diets for Atlantic salmon (*Salmo salar*) fry. *Aquaculture*, 145(1-4): 205-212.
- 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 (4): 3020 -3038.
- Dauksas, E., Falch, E., Slizytė, R., Rustad, T., (2005). Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (*Gadus morhua*) by-products. *Process Biochemistry*, 40: 2659-2670.
- Dumay, J., Donnay-Moreno, C., Barnathan, G., Jaouen, P., Bergé, J. P. (2006). Improvement of lipid and phospholipid recoveries from sardine (*Sardina pilchardus*) viscera using industrial proteases. *Process Biochemistry*, 41: 2327-2332.
- FAO/WHO (1990). Energy and protein requirements. Report of joint FAO/WHO/UNU Expert Consultation Technical Report. Geneva, FAO/WHO and United Nations University
- 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.
- Guérard, F., Guimas, L., Binet, A. (2002). Production of tuna waste hydrolysates by a commercial neutral protease preparation. *J Mol Catal B-Enzym.*, 19-20: 489-98.
- Kechaou, E.,S., Dumay, J., Donnay-Moreno, C., Jaouen, P., Gougou, 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. *J Biosci Bioeng.*, 107(2): 158-64.
- Liaset, B., Nortvedt, R., Lied, E., Espe, M. (2002). Studies on the nitrogen recovery in enzymatic hydrolysis of Atlantic salmon (*Salmo salar*, L.) frames by Protamex™ protease. *Process Biochemistry*, 37: 1263-1269.
- Mbatia, B., Adlercreutz, D., Adlercreutz, P., Mahadhy, A. (2010). Enzymatic oil extraction and positional analysis of ω-3 fatty acids in Nile perch and salmon heads. *Process Biochemistry*, 45: 815-819.
- Nguyen, H. T. M., 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.
- Nguyen, H. T. M., Pérez-Gálvez, R., Bergé, J. P. (2012). Effect of diets containing tuna head hydrolysates on the survival and growth of shrimp *Penaeus vannamei*. *Aquaculture*. 324-325:127-134.
- Noriega-Rodríguez, J.A., Ortega-García, J., Angulo-Guerrero, O; García, H. S., Medina-Juárez, L. A., Gámez-Mezac, N. (2009). Oil production from sardine (*Sardinops sagax caerulea*). *CyTA - Journal of Food*. Vol. 7 (3): 173-179.
- Refstie, S., Olli, J. J., Standal, H. (2004). Feed intake, growth and protein utilization by post-smolt Atlantic salmon (*Salmo salar*) in response to graded levels of fish protein hydrolysate in the diet. *Aquaculture*, 239: 331-349
- 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.
- Sathivel, S., Smiley, S., Prinyawiwatkul, W., Bechtel, P. J. (2005). Functional and nutritional properties of red salmon (*Oncorhynchus nerka*) enzymatic hydrolysates. *Food Science*, 70(6): 401-406.
- Shahidi, F., Han, X.,Q, Synowieck, J. (1995). Production and characteristics of protein

- hydrolysates from capelin (*Mallotus villosus*). *Food Chem*, 53: 285-93.
- Shimada, Y., Maruyama, K., Sugihara, A., Moriyama, S., Tominaga, Y. (1997). Purification of docosahexaenoic acid from tuna oil by a two-step enzymatic method: hydrolysis and selective esterification. *Journal of the American Oil Chemists' Society*.74:1441-1446.
- Šližyte, R., Dauksas, E., Falch, E., Storro, I., Rustad, T. (2005). Yield and composition of different fractions obtained after enzymatic hydrolysis of cod (*Gadus morhua*) by-products. *Process Biochem.*, 40: 1415-1424.
- Stansby, M.E., Schlenk, H., Gruger, E.H. (1990). Fatty acid composition of fish. In Stansby, M.E, *Fish oil in nutrition*. 6-39. New York: Van Nostrand Reinhold.
- Tang, H.G., Wu, T.X., Zhao, Z.Y., Pan, X.D. (2008). Effects of fish protein hydrolysate on growth performance and humoral immune response in large yellow croaker (*Pseudosciaena crocea* R.). *Zhejiang Univ Sci B.*, 9: 684-690.
- Yu, S.Y, Tan, L. (1990). Acceptability of crackers ('keropok') with fish protein hydrolysate. *International J Food Sci. Tech.*, 25: 204-8.