Nutritional and functional properties of protein hydrolysate from Tra fish by-products

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Abstract

The nutritional and functional properties, molecular weight distribution of protein hydrolysate from Tra fish by-products were studied. The protein hydrolysate was produced by enzymatic hydrolysis of Tra fish by-products with Protamex 0,5% at the temperature of 50 ^{o}C in 6 h. The study results showed that the Tra fish by-products protein hydrolysate had a moisture content of 6,7%, protein content of 65.8%, lipid content of 1,6% and ash content of 9,5 %. The protein hydrolysate had a total amino acid content of 52,81 g/100g of protein and essential amino acid content of 16,25 g/100g of protein. The ratio of essential amino acids to total amino acids was 30,76%. The protein hydrolysate was rich in glycine, glutamic, proline, alanine, aspartic and leucine. The most of peptides (68,25%) in Tra fish by-products protein hydrolysate had a molecular weight between 360 Da and 2.500 Da. The protein hydrolysate had solubility in the range of 90,3% - 98,2%. The solubility of protein hydrolysate was the lowest value at pH 4 and the highest value at pH 10. The foaming capacity of protein hydrolysate ranged from 19,5% to 27,8%. The emulsifying capacity of protein hydrolysate was 12,8 – 17,3 mL/g. The protein hydrolysate of Tra fish by-products possesses high nutritional value and good functional properties. The study results suggested that the protein hydrolysate from Tra fish by-products could be used as a promising food ingredient and protein source in food systems.

Received 27/05/2022
Accepted 08/09/2022
Published 01/11/2023

Keywords

Fish protein hydrolysate, functional properties, molecular weight distribution, nutritional property, Tra fish by-products.

1 Introduction

Seafood processing industry contributes up to 60% by-products including head, frames, viscera, skin, trimmings, fins, and roes, and only 40% of fish products for human consumption [1]. The Tra fish processing industry discards a large amount of by-products which contain a rich source of protein. The high protein content in fish by-products make them more perishable, and may cause undesirable pollution to the environment. Production of protein hydrolysate from fish by-products by enzymatic hydrolysis is one solution to add value to fish by-products. Enzymatic hydrolysis is the process of breaking down the peptide bonds of the parent protein by 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 effective 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 contain a high content of amino acids and are used as available sources of protein for humans due to their good functional properties. Therefore, the study on nutritional and functional properties of protein hydrolysates from Tra fish by-products was necessary. This study aimed to produce

https://doi.org/10.55401/a4h7mm24

the protein hydrolysate from Tra fish by-products and determine the nutritional property and functional properties such as solubility, foaming capacity and emulsifying capacity. 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, stability and emulsion formation in some products such as beer, fresh cream, margarine and sausages. Fish protein hydrolysates have been incorporated into different food systems such as cereal products, fish and meat products, desserts and crackers [3].

2 Materials and methods

2.1 Tra fish by-products

Tra (*Pangasius hypophthalmus*) fish by-products (e.g. heads and frames) were provided by Nam Viet, a seafood processing company in An Giang, Vietnam. Tra fish by-products 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 Protamex

Protamex is a *Bacillus* protease complex developed for the hydrolysis of food proteins. Protamex was produced by Novozymes A/S, (Bagsvaerd Denmark). The optimal working conditions for Protamex are reported to be at pH of 5.5 - 7.5 and at a temperature of 35-60 °C.

2.3 Preparation of protein hydrolysate from Tra fish by-products

Frozen Tra fish by-products was thawed in a chiller at 4 °C overnight. Tra fish by-products was hydrolyzed with the water/materials ratio of 1/1 using Protamex 0.5% of the weight of materials (w/w) at 50 °C and natural pH of materials (pH 6.5) for 6 h. The hydrolysis process was carried out by using a water bath (Memmert WNB29, Germany). The reaction was stopped by heating the mixture in a water bath at 85 °C for 15 mins in order to deactivate the enzymes. The mixture was then filtered through a mesh to remove the solid fraction (fish bones). The filtrate was centrifuged at 10000 rpm for 20 min (Hermle Z323, Germany) to separate the fish oil, liquid protein hydrolysate and sludge. The liquid protein hydrolysate was then spray-dried using a spray drier (MSPS - 01) 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 the Folch method [4]. Amino acid composition was determined according to a previous study [5].

The molecular weight distribution of peptides in the hydrolysate was analysed by gel filtration chromatography. The molecular weight fractions were separated using a high-performance liquid chromatography (HPLC) system equipped with a size exclusion column (Superdex Peptide 10/300 GL, GE Healthcare UK Ltd, Chalfont, UK) with 0.1 % trifluoroacetic acid in 30 % of acetonitrile as mobile phase, the flow rate was 0.5 mL/min. Chromatography was monitored by measuring the absorbance at 205 nm. The molecular weight ranges of different fractions were based on the retention times of the collected fractions and determined from a standard curve.

The protein solubility of the protein hydrolysate was determined at pH 2, 4, 7 and 10. Solubility of fish protein hydrolysate was determined according to a previous study with slight modifications [2]. 1g of protein hydrolysate samples were dispersed in 100 mL of deionized water. The solution was adjusted to different pHs levels (2, 4, 7 and 10) with either 6 N HCl or 6 N NaOH. The mixture was then stirred at room temperature for 10 mins and centrifuged at 8000 g for 10 mins. The protein content in the supernatant was determined using 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) ×100.

Foaming capacity (FC) was determined as follows: Fish protein hydrolysate (3 g) was dispersed in 100 mL of distilled water. The solution was adjusted to different pH levels (2, 4, 7 and 10) with either 6 N HCl or 6 N NaOH. The mixture was homogenized for 1 min using a homogenizer at high speed (10000 rpm). The

mixture was then poured into a 250 mL graduated cylinder and the total volume was read [6]. Foaming capability was expressed as the percentage of volume increase upon whipping. Foaming capability was calculated as follows:

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

The emulsification capacity (EC) was determined as described in a previous study with slight modifications [7]. Protein hydrolysate samples (0.5 g) and 30 mL of vegetable oil were added to 60 mL of NaCl solution (30 g/L). The pH was adjusted to 2, 4, 7 and 10 with either 6 N HCl or 6 N NaOH. The mixture was homogenized at 9500 rpm for 30 min. Then, another 30 mL of oil was added over 1.5 min and mixed for a further 30 seconds. The mixture was transferred to centrifuge tubes, placed in a water bath at 85 °C for 15 min, and then centrifuged at 3,000 rpm for 30 min. The emulsification capacity was calculated by the following equation: EC (mL/g) = (VA – VR)/WS where VA was the volume of oil added to form an emulsion (mL), VR was the volume of oil released after centrifugation (mL), WS was the weight of sample (g).

2.5 Statistical analysis

The experiments were carried out in triplicates. The data obtained were subjected to Oneway 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 Tra fish by-products and protein hydrolysate

The proximate composition of Tra fish by-products and protein hydrolysate produced from Tra fish by-products is shown in Table 1.

Table 1 Proximate composition of Tra fish by-products and protein hydrolysate produced from Tra fish by-products

Components (%)	Tra fish by-products	Protein hydrolysate
Moisture	63.1 ± 0.3	6.7 ± 0.2
Protein	14.3 ± 0.1	65.8 ± 0.3
Lipid	13.2 ± 0.1	1.6 ± 0.1
Ash	6.7 ± 0.2	9.5 ± 0.2

The study results showed that the moisture content of Tra fish by-products was 63.1%, which was lower than that of tilapia waste (66.29%) [8]. The protein content of Tra fish by-products (14.3%) was higher than those of sardine head (13.7%) [9] and mackerel head (12.3%) [10]. Lipid content of Tra fish by-products (13.2%) was far higher than that of tilapia waste (4.51%) [8]. Ash content (6.7%) of Tra fish by-products was lower than that of tilapia waste (8.6%) [8]. The study result showed that the Tra fish by-products was a good source of protein which can be used for production of protein hydrolysate.

The protein hydrolysate produced from Tra fish by-products had significantly higher protein content (65.8%) than Tra fish by-products (14.3%). The high protein content of hydrolysate resulted from the solubilisation of protein during hydrolysis and removal of insoluble undigested substances by centrifugation after hydrolysis. Thus, the protein hydrolysate could be an essential source of protein supplements for human nutrition. The protein content of hydrolysate from Tra fish by-products was 65.8%, which was higher than salmon head protein hydrolysates (62.3-64.8%) [11], yet lower than tilapia waste protein hydrolysate (82.19%) [8] and shortfin scad waste hydrolysate (73.08%) [12].

The lipid content was significantly different between Tra fish by-products (13.2%) and protein hydrolysate from Tra fish by-products (1.6%). The lipid content in protein hydrolysate depends on the lipid separation process at the centrifugation stage after hydrolysis. Lipid content in protein hydrolysate from Tra fish by-products was low because the lipid was separated mechanically during the centrifugation process. The lipid content of protein hydrolysate from Tra fish by-products was 1.6%, which was lower than shortfin scad waste hydrolysate (7,55%) [12]. The low lipid content of protein hydrolysate might enhance the stability of the hydrolysate towards lipid oxidation.

The ash content of protein hydrolysate from Tra fish by-products was 9.5%, which was lower than that showed for shortfin scad waste hydrolysate (10.4%) [12]. The moisture content of protein hydrolysate from Tra fish Tra fish by-products was 6.7%. Most studies have demonstrated that protein hydrolysates from fish by-products contain moisture <10 % [12-14].

3.2 Amino acid composition of the protein hydrolysate

Amino acid composition of the protein hydrolysate from Tra fish by-products is indicated in Table 2. The composition and content of amino acids indicated the nutritional quality of protein hydrolysate.

Table 2 Amino acid composition of the protein hydrolysate from Tra fish by-products

Amino acid composition	Content (g/100g of protein)	Reference for essential amino acids * (g/100g of protein)
Alanine	4.86 ± 0.06	
Aspartic	4.85 ± 0.17	
Glutamic	6.50 ± 0.23	
Glycine	9.64 ± 0.11	
Histidine	1.79 ± 0.04	1.6
Hydroxyproline	2.51 ± 0.06	
Isoleucine	1.84 ± 0,17	1.3
Leucine	3.10 ± 0.06	1.9
Lysine	1.80 ± 0.23	1.6
Methionine	1.25 ± 0.02	1.7
Phenylalanine	1.78 ± 0.03	1.9
Proline	5.43 ± 0.11	
Serine	2.29 ± 0.15	
Threonine	2.54 ± 0.06	0.9
Tyrosine	0.49 ± 0.04	
Valine	2.14 ± 0.11	1.3
Total amino acids	52.81 ± 1.60	
Essential amino acids	16.25 ± 0.68	
Essential amino acids/ Total amino acids (%)	30.76 ± 0.54	

*Suggested profile of essential amino acid requirements for adults (FAO/WHO, 1985).

The protein hydrolysate from Tra fish by-products had a total amino acid content of 52.81 g/100g of protein and essential amino acid content of 16.25 g/100g of protein. The ratio of essential amino acids to total amino acids was 30.76%. The content of essential amino acids indicates the potential of hydrolysate to serve as a useful source of nutrition. This result was similar to a previous study on protein hydrolysate from the snapper head, which showed that the ratio of essential amino acids to total amino acids was 31.12 % [15]. The protein hydrolysate from Tra fish by-products was rich in glycine, followed by glutamic, proline, alanine and aspartic, along with low contents of tyrosine and methionine. A previous study showed that glutamic was the largest component in protein hydrolysate from Tilapia by-products in followed by glycine, aspartic, alanine and proline [14]. Among all amino acids, aspartic and glutamic acid have been found to be the most abundant in most reported fish protein hydrolysates [13]. The amino acid content of protein hydrolysate from Tra fish by-products was lower than protein hydrolysate from Tilapia by-products (55.213 g of total amino acids/100 g protein; 19.915 g of essential amino acids/100 g protein) [14]. Fish protein hydrolysates of different raw materials contain various amino acid composition. The difference in amino acid composition in hydrolysates is also determined by enzyme specificity and hydrolysis conditions. The nutritional value of any ingredients depends on the capacity of protein to meet an organism's requirements based on the essential amino acids [16]. The amino acid composition of protein hydrolysate in this study revealed that the protein hydrolysate from Tra fish by-products contained a higher content of essential amino acids compared to the suggested pattern by FAO/WHO for the adult requirements except for low contents of methionine and phenylalanine [17]. Therefore, this study indicated that the protein hydrolysates from Tra fish by-products had high nutritional values and could be incorporated into other products as a dietary protein supplement. Tra fish by-products protein hydrolysate could be targeted as alternative products or ingredients of high nutritional quality with potential application in the food industry.

3.3 Molecular weight distribution of protein hydrolysate

Molecular weight distribution of protein hydrolysate from Tra fish by-products is shown in Table 3.

Table 3 Molecular weight distribution of Tra fish by-products protein hydrolysate

Molecular weight distribution	(%)
MW > 6500 Da	3.73
5700 Da < MW < 6500 Da	1.74
2500 Da < MW < 5700 Da	7.77
1300 Da < MW < 2500 Da	12.45
900 Da < MW < 1300 Da	21.05
600 Da < MW < 900 Da	21.17
360 Da < MW < 600 Da	13.58
200 Da < MW < 360 Da	6.60

130 Da < MW < 200 Da	7.36
MW <130 Da	4.23

After 6 h of hydrolysis, the Tra fish by-products protein hydrolysate contained polypeptides, oligopeptides and free amino acids of various molecular sizes. After 6 h of enzymatic hydrolysis with Protamex, the percentage of molecules with molecular weight < 130 Da was 4.23%, between 130 and 200 Da was 7.36%, between 200 and 360 Da was 6.6%, between 360 and 600 Da was 13.58%, between 600 and 900 Da was 21.17%, between 900 and 1300 Da was 21.05%, between 1300 and 2500 Da was 12.45%, between 2500 and 5700 Da was 7.77%, between 5700 and 6500 Da was 1.74%, upper 6.500 Da was 3.73% of all molecules contained in the hydrolysate. Most of peptides (68.25%) with a molecular weight between 360 and 2500 Da were detected in protein hydrolysate from Tra fish by-products.

The study results indicated that Protamex enzyme was able to produce small-sized peptides. Fish protein hydrolysate with high nutritional value should be rich in low molecular weight peptides. The low molecular weight peptides in the protein hydrolysate from Tra fish by-products indicated the efficacy of this protein hydrolysate as a source of bioactive peptides. The successful production of such desired peptides from Tra fish by-products indicated its potential application in functional food.

3.4 Solubility of the protein hydrolysate

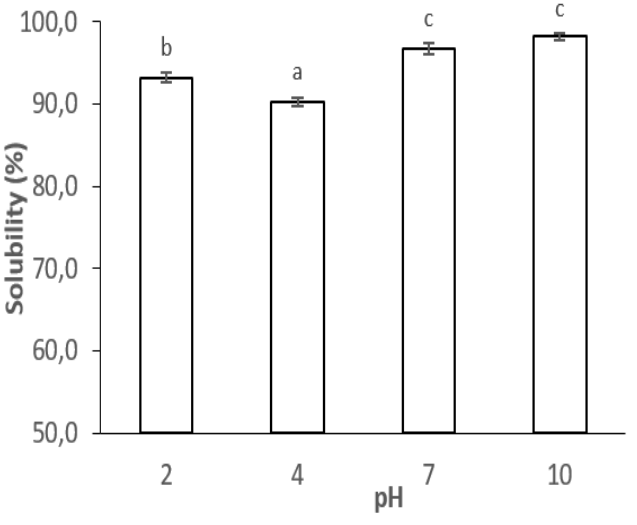

Fig. 1 Sobubility of protein hydrolysate from Tra fish by-products at different pH values. Different letters show significant differences ($p < 0.05$)

The solubility is one of the most important functional properties of fish protein hydrolysates. Good solubility of proteins is essential in many functional applications, especially for foams and emulsions. The high solubility of protein hydrolysates showed potential applications in food industry [6]. The solubility of protein hydrolysate from Tra fish by-products at different pH values is displayed in Figure 1.

The study results indicated that the solubility of protein hydrolysate from Tra fish by-products at different pH values were 93.2%, 90.3%, 96.7% and 98.2% for pH 2, pH 4, pH 7 and pH 10 respectively. The solubility of protein hydrolysate was the highest at pH 10 and the lowest at pH 4. There was a significant difference in solubility of protein hydrolysate from Tra fish by-products between pH 2 and pH 4 as well as between pH 4 and pH 7. However, no significant difference in solubility was observed between pH 7 and pH 10.

This result was similar to a previous study on salmon by-products hydrolysates, which showed that the solubility of salmon by-products hydrolysates was high at pH 6 -7 (more than 90%) and was low at pH 3 -4 [18]. The solubility of shortfin scad waste hydrolysate were 85.45%, 87.99% and 92.98% for pH 4, pH 7 and pH 10, respectively [12]. The solubility of cobia frame hydrolysates was in the range of 85-86% [6]. The influence of pH on the solubility of protein hydrolysates could be attributed to the increasing net charge of peptides when pH moves away from isoelectric point. Protein hydrolysates display low solubility at their isoelectric point. Protein solubility increased when more protein functional groups are ionised, and protein-water interactions are enhanced with changes in pH away from the isoelectric point [12]. High solubility of protein hydrolysate from Tra fish by-products was due to the cleavage of proteins molecules into smaller peptides that have more polar residues, with the ability to form hydrogen bonds with water and enhance solubility [2,18]. The protein hydrolysate from Tra fish by-products with high solubility indicated potential applications in formulated food industry.

3.5. Foaming capacity and emulsifying capacity of protein hydrolysate

Foaming capacity and emulsifying capacity of protein hydrolysate from Tra fish by-products at different pH values are shown in Figure 2.

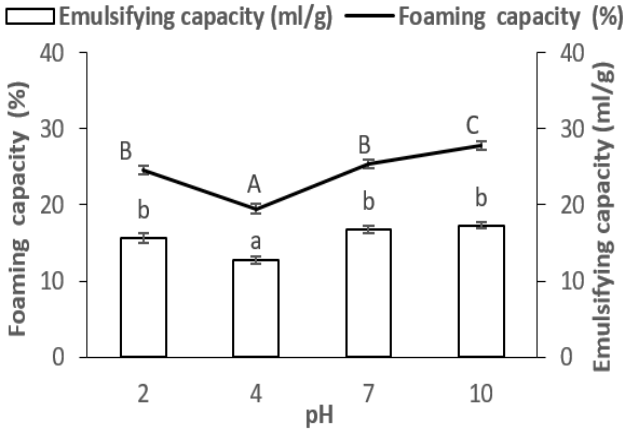

Fig. 2 Foaming capacity and emulsifying capacity of protein hydrolysate from Tra fish by-products at different pH values. Different letters show significant differences ($p < 0.05$).

The lowest foaming capacity of protein hydrolysate from Tra fish by-products was found at pH 4.0 (19.5%). Similar results were also reported for protein hydrolysates from rainbow trout viscera and poultry by-products in a previous study, which showed that the foaming capacity of protein hydrolysates was the lowest at pH 4 [19]. The minimum foaming capacity at pH 4 may be due to proximity to the isoelectric point of the proteins. The foaming capacity of protein hydrolysate from Tra fish by-products reached a maximum value (27.8%) at pH 10. The round scad muscle hydrolysates had foaming capacity from 23.33% to 70% [20]. The capelin protein hydrolysate possessed the foaming capacity of 90 % [21].

The emulsifying capacity of protein hydrolysate from Tra fish by-products at different pH values were 15.7%, 12.8%, 16.8% and 17.3% for pH 2, pH 4, pH 7 and pH 10 respectively. The emulsifying capacity of protein hydrolysate from Tra fish by-products was higher than the cobia protein hydrolysates (3 -12 mL/g) [6] but lower than the carp skin protein hydrolysates (20 - 38 mL/g) [22] and catfish frame hydrolysates (17.2 - 29.8 mL/g) [23]. The minimum value of emulsifying capacity was observed at pH 4.0, probably because this pH is close to the isoelectric point of fish proteins, resulting in a reduction of their emulsifying property. The emulsifying capacity of protein hydrolysate from Tra fish by-products reached the highest value at pH 10. The alkaline pH enhanced emulsifying properties due to the unfolding of the polypeptides because of the negative charges in this pH range. The higher emulsifying capacity of the hydrolysate accompanied the increased solubility [19].

4 Conclusion

The protein hydrolysate produced from Tra fish by-products had a high nutritional value with protein content of 65.8% and essential amino acid content of 16.25 g/100g of protein. pH had a significant effect on the solubility, foaming capacity and emulsifying capacity of protein hydrolysate. The protein hydrolysate from Tra fish by-products showed the highest values of solubility (98.2%), foaming capacity (27.8%) and emulsifying property (17.3%) at pH 10. On the contrary, the lowest values of solubility, foaming capacity and emulsifying property were obtained at pH 4.0. From this study, it is evident that the protein hydrolysate from Tra fish by-products could be used as a good source of desirable peptides and essential amino acids. This protein hydrolysate could be used as a functional ingredients in food industry for human consumption.

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Đặc tính dinh dưỡng và chức năng của sản phẩm thủy phân protein từ phụ phẩm cá tra

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Tóm tắt Đặc tính dinh dưỡng và chức năng, sự phân bố trọng lượng phân tử của sản phẩm thủy phân protein từ phụ phẩm cá tra đã được nghiên cứu. Sản phẩm thủy phân protein được sản xuất bằng sự thủy phân phụ phẩm cá tra bởi enzyme Protamex 0,5% ở nhiệt độ 50 ^{o}C trong 6 giờ. Kết quả nghiên cứu đã cho thấy sản phẩm thủy phân protein phụ phẩm cá tra có hàm lượng ẩm 6,7%, protein 65,8%, lipid 1,6% và tro 9,5%. Sản phẩm thủy phân protein có hàm lượng axit amin tổng số là 52,81 g/100g protein và hàm lượng axit amin thiết yếu là 16,25 g/100g protein. Tỉ lệ axit amin thiết yếu trên tổng số axit amin là 30,76%. Sản phẩm thủy phân protein giàu glycine, glutamic, proline, alanine, aspartic và leucine. Hầu hết các peptit (68,25%) trong sản phẩm thủy phân protein phụ phẩm cá tra có trọng lượng phân tử từ 360 Da đến 2500 Da. Sản phẩm thủy phân protein có độ hòa tan trong khoảng 90,3% - 98,2%. Độ hòa tan của sản phẩm thủy phân protein thấp nhất ở pH 4 và cao nhất ở pH 10. Khả năng tạo bọt của sản phẩm thủy phân protein trong khoảng từ 19,5% đến 27,8%. Khả năng nhũ hóa của sản phẩm thủy phân protein là 12,8 - 17,3 mL/g. Sản phẩm thủy phân protein phụ phẩm cá tra có giá trị dinh dưỡng cao và có đặc tính chức năng tốt. Kết quả nghiên cứu cho thấy sản phẩm thủy phân protein phụ phẩm cá tra có thể được sử dụng làm nguồn protein trong hệ thực phẩm và là thành phần thực phẩm tiềm năng đầy hứa hẹn.

Từ khóa Sản phẩm thủy phân protein cá, đặc tính chức năng, phân bố trọng lượng phân tử, đặc tính dinh dưỡng, phụ phẩm cá tra.