Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 87 (2004) 97–102
Food Chemistry
www.elsevier.com/locate/foodchem

Amino acid composition and anti-anaemia action of hydrolyzed offal protein from *Harengula Zunasi* Bleeker

Deng Shang-gui [a,b,*], Peng Zhi-ying [a], Chen Fang [c], Yang Ping [b], Wu Tie [c]

[a] *Food and Bioengineering College, South China University of Technology, Guangzhou 510640, China*
[b] *Department of Food Science and Engineering, Zhanjiang Ocean University, Zhanjiang 524025, China*
[c] *Department of pharmacology, Guangdong Medicine College, Zhanjiang 524023, China*

Received 25 April 2003; received in revised form 28 October 2003; accepted 28 October 2003

Abstract

A hydrolyzed offal protein (HOP) from *Harengula Zunasi* offal was produced according to the poly-enzymatic method. Its amino acid composition and in vivo anti-anaemia action were investigated. Chemical analysis showed that the hydrolyzed offal protein contained 16.0% protein, 4.21% fat, 76.28% moisture and 3.39% ash. Compared with the amino acid profiles recommended by FAO/WHO, the protein quality of the hydrolyzed offal protein was fairly high, due to its high contents of essential amino acids, including isoleucine, leucine, lysine, methionine, cystine, and threonine, valine, that covered 91–100% of those suggested by FAO/WHO. Particularly, the high amount of lysine in the hydrolyzed offal protein may provide good protein supplementation for vegetable foods, in which lysine is commonly a limiting amino acid. Animal experiments revealed that the hydrolyzed offal protein inhibited mice anaemia caused by an injection of cyclophosphamide. The hydrolyzed offal protein could significantly inhibit the decreases in red blood cells, hematocrit, hemoglobin and platelets, but not white blood cells of the mice caused by cyclophosphamide. Presumably, its anti-anaemia action might be due to prevention of the functional groups of protein (including amido, mercapto, hydroxyl group and carboxyl groups in mouse body) undergoing the reaction of alkylation with cyclophosphamide. In addition, the anti-anaemia action of the hydrolyzed offal protein might also be due to nutritional functions of minerals (calcium, phosphorus and iron). The results of the present study provide some technical information and suggestions for the seafood industry for more effective utilization of fish offal.

Keywords: Hydrolyzed offal protein; *Harengula Zunasi* Bleeker; Amino acid composition; Anti-anaemia; In vivo

1. Introduction

Harengula Zunasi Bleeker, a salt water fish, is mainly distributed in the coastal areas of the Philippines, Japan, Korea and China. With the annual harvest ranging from 30 to 40 thousand tons, it is not appropriate to eat it raw or store it for a long time due to its small body and small bone and high contents of fat and dark muscle. The fish is universally processed as salt-dried fish or fresh-dried fish, with a small quantity in cans or fishmeal. In order to make more effective use of the protein of the fish, extensive studies have been carried out using modern biotechnology, Deng, Zhang, and Luo (1996) reported that a hydrolyzed protein containing 1.5 g of α-amino nitrogen per 100 ml could be made from *Harengula Zunasi* Bleeker by the joint treatments of B. Subctls neutral proteinase and pepsin (i.e., bi-enzyme method). The brown colour and fishy odour of the hydrolyzed protein could be removed by the joint actions of β-CD (β-cyclodextrin) and activated charcoal. In addition, the effective removal of the fishy odour and bitter taste of the hydrolyzed protein could also be achieved by using yeast powder and flavorease (endoproteinase and exopeptidase from *Aspergillus oryzae*) (Deng & Zhang, 1998). Deng (2000a) also found that the ratio of soluble to insoluble protein in the fish muscle was 2–9 and the denaturalization temperature of the soluble protein ranged from 60 to 70 °C. A protein concentrate was produced from the dark muscle of the fish by using the

* Corresponding author: Tel.: +86-759-2382026.
E-mail address: dengshanggui@163.com (D. Shang-gui).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.10.024

method of Soviet Extraction (Deng, 2000b, 2001), which is the first report in China on the utilization of the dark muscle to produce fish protein concentrate (FPC). In addition, a protein drink was obtained from the fish muscle. Its fishy odour, bitter and other unpleasant tastes could be removed by using physical and chemical and biochemical methods (Deng & Zhang, China patent 00101517). Through the poly-enzymatic method (alkaline protease, neutral protease and flavorase), hydrolyzed offal protein (HOP) was also developed from *Harengula Zunasi* (Deng, Peng, & Yang, 2002a, 2002b; Deng & Zhang, China patent 00118904). For more effective utilization of the protein of *Harengula Zunasi* and its offal, we focus on the amino acid composition of the HOP processed by the poly-enzymatic method and its anti-anaemia action in mice anaemia induced by cyclophosphamide. Through this work, it is hoped to provide some fundamental information and recommendations for the all-around utilization of the fish offal.

2. Materials and methods

2.1. Raw materials

Harengula Zunasi Bleeker was obtained from a local supplier near the campus from September to December in 2002. The enzymes used for the hydrolysis were B.Subctls alkaline protease (120,000 IU ml^{-1}), neutral protease (100,000 IU g^{-1}) and flavorase (1000 l®), commercial proteases, respectively obtained from Wuxi Jienengke Bioengineering Co. Ltd. (China) and NoVo Nordisk Co. Ltd. (Denmark). Cyclophosphamide (No. 000304) was chemical grade and obtained from Shanghai Hualian pharmacy Co. Ltd. (China).

2.2. Preparation of offal for enzymatic hydrolysis

The fish, after washing, were gutted and deheaded immediately upon arrival at the laboratory and deboned using a double-drum fish deboner. So, the offal for enzymatic hydrolysis consisted of head, bone and gut. The offal was then packed in PVC bags and stored at −18 °C until required for further use. The offal was mixed with four volumes of distilled water and homogenized for 60 s using a Waring blender, and the pH of the mixture was adjusted to 7.0 with 1 N NaOH. The offal was raised to 50 °C then placed into the 50 °C-constant temperature reactor.

2.3. Enzymatic offal protein hydrolysis

The production of an HOP was performed according to the poly-enzymatic method of Deng and Zhang, China patent 00118904. Previous work had shown that the optimum enzymatic conditions were pH 7.0, 50 °C and 120 min, simultaneously using 1.5% alkaline and 1.5% neutral protease, and then 2% flavorase for 60 min. After hydrolysis, the process was terminated by raised the hydrolysis temperature to 90 °C for 20 min (Adler-Nissen, 1986). After the mixture had cooled down, it was filtered through a 100-hole nylon to remove the bone from the hydrolysate, and centrifuged at 4500 rpm for 20 min to separate the soluble from the insoluble fraction. The hydrolysate was then placed in a concentrate can until further processing.

2.4. Production of HOP

Hydrolyzed offal protein, for chemical analysis, was obtained from the liquid hydrolysate, being concentrated to a soluble solids content of 20% using a concentrate can under vacuum press but, for animal test, it had to be further concentrated to a soluble solids content of 30%.

2.5. Proximate analysis

Crude protein and fat contents were determined according to the micro-Kjeldadl and Soxhlet methods of AOAC (1990), respectively. Moisture was determined by using the air oven AOAC method (1990) and ash by using the basic AOAC method (1990) heating the samples in the furnace at 550 °C for 8–12 h. The soluble solids content of HOP was determined by a dioptrometer.

2.6. Amino acid analysis

The PICO TAG method, with modification, was employed for determining the amino acid profile of the hydrolysate (Bidlingmeyer, Cohen, & Tarvin, 1987). HOP (weight equivalent to 4% protein) was treated with 6 M HCl (15 ml) and placed in the oven at 110 °C for 24 h. Ten millilitres of internal standard were added to the mixture. After derivatisation, 100 ml PICO TAG diluent were added and mixed. 100 ml sample were then injected into the HPLC and analysed with of Waters PICO TAG amino acid analyser. Then, tryptophan (Trp) was determined using the basic hydrolysis method (Deng et al., 2002a, 2002b). HOP (weight equivalent to 1–2 mg Trp) was treated with 4.2 M NaOH (100 ml) and 0.3 ml thioglycerin and placed in the oven at 110 °C for 24 h; 7 ml 6 M HCl were then added to the mixture and the pH adjusted to 4.5 using pH 4.2 citric acid buffer solution and the mixture was made up to a certain volume. Trp content was determined by colorimetric analysis at 440 nm under the conditions of pH 5.0–5.5, columniation temperature 55 °C, reactor temperature 100 °C, reaction time 10–15 min.

2.7. Inorganic analysis (Deng et al., 2002a and Deng et al., 2002b)

Hydrolyzed offal protein (approximately 1.5 g) was treated with a 20 ml mixture of perchloric acid and nitric acid (1:4) and stood overnight. The mixture was then digested to approximate dryness and cooled to the room temperature. The cooled mixture was made up to 100 ml using nitric acid and the acidity kept to 1% until required for further use. Iron was determined using atomic absorption analysis and the other minerals determined using plasma chromatography (IRIS/AP, TJA Co., USA).

2.8. In vivo anti-anaemia action of HOP

2.8.1. Mice and model

Ordinary-grade male Kunming mice, body weight 20 ± 2 g, were obtained from the Animal Center of Guangdong Medicine College and divided into three different groups at random, consisting of 11 mice in each group. The first group (group A), as a control, was fed daily by P.O (i.e., irrigated into stomach) with physiological saline (0.1 ml/kg body weight). Over the following 21 days, the second group (group B), as a model, was daily injected with cyclophosphamide (7.5 mg/kg body weight) into retineum in the forenoon and administered by P.O with physiological saline (0.1 ml/kg body weight) in the afternoon over the following 21 days. The third group (group C), as a trial, was daily injected with cyclophosphamide (7.5 mg/kg body weight) into the retineum in the forenoon and administered by P.O with HOP (0.1 ml/kg body weight) in the afternoon over the following 21 days. All of the mice were weighed every week until sacrifice on the 22nd day.

2.8.2. Haematology analysis

Blood was removed at the end of giving medicine. Haematology analyses, including red blood cells (RBC, 10^{12}/l), hemoglobin (Hb, g/l), hematocrit (HCT,%), white blood cells (WBC, 10^9/l) and platelets (PLT, 10^9/l), were carried out using the Coulter JT made by Beckman Coulter Co., USA.

2.8.3. Indices of the liver, spleen and thymus

The liver, spleen and thymus, from all mice sacrificed on the 22nd day, were removed and weighed. The liver, spleen and thymus indices were calculated as follows:

liver index = liver weight of mice (g)/body weight of mice (10 g),

spleen index = spleen weight of mice (g)/body weight of mice (10 g),

thymus index = thymus weight of mice (g)/body weight of mice (10 g).

2.8.4. Statistical analysis

Significances of results were tested by *t*-test.

3. Results and discussion

3.1. Proximate composition of Harengula Zunasi's offal and HOP

As shown in Table 1, fish offal, with 14.8% of crude protein, could be regarded as a protein-rich resource with a complicated composition, including mainly cartilage mucin, collagen, cartilage protein, elastin, sarcoplasmic protein, fibrin, actin, keratoprotein and mucus protein. Due to the complexity of offal protein composition and specificity of enzyme, it is difficult to hydrolyze the protein of the offal into HOP by the action of one or two enzymes. So it is essential to develop new methods and techniques to resolve the problem. Moreover, it was necessary to process the offal over time, due to the high content of fat, especially of polyunsaturated fatty acids in the offal, that made easily perished and acidified. In addition, the high amount of ash, including mainly calcium, iron and phosphorus, could be utilized as an inorganic source by proper processing. Table 1 shows the proximate composition of offal and HOP (China patent 00118904). A significant difference was observed between the samples in the contents of crude protein, fat and ash. It was found that the crude ash and fat contents decreased while the crude protein content increased after treatment by the poly-enzymatic method. The crude protein increased by 8.1% when the offal was prepared into HOP, due to the addition of several enzymes. In the meantime, the ash and crude fat decreased by 31.8% and 37.4%, respectively, because of the removing of fishbone and fat from the offal hydrolysate. With a high content of protein (Table 1), HOP is clearly a protein resource, rich in amino acids and peptides from offal muscle protein after hydrolysis by the poly-enzymatic method.

3.2. Amino acid composition and mineral contents of HOP

Table 2 shows the amino acid profile of HOP. Several outstanding characteristics of HOP can be found from the results: (1) the ratios of fresh tasting amino acids (asparagine and glutamic acid) and sweet amino acids (glycine and alanine) to total amino acids were as high as 25.9% and 15.2%, respectively; (2) the lysine content in HOP was rich (0.626 g/100 ml) and might provide good protein supplementation for vegetal foods, in many of which lysine is regarded as a limiting amino

Table 1
Proximate composition of *Harengula Zunasi*'s offal and HOP

Material	Moisture	Ash	Crude protein	Crude fat
Offal	74.26	4.97	14.8	6.73
HOP	76.28	3.39	16.0	4.21

Table 2
Amino acid composition of HOP

Amino acid	g/100 ml HOP	mg/g protein	Amino acid	g/100 ml HOP	mg/g protein
Cysteine	0.440	48.1	Tyrosine	0.097	10.7
Lysine	0.626	68.5	Methionine	0.179	19.6
Arginine	0.459	50.2	Valine	0.450	49.3
Asparagine acid	0.798	87.4	Phenylalanine	0.321	35.1
Glutamic acid	1.11	121.6	Leucine	0.627	68.6
Serine	0.270	29.5	Isoleucine	0.372	40.6
Glycine	0.566	62.0	Histidine	0.151	16.5
Threonine	0.334	36.5	Tryptophan	0.063	6.9
Alanine	0.554	60.7	Proline	0.351	38.5

Table 3
Essential amino acid composition of HOP compared with the FAO/WHO pattern[a]

Amino acids	HOP (mg/g crude protein)	FAO/WHO pattern[a]	Percentage (%)
Isoleucine	40.6	40	>100
Leucine	68.6	70	98
Lysine	68.5	55	>100
Methionine + cystine	67.7	35	>100
Phenylalanine + tyrosine	45.8	60	76
Threonine	36.5	40	91
Tryptophan	6.9	10	69
Valine	49.3	50	98
Total	384	360	

[a] From [28, 29] (Copyright Food and Agriculture Organization of the United Nations, 1973).

acid; (3) the essential amino acids were rich in HOP with the ratio of the content of essential amino acids to the total amino acids being 40.3%. In addition, total content of amino acids was 7.37 g/100 ml in HOP.

Table 3 shows that HOP contents of isoleucine, leucine, lysine, methionine, cystine, threonine and valine were comparable with that of the FAO/WHO amino acid reference pattern (1973) established for humans. The first limiting amino acid of HOP is found to be tryptophan. The high contents of hydrophobic amino acids (isoleucine, leucine and lysine) in HOP were important, due to their good effects on the physical and functional properties of food proteins. HOP supplied a higher proportion of the amino acids required for humans than the non-hydrolyzed fish protein, and the quantities of essential amino acids, isoleucine, leucine, lysine, methionine, cystine, threonine and valine covered 91–100% of the FAO/WHO ideal (1973).

Table 4 shows the mineral contents of HOP. Among the four inorganic elements determined in HOP, the content of phosphorus was the highest and amounted to 69.2 mg/100 g, four times that of the similar product-black shrimp sauce (Zhang, Deng, & Hong, 2000) and twice that (300 mg/100 g) of the gravy (Huang, 1996). The calcium in HOP, amounting to 33.7 mg/100 g, might be easily absorbed by the human body (Zhang et al., 2000). In addition, the content of iron in HOP (0.64 mg/100 g) was also high.

Table 4
Mineral content of HOP (mg/100 g)

Mineral	Ca	Fe	Cu	P
HOP	33.7	0.64	0.03	69.2

3.3. In vivo anti-anaemia action of HOP

3.3.1. Effect of HOP on body weight of cyclophosphamide mice

Table 5 shows the effect of HOP on body weight of cyclophosphamide mice. No significant difference was observed in the body weights of the mice among groups A, B and C, revealing that cyclophosphamide (7.5 mg/kg/d) and HOP (0.1 ml/kg/d) had no significant effect on the body weights of mice. As shown in Table 5, cyclophosphamide showed a trend of lightening the mouse body weight; however, HOP had the anti-action of lightening mouse body weight caused by cyclophosphamide.

3.3.2. Effect of HOP on indices of thymus, liver and spleen of cyclophosphamide mice

Table 6 shows that the viscera indices of cyclophosphamide mice were affected by HOP after the mice had been daily injected with cyclophosphamide into the retineum and administered, by P.O with HOP over the

Table 5
Effect of HOP on body weight (g) of cyclophosphamide mice ($x \pm s$)

Group	Dose (/kg)	Mice (*n*)	Body weight[a]			
			Pre-medicine	1st week	2nd week	3rd week
A	0	10	23.8 ± 1.8	27.8 ± 1.7	30.1 ± 2.0	32.0 ± 3.3
B	7.5 mg	10	23.6 ± 2.2	26.8 ± 3.7	27.4 ± 4.4	29.1 ± 5.7
C	0.1 ml	9	24.1 ± 1.6	27.8 ± 1.6	29.1 ± 2.9	29.4 ± 2.5

[a] $p < 0.05$, compared with group A.

Table 6
Effect of HOP on the viscera indices of cyclophosphamide mice

Group	Dose (/kg)	Mice (*n*)	Body weight	Liver index	Spleen index	Thymus index
A	0	10	32.5 ± 3.3	0.45 ± 0.05	0.025 ± 0.005	0.028 ± 0.007
B	7.5 mg	10	29.1 ± 5.7	0.46 ± 0.06	$0.041 \pm 0.016^{a,b}$	$0.020 \pm 0.008^{a,b}$
C	0.1 ml	9	29.4 ± 2.5	0.45 ± 0.05	$0.037 \pm 0.007^{a,b}$	0.020 ± 0.010^{a}

[a] $p < 0.05$, compared with group A.
[b] $p < 0.01$ compared with group A.

following 21 days. Significant ($p < 0.05$ or 0.01) differences in spleen (significant increase) and thymus indices (significant decrease) were observed between groups B and A or C and A, whereas HOP showed no marked effect on liver index of mouse. As shown in Table 6, cyclophosphamide had significant effects on mouse thymus and spleen, and it was possible that the enlargement of spleen was caused by anaemia. These results revealed that cyclophosphamide had a significant restraining action on the immunity system of mice and HOP had no anti-action of the restraining immunity caused by cyclophosphamide.

3.3.3. Effect of HOP on haematologic parameters of cyclophosphamide mice

Table 7 shows the effect of HOP on haematologic parameters of cyclophosphamide mice. Compared with those of group A, the haematologic parameters, including RBC, HCT and WBC, of group B decreased significantly ($p < 0.01$ or 0.05) after the mice had been daily injected with cyclophosphamide into the retineum over the following 21 days. Meanwhile, there was a decreasing trend in the parameters Hb and PLT for group B. These results revealed that the anaemia model mice were obtained through injection with cyclophosphamide into the retineum for 21 days.

Table 7, shows significant difference ($p < 0.05$ or 0.01) in parameters RBC and HCT between groups B and A, whereas there is no significant difference between groups C and A. In addition, there was no decreasing trend for group C in parameters Hb and PLT, but there was significant ($p < 0.05$ or 0.01) difference in WBC between groups C and A. These results revealed that HOP could significantly prevent the decreases in RBC, HCT, Hb and PLT, but could not prevent the decrease in WBC caused by cyclophosphamide. The main reason why the action of HOP on WBC follows a different trend is that WBC is more seriously injured by cyclophosphamide than RBC and PLT.

3.4. Anti-anaemia mechanism of HOP

The HOP anti-anaemia action caused by cyclophosphamide might be related to the cyclophosphamide anaemia mechanism and HOP composition. Cyclophosphamide, an alkylation medicine, which can cause alkylation of cell functional groups, such as DNA, amido, mercapto, hydroxyl and carboxyl groups in protein, acts by restraining medulla hemopoietic function and causing leucopenia by decreasing white blood cell numbers in blood. Particularly, a high dose of cyclophosphamide can also lay significant restraints on building blood in the medulla and extramedulla.

Table 7
Effect of HOP on haematological parameters of cyclophosphamide mice ($x \pm s$)

Group	Dose (/kg)	Mice (n)	RBC (10^{12}/l)	Hb (g/l)	HCT (%)	WBC (10^9/l)	PLT (10^9/l)
A	0	10	9.15 ± 0.36	144 ± 8.0	0.382 ± 0.02	5.85 ± 1.17	797 ± 144.4
B	7.5mg	10	$8.41 \pm 0.56^{a,b}$	138 ± 11.0	$0.349 \pm 0.02^{a,b}$	4.67 ± 1.30^{a}	695 ± 155.3
C	0.1ml	9	8.97 ± 0.42	145 ± 5.9	0.377 ± 0.02	$3.21 \pm 0.79^{a,b}$	780 ± 141.9

The abbreviated headings RBC, Hb, HCT, WBC and PLT are red blood cells, hemoglobin, hematocrit, white blood cells and platelets, respectively.
[a] $p < 0.05$ compared with A.
[b] $p < 0.01$ compared with A.

Hydrolyzed offal protein, a hydrolyzed protein, prepared from *Harengula Zunasi* offal through the polyenzymatic method, has a lot of free functional groups, such as amido, mercapto, hydroxyl and carboxyl groups. These free functional groups in HOP may prevent the same groups in mouse body from reacting by alkylation with cyclophosphamide. In addition, HOP is rich in calcium, phosphorus and iron, which elements are necessary for cells building blood to breed and grow and synthesize hemoglobin. Therefore, HOP has a significant anti-action to the decreases in RBC, HCT, Hb and PLT, but shows no significant prevention of the decrease in WBC caused by cyclophosphamide.

4. Conclusions

Proximate analysis revealed that the HOP contained 16.0% protein, 4.21% fat, 76.28% moisture and 3.39% ash. Protein quality of HOP was found to be high because the quantities of essential amino acids, isoleucine, leucine, lysine, methionine, cystine, threonine, and valine covered 91 to 100% of the FAO/WHO ideal (1973). The lysine content of HOP was rich and could provide a good supplementation for the protein of vegetal foods, in many of which lysine is regarded as a limiting amino acid, although HOP chemical score was only 69 and the first limiting amino acid was tryptophan.

The anaemia mouse model was obtained with mice being injected daily with cyclophosphamide (7.5 mg/kg body weight) into the retineum over the following 21 days. HOP had a definite anti-anaemia action in vivo because it could significantly prevent the decreases in RBC, HCT, Hb and PLT. However, it showed no significant anti-action to the decrease in WBC caused by cyclophosphamide. The possible reason for the anti-anaemia action of HOP might be that HOP could prevent the functional groups (including amido, mercapto, hydroxyl and carboxyl) of protein in mouse body from reacting by alkylation with cyclophosphamide. In addition, it had nutritional contributions from calcium, phosphorus and iron.

References

Adler-Nissen (1986). Relation of structure to taste of peptides and peptide mixtures. In *Protein tailoring for food and medical uses* (pp. 97–102). New York: Marcel Dekker.

AOAC. (1990). *Official methods of analysis* (14th ed.). Washington, DC: Association of Official Analytical Chemistry.

Bidlingmeyer, B. A., Cohen, S. A., Tarvin, T. L., et al. (1987). A new, rapid, high sensitivity analysis of amino acid in food type samples. *Journal of Association Official Analytical Chemistry, 70*(2), 241–247.

Deng, S. G. (2000a). Study on traits of muscle extract from *Harengula Zunasi* during heating. *Journal of Fujian Fisheries* (1), 17–22.

Deng, S. G. (2000b). Processing of fish protein concentrate from *Harengula Zunasi*. *Journal of Shanghai Fisheries University, 9*(1), 40–44.

Deng, S. G. (2001). Processing of fish protein concentrate from *Harengula Zunasi*. The American Chemical Society (Chemistry Abstracts) *134*(10), 655.

Deng, S. G., Peng, Z. Y., Yang, P., et al. (2002a). Application of multi-enzymatic method in fermented fish sauce production from *Harengula Zunasi*'s offal. *Food and Fermentation Industries, 28*(2), 32–36.

Deng, S. G., Yang, P., Pang, Y. S., et al. (2002b). Preparing of shrimp-head paste from penaeus momodn's offal. *Food and Fermentation Industries, 28*(10), 80–83.

Deng, S. G., & Zhang, C. H. (1998). Technological study on preparing of aquatic hydrolyzed animal protein by bienzyme method. *Journal of Fisheries of China, 22*(4), 352–356.

Deng, S. G., & Zhang, C. H. Preparing method of protein drink from *Harengula Zunasi*. China patent 00101517.

Deng, S. G. & Zhang, C. H. Multi-enzymatic method of fish and fish gravy of preparing technique. China patent 00118904.

Deng, S. G., Zhang, C. H., Luo, H. W., et al. (1996). The preparing of hydrolsate of *Harengula Zunasi* protein. *Journal of Zhanjiang Fisheries College, 16*(2), 58–61.

FAO/WHO. (1973). Energy and protein requirements. Rome: Food and Agricultural Organization of the United Nations.

Huang, Z. B. (1996). *Fish gravy and hydrolyzed protein* (1st ed.). Beijing: Agriculture Press, 39p.

Zhang, C. H., Deng, S. G., & Hong, P. Z. (2000). Applied studies on rapid technology of shrimp tissues in nature sea condiment. *Food and Ferment Industry, 26*(2), 36–39.