Jorjani Biomedicine Journal. 2018; 6(3): P 24-39.

Evaluation of Different Proportions of Distilled Water to Substrate on Functional Properties, Antioxidant and Nutritional Quality of Bigeye Ilisha (Ilisha Megaloptera) protein hydrolysate

Aniseh Jamshidi[1], Bahareh Shabanpour[*2], Parastoo Pourashouri[3], Mojtaba Raeisi[4]

1. PhD Student of Fish Processing, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
2. Professor of Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
3. Assistant Professor of Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
4. Assistant Professor, Cereal Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Article Type:

Original Article

Article History:

Received: 25 Jun. 2018
Revised: 29 Aug. 2018
Accepted: 29 Sep. 2018

****Correspondence:***

Bahareh Shabanpour, Department of Fisheries, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
b_shabanpour@yahoo.com

Abstract

Background and objectives: Production of fish protein hydrolysate is a method for converting the low-value economical underutilized fish species to value-added products. This study aimed to evaluate the different proportions of distilled water to the substrate on the functional characteristics, antioxidant and nutritional quality of fish protein hydrolysate of bigeye ilisha (Ilisha megaloptera) produced by enzymatic hydrolysis.

Methods: After defatting of minced fish, the hydrolysis process was carried out using three different 4:1, 5:1 and 6:1 distilled water to substrate proportions by using alcalase enzyme in three replications. The protein hydrolysate samples were analyzed for approximate composition (soluble protein, moisture, fat and ash), functional characteristics (solubility, foam capacity, and foam stability), antioxidant properties (DPPH radical scavenging activity and reducing power) and mineral composition.

Results: In this study, the ratio of distilled water to the substrate affected the protein hydrolysate properties and the highest amounts of hydrolysis degree and DPPH radical scavenging activity were observed in samples with 5:1 ratio. Fish protein hydrolysate obtained from 4:1 ratio had the highest amount of soluble protein, and no significant difference was observed in term of solubility with samples obtained by 5:1 ratio. Moreover, there were no significant differences in terms of lightness and foam capacity of samples obtained from 4:1 and 6:1 proportions.

Conclusion: According to the results of the study, using a 4:1 ratio of distilled water to substrate led to the production of fish protein hydrolysates from bigeye ilisha with higher functional properties and nutritional composition.

Keywords: Alcalase Enzyme, Fish Protein Hydrolysate, Bigeye Ilisha (Ilisha Megaloptera), Functional Properties, Antioxidant Properties

Introduction

There is an increased demand for protein in the world due to population increase and changing of food preferences. Having a high digestibility, fish is one of the great sources of protein. Some species of tiny fish species that are classified as high-fat fish, such as yellow stripe scad (Selaroides leptolepis) and kilka (Clupeonella engrauliformis), are known as low-consumption species (1). On the other hand, the waste from fish processing factories is also rich in protein sources that are economically disadvantageous and cause inappropriate waste, producing one of the major environmental contaminants of the world (2). One of the biological techniques for using low-value fish waste and low-consumption and protein-rich fish species is the enzymatic hydrolysis of fish proteins that results in the production of a biologically active protein hydrolysate. The fish protein hydrolysate is the result of the enzymatic degradation and the conversion of proteins to smaller peptides and can be widely used in foods. The major biological activity of fish protein hydrolysate is antioxidant and antihypertensive activities (3). Due to the presence of bioactive peptides, chondroitin sulfate, and antioxidant properties, these materials are suitable for cancer treatment. On the other hand, they have high digestibility due to the shortage of the peptide chain and can be used as a protein supplement for human, livestock and aquatic food (1).

Protein hydrolysis affects molecular size, hydrophilic groups, and hydrolyzed polar groups, and directly influences the functional characteristics and their application as food additives. The degree of hydrolysis has a direct effect on the solubility of the protein hydrolysate. In general, the most useful feature of the protein hydrolysate in the food industry is its high solubility over a wide range of pH (4). On the other hand, the solubility of the protein hydrolysate affects its other functional properties, including the foam production capacity and emulsifying power. It has been proven that along with the functional characteristics, the protein hydrolysate has different antioxidant properties, depending on the combination of free amino acids and their peptides (5).

In recent years, extensive studies have been conducted on the hydrolysis of proteins and the effective factors involved in its optimal production method. Pacheco-Aguilar et al. (6) prepared protein hydrolysate with various levels of hydrolysis (10%, 15%, and 20%) from Atlantic salmon (Merluccius productus). Evaluating the effect of different pH levels on its functional characteristics, these researchers marked that the solubility of all treatments was approximately 100% and different degrees of hydrolysis had no significant effect on the amount of emulsifying activity of protein hydrolysates produced. Ovissipour et al. (7) applied different enzymes (alcalase, proteomics, nutrase, flavourzyme, and trypsin), pH levels (3.3 and 12), and temperatures (70 and 85°C) to produce protein hydrolysate from Persian sturgeon (acipenser persicus), concluding that the hydrolysis conditions had a significant effect on the properties of protein hydrolysates produced, and combination of alcalase enzyme with low pH and high temperature increased the hydrolysis degree and led to a higher protein recovery.

In another research, Klomklao S, Benjakul (5) carried out the optimization of the production

process of protein hydrolysate from tuna (Katsuwonus pelmais) based on alcalase enzyme concentration, reaction time and waste to buffer ratio. According to their results, the optimal hydrolysis condition was determined at 3% enzyme, 20 min reaction time and 1:2 waste to buffer ratio (weight/volume). According to studies, the ratio of distilled water (or buffer) to the substrate can be one of the factors affecting the hydrolysis process conditions. Based on various ratios, it is possible to prepare a protein hydrolysate with different degrees of hydrolysis. The bigeye ilisha (Ilisha megaloptera) is a species of ray-finned fish in the family Clupeidae and one of the less-consumed and discarded by-catch species. This fish does not have human consumption and is mostly used to produce silage and animal food or is returned to the sea with other bycatch catches. Since this fish makes up 2.5% of the mean catch weight of bycatch (northwest of the Persian Gulf-Khuzestan province) (9), it has a considerable potential to produce side products (fish oil, protein hydrolysate and gelatin).

According to the literature, there is no report on protein hydrolysate produced from bigeye ilisha and evaluation of its functional and antioxidant characteristics. With this background in mind, this study aimed to produce protein hydrolysate from low-consumed fish of bigeye ilisha and assess its antioxidant and functional properties to be applied in various food combinations with the use of different distilled water to substrate proportions.

Materials and Methods

Chemical Compounds

The alcalase enzyme was purchased from the Novozyme Co. (Denmark), whereas DPPH reagent and ascorbic acid were acquired from Sigma Co. (Germany). Other chemicals and solvents used were bought from Tetrachem and Fluka companies with laboratory grades. It should be noted that double distilled water was applied for distillation in all solutions.

Research Design and Classification

In order to produce protein hydrolysate and evaluate the effect of various proportions of distilled water to the substrate, research design and classification of samples were initiated by preparing the bigeye ilisha (Ilisha megaloptera) fish with approximate weight of 45 grams from local market of Abadan, Iran. The fish were immediately frozen and transferred to the processing laboratory of Gorgan University of Agricultural Sciences and Natural Resources after suitable freeze-up (1:3 fish to ice ratio). After thawing of fish at room temperature, manual filleting (without separation of skin) was carried out by cutting heads and gutting the fish. After washing, the fillets were minced with the aid of a meat grinder (Model: CNFW2, Bush Co., Slovenia) with a diameter of 0.4 cm, and the products were maintained in a freezer at -20°C after packing and until the hydrolysis process.

At this stage, the defatting of the minced fish was performed according to the method by Thiansilakul et al. (10). To this end, the minced fish was mixed with isopropanol at a ratio of 1:2 (w/v), and after proper stirring, the mixture was heated for 30 minutes at 70°C. After separating the fatty masses that were collected at the surface, the defatted minced meat was washed in two phases with five times the volume of distilled water, and the dewatering operation was carried out manually. Afterwards, the defatted minced meat was dried for 24 hours at room temperature (25°C). In order to perform the

hydrolysis process, the defatted samples were divided into three groups with three replications, mixed with distilled water at various ratios of 4:1, 5:1, and 6:1 (w/v), and was heated at the temperature of 85°C for 20 min in Bain-Marie (Model: WNB14, Memmert Co., Germany) (2).

After cooling the mixture in water and ice, the pH of the mixture was adjusted using sodium hydroxide solution (0.2 N) at 5.8 pH (optimum pH for alcalase enzyme activity), and the alcalase enzyme was added to 2% amount of the total protein content of the fish. The enzymatic hydrolysis process was performed in a shaking incubator (Model: KS4000ic, IKA CO., Germany) at 200 rpm at 60°C for two hours. In the end, the hydrolysis process was completed by heating the hydrolyzed mixture at the temperature of 95°C for 20 min in Bain-Marie. The temperature of the mixture was reduced by using a water and ice bath. To obtain the protein of the hydrolyzed solution, the sample was centrifuged in a refrigerated centrifuge (Model: 5810 R, Eppendorf Co., Germany) for 20 min at 8000 rpm and at 10°C. The supernatant was dried after being collected using a freeze-drying machine (Model: ALPHA 1-2 LD PLUS, Christ, Germany), and the protein hydrolysates were maintained in a dark air-tight container at -20°C until use (2).

Hydrolysis Degree Estimation

In order to measure the degree of hydrolysis, 50 ml of trichloroacetic acid solution (20% w/v) was added to the protein hydrolysate solution, and the concentration of the protein solution in trichloroacetic acid was determined after centrifuge (20°C at 1000 rpm) using Biuret method. The degree of hydrolysis was estimated based on equation 1 (11).

(1)

$$hydrolysis\ degree\ (\%) = \frac{Soluble\ nitrogen\ in\ 10\%\ TCA}{Total\ nitrogen}$$

Proximate Analysis

Moisture, fat, ash and protein values were assessed using the AOAC method (12). In addition, the moisture content was expressed on a dry weight basis.

Colorimetry

To measure the color of the samples, some of the protein hydrolysates were deposited on the plate and the color was measured using a colorimetric device (Model: CAM 500, Lovibond Co., UK). In this regard, the variable of L* is for expressing the lightness index from zero (black dimension) to 100 (white dimension), whereas the a* and b* indexes are for expressing the red-green dimension (+a* showing more red dimensions and –a* showing more green dimensions) and the yellow-blue dimension (+b* for more yellow dimensions and –b* for more blue dimensions) (8).

Evaluation of Functional Properties

Solubility

At this stage, five gr of protein hydrolysate was dissolved in 50 ml of sodium chloride solution (0.1 M). The obtained solution was stirred at room temperature for one hour and was then centrifuged at 2560 g for 30 min.

The resulting supernatant was passed through a Whatman filter paper 1001, and nitrogen content was calculated according to the Kjeldahl method (Model: KBL 20S, Gerhardt Co., Germany). Moreover, the solubility of nitrogen was estimated based on equation 2 (10).

(2)

$$Solubility\ (\%) = \frac{Protein\ content\ in\ supernatant}{Total\ protein\ content\ in\ sample}$$

Foam Production Property

In order to estimate the foam production property, 20 ml of the hydrolysis solution was homogenized in a 50 ml tube at 16000 rpm for one min (Model: ULTRA-TURAX T25 digital, IKA, Germany), and its total volume was measured at times of zero and 60 min after stirring. The foam capacity was expressed based on the height of the foam created at time zero, whereas its stability was determined based on foam height after 60 min according to equation 3 (4).

(3)

$$Foam\ created\ (\%) = \frac{Primary\ volume - secondary\ volume\ after\ homogenization\ at\ different\ times}{Primary\ volume}$$

Evaluation of Antioxidant Properties

Estimation of DPPH Radical Scavenging Activity

According to the method by Egerton et al. (1), the protein hydrolysate was dissolved in distilled water until a concentration of 40 mg/mL of protein was obtained. Afterwards, one ml of DPPH (0.2 mM) was added to four ml of the sample solution, followed by analysis with Vertex and 30 min of incubation in dark. Ultimately, sample absorption amount was read at 517 nm with the help of a spectrophotometer (Model: S12, Biochrom Co., UK). Following that, the level of radical scavenging activity was estimated using equation 4.

(4)

$$(\%)\ DPPH\ radical\ scavenging\ activity = (1 - \frac{Sample\ absorption\ amount}{Control\ absoprtion\ amount} \times 100$$

The control sample was prepared using the same technique. However, the sample was replaced by distilled water, and the positive control sample was obtained applying ascorbic acid. In this regard, the lower absorption rate was indicative of radical scavenging activity and higher DPPH.

Measuring the Regeneration Power

About 0.5 ml of protein hydrolysate solution was mixed with 2.5 ml phosphate buffer (M 0.2) and 0.5 ml of 1% potassium ferricyanide and the resulting mixture was incubated at 50°C for 20 min. After adding 2.5 ml of 10% trichloroacetic acid and centrifuging at 3000 rpm for 10 min, the supernatant was removed, which was then mixed with distilled water and 1% ferric chloride. After that, the absorption amount was read at 700 nm (10).

Measuring the Amount of Mineral Compositions

The amount of mineral compositions of the samples (calcium, cadmium, copper, iron, potassium, magnesium, manganese, sodium, nickel, phosphorus and sulfur) was measured by a diffusion spectrophotometer coupled with plasma optical emission (Model: 4300 DV, Perkin-Elmer Co., USA) based on the AOAC method (12). To this end, four g of the sample was mixed with four ml of 70% nitric acid, and after incubation, the sample size increased to 10 ml with distilled water for sample digestion. Afterwards, the prepared samples were analyzed (10). The condition of the device was adjusted at 15 L/min based on the argon flow rate to plasma, and the concentration of mineral compositions was expressed in mg/kg.

Statistical Analysis

In total, nine samples were taken from the produced treatments randomly, and the Kolmogorov-Smirnov test was applied to evaluate the normal distribution of the data. The results obtained from chemical tests were analyzed in SPSS using one-way ANOVA. In addition, Tukey's multiple range test was used to compare the differences in pairs of mean at the significance level of 0.05. The relevant samples were drawn in Excel.

Results

According to Table 1, there was a significant difference between treatments of protein hydrolysate in terms of amounts of hydrolysis degree, soluble protein, and solubility ($P<0.05$). The treatment with 1:5 ratio had the highest degree of hydrolysis, whereas the treatment with 6:1 ratio had the lowest rate in this regard. Meanwhile, there was no significant between 4:1 ratio treatment and other treatments. The protein hydrolysate obtained from the 4:1 distilled water to substrate proportion had the highest level of soluble protein, compared to other treatments. In addition, the solubility of the protein hydrolysate obtained from the 4:1 and 5:1 proportions were higher, compared to the 6:1 ratio. However, the difference was not significant. Similarly, no significant difference was observed in the level of moisture, fat, and ash of the protein hydrolysates of bigeye ilisha with various water to substrate proportions ($P>0.05$).

Table 1. Effect of various waster to substrate proportions on approximate analytic values, degree of hydrolysis, and solubility of protein hydrolysate of bigeye ilisha (Ilisha megaloptera)

Variable	4:1 ratio	5:1 ratio	6:1 ratio	Sig
Degree of hydrolysis (%)	39.1±59.79ab	44.0±06.82a	37.0±27.53b	0.017*
Soluble protein (mg/mL)	55.0±32.17a	45.0±61.89b	45.0±29.14b	0.00*
Moisture (%)	5.0±66.47a	4.0±80.13a	5.0±16.73a	0.519ns
Fat (%)	1.0±54.07a	1.0±68.04a	1.0±65.08a	0.374ns
Ash (%)	2.0±65.45a	2.0±48.11a	2.0±57.25a	0.932ns
Solubility (%)	77.0±75.24a	81.1±72.55a	69.1±88.12b	0.001*

Data are presented as the mean of three replications with ± standard deviation. Different letters in each row show the significant difference between the values of approximate analytical indexes, the degree of hydrolysis, and solubility of the protein in treatments.

The effect of various distilled water to substrate proportions on the indexes of the color of the protein hydrolysates produced is presented in Figure 1. It seems that the mentioned variable had a significant impact only on the lightness of the protein hydrolysates. In this regard, the highest and lowest levels of lightness were related to treatments with 6:1 and 5:1 proportions, respectively. Meanwhile, there was no significant difference between treatments with 4:1 ratio and other treatments. In addition, no significant difference was observed in values of redness and yellowness of protein hydrolysates (P>0.05).

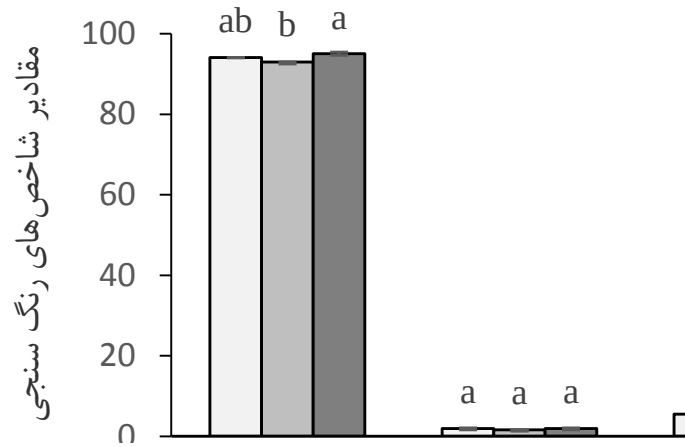

Figure 1. Effect of various distilled water to substrate proportions on values of colorimetry indexes in protein hydrolysates of bigeye ilisha (Ilisha megaloptera)

Data are presented as the mean of three replications with ± standard deviation. Different letters in the figure show the significant difference between the values of colorimetry indexes in treatments.

The effect of various distilled water to substrate proportions on values of foam capacity and stability obtained from the protein hydrolysates is shown in Figure 2.

The foam capacity produced in treatment with 5:1 ratio was significantly lower, compared to treatments with 4:1 and 6:1 proportions ($P<0.05$). In terms of foam stability after 60 min, no significant difference was observed between treatments of protein hydrolysate and various distilled water to substrate proportions ($P>0.05$).

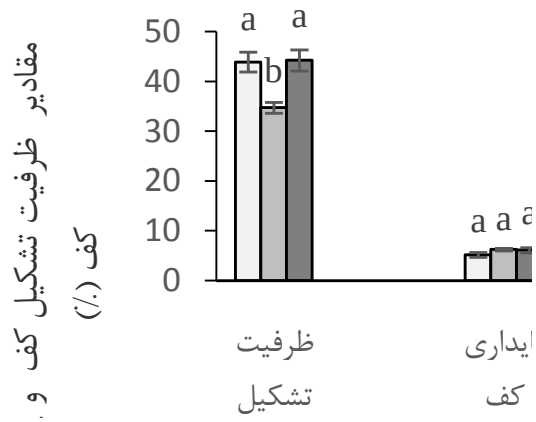

Figure 2. Effect of various distilled water to substrate proportions on values of foam capacity and stability in protein hydrolysates of bigeye ilisha (Ilisha megaloptera)

Data are presented as mean of three replications with ± standard deviation. Different letters in the figure show the significant difference between the values of foam capacity and stability in treatments.

Values of antioxidant properties of protein hydrolysates of bigeye ilisha are shown in Table 2. In this regard, treatment with 5:1 ratio followed by treatment with 4:1 ratio had the highest values of DPPH radical scavenging activity in the protein hydrolysates. On the other hand, the lowest level of DPPH radical scavenging activity was related to the treatment with 6:1 ratio ($P<0.05$). In this regard, no significant difference was observed in the reducing power of protein hydrolysates and various distilled water to substrate proportions ($P>0.05$).

Data are presented as the mean of three replications with ± standard deviation. Different letters in each row show the significant difference between the values of antioxidant properties in treatments.

According to Table 3, various treatments of protein hydrolysates of bigeye ilisha yielded different values of mineral compositions. According to the results, it seems that the increase of distilled water to substrate proportions was associated with higher values of mineral compositions of magnesium, calcium, nickel, and sulfur. In this respect, protein hydrolysates obtained from 4:1 ratio had the lowest amount of compositions, whereas protein hydrolysates obtained from 6:1 ratio had the highest level of compositions. In addition, various changes were observed in the values of iron, copper, sodium, potassium, manganese, and cadmium in protein hydrolysates obtained from various distilled water to substrate proportions.

Table 2. Effect of various distilled water to substrate proportions on antioxidant properties of protein hydrolysate of bigeye ilisha (*Ilisha megaloptera)*

Variable	4:1 ratio	5:1 ratio	6:1 ratio	Sig
DPPH radical scavenging activity	86.1±65.23[b]	97.0±0.943[a]	80.0±64.12[c]	0.00[*]
Reducing power	0.0±811.001[a]	0.0±820.008[a]	0.0±803.010[a]	0.391[ns]

Table 3. Effect of various distilled water to substrate proportions on values of mineral compositions of protein hydrolysate in bigeye ilisha (Ilisha megaloptera)

Compositions	4:1 ratio	5:1 ratio	6:1 ratio
Ca	930	966	1062
Cd	0.19	0.18	0.2
Cu	9	6	6
Fe	166	186	175
K	9164	9112	8912
Mg	378	406	512
Mn	<5	<5	<5
Na	19821	20525	18799
Ni	6111	6150	6453
P	7111	7150	7453
S	4972	5114	6903

Data are presented as one repeat due to the high accuracy of the instrument.

Discussion

One of the most important factors that strongly depends on the efficiency of the hydrolytic process of protein is the degree of hydrolysis, which shows the rate of breaking of protein bonds. Protein hydrolysates from the 5:1 distilled water to the substrate proportion had the highest level of hydrolysis while the protein hydrolysates produced by the 6:1 ratio of distilled water to the substrate had the lowest degree of hydrolysis. On the other hand, the samples obtained from 4:1 ratio showed the average degree of hydrolysis (Table 1). It seems that the degree of hydrolysis increased by increasing the distilled water to substrate proportion to a specific value, which was 5:1 in the present study. But after that, a further increase in the level of this ratio led to a reduction in the degree of hydrolysis due to a decrease in the concentration of peptide bonds available for the enzyme. Therefore, the ratio of distilled water to the substrate should be at a certain level so that the activity of the enzyme progresses to the maximum and the final product is optimized (2). In a study by Klomklao and Benjakul (8) on Tuna Fish waste, the mentioned ratio was reported at 1:2, whereas it was 1:3 in the research by Klomklao et al. (13) on Leiognathus lineolatus.

The mean level of protein in the fillets of bigeye ilisha was 21.07±0.6%, which was used to estimate the level of enzymes required for performing the hydrolysis process. The protein hydrolysates obtained had a protein amount within the range of 45.29-55.32 mg/mL (Table 1), which suggests a high increase in the amount of protein in the

hydrolyzed product and can be considered as a source of essential proteins. High levels of protein might be due to protein solubility during the hydrolysis process, the removal of non-protein insoluble substances that were not digested during the hydrolysis process, and the partial removal of fat after the hydrolysis process (13). This increase in protein content is actually a kind of protein enrichment. Other research results have also reported high protein content in the protein hydrolysate products (2, 14).

In the current research, protein hydrolysates with 4:1 ratio of distilled water to substrate showed a higher protein content, compared to protein hydrolysates obtained from 5:1 and 6:1 proportions. It seems that the amount of soluble protein decreased with increasing proportion of distilled water to the substrate, which is in congruence with the results obtained by Egerton et al. (1) and Klomklao and Benjakul (5). In various studies on the tissue hydrolysis of fish using protease enzymes, it has been stipulated that the functional properties and nutritional value of protein hydrolysate depend on various factors, including the type of fish, the type and amount of enzyme used, pH, temperature, degree of hydrolysis, and hydrolysis time (3).

There was no significant difference between protein hydrolysates in terms of moisture, fat and ash levels (Table 1). In this regard, the level of protein in the protein hydrolysates was within the range of 1.54-1.68, which was significantly low in bigeye ilisha considering the fact that it is among the high-fat fish. The results of other researchers also indicated low contents of fat in a hydrolyzed product, some of which reported a fat percentage below 1% (13, 15).

Protein hydrolysate is typically considered a low-fat product, which can prevent or reduce the oxidation of fat and protein in fish over time due to the antioxidant properties. In this respect, our findings are in line with the results obtained by Ovissipour et al. (16) and Pacheco-Aguilar et al. (6). For instance, Ovissipour et al. (16) evaluated the protein hydrolysate from belly contents of Thunnus albacares using microbial protease enzymes, including alcalase, proteomics, and flavourzyme. These researchers concluded that the protein hydrolysates obtained from alcalase enzyme had a higher level of hydrolysis, compared to protein hydrolysates obtained from the other two enzymes. However, no significant difference was observed between the produced samples in terms of fat, moisture, and ash. Pacheco-Aguilar et al. (6) assessed the functional features of protein hydrolysate from Merluccius productus, reporting no significant difference between the samples regarding moisture, protein and ash levels.

The protein color depends on the type of raw material and its extraction steps. Generally, color has no effect on the functional properties of the protein. The level of lightness index in the treatments produced in 6:1 ratio was higher, compared to the of 5:1 ratio (Figure 1). This variable had no significant difference in treatments obtained by 4:1 ratio, compared to other treatments. In terms of comparison of the level of hydrolysis to the level of lightness index, the protein hydrolysates obtained from the 5:1 ratio had the highest level of hydrolysis and the lowest level of lightness. In addition, the levels of redness and yellowness in the hydrolysate proteins using various distilled water to substrate proportions had no significant

difference. Similar results were obtained by other studies in terms of the level of hydrolysis degree and its effect on colorimetry index through testing different types of fish and hydrolysis degrees (13, 17). In the study by You et al. (18) increase of hydrolysis degree from 18% to 33% led to a significant reduction in the lightness index from 29.1 to 23.2 and an increase in the redness index.

Dong et al. (19) evaluated the antioxidant and biochemical properties of protein hydrolysate in Hypophthalmichthys molitrix, expressing that the level of lightness in the protein hydrolysate of Hypophthalmichthys molitrix significantly decreased after four hours of hydrolysis using alcalase and flavourzyme enzymes. The results of the mentioned research approved that the degree of hydrolysis significantly affected the color intensity of the protein hydrolysate. Therefore, the difference in the color of the protein hydrolysates depends on the combination of the primary raw material and the conditions of the hydrolysis process, and is probably due to changes in the degree of hydrolysis, as well as in the size of the peptides and amino acid sequences of the protein hydrolysates (8, 18).

Solubility is one of the most important functional properties of a protein hydrolysate. High solubility levels are required in many applications of proteins, especially emulsions, foams, and gels. The solubility of proteins provides a homogeneous dispersion of molecules within the colloidal system and improves interfacial properties (10, 14). The highest solubility of protein hydrolysate was related to the protein hydrolysates obtained from 4:1 and 5:1 proportions (Table 1). In the treatment with 6:1 ratio, there was a significant reduction in solubility, compared to other treatments. The higher solubility level of protein hydrolysate in treatments with 5:1 and 4:1 proportions, compared to 6:1 ratio, might be due to a higher degree of hydrolysis.

In a research by Dong et al. (19) and Gbogouri et al., there was a linear relationship between the degree of hydrolysis and solubility. The solubility increased by increasing the degree of hydrolysis since it led to a decreased length of the peptide chains, and smaller peptides were able to form hydrogen bonds with water by creating greater polarity, which increased solubility. The high solubility of protein hydrolysates suggests the potential for this compound to be used in the formulation of food systems by providing an attractive appearance and gentle mouthfeel for the production of new products (20). In this respect, our findings are consistent with the results obtained by Liu et al. (21), who reported increased solubility along with an elevated degree of hydrolysis.

The amount of expansion created in foam after applying perturbation at time zero was indicative of the foam production ability of protein hydrolysate. In the present study, the highest rate of foam capacity in protein hydrolysate was related to 4:1 and 6:1 proportions, whereas the lowest rate was observed in the protein hydrolysate obtained from 5:1 ratio (Figure 2). Meanwhile, no significant difference was observed in the level of foam stability among the treatments. High degree of hydrolysis increases the solubility of the protein. Nonetheless, it has negative effects on protein's functional features, such as foam production ability and emulsifying properties. In this regard, it could be stated that increased level of hydrolysis results in a reduced molecular size of peptides obtained, and a higher amount of the peptides are dissolved in water by creating hydrogen

bonds with water molecules. Therefore, the peptides obtained do not get the chance to employ their functional properties (19).

In the current study, the treatment with 5:1 ratio had the highest hydrolysis degree and the lowest foam capacity. The foam stability level of the protein hydrolysate refers to foam stability 60 min after perturbation. In the current research, no significant difference was observed among the treatments regarding foam stability, and all three treatments were homogeneous in this regard. Similar results were obtained by Liu et al. (21), who reported the level of foams produced from protein hydrolysate from the waste of fish production factory. Sanchez and Patino (22) pointed out that increased concentration of protein led to higher air diffusion and foam production levels. In another research by Thiansilakul et al. (10), after 10 min, the protein hydrolysate with 3% concentration had the highest foam stability level. After that, minor differences were observed among the protein hydrolysates with various concentrations regarding foam stability level.

DPPH determines the ability of a substrate to transport electrons or hydrogen atoms that can react with free radicals to produce more stable compounds. Reducing powers are measured to assess the electron donation capability of hydrolyzed compounds to free radicals. With this electron donation, an oxidized antioxidant molecule can be able to reconstruct itself (23). The antioxidant property of the protein hydrolysate is due to high amounts of tyrosine, trypsin, methionine, lysine, cysteine, and histidine amino acids, which, as a proton or hydrogen donor, has a positive charge for reacting with single electrons in free radicals. The process of hydrolysis exposes more amino acids by opening the structure of proteins and leads to the improved antioxidant activity of the protein hydrolysate, compared to the original protein.

In the current research, there was a significant difference between protein hydrolysates obtained from various distilled water to substrate proportions regarding the level of free radical inhibition. The protein hydrolysates obtained from 5:1 ratio followed by the protein hydrolysates resulting from the 4:1 ratio showed the highest levels of radical scavenging ability, respectively (Table 2). On the other hand, treatment obtained from 6:1 ratio had the lowest radical scavenging ability, compared to other treatments. According to the results, no significant difference was observed among the treatments obtained from various distilled water to substrate proportions in terms of reducing power (Table 2). While all three products produced protein hydrolysate with an appropriate reducing power, no advantage was observed for any of the treatments after their comparison. In general, the accurate identification of the antioxidant function of the protein hydrolysate is in accordance with hydrolysis progress, and various reports exist in this regard.

Thiansilakul et al. (10) reported that the activity of DPPH radical scavenging activity of protein hydrolysate produced from round scad increased by per each hydrolysis degree using the alcalase enzyme. Klompong et al. (4) evaluated the protein hydrolysate of Selaroides leptolepis fish using alcalase and flavourzyme enzymes and various hydrolysis degrees, concluding that increased degree of hydrolysis reduced the free radical inhibition activity and reducing power. However, better

free radical scavenging ability was detected at low hydrolysis degrees of hydrolyzed products. Khantaphant et al (24) showed that the increase in the reducing power of Fe+2 occurred by protein hydrolysate of brownstripe red snapper when there was an increase in the hydrolysis degree of the treatments, and this feature prevented the oxidation progress. Morales-Medina et al (25) reported that a higher degree of hydrolysis in protein hydrolysates was obtained from Trachurus mediterraneus and Sardina pilchardus reduced the DPPH radical scavenging activity and decreased the reducing power. According to these results, it seems that the protein hydrolysate obtained from bigeye ilisha had the power to donate an electron to free radicals and reduce the oxidation activity, the level of activity of which depends on the degree of hydrolysis, process condition and type of enzyme used.

The protein hydrolysate obtained from bigeye ilisha with the use of various distilled water to substrate proportions had various mineral compositions, as shown in Table 3. Sodium, potassium, calcium, and magnesium were observed at higher levels while copper and manganese were very low. In addition, the amount of iron was moderate. These observed metals can act as pro-oxidants in the protein hydrolysate and provide the conditions for oxidation of this compound. The transfer of metal ions, especially copper and iron, is one of the main factors of the oxidation process catalyzers (10).

By increasing the distilled water to substrate proportion, the amounts of magnesium, calcium, nickel, and sulfur minerals increased in the protein hydrolysates, in a way that protein hydrolysate obtained from 4:1 ratio had the lowest amount of these elements, whereas the protein hydrolysates obtained from 6:1 ratio had the highest level of these compositions. However, changes in other elements had no specific trend. Sathivel et al. (26) evaluated the biochemical and functional properties of wastes of Clupea harengus, reporting that potassium, magnesium, potassium, sodium, sulfur and calcium were abundantly found in protein hydrolysate from Clupeidae and wastes of fish, amounts of which could be different and changed depending on the substrate used. Moreover, the protein hydrolysate had proper amounts of sodium chloride to maintain or adjust the pH during adjusting the pH value for the hydrolysis process, which limits the application of protein hydrolysate (27). Evaluating the combinations, functional features, and antioxidant activity of protein hydrolysate from Decapterus maruadsi, Thiansilakul et al. (10) expressed that the amount of sodium, potassium, calcium, and magnesium in the protein hydrolysate was found in high amounts while nickel was not observed.

The production of protein hydrolysates provides the basis for the use of low-protein sources and their conversion into a protein-rich protein product. The conditions of the hydrolysis process, including the proportion of distilled water to the substrate, had a significant effect on the functional and antioxidant properties of the protein hydrolysate produced. Protein hydrolysates from bigeye ilisha with 4:1 ratio of distilled water to substrate showed the highest amount of soluble protein and solubility. In terms of hydrolysis degree and their functional features, there was no significant difference with protein hydrolysates obtained from 5:1 ratio. As a result, the production of protein hydrolysate from bigeye ilisha with 4:1 ratio of distilled water to the substrate is recommended to produce protein hydrolysates

with high functional properties and 5:1 ratio in order to produce protein hydrolysates with high antioxidant properties.

Acknowledgements

This article was extracted from a doctoral dissertation with the code of 9221064102, financially supported by Gorgan University of Medical Sciences and Golestan University of Medical Sciences.

Declarations

Funding source(s)

Ethics approvals and consent to participate

Conflict of interest

Authors' contributions

References

1. Egerton S, Culloty S, Whooley J, Stanton C, Ross RP. Characterization of protein hydrolysates from blue whiting (Micromesistius poutassou) and their application in beverage fortification. Food chemistry 2018; 245: 698-706.

2. Ovissipour M, Rasco B, Ghorban Shiroodi S, Modanlow M, Gholami S, Nemati M. Anioxidant activity of protein hydrolysates from whole anchovy sprat (Clupeonella engrauliformis) prepared using endogenous enzymes and commercial proteases. Journal of Science and Food Agricultural 2012; 93: 1718- 1726.

3. Chilamaiah M, Kumar BD, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: A review. Journal of Food Chemistry 2012; 135: 3020-3038.

4. Klompong V, Benjakul S, Kantachote D, Shahidi F. Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (Selaroides leptolepis) as influenced by the degree of hydrolysis and enzyme type. Journal of Food Chemistry 2007; 102: 1317-1327.

5. Klomklao S, Benjakul S. Protein hydrolysates prepared from the viscera of skipjack tuna (Katsuwonus pelmamis): antioxidative activity and functional properties. Turkish Journal of Fisheries and Aquatic Sciences 2018; 18: 69-79.

6. Pacheco-Aguilar R, Mazorra-Manzano MA, Ramirez-Suarez JC. Functional properties of fish protein hydrolysates from pacific whiting (Merluccius Productus) muscle produced by a commercial protease. Journal of Food Chemistry 2008; 109: 782- 789.

7. Ovissipour M, Safari R, Motamedzadegan A, Shabanpour B. Chemical and biochemical hydrolysis of Persian sturgeon (Acipenser

persicus) visceral protein. Food and Bioprocess Technology 2009, 5: 460-465.

8. Klomklao S, Benjakul S. Utilization of tuna processing byproducts: Protein hydrolysate from skipjack tuna (Katsuwonus pelamis) viscera. Journal of Food Processing and Preservation 2017; 41: 1-8.

9. Hosseininejad SH, Mohammadi GHH, Eskandar, GHR, Khodadadi M. Estimated the implicit catching rate of traditional moorage trawls in Khuzestan province. Sea Biology Journal of Islamic Azad University of Ahvaz 2010; 2: 1-10. (Persian)

10. Thiansilakul Y, Benjakul S, Shahidi F. Composition, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (Decapterus maruadsi). Journal of Food Chemistry 2007; 103: 1385-1394.

11. Hoyle N, Merritt JH. Quality of fish protein hydrolysates from herring (Clupea harengus). Journal of Food Science 1994; 59: 76-79.

12. AOAC. Official methods of analysis (17th ed.). Gaithersberg, Maryland: Association of Official Chemists, 2000.

13. Klomklao S, Kishimura H, Benjakul S. Use of viscera extract from hybrid catfish (Clarias macrocephalus× Clarias gariepinus) for the production of protein hydrolysate from toothed ponyfish (Gazza minuta) muscle. Food chemistry 2013; 136: 1006-1012.

14. Kristinsson HG, Rasco BA. Fish protein hydrolysates: Production, biochemical and functional properties. Critical Reviews in Food Science and Nutrition 2000; 40: 43–81.

15. Slizyte R, Dauksas E, Falch E, Storro I, Rustad T. Characteristics of protein fractions generated from cod (Gadus morhua) by-products. Process Biochemistry 2005; 40: 2021–2033.

16. Ovissipour MR, Abedian Kenari A, Motamedzadegan A, Nazari RM. Investigation of protein hydrolysate properties in viscera tuna (Thunnus albacares) using commercial enzymes. Journal of Iranian Food Science and Technology Researches 2010; 6: 68-76. (Persian)

17. Gbogouri GA, Linder M, Fanni J, Parmentier M. Influence of hydrolysis degree on the functional properties of salmon byproduct hydrolysates. Journal of Food Science 2004; 69: 615–622.

18. You L, Zhao M, Cui C, Zhao H, Yang B. Effect of degree of hydrolysis on the antioxidant activity of loach (Misgurnus anguillicaudatus) protein hydrolysates. Journal of Innovative Food Science and Emerging Technologies 2009; 10: 235-240.

19. Dong S, Zeng M, Wang D, Liu Z, Zhao Y, Yang H. Antioxidant and biochemical properties of protein hydrolysates prepared from silver carp (Hypophthalmichthys molitrix). Journal of food chemistry 2008; 107: 1485-1493.

20. Peterson BR. The impact of the enzymatic hydrolysis process on recovery and use of proteins. In G. G. Birch, N. Blakebrough, and K. J. Parker (Eds.), Enzymes and food processing (pp. 269–299). London, UK: Elsevier Applied Science Publishers 1981.

21. Liu Y, Li X, Chen Z, Yu J, Wang F, Wang J. Characterization of structural and functional properties of fish protein hydrolysates from surimi processing by-products. Food chemistry 2014; 151: 459-465.

22. Sánchez CC, Patino JMR. Interfacial, foaming and emulsifying characteristics of sodium caseinate as influenced by protein concentration in solution. Journal of Food Hydrocolloids 2005; 19: 407–416.

23. Shahidi F, Zhong Y. Lipid oxidation and improving the oxidative stability. Chemical Society Reviews 2010; 39: 4067-4079.

24. Khantaphant S, Benjakul S, Ghomi MR. The effects of pretreatments on antioxidative activities

of protein hydrolysate from the muscle of brownstripe red snapper (Lutjanus vitta). Journal of LWT- Food Science and Technology 2011; 44: 1139-1148.

25. Morales-Medina R, Tamm F, Guadix AM, Guadix EM, Drusch S. Functional and antioxidant properties of hydrolysates of sardine (S. pilchardus) and horse mackerel (T. mediterraneus) for the microencapsulation of fish oil by spray-drying. Food chemistry 2016; 194: 1208-1216.

26. Sathivel S, Bechtel PJ, Babbitt J, Smiley S, Crapo C, Reppond KD, Prinyawiwatkul W. Biochemical and functional properties of herring (Clupea harengus) by-product hydrolysates. Journal of Food Science 2003; 68: 2196-2200.

27. Picot L, Bordenave S, Didelot S, Fruitier-Arnaudin I, Sannier F, Thorkelsson G. Antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines. Process Biochemistry 2006; 41: 1217–1222.

How to cite:
Jamshidi A, Shabanpour B, Pourashouri P, Raeisi M. Evaluation of Different Proportions of Distilled Water to Substrate on Functional Properties, Antioxidant and Nutritional Quality of Bigeye Ilisha (Ilisha Megaloptera) protein hydrolysate. Jorjani Biomedicine Journal. 2018; 6(3): 24-39.