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Scientific Research

Investigating the antioxidant properties of hydrolyzed protein with different molecular weights obtained from Caspian Sea mullet wastes

Seyed Mahmoud Rabiei[1], Sakineh Yeganeh[2*], Mina Esmaeili Kharyeki[3]

1- M.Sc. graduated, Department of Fisheries, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

2- Professor, Department of Fisheries, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

3- Assistant Professor, Department of Fisheries, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

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*Corresponding Author E-

skyeganeh@gmail.com;
s.yeganeh@sanru.ac.ir

ABSTRACT

Aquatic wastes management through the production of value-added products is very important. Among these products with many properties (including foaming, emulsifying, antioxidant, antibacterial, etc.), hydrolyzed proteins can be mentioned. Various factors, including molecular weight and concentration of hydrolyzed proteins, can affect the mentioned properties. Therefore, in the present study, the antioxidant properties of hydrolyzed protein obtained from Caspian Sea mullet wastes were investigated in different ranges of molecular weight and different concentrations. For this purpose, fish waste was first hydrolyzed with alcalase (1% concentration) at 55°C and pH = 8 for 120 minutes (Optimum conditions based on pre-test). Then, the hydrolyzed protein solution was divided into molecular weights of less than 3, between 3 and 10 kDa, and more than 10 kDa with 3 and 10 kDa ultrafilters. In order to evaluate the effect of molecular weight and concentration on the antioxidant activity of hydrolyzed protein, the indices of DPPH and ABTS free radicals scavenging power and ferric ion reducing power were used. Evaluation of DPPH and ABTS radicals scavenging activities showed that there is a significant difference between different molecular weights, and the highest and lowest values were recorded in molecular weights of 3-10 and less than 3 kDa, respectively (p<0.05). The lowest IC50 values for DPPH and ABTS radicals scavenging power were also obtained for sample with molecular weight between 3 and 10 kDa (p<0.05). The free radical scavenging power and the ferric ion reduction power increased with increasing concentration in all molecular weights (p<0.05). Among the different fractions, the sample with a molecular weight of more than 10 kDa showed the highest ferric ion reducing power (p<0.05). According to the results, it can be stated that hydrolyzed protein produced in this research can be used as an antioxidant compound (especially in the molecular weight of 3-10 kDa).

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1-Introduction

Proteins are one of the basic and essential components of the tissues of living organisms, which play a major role in biological activities and physiological processes related to cells. they do [1]. in Proteins are an important macronutrient as a source of energy and amino acids. Many physiological and functional properties of proteins are related to bioactive peptides in their structure. Research has shown that most bioactive peptides are inactive in their parent protein sequenceND [2] But PQ from being released during the digestive digestion process or by using different processing methods, its physiological activity They show [3]. Hydrolyzed proteins resulting from hydrolyzed (chemical, biochemical and autolysis) are protein substrates. One of the most efficient techniques for the production of these compounds is the enzymatic hydrolysis method, which is one of the most interesting techniques for the modification of proteins [4]. to In general, enzymes of microbial origin have more advantages than other enzymes, including more stability in heat and pH mentioned above and appropriate proteolytic properties [5]. The proteins resulting from this method are a mixture of different peptide parts (di, tri and oligopeptide) with a diverse range of molecular weights, which increase the solubility of the protein by producing hydrophilic groups and thus improve the functional properties and bioactivity of the protein. Water soluble proteins have functional properties (solubility, emulsifier activity index and emulsion stability, foaming capacity and foam stability, oil absorption capacity and water retention capacity) and antioxidant activity [6 and 7]. Various factors, including the type of protein source and enzyme [6 and 8], the ratio of enzyme to substrate, temperature, time [9], the degree of dehydration [8, 10 and 11], Molecular weight of the resulting peptides [12 and 13] and pH They can put the functional properties and antioxidant activity of these proteins under the tire.

Due to the presence of double bonds in the tissue and especially in fish oil, this food item is highly prone to oxidative spoilage. Free radicals and active oxygen can be mentioned among the compounds resulting from oxidative damage. These radicals lead to undesirable chemical and biological reactions. Avoid today Oxidation of lipids has caused the most concern in the food industry and this has become a very important issue. [14]. Fat oxidation products are potentially harmful to human health, and frequent consumption of these foods may lead to cancer, cardiovascular diseases, and Alzheimer's disease. The use of natural antioxidants in order to prevent oxidative spoilage and reduce diseases caused by this process in food products has become one of the important goals of the food industry. [15]. Therefore, effective control and rapid removal of excess free radicals is critical to maintain the health of the organism and improve its performance. Food supplements with antioxidant properties can improve the health of living beings [9].

Since one of the goals of the production of these proteins is the production of materials with high added value, therefore, in order to commercialize and reduce production costs, choosing the type of substrate is very important. Usually, protein wastes are used for such purposes, depending on the desired efficiency. According to the statistical yearbook of Iranian fisheries (1397-1401) in the three northern provinces of Iran in 1401, the total catch was 32,515 tons, and 13,396 tons of it were bony fish, of which mullets are a part of this catch. [17]. There are two important species of mullet, narrow-nosed mullet.*Liza jumping*(and golden mullet)*Liza couple*) are caught between October and April [18]. Therefore, a large amount of waste is produced from the consumption of these fish, and their optimal use is very

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important. According to the explanations provided, a research was conducted with the aim of investigating the antioxidant properties of hydrolyzed protein prepared from Caspian sea mullet wastes, in which, based on the degree of hydrolyzing and antioxidant properties, the best time for hydrolyzing these fish wastes with alcalase enzyme was 120 minutes (19). And the present research was carried out as a continuation of the aforementioned research with the aim of investigating the antioxidant properties of water-soluble protein prepared from Caspian sea mullet wastes in different molecular weights and concentrations.

2- Materials and methods

2-1- Preparation of mullet fish waste

20 pieces of mullet (*Liza couple* and *Liza jumping*) with a weighted average of 50 ± 300 grams was procured from the fishmongers' market in Babol city and was quickly transported to the research laboratory in Unilith boxes with ice (1:3 weight/weight). After washing with cold water (to remove impurities and mucus from the surface of the fish), the heads, steaming and emptying of the contents of the stomach of the fish were done. In this research, the head, fins and stomach contents of mullet fish were used as fish waste in the further stages of the research. The fish wastes were completely ground with a meat grinder and after packaging, they were stored in the freezer (-20 degrees Celsius) until the day of the experiment.

2-2- Dewatering of mullet fish waste

In order to prepare the samples, first, for each treatment, 100 grams of mullet wastes were defrosted at a temperature of 4 degrees Celsius and with distilled water at a ratio of 1: 2 (v/w) were mixed and homogenized with a stirrer for 2 minutes. In order to deactivate the internal enzymes, the samples were placed in a hot water bath at a temperature of 90°C for 10 minutes. The amount of alkalase enzyme (2.4 L, Novozymes, Denmark) 1.5 percent (volume/weight), temperature 55 degrees Celsius, pH The equivalent of 8 and the time of 120 minutes (the conditions of the dehydration reaction based on the optimal limit specified in the authors' previous study (19)) were considered. After the end of the dehydration reaction, the samples were soaked in a water bath for 10 minutes in order to deactivate the alkalase enzyme.Memmert WNB 29, Germany) were placed at a temperature of 90 °C. In order to separate the insoluble substances from the soluble proteins, the samples were subjected to centrifugation (20 minutes at g8000) in a refrigerated centrifugeSigma, 2-16KL) were placed and finally the supernatants were separated by a sampler and stored in a freezer at -20°C for relevant analyzes [19]. The amount of soluble protein and the degree of water absorption of the best water quality protein is 0.58 ± 18.73 mg/ml and 0.72 ± 37.53% were calculated. Abkaft product, using 3 and 10 kilodalton ultrafilters (Amicon Centrifugal Filter, Merck) were divided into parts smaller than 3 kDa, between 3 and 10 kDa and larger than 10 kDa. For this purpose, the watered protein solution was first filtered using 10 kilodalton ultrafilters and using a refrigerated centrifuge (5000 g, temperature 4 degrees Celsius and for 40 minutes). The solution remaining on top of the filter was considered as a sample of more than 10 kDa. The solution passed through the filter was separated again with a 3 kilodalton ultrafilter and the method mentioned above. The solution remaining on top of the filter was considered as a fraction of 3 to 10 kDa, and the solution passed through the filter was separated as a sample of less than 3 kDa. And then antioxidant activity was measured in different ways for each of the fractions.

2-3- Measurement of free radical inhibitory power DPPH

First, based on the concentration of soluble protein, different concentrations (0.5, 1, 1.5, 2 and 2.5 milligrams per milliliter) were prepared from the hydrolyzed protein sample, and to perform this test, the same

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amount of 0.1 millimolar solution was added to a certain volume of the sample solution. DPPH was added in methanol. The resulting mixture was shaken well and placed in the dark for 15 to 30 minutes. Then the absorption of the mixture by a spectrophotometer (Spectrophotometer UV-M51 UV/Vis, Italy) was read at a wavelength of 517 nm. The percentage of radical inhibition was calculated according to the following equation [9] in this relation Ab Control absorbance at 517 nm wavelength and As The absorption of the sample is at the wavelength of 517 nm.

$$DPPH\ (\%) = \frac{Ab - As}{Ab} \times 100$$

2-4- Measuring the reducing power of trivalent iron ion

First, based on the concentration of soluble protein, different concentrations (1, 2 and 3 mg/ml) of hydrolyzed protein samples were prepared and to check this feature, 1 milliliter of hydrolyzed protein with different concentrations was mixed with 2.5 milliliters of 0.2 M phosphate buffer (with =6.6pH) and 2.5 milliliters of 1% potassium ferricinide solution were mixed. Then the obtained mixture was incubated for 20 minutes at 50 degrees Celsius and then 2.5 ml of 10% trichloroacetic acid solution was added to it. In the next step, this solution was centrifuged for 10 minutes at 3000 rpm and the supernatant was mixed with 2.5 ml of distilled water and 2.5 ml of 0.1% ferric chloride solution. The absorbance of the resulting solution was read at a wavelength of 700 nm. The higher the absorption, the higher the reducing power of hydrolyzed protein [22].

5-2- Measuring the power of radical inhibitory activity ABTS

First, based on the concentration of soluble protein, the concentration (0.5, 1, 1.5, 2 and 2.5 mg/ml) milligrams prepared in milliliters of water-soluble protein sample and in order to measure the inhibitory activity of the method German et al. (2011) was used [23]. 7 mM solution ABTS It was prepared in 2.45 mM potassium persulfate and kept for 16 hours at room temperature in a dark place. After the desired time, dilution with distilled water until reaching the absorption rate of 0.02±0.7 was performed at a wavelength of 734 nm. Then 20 microliters of the sample was diluted with 980 microliters of the solution ABTS It was mixed and incubated for 10 minutes in a dark place at a temperature of 30 degrees Celsius. After the desired time, the absorbance of the samples was read at a wavelength of 734 nm. In order to compare, using different concentrations of ascorbic acid and the percentage of radical inhibition ABTS Based on the following relationship, it was calculated that in this relationship Ab Absorption of the witness at a wavelength of 734 nm and As The absorption of the sample is at the wavelength of 734 nm.

$$ABTS\ (\%) = \frac{Ab - As}{Ab} \times 100$$

6-2- Statistical analysis

The present research was conducted in the form of a completely random design. All indices were determined with at least three repetitions and after checking the normality of the data with the Shapiro-Wilk test, comparing the averages to determine the effect of the molecular weight range on the antioxidant activity of hydrolyzed protein with one-way analysis of variance and to check the significant difference between the averages, Duncan's test was used at the 5% level. All analyzes using software SPSS $_{20}$ done

3- Results

3-1- The ability to inhibit free radicals DPPH

The results of measuring the power of free radical inhibition DPPH In molecular weights less than 3, between 3 and 10 and above 10 kilodaltons (Table 1), it showed that with increasing concentration, the

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inhibitory power also increases in all weights (0.05).*p*<). In all concentrations, a significant difference was observed between different molecular weights, and the highest and lowest improvement rates were observed in molecular weights of 10-3 and less than 3 kilodaltons (0.05*p*<). At the concentration of 1 mg/ml, no significant difference was observed between treatments with molecular weight of 10-3 and over 10 kilodaltons (0.05*p*>).

Table1: DPPH radical scavenging activity (%) of protein hydrolysate in different molecular weight

Molecular weight (kDa)	Concentration (mg/ml)				
	0.5	1	1.5	2	2.5
<3	15.05±0.61 Aa	42.70±1.23 Not	55.29±1.27 That	62.31±1.71 And	78.48±1.06 Yes
3-10	27.92±0.88 And	50.20±1.03 Bb	70.37±0.63 Cc	80.65±0.61 Dc	97.06±2.97 Ec
>10	25.04±0.34 Ab	50.73±0.53 Bb	62.94±0.61 Cb	73.98±0.46 Db	90.12±1.39 Eb

Fromfferent uppercase and lowercase letters in each raw and column show significant difference among different concentrations and molecular weight, respectively (*p*< 0.05).

Calculation results IC_{50} showed (Table 2) that there was a significant difference between different molecular weights, so that the lowest and highest amount of protein needed to reach IC_{50} Beretib was registered in the molecular weight of 3 to 10 kilodaltons and less than 3 kilodaltons (05/0*p*<).

Table 2: IC_{50} value for DPPH radical scavenging activity

Molecular weight (kDa)	>10	3-10	<3
IC_{50} (mg/ml)	0.99±0.010[b]	0.90±0.007[a]	1.27±0.020[c]

Fromfferent letters show significant difference (*p*<0.05).

3-2- Reducing power of trivalent iron ion (ferric ion)

The results of measuring the reducing power of trivalent iron ions are shown in Table 3. According to this table, with the increase in concentration, the reduction power of trivalent iron ion in all molecular weights increased significantly, so that the highest amount was observed in the concentration of 3 mg/ml (0.05).*p*<). In all concentrations, a significant difference was observed between different molecular weights, and with the increase in molecular weight, the reducing power increased significantly, and the highest value was obtained in a molecular weight of more than 10 kilodaltons (0.05).*p*<).

Table 3: ferric ion (Fe^{+3}) reduction power of protein hydrolysate in different molecular weight

Molecular weight (kDa)	Concentration (mg/ml)		
	1	2	3
<3	0.160±0.003 Aa	0.262±0.008 Not	0.323±0.011 That

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3-10	0.267±0.004^{Ab}	0.354±0.004 ^{Bb}	0.432±0.003 ^{Cb}
>10	0.283±0.003 ^{And}	0.367±0.007 ^{Bc}	0.493±0.008 ^{Cc}

Fromfferent uppercase and lowercase letters in each raw and column show significant difference among different concentrations and molecular weight, respectively (p<0.05).

3-3- Radical inhibitory activity ABTS

The results of measuring the power of free radical inhibition ABTS In molecular weights less than 3, between 3 and 10 and above 10 kilodaltons (Table 4), it showed that with the increase in concentration, the inhibitory power also increased in all weights and the highest amount was observed in the concentration of 2.5 mg/ml (0.05).p<). In all concentrations, a significant difference was observed between different molecular weights, and the highest and lowest inhibitory power of Botretib was recorded in the molecular weight of 10-3 and less than 3 kilodaltons (0.05p<).

Table 4: ABTS radical scavenging activity (%) of protein hydrolysate in different molecular weight

Molecular weight (kDa)	Concentration (mg/ml)				
	0.5	1	1.5	2	2.5
<3	3.39±0.92^{Aa}	24.93±0.71 ^{Not}	34.66±2.00 That	54.17±1.08 And	70.09±0.56 ^{Yes}
3-10	13.88±0.43 And	38.68±0.70 ^{Bc}	62.48±0.08 ^{Cc}	76.14±2.88 ^{Dc}	82.94±1.41 ^{Ec}
>10	9.20±0.57 ^{Ab}	30.79±0.43 ^{Bb}	54.45±0.07 ^{Cb}	68.11±0.85 ^{Db}	77.60±1.27 ^{Eb}

Fromfferent uppercase and lowercase letters in each raw and column show significant difference among different concentrations and molecular weight, respectively (p<0.05).

The results of the measurement IC_{50} It showed that there is a significant difference between different molecular weights (05/0p<) And the molecular weight of 3 to 10 kilodaltons is the minimum required protein to reach IC_{50} (Table 5)

Table 5: IC_{50} value for ABTS radical scavenging activity

Molecular weight (kDa)	>10	3-10	<3
IC_{50} (mg/ml)	1.35±0.01^{b}	1.10±0.01^{a}	1.78±0.02^{c}

Fromfferent letters in each raw show significant difference (p<0.05).

4-Discussion

Free radical removal is done by antioxidants. Therefore, measuring its amount can indicate the antioxidant status of a compound. DPPH A free radical is stable and will be inhibited when it receives electrons from an antioxidant [24]. Therefore, it is expected that in the case of mullet waste protein acting as an antioxidant, with increasing concentration of hydrolytic protein in the present research, the inhibitory power will increase. DPPH Increase and amount of free radicals DPPH be reduced Changes in the size,

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quantity and composition of free amino acids and small peptides affect the antioxidant activity. Shorter peptides have a wider effect on antioxidant activity and this is achieved with a higher degree of dehydration. But finally, the sequence of peptides, their composition and hydrophobicity percentage determine the antioxidant properties of the isolated peptides [25-27]. As in the results of measuring the free radical inhibitory power DPPH It was observed in different molecular weights, with increasing concentration, the inhibitory power also increased in all weights. Also In the molecular weight between 3-10 kilodaltons, the minimum protein required to reach IC_{50} there was In a study on the protein of Alaska pollack, it was found that between different molecular weights below 1, 1 to 3, 3 to 5, 5 to 10 and 10 to 30 kilodaltons, the antioxidant property of removing free radicals is the highest in the weight below 1 kilodalton. [28]. In research Gobogouri et al. (2004) in the digestion of salmon head using alkalase, the molecular weight of peptides affected the antioxidant properties, and generally 4.2 to 13.2 kilodaltons were reported as the optimal weight. [29]. In wheat protein hydrolysis, investigation of radical scavenging DPPH It showed that by increasing the extraction time and reducing the size of the produced peptides, the ability to remove free radicals of the peptides increased. [30]. In the study Lahartet al. (2011) regarding casein dehydration, with the progress of dehydration, the molecular weight of the resulting dehydrated protein decreased and the antioxidant activity increased with the decrease in molecular weight. [31]. Different enzymes break different bonds and have the ability to create different molecular weights, and finally, different weights will have different effects on the antioxidant activity, which is affected by the properties of the peptides obtained from hydrolysis. [31]. For example in research That et al. (2008) molecular weight of 500 to 1500 daltons had the most antioxidant properties, including free radical inhibition. [32]. In the study Bougatef et al. (2009) between the weights of less than 3500, 6500 to 12200 and more than 12200 daltons of peptides obtained from shark muscle protein hydrolysis, molecular weight of 3500 daltons showed the best antioxidant status [33]. In addition, in the study Dong et al. (2013) three molecular weights less than 1, 1-5 and above 5 kilodaltons were extracted from silver carp fish protein and it was found that molecular weight higher than 5 kilodaltons has the greatest free radical inhibition power. DPPH has the They also used different weights as the optimal weight for different antioxidant indices such as radical inhibition ABTS and the power of reducing iron stated that it indicates the different characteristics of peptides in different molecular weights [34].

Iron ion is the key active ferrotype responsible for the formation of oxidants in cells, which leads to increased levels of lipid peroxidation [35]. Carboxyl and amine peptide groups in water-soluble proteins prevent lipid oxidation by chelating metal ions, including ferrous iron ions, and prevent the formation of oxidants [36]. The reducing power of a compound can be a significant indicator to show the antioxidant potential of that compound. Iron reducing power III (Frick) shows the ability of electron donation by hydrolytic protein as an antioxidant in order to stabilize free radicals [36]. Therefore, the antioxidant power of hydrolytic protein peptides through iron conversion III to iron II can be investigated [38]. The characteristics of the peptides obtained from the extraction are variable due to the extraction conditions and the type of enzyme used. Also, the antioxidant activity of hydrolyzed protein depends on several factors, including the type of enzyme, the degree of hydrolysis, the solubility of proteins, peptides, and the presence of free amino acids [39]. Therefore, different

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antioxidant activities may be observed in different studies. An increase in the amount of dehydration will not always lead to an increase in antioxidant properties, and the reason for the decrease in ferric reduction capacity with the decrease in molecular weight in the present study is probably related to the excessive amount of free amino acid. Because peptides are more effective than free amino acids, and with increasing conversion of peptides to free amino acids during the dehydration process, this effectiveness decreases. The characteristics of the peptides isolated from the protein hydrocatheter, such as their sequence and composition, are variable in different hydrocatheter and may not have the properties of metal ion chelation through hydrogen or electron donation. For example, hydrophobic amino acids such as histidine, proline, methionine, cysteine, tyrosine and phenylalanine improve the antioxidant activity of peptides [20, 25, 27 and 40]. Another reason for reducing the antioxidant capacity is the excessive reduction of peptides, which was mentioned in this research, but in general, the present research is in agreement with the results of the research. Bakshanet al. (2014) had a discrepancy [41]. Nahvi et al. (2017) similarly to the present research showed that with the increase in the concentration of the protein of hydrocafate, the reducing power of trivalent iron increases under the same conditions of hydrocafate [42]. Also Dey and Dora (2011) digested shrimp waste and showed that the antioxidant compounds in the resulting digested protein show greater reducing power at higher concentrations [14]. Study results Suetsuna(2000) during which the shrimp muscle was digested using protease was also consistent with the results of these studies and confirmed this issue [43]. Protein hydrolyzates include groups of peptides or proteins, and by donating hydrogen and reacting with radicals, it turns them into stable products. As a result, the reaction of radical chains stops [44]. A significant difference was observed in the reduction power of trivalent iron ion in all concentrations between different molecular weights in the present research and with increasing molecular weight, this index increased significantly. The molecular weight of more than 10 kilodaltons was more suitable in the reducing power index of trivalent iron. Bougatef et al. (2010) stated that the size of sardine hydrolytic protein peptides affects the reducing power [38]. In research That et al. (2008) found that the molecular weight of 500 to 1500 daltons has the highest reducing power [32]. Also in the study Bougatef et al. (2009) between the weights of 3500, 6500 to 12200 and 12200 daltons of peptides obtained from shark muscle protein hydrolysis, the molecular weight of 3500 daltons showed the highest iron reduction power [33]. Dong et al. (2013) compared silver carp hydrolytic protein in three ranges of molecular weight less than 1, 1 to 5 and above 5 kilodaltons, reported that 1 to 5 kilodaltons is the optimal weight to increase iron reduction power [34]. These researches confirm that different molecular weights of peptides create different characteristics that ultimately lead to different antioxidant properties.

Free radical inhibition index ABTS It is used to determine the antioxidant activity of hydrogen-donating antioxidants (blocking aqueous phase radicals) and breaking the chain of antioxidants (blocking fatty proxyl radicals) [45]. According to the results of the present research, the molecular weight of 3 to 10 kilodaltons has the greatest free radical inhibitory power. ABTS and with increasing and decreasing molecular weight of the peptide, showed the ability to inhibit free radicals ABTS, a decrease was observed. Also, the least amount of protein needed to reach IC_{50} In the same weight range, it was observed that it indicates the range of optimal molecular weight of the water-distilled protein obtained from the water-dilution of mullet wastes by alcalase enzyme to be used as an antioxidant. In the

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present study, increasing the power of free radical inhibition ABTS Under the influence of higher protein concentrations, water solubility can be due to the fact that by increasing the availability and exposure of the hydrophilic carboxyl and amino groups of proteins to water molecules, more bonds are formed between peptides and water, and finally the solubility of the protein increases. These results with the results of the study Nahvi et al. (2017) was consistent, so that in this research, with increasing the concentration of kilka fish digested protein up to 5 mg/ml, the percentage of radical inhibition ABTS increased [42]. By combining with free radicals and turning them into safe products, as well as by breaking some reactions, hydrolytic protein protects cells against oxidation [46]. In research Ramezanzadeh et al. (2016) salmon skin gelatin was hydrolyzed using alcalase enzyme and between the concentrations of 1, 2, 5 and 10 mg/ml, the concentration of 10 mg/ml of hydrolyzed protein was the optimal concentration in terms of radical inhibitory properties. ABTS It was suggested [47] that with other researches including Sauceret al. (2013) [48], Godinhoet al. (2013) [49] and Intersectionet al. (2012) [50] was similar. Different peptides with different molecular size and amino acid sequences may show different antioxidant activity [13].. Through the ultrafiltration process, the sequence of amino acids can be separated based on molecular weight. A higher degree of hydrolysis indicates that more peptide bonds are broken and lower molecular weight peptides are produced. Finally, increasing the degree of hydrolysis leads to an increase in the free head of hydrophilic carboxyl and amine groups, which leads to greater solubility of peptides. Although in the present research, by reducing the molecular weight to less than 3 kilodaltons, the free radical inhibition power ABTS decreased Therefore, according to this result, in the present study, it can be stated that reducing the molecular weight of peptides does not mean more effective and that peptides in the weight range show more favorable antioxidant effects.

Therefore, the difference in reducing, chelating and radical-inhibiting power between hydrolytic proteins can be considered due to the presence of specific peptides with a specific amino acid sequence and specific molecular weight. For example, it has been reported that the antioxidant activity of peptides with a molecular weight of 500 to 1500 daltons is stronger than peptides with a molecular weight higher than 1500 and peptides with a molecular weight of less than 500 daltons [32]. In the study Chai et al. (2015) Free radical scavenging activity ABTS in the weights of 3 to 10, less than 3 and more than 10 kilodaltons in peptides obtained from fish broth Blue spotted stingray (*Taeni career lymma*) was evaluated and the results showed that the molecular weight of 3 to 10 kilodaltons has more power to inhibit free radicals. ABTS has [51]. Dong et al. (2013) also compared the inhibition activity ABTS Between three molecular weights of less than 1, 1 to 5, and more than 5 kilodaltons of silver carp protein, the weight of less than 1 kilodalton is the optimal weight in order to increase the inhibition power. ABTS reported [34].

5-Conclusion

Since the range of molecular weight and size of the peptides obtained from water extraction is of great importance and is related to the level of antioxidant activity, in this study, the water extraction protein obtained from Caspian sea mullet wastes was classified into different ranges of molecular weight using different ultrafilters and sepsis was evaluated. Comparing the antioxidant properties of hydrolyzed protein with different molecular weights showed that the molecular weight of 3 to 10 kilodaltons in the case of two indicators of free radical removal. DPPH and ABTS And the molecular weight of more than 10 kilodaltons were more suitable in trivalent iron reducing power index. Also, the

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antioxidant activity of all the samples was dependent on the concentration and an increasing trend was observed with increasing concentration. In general, it seems that the use of hydrolyzed protein obtained from Caspian sea mullet waste, especially with a molecular weight of 3 to 10 kilodaltons, can lead to the optimal use of these fish wastes.

5-Resources

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مقاله علمی_پژوهشی

بررسی خواص آنتی‌اکسیدانی پروتئین آبکافتی با وزن‌های مولکولی مختلف حاصل از ضایعات کفال ماهیان دریای خزر

سید محمود ربیعی[۱]، سکینه یگانه[۲]*، مینا اسمعیلی خاریکی[۳]

۱- دانش‌آموخته کارشناسی ارشد فرآوری محصولات شیلاتی، گروه شیلات، دانشکده علوم دامی و شیلات، دانشگاه علوم کشاورزی و منابع طبیعی ساری. ایران.

۲- استاد، گروه شیلات، دانشکده علوم دامی و شیلات، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ایران.

۳- استادیار، گروه شیلات، دانشکده علوم دامی و شیلات، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ایران.

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وزن مولکولی،

الترافیلتر

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* مسئول مکاتبات:

skyeganeh@gmail.com;
s.yeganeh@sanru.ac.ir

چکیده

مدیریت ضایعات آبزیان از طریق تولید محصولات دارای ارزش افزوده از اهمیت زیادی برخوردار است. از جمله محصولات دارای ارزش افزوده با خواص بسیار متعدد (از جمله خواص کف‌زایی، امولسیفایری، آنتی‌اکسیدانی، ضد باکتریایی و ...) می‌توان به پروتئین‌های آبکافتی اشاره کرد. عوامل مختلفی از جمله وزن مولکولی و غلظت پروتئین‌های آبکافتی می‌توانند بر خواص مذکور تاثیرگذار باشند. لذا در پژوهش حاضر خواص آنتی‌اکسیدانی پروتئین آبکافتی حاصل از ضایعات کفال ماهیان دریای خزر در محدوده های مختلف وزن مولکولی و غلظت‌های مختلف مورد بررسی قرار گرفت. بدین منظور ابتدا آبکافت ضایعات ماهی با آلکالاز (غلظت ۱٪) در دمای ۵۵ درجه سانتی‌گراد و pH=۸ به مدت ۱۲۰ دقیقه انجام شد (شرایط بهینه بر اساس پیش‌آزمون). سپس محلول پروتئین آبکافت‌شده با الترافیلترهای ۳ و ۱۰ کیلودالتون به وزن‌های مولکولی کمتر از ۳، بین ۳ تا۱۰ و بیشتر از ۱۰ کیلودالتون تقسیم گردید. به منظور ارزیابی تأثیر وزن مولکولی و غلظت بر فعالیت آنتی‌اکسیدانی پروتئین آبکافتی، از شاخص‌های قدرت مهار رادیکال‌های آزاد DPPH، ABTS و قدرت کاهندگی یون فریک استفاده شد. سنجش قدرت مهار رادیکال‌های آزاد DPPH و ABTS نشان داد که بین وزن‌های مولکولی مختلف اختلاف معنی‌داری وجود دارد و بیشترین و کمترین میزان به‌ترتیب در وزن مولکولی ۳-۱۰ و کمتر از ۳ کیلودالتون ثبت شد (p<۰/۰۵). کمترین میزان IC_{50} برای قدرت مهار رادیکال آزاد DPPH و ABTS نیز برای نمونه با وزن مولکولی بین ۳ تا ۱۰ کیلودالتون محاسبه گردید (p<۰/۰۵). قدرت مهار رادیکال‌های آزاد و قدرت کاهندگی آهن با افزایش غلظت، در تمامی وزن‌های مولکولی بیشتر شدند (p<۰/۰۵). در بین فرکشن‌های مختلف نیز نمونه با وزن مولکولی بیش از ۱۰ کیلودالتون بیشترین قدرت کاهندگی آهن را نشان داد (p<۰/۰۵). با توجه به نتایج، می‌توان اظهار داشت پروتئین آبکافتی تولیدشده در این تحقیق، به عنوان یک ترکیب آنتی‌اکسیدانی (به ویژه در وزن مولکولی ۳-۱۰ کیلودالتون) قابل استفاده می‌باشد.

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