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Evaluation the antioxidant properties of purified bioactive peptides from the wastes of skipjack fish (*Katsuwonus pelamis*) processing, by pepsin and trypsin digestive enzymes

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ABSTRACT

In fish processing industries, 50-70% of primary fish are produced as waste, while they are rich sources of protein and essential amino acids. The optimal use of these wastes and the production of compounds with high added value that have significant health-giving properties is one of the important challenges of fish processing industries. In this research, the effect of hydrolysis conditions (time: 30-300 minutes and enzyme concentration 0.5-3%) and type of protease (pepsin and trypsin) on the degree of hydrolysis and antioxidant properties (DPPH radical scavenging activity, Fe chelating activity, No radical scavenging activity, total antioxidant capacity and Fe reducing power) of hydrolyzed protein obtained from Skipjack viscera were evaluated using response surface methodology. The results showed that the optimal conditions for the production of hydrolyzed protein with the maximum antioxidant properties with pepsin and trypsin enzymes were respectively: hydrolysis time of 179.09 and 143.62 minutes and enzyme concentration of 2.63 and 1.94 %; In this condition, the degree of hydrolysis of the hydrolyzed proteins resulting from the activity of trypsin was calculated to be higher than that of pepsin. Comparing the antioxidant properties of the hydrolysates obtained from the two enzymes used showed that the hydrolyzed protein obtained from trypsin had a stronger antioxidant potential than pepsin. Therefore, it can be stated that the hydrolyzed protein of the Skipjack viscera using trypsin enzyme as a health-giving supplement and with high added value can be used in the production of functional food products and health supplements for athletes and elderly people.

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1. Introduction

Today, it is known that many metabolic diseases are related to the increase in body inflammation, cell oxidation, glucose level and blood pressure [1]. Oxidation plays a beneficial role in cells in that intracellular reactive oxygen species are able to regulate and direct the signal of several immune system activation pathways against inflammation. However, the imbalance of intracellular reactive oxygen species can cause cell damage, which leads to many adverse effects, including cardiovascular diseases, in the long term [2]. For many years, synthetic antioxidant compounds have been used to control oxidative stress, but today identificationTheir adverse effects and pathogenicity have caused the limited use of these compounds and attempts to identify natural antioxidant compounds. Among natural antioxidant compounds, bioactive peptides derived from food proteins usually contain 2-20 amino acids, which todayTheir antioxidant properties have been widely studied. In addition to antioxidant potential, these peptides have various health-giving properties such as anti-inflammatory, anti-hypertensive and antimicrobial [3]. On the other hand, in the 20th centuryFirst, food is not only a source of essential nutrients for the body, but also a source of bioactive compounds that have a positive effect on The body is being consideredto be [4]. Hydrolyzed proteins are considered as one of these nutrient sources, which are a source of bioactive peptides with multiple health-giving properties [5].Various researches have shown that the type of protease enzyme, hydrolysis conditions and protein source play an important role in the health-giving properties of the produced bioactive peptides.]6[. Many plant and animal sources are suitable for the production of hydrolyzed protein and bioactive peptides, includingfenugreek]7[، goat milk]8[and mung bean]9[have been producedBut in order to reduce the cost of the final product and sustainable and proper management of food resources, most of the hydrolyzed proteins are produced from food industry processing waste [10]. Aquatic processing industries, especially fish, is one of the manufacturing industries with the highest amount of waste, and the increase in demand for processed fish products has turned the use of processing waste into one of the main concerns of producers [11]. Although there is no clear and specific definition for waste from fish processing, about 50-70% of primary fish (head, spines, fins, skin, guts and viscera) are considered as waste [12]. About 4 million tons of tuna fish are caught in the world every year, among which is Hoover Muscat with the scientific name *Katsuwonus the suitor* from the highest The amount of catch is good and it has the highest amount of consumption in the production of canned fish; Therefore, it constitutes a large part of the waste of canned fish factories and is a suitable option for the production of hydrolyzed protein]13[. In recent years, hydrolyzed proteins with significant antioxidant capacity from fish processing waste such as sardine fish head]14[، White skin, intestines and viscera[1]]15[, fish skin and leather jacket[2]]16[have been produced that have the potential to be used in the production of ultra-profitable products.

Therefore, according to the mentioned cases, the purpose of this research is to investigate the effect of hydrolysis time and the type of protease (pepsin and trypsin) in the production of bioactive peptides from the intestine and viscera of Hoover Muscat fish and to investigate the degree of hydrolysis and its characteristics.Antioxidant (Radical

[1]- cuttlefish

[2]- leatherjacket

scavenging activity[3]DPPH, iron ion reduction, iron ion chelation, nitric oxide radical inhibition, etcAntioxidant The whole) was it.

2- Materials and methods

2-1- Materials

Pepsin, trypsin, trichloroacetic acid, DPPH, Coomassie Brilliant Blue (G250), ferric chloride, potassium ferricyanide, iron dichloride and ferrosin were obtained from Merck, Germany. Other chemical substances were also obtained with laboratory purity from Sigma Company of America.

2-2- sample preparation

FirstThe intestines and viscera of Hoover Muscati fish obtained from the processing waste of Sahil Said Kanarak (Tafeh) were washed with distilled water and completely minced. Then, to deactivate the internal enzymes of the fish, for a period of time20 They were heated at 95°C for 10 minutes in a bain-marie and placed in an ice water bath for 10 minutes to reduce the temperature. The samples were dried by placing them in a 30°C oven for 24 hours. In the next step, the samples are milled and They were passed through a sieve with 40 mesh. Finally, the samples were stored in polyethylene bags in a freezer at -20 degrees Celsius for further tests.]17[.

2-3- Fat removal

In order to degrease, the powders were mixed with hexane at a ratio of 1:10 (weight/volume) and stirred for 5 hours at 25°C. Then, using a Buchner funnel, hexane is separated and in the oven30 °C for 5 hours. The resulting powder is ground again and passed through a sieve with 40 mesh]16[.

2-4- protein extraction

For protein extraction, defatted powdermixed with distilled water at a ratio of 1:10 andpH It was adjusted to pH=10 using 1 normal soda and stirred for 1 hour using a magnetic stirrer at 25°C. Then the resulting solution was centrifuged at 7000×rpm for 20 minutes and the resulting supernatant was collected. ThenpH Supernatant to 4=pH (pH isoelectric) and centrifuged at 8000×rpm for 20 minutes to precipitate the proteins. Finally, the resulting protein precipitate was washed twice with distilled water and rpm×7000, was centrifuged again for 5 minutes. The resulting protein isolate is dried using a dry freeze dryer and kept in the freezer until the tests are performed-20 degrees Celsiuswas kept]18[.

5-2- Preparation of hydrolyzed protein

The resulting protein isolate was dissolved at a concentration (w/v) of 5% in 0.2 M M phosphate buffer, pH = 7.4 for trypsin and pH = 2 for pepsin, and was stirred for 30 minutes at room temperature to fully hydrate it. Then it was placed in a shaker incubator at 200 rpm at a temperature of 37 degrees Celsius. Enzyme was added to the samples in the proportions mentioned in Table 1 and hydrolysis was done according to the times specified in this table. Finally, to inactivate the enzyme, the erlens were placed in a 90°C water bath for 10 minutes and cooled to ambient temperature using an ice water bath. Then the samples were centrifuged at 8000 × rpm for 20 minutes. The supernatant was separated and dried using a freeze dryer and kept at -20°C until the next tests.]19[.

[3]- 1,1-diphenyl-2-picrylhydrazyl

6-2-Measurement of chemical compounds

Measuring the amount of moisture, protein, ash and fat using the methodAOAC 2000, Done]20[.

2-7- Determining the degree of hydrolysis

suspension of hydrolyzed protein and trichloroacetic acid (M0.44) in a volume ratio of 1:1 mixed and for a long timemin 15 minutes at temperature 4Cwere incubated Then, mix inrpm10,000 and for the durationmin10 centrifuged. The amount of protein in the supernatant containing trichloroacetic acidM 0.22 was determined by Bradford method]21[. Finally, the degree of hydrolysis was determined using formula 1:

(1)

$$Degree\ of\ hydrolysis(\%) = \frac{protein\ \ in\ supernat}{protein\ in\ hydrol} 100$$

2-8- Examining the antioxidant properties of hydrolyzed protein

2-8-1- Free radical inhibition activityDPPH

First, the resulting hydrolyzates in distilled water (mg/ml10) were solved then,ml1.5 ml of each sample with51/ml of ethanol solutionDPPH (mM0.15) were mixed and vortexed for 20 seconds. In the next step, the resulting mixture was stored in the dark for 30 minutes and then inrpmCentrifuged at 4000 for 10 minutes. Absorption of the supernatant solution at wavelengthnm517 were read]16[. Radical scavenging percentageDPPH free It was calculated using formula 2:

(2) $(\%) = \left[\frac{Ablank - AsampleAblank - Asample}{Ablank \qquad Ablank}\right] \times 100$

2-8-2- Determining the reducing power of iron ion

To determine the reducing power of iron ion of hydrolyzed samples,50.0 ml of sample dissolved in distilled water (in concentrationmg/ml10) with50.0 ml of phosphate bufferM2/0 (6/6=pH) and 0.5 ml of potassium ferricyanide(W/V)1% mixture and at 50 °C for20 Incubated for a minute. Then,5Add 0.0 ml of 10% trichloroacetic acid solution to the mixture and keep it for a long time10 Minutes inrpm2500 It was centrifuged. Eventually,1 ml of supernatant with1 ml of distilled water and20.0 ml of ferric chloride(W/V) 0.1% was mixed. Sample absorption innm700 after10 The minutes of keeping the mixture at ambient temperature were read. An increase in the absorption of the reaction mixture indicates an increase in the reducing power]22[.

2-8-3- Iron ion chelating activity

To evaluate the iron ion chelating activity,1 mlSample dissolved in distilled water (concentrationmg/ml40) with050.0 ml of iron dichloride solution (Mm2) and851/ml of double distilled water was mixed. Then,10.0 ml of ferrozin solution (Mm5) added and the mixture was vigorously stirred. Absorption after 10 minutes of keeping the mixture at ambient temperaturenm 562 read]23[. Double distilled water was used as a control sample. The chelating activity of the samples was calculated using equation 3**:**

(3) **Chelating effect (%) =** $\frac{(Acontrol - Asample)}{Acontrol} \times 100$

2-8-4- CapacityAntioxidant Total

This method is based on the reduction of 6-valent molybdenum to 5-valent molybdenum, which is associated with the formation of a green phosphomolybdenum complex in an acidic environment. In this method, 0.1 ml of the sample dissolved in distilled water (concentrationmg/ml10) With1 ml of reagent (sulfuric acidM 0.6, sodium phosphateMm 28 and ammonium molybdatemM 4) pour into Eppendorf tube and in water bath for 90 minutes90 degrees Celsius it placed. After cooling, absorb the samples innm 695 were read. Double distilled water was used as control sample. More absorption indicates capacityAntioxidant The whole is more]24[.

2-8-5- nitric oxide radical inhibition activity

For this purpose, 60 microliters of sodium nitroperoxide in bufferPBS[4] Withµl 60 samples dissolved in distilled water (concentrationmg/ml10) was mixed. then at 25 °C for a period of timemin It was incubated for 150 minutes. Then add 120 microliters of grease reagent to it and absorb the resulting solutionnm 546 were read]25[. The nitric oxide radical scavenging activity of the samples was calculated using equation 4:

(4)

$$NO\ chelating\ activity = \frac{(Acontrol - Asample)}{Acontrol} \times$$

$$NO\ chelating\ activity = \frac{(Acontrol - Asample)}{Acontrol} \times$$

100

9-2- Statistical analysis

Optimization of hydrolysis conditions was done with Design expert software version 7 and response surface method in the form of central compound design. The independent variables of time (30-300 minutes) and the ratio of enzyme to substrate (0.5-3%) and the investigated response are iron ion reduction power, DPPH radical scavenging activity, capacityAntioxidant total, iron ion chelating activity, nitric oxide radical scavenging activity and degree of hydrolysis were considered. To compare the average of fat, moisture, ash and protein, Duncan's multi-range test was used at the 95% confidence level. All tests were performed in three repetitions.

3. Results and Discussion

3-1- Chemical compounds

The amount of ash, moisture and protein of non-defatted, defatted intestines and viscera powder and the resulting protein isolate are listed in Table 1.The amount of protein in the non-defatted powder was 43.75%, which increased to 87.5% after the extraction process. The increase of the isolated protein compared to the primary intestines and viscera powder, in addition to the reduction of the amount of fat during the degreasing process, can be due to the removal of a large part of non-protein compounds at pH=10. The amount of fat in the non-defatted powder was 21.25%, which decreased to 7.06% and 4.10% after the defatting and protein extraction stages. The amount of hydrolyzed protein fat was significantly lower than the original powder of intestines and fish viscera. The decrease in fat content can be due to the processes of defatting and protein extraction in isoelectric pH. The low amount of fat (less than 5%) in the protein isolate is the result of the good efficiency of hexane in degreasing the intestines and viscera of fish. Also, the amount of moisture and ash has decreased and after the extraction of better protein, they decreased from 8.03% and 8.4% to 5.2% and 3.15%. The moisture reduction process during the protein extraction process can also be the result of using an oven with a temperature of 30 degrees Celsius after the degreasing process in order to remove the remaining hexane and also using a freeze dryer to dry the protein isolate. On the other hand, reducing the amount of ash can also be due to the protein extraction process, which removes many non-protein organic compounds and mineral salts [19].

[4]– Phosphate-buffered saline

Table 1. chemical composition of Undefatted, defatted powder and protein isolate of

	Ash (%)	Fat (%)	Moisture (%)	Protein (%)
Undefatted powder	0.16 [a] ±5.4	0.12[a]±21.25	0.35[a]±8.03	0.12[c]±43.75
Defatted powder	0.12 [b] ±4.36	0.11[b]±7.06	0.26[b]±7.2	0.16[b]±61.25
Protein isolate	0.11 [c] ±1.15	0.23[c]±4.10	0.25[c]±5.3	87.5±0.24[a]

Means with the same letters in each column show no significant difference at the 0.05 level

2-3- The effect of the ratio of enzyme to substrate, time and type of protease Degree of hydrolysis

The results of variance analysis of the degree of hydrolysis of hydrolyzed proteins from pepsin and trypsin are shown in (Table 2). According to the results of all investigated variables except A^2 had a significant effect on the degree of hydrolysis of the hydrolyzed protein produced by pepsin; On the other hand, the hydrolyzed protein produced by trypsin is also the only variableB^2 and AB had no significant effect(05/0>p). The relationship between the degree of hydrolysis and the parameters of the hydrolysis reaction resulting from both pepsin and trypsin enzymes was of the quadratic type, and their regression coefficient was the best:2199/0R^2= And9530/0R^2=. On the other hand, the lack of fit factor[5] The hydrolysis reaction with both enzymes was not significant, indicating the ability

The top of the model predicts the effect of the examined variables on the degree of hydrolysis. The results of analysis of variance showed that the relationThe investigated variables in the process of hydrolysis with pepsin and trypsin and the degree of hydrolysis best follow the relationship (5) and (6).

(5)

degree of hydrolysis =+ *2.37 + 15.76 A + 2.33 B - 2.94 AB - 0.60 A2 - 2.06 B2*

-

-

(6)

degree of hydrolysis = + 4.19 + 18.09 A + 1.64 B – 1.32 AB – 1.74 A2 – 1.94 B2

[5]- Lack of fit

Table 2. ANOVA of degree of hydrolysis of protein hydrolysate with pepsin and trypsin

	Sum of squares	df	P value	Estimate the coefficient
	Hydrolysis with pepsin **Hydrolysis with trypsin**			
	ANOVA of degree of hydrolysis			
Model	146.79 198.60	5 5	0.0025 0.0085	15.76 18.09
A-time	44.84 140.37	1 1	0.0026 0.0013	2.37 4.19
B- E/S	43.51 21.64	1 1	0.0028 0.0422	2.33 1.64
AB	34.57 6.92	1 1	0.0045 0.2111	-2.94 -1.32
A^2	2.04 17.02	1 1	0.2885 0.0743	-0.60 -1.74
B^2	23.87 21.28	1 1	0.0097 0.0535	-2.06 -1.94
Lac of fit	0.17 16.48	3 3	0.3647 0.0847	

* A: hydrolysis time B: enzyme to substrate ratio

According to Figure 1A, at constant times, the degree of hydrolysis increased by increasing the concentration of the enzyme up to 2.16%, and then with a further increase in the concentration of the enzyme up to 2.86%, the degree of hydrolysis increased with a constant slope and reached 86. It reached 15 percent. On the other hand, at constant concentrations of pepsin enzyme, increasing the hydrolysis time increased the degree of hydrolysis, but after 212 minutes, there was a constant increase in the slope. In general, the highest degree of hydrolysis (17.30 percent) with pepsin enzyme in the hydrolysis time of 244.27 minutes and the concentration of the enzyme is 1.65 percent and the lowest degree of hydrolysis (5.87 percent) in the time of 73.89 minutes and the concentration of the enzyme is 88 0.0% was obtained. In hydrolysis with trypsin enzyme (Figure 1B), at constant enzyme concentrations up to 2.16%, increasing the hydrolysis time increased the degree of hydrolysis to 18.03%, but at higher concentrations of the enzyme, increasing the hydrolysis time increased the degree of hydrolysis with a constant slope. slow down On the other hand, at fixed times and less than 212 minutes of hydrolysis with trypsin enzyme, increasing the concentration of the enzyme also increased the degree of hydrolysis, but at longer times of hydrolysis, this increase had a constant slope. In general, the highest amount of hydrolysis degree (20.33 percent) during the hydrolysis time was 247.76 and the enzyme concentration was 1.75 percent, and the lowest degree of hydrolysis (7.52 percent) was during the hydrolysis time of 69.86 minutes and the enzyme concentration was 0.89. The percentage was obtained. Therefore, in the comparison between pepsin and trypsin enzymes, the highest degree of hydrolysis was obtained using trypsin enzyme. Studies have shown that the steady increase in the degree of hydrolysis with increasing time can be due to the excessive hydrolysis of the protein substrate and the inhibitory effect of the produced peptides [26], reducing the number of peptide bonds available for hydrolysis, as well as deactivating the enzyme. be a protease [27]. Similar to these results, Sharaf

et al. (2013) reported with the hydrolysis of waste protein after cooking Hoover fish that the degree of hydrolysis decreased with an excessive increase in the hydrolysis time and reached 3 percent [28]. On the other hand, Yu et al. (2009) reported with the hydrolysis of the guts and viscera of Sargandeh carp that increasing the hydrolysis time up to 20 hours increased the degree of hydrolysis from 18 to 33% [29].

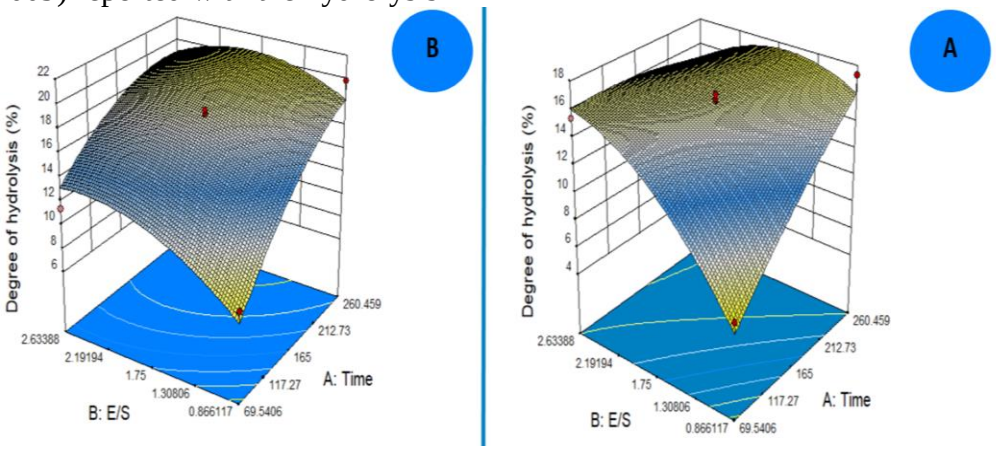

Figure 1- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on degree of hydrolysis of protein hydrolysate of skipjack viscera (A: hydrolysis with pepsin, B: hydrolysis with trypsin)

3-3- Effect of time, ratio of enzyme to substrate and type of protease on radical scavenging activityDPPH

Variance analysis results of the effect of time, enzyme to substrate ratio and protease type on radical scavenging activityDPPH It is mentioned in Table 3. A variables^2 and B^2 on radical scavenging activityDPPH The hydrolyzates obtained from the activity of both pepsin and trypsin enzymes were significant, and the AB time variable was only on the activity of radical scavenging.DPPH The hydrolyzates obtained from pepsin were significant and other variables had no significant effect on the hydrolysates obtained from both enzymes ($p<0.05$). Radical scavenging activityDPPH with the independent variables investigated in the hydrolysis with both enzymes, it was quadratic and the correlation coefficient in the case of hydrolyzates obtained from pepsin and trypsin was R 0.9184 respectively.^{2}= and R 0.9671^2= was obtained, which indicates the high ability of the model in predicting the effect of the variables of the hydrolysis process with both pepsin and trypsin enzymes on radical scavenging activityDPPH is. On the other hand, the non-significance of the lack of fit index shows that the desired model has been able to describe the changes in the amount of data well. Radical scavenging relationshipDPPH With the variables of the hydrolysis process with pepsin and trypsin, it follows equations (7) and (8).

(7)

DPPH radical scavenging activity =+ 2.01 + 42.91 A + 0.60 B + 0.55 AB – 7.57 A2 – 8.25 B2

(8)

DPPH radical scavenging activity= + 2.75 + 51.57 = A + 3.08 B + 1.05 AB – 9.21 A2 – 8.65 B2

Table 3. ANOVA of DPPH radical scavenging activity of protein hydrolysate with pepsin and trypsin

	Sum of squares	df	value P	Estimate the coefficient
				Hydrolysis with pepsin Hydrolysis with trypsin
Model	584.29 837.88	5 5	0.0010 0.0095	42.91 51.57
A-time	32.30 60.36	1 1	0.0358 0.1003	2.01 2.75
B- E/S	2.91 75.78	1 1	0.4314 0.0738	0.60 3.08
AB	1.22 4.43	1 1	0.6032 0.6089	0.55 1.05
A^2	323.46 479.44	1 1	0.0003 0.0024	-7.57 -9.21
B^2	384.43 422.20	1 1	0.0002 0.0031	-8.25 -8.65
Lac of fit	19.71 24.59	3 3	0.0711 0.0641	

* A: hydrolysis time B: enzyme to substrate ratio

According to Figure 2A, at constant enzyme amounts, increasing the hydrolysis time up to 165 minutes increases the radical scavenging activity.DPPH The resulting hydrolyzed protein was 42.84%, but increasing the hydrolysis time had a negative effect on the ability of the produced hydrolyzates to inhibit DPPH radical up to 30.09%. On the other hand, increasing the concentration of the enzyme up to 1.75% during the constant hydrolysis times increases the radical scavenging activity.DPPH up to 40.78%, and the further increase in the concentration of the enzyme decreases the radical inhibition abilityDPPH Hydrolyzed protein was obtained. In general, the highest level of radical scavenging activityDPPH It was obtained by pepsin enzyme (43.02%) in the hydrolysis time of 182.97 minutes and the enzyme concentration was 1.75%. In the case of the hydrolyzates resulting from trypsin activity (Figure 2B), at fixed hydrolysis times, increasing the enzyme concentration up to 1.75% increases the radical scavenging activity.DPPH increased to 51.29%, but increasing the concentration of the enzyme had a negative effect on the antioxidant capacity of the hydrolyzed products, so that their ability to inhibit radicalsDPPH It decreased to 41.11%. On the other hand, in constant enzyme concentrations, with increasing time up to 165 minutes, radical scavenging activityDPPH Trypsin hydrolyzates increased and reached 49.24%, but further increase in hydrolysis time had a negative effect. In general, according to the graph, the maximum amount of radical scavenging activityDPPH (52.06 percent) was obtained in the hydrolysis time of 180.70 minutes and the enzyme concentration was 1.96 percent. negative effectThe excessive increase in the hydrolysis time on the antioxidant capacity of the hydrolyzed protein can be due to the progress of the hydrolysis process and the excessive effect of proteases on the antioxidant peptides produced in the early stages and their destruction, which reduces the capacity of the hydrolyzed protein in radical inhibition.DPPH has been [30].Decreased radical scavenging

activityDPPH Hydrolyzed protein with increasing enzyme concentration probably due toExcessive hydrolysis of the protein by the enzyme leads to the complete release of hydrophilic amino acids, which interacts with the lipid-soluble radical.DPPH It makes it difficult [31]. Considering that both enzymes used in this study were endopeptidase, the higher radical scavenging activityDPPH The hydrolyzates obtained from trypsin compared to pepsin can indicate More release of lipid-soluble radical scavenging peptidesDPPH by trypsin. In general, various studies have shown that various factors play a role in the free radical inhibition ability of hydrolyzed protein, includingProtease type, amino acid composition and degree of hydrolysis [23].In accordance with these resultsOysipour et al. (2009) also found the negative effect of excessive hydrolysis time (more than 180 minutes) on radical scavenging activity.DPPH reported the hydrolyzed protein of salmon waste [32]. Also, similar to these results, Kaveh et al. (2018) stated that increasing the concentration of the enzyme up to 2% increased the DPPH radical inhibition activity of hydrolyzed fenugreek protein with pancreatin enzyme and further increasing the concentration of the enzyme decreased this ability.19[.

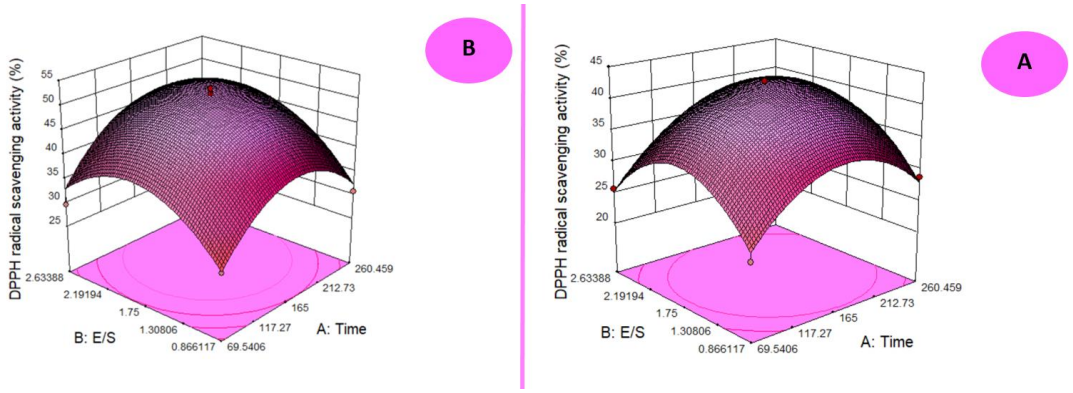

Figure 2- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on DPPH radical scavenging activity of protein hydrolysate of skipjack viscera (A: hydrolysis with pepsin, B: hydrolysis with trypsin)

3-4- The effect of time, the ratio of enzyme to substrate and the type of protease The rejuvenating power of iron ion

Iron ion reduction power test to evaluate the potential of an antioxidant compound in ion transformationFe^{3+} ToFe^{2+} is used The results of variance analysis of iron ion reduction power of hydrolyzed protein are shown in Table 4. According to the results, the variablesA^2 AndB^2 Pepsin and trypsin had a significant effect on the slowness regeneration activity of hydrolyzed protein, and the time variable had a significant effect only on the hydrolyzate produced by trypsin. Other variables had no significant effect on the reductive ability of any of the hydrolyzed proteins(05/0>p). The reducing power of both hydrolyzed iron ions from pepsin and trypsin had quadratic relationships with the reaction parameters, and their regression coefficient was the best.8125/0R^2= And8983/0R^2=. The misfit factor of the defined model for both hydrolyzates was not significant, which indicates the appropriate ability of the model in predicting the effect of process variables. Analysis of variance of the results showed that the relationship between the reducing power of iron ionAnd the variables of the process in hydrolysis with pepsin and trypsin enzymes are in relation (9) and (10).

(9) reducing power of iron ion = 0.025 - 0.41+ A + 0.045 B - 4.5 AB + 0.12 A^2 + 0.13 B2

(10) *reducing power of iron ion = 0.079 – 0.46+ A — 0.028 B – 2 AB — 0.10 A2 – 0.12 B2*

Table 4. ANOVA of Fe reducing power of protein hydrolysate with pepsin and trypsin

	Sum of squares	df	value P	Estimate the coefficient
				Hydrolysis with pepsin Hydrolysis with trypsin
Model	0.16 0.16	5 5	0.0160 0.0467	0.41 0.46
A-time	5 0.050	1 1	0.2944 0.0494	-0.025 -0.079
B- E/S	0.016 6.13	1 1	0.0906 0.4091	0.045 0.028
AB	8.1 1.6	1 1	0.8873 0.9651	-4.5 -2
A^2	0.085 0.057	1 1	0.0047 0.0408	0.12 0.10
B^2	0.096 0.081	1 1	0.0037 0.0220	0.13 0.12
Lac of fit	0.018 0.038	3 3	0.0891 0.1130	

* A: hydrolysis time B: enzyme to substrate ratio

According to Figure 3A, at fixed times of hydrolysis, increasing the concentration of enzyme up to 1.75% decreased the reducing power of pepsin hydrolyzates, but with a further increase in enzyme concentration, the reducing power increased to 0.7055 (absorbance at 700 nm) arrived. On the other hand, at a fixed concentration of pepsin enzyme, increasing the hydrolysis time up to 165 minutes decreased the reducing power of iron ions, and further increasing the time increased the reducing power of hydrolyzed protein from pepsin to 0.581 (absorbance at 700 nm). In general, the highest level of reducing power of iron ion (0.712, absorbance at 700 nm) was obtained in the hydrolyzates obtained from pepsin in the hydrolysis time of 73.21 minutes and the enzyme concentration was 2.60%. In the case of trypsin hydrolyzates (Figure 3B), at constant enzyme concentration, increasing the hydrolysis time up to 165 minutes decreased the reducing power of iron ions, but increasing the time had a positive effect as it increased the reducing power of iron ions of the resulting hydrolyzed protein. from trypsin to 0.612 (absorbance at 700 nm). In the fixed times of hydrolysis with trypsin enzyme, increasing the enzyme concentration up to 1.75% decreased the reducing power of hydrolyzed iron ions, but further increasing the enzyme concentration

increased the antioxidant property up to 0.773 (absorbance at 700 nm). In general, the highest amount of reducing power of iron ion (0.785, absorbance at 700 nm) was obtained in the hydrolyzates obtained from trypsin in the hydrolysis time of 71.83 minutes and the enzyme concentration was 2.63%. In general, studies have shown that with the passage of time and the progress of the protein hydrolysis process, the structure, size, composition and sequence of the resulting bioactive peptides change, which has a significant effect on the reductive power of iron ion [14]. The increase in the reductive power of hydrolyzed protein during hydrolysis and the appropriate enzyme concentration can be related to the release of amino acids with high reductive power such as methionine, tryptophan and lysine. Studies have shown thatPepsin enzyme preferentially breaks the bonds adjacent to the amino acids phenylalanine, tyrosine and tryptophan, by breaking these peptide bonds and releasing them from the main structure of proteins, their molecular weight is reduced, and then with the progress of the process of producing peptides with the ability to donate electrons for the reduction of trivalent iron ions to Bicapacitance increases thus leading to an increase

The reducing power of iron ion is hydrolyzed protein [22]. These results are similar to the findings of Khantafant et al. (2011) andAhmadi et al. (2019) in hydrolysisRedfish muscle protein[6] And the intestines and intestines are carp fish [34 and 33]. Also, Maqsoodlou et al. (2018) reported with the hydrolysis of bee pollen protein with pepsin enzyme that hydrolysis up to 2.5 hours decreased the reductive power of the hydrolyzed products, but increasing the hydrolysis time increased the reductive property [35].

6- Brown stripe red snapper

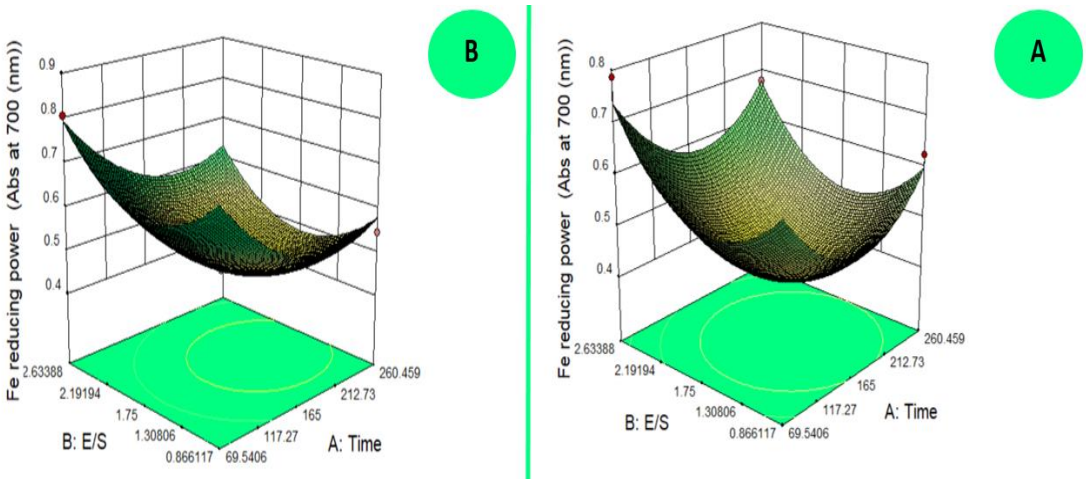

Figure 3- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on Fe reducing power of protein hydrolysate of skipjack viscera (A: hydrolysis with pepsin, B: hydrolysis with trypsin)

5-3- The effect of time, the ratio of enzyme to substrate and the type of protease Iron ion chelating activity

in Fe ion oxidation reactions^{2+} It acts as a catalyst that leads to the rapid conversion of superoxide anion into hydroxyl radicals. Produced hydroxyl radicals react with nearby biological molecules and cause damage to cells and tissues and the occurrence of dangerous diseases such as cancer [36].The results of variance analysis of hydrolysis with pepsin and trypsin enzymes are listed in Table 5; According to these results, time variables, AB andB^2 On the chelation activity of iron ion hydrolyzates obtained from pepsin and enzyme concentration variablesA^2 had a significant effect on the iron ion chelating activity of hydrolyzed protein obtained from trypsin and other variables had no significant effect(05/0>p). Reaction parameters with chelating activity of both hydrolyzates resulting from pepsin and trypsin, quadratic and better interfaces with regression coefficient9407/0R^2= And9291/0R^2= they had. The non-significance of the misfit factor also indicates the high ability of the model in predicting the effect of process variables on the investigated response (iron ion chelation). According to the results of relational variance analysis of iron ion chelating activityAnd the variables of the hydrolysis process with pepsin and trypsin enzymes are in relation (11) and (12).

(11)

$$\text{فعالیت شلاته‌کنندگی یون آهن} = +35.71 - 2.44\,A - 0.61\,B + 2.64\,AB - 1.10\,A^2 + 4.26\,B^2$$

(12)

$$\text{فعالیت شلاته‌کنندگی یون آهن} = +40.38 + 0.72\,A + 5.01\,B - 0.36\,AB - 4.80\,A^2 + 1.55B^2$$

Table 5. ANOVA of Fe chelating activity of protein hydrolysate with pepsin and trypsin

Sum of squares	df	value P	Estimate the coefficient

				Hydrolysis with pepsin **Hydrolysis with trypsin**
Model	215.06 390.36	5 5	0.0044 0.0067	35.71 40.38
A-time	47.64 4.19	1 1	0.0086 0.4398	-2.44 0.72
B- E/S	2.96 201.01	1 1	0.3437 0.0021	-0.61 5.01
AB	27.93 0.51	1 1	0.0238 0.7814	2.64 -0.36
A^2	6.78 130.35	1 1	0.1746 0.0055	-1.10 -4.80
B^2	102.43 13.58	1 1	0.001 0.1916	4.26 1.55
Lac of fit	11.98 29.61	3 3	0.1697 0.0980	

* A: hydrolysis time B: enzyme to substrate ratio

According to Figure 4A, at fixed times of hydrolysis, increasing the enzyme concentration up to 1.75%, the amount of chelating activity of the hydrolysed iron ions from pepsin decreased, further increasing the enzyme concentration did not have a significant effect on it. On the other hand, at constant enzyme concentrations, the chelating activity of iron ions decreased with increasing hydrolysis time. In general, according to the diagram, in the hydrolysis with pepsin enzyme, the highest level of iron ion chelating activity (44.15%) was obtained in the hydrolysis time of 72.3169 minutes and the enzyme concentration was 0.89%. In the hydrolysis with trypsin enzyme (Figure 4B), at fixed times, the iron ion chelating activity of the hydrolyzed protein increased with increasing enzyme concentration. On the other hand, in constant enzyme concentrations, increasing the hydrolysis time up to 165 minutes, the iron ion chelating activity of trypsin hydrolyzates increased and decreased with further increase in time. In general, the highest amount of iron ion chelating activity (46.33%) was obtained by hydrolysis with trypsin at the hydrolysis time of 154.68 minutes and the enzyme concentration was 2.57%. The negative effect of excessive increase in hydrolysis time on iron ion chelating activity, probably due to the decrease in enzyme efficiency in the production of peptides with appropriate antioxidant capacity due to the releaseEnzyme inhibitory compounds during hydrolysis are long; Also, the hydrolysis and decomposition of peptides with suitable iron ion chelating properties produced early in the hydrolysis process can be another reason

for the negative effect of hydrolysis over long periods of time on iron ion chelating activity [32]. The positive effect of increasing enzyme concentration on chelating activity of hydrolyzed protein can be due toIncreasing the amount of active enzyme sites in the environment for protein hydrolysis and as a result breaking more peptide bonds and increasing the solubility of the produced peptides, which have a positive effect on the chelating activity of the resulting peptides [37]. In general, studies have shown that many factors have an effect on the chelation activity of hydrolyzed protein, the most important of which are: the nature and amino acid composition of the primary protein, the type of protease enzyme, and the degree of hydrolysis [38]. These results are similar to the findings of Kaveh et al (2022) in the hydrolysis of fenugreek seed protein with pepsin and trypsin enzymes, who reported that increasing the hydrolysis time up to 150 minutes increased the iron ion chelating activity of the produced hydrolyzates, but a further increase in the hydrolysis time did not have a significant effect on the chelating property [7]. Also, these results are in agreement with the findings of Klampang et al. (2007), Bayram et al. (2008) and Jamdar et al. (2010) respectively in protein hydrolysis of yellow tail fish.[7]protein is whey and peanut [41-39].

[7] - yellow stripe trevally

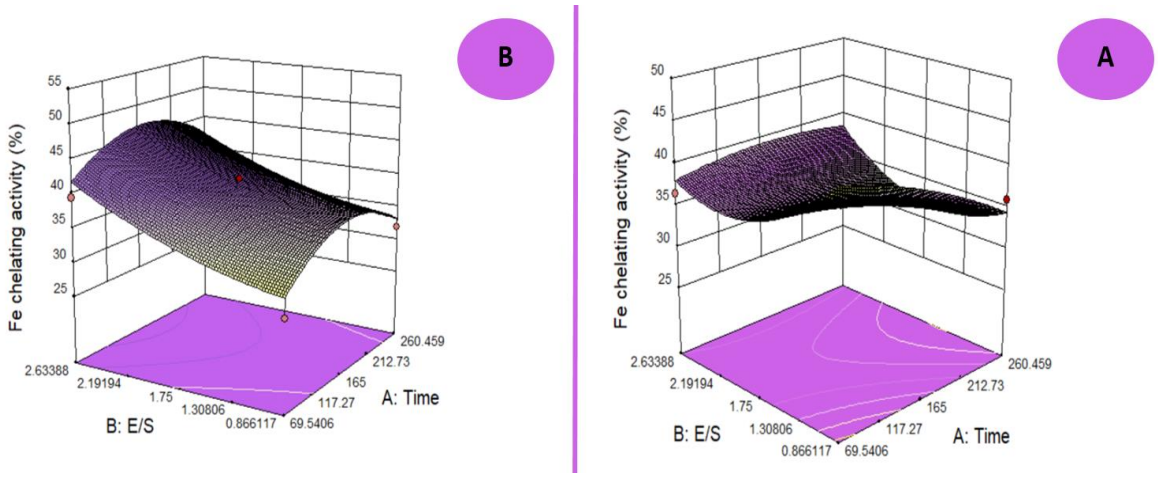

Figure 4- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on Fe chelating activity of protein hydrolysate of skipjack viscera (A: hydrolysis with pepsin, B: hydrolysis with trypsin)

3-6- The effect of time, the ratio of enzyme to substrate and the type of protease

Total antioxidant capacity

The total antioxidant capacity test evaluates the total antioxidant power of a compound, which includes the antioxidant power of both fat-soluble and water-soluble compounds. This method is based on the reduction of 6-valent molybdenum to 5-valent molybdenum, which causes the formation of a green phosphomolybdenum complex in an acidic environment [42]. The results of variance analysis of total antioxidant capacity are listed in Table 6. In hydrolysis with the enzyme pepsin, the only variable is the concentration of the enzyme, and in the case of trypsin, the only variableA^2 had a significant effect on the antioxidant capacity of the total hydrolyzates produced. Other investigated variables did not show any significant effect (>0.05).p). Reaction parameters with the total antioxidant capacity of both hydrolysates obtained from pepsin and trypsin, better quadratic interfaces with their regression coefficient $0.8557R^2$= and $0.8844R^2$= had The non-significance of the misfit index also shows the high ability of the model in predicting the range of valueswas investigated. The relationship between the total antioxidant capacity and the investigated variables in hydrolysis with pepsin and trypsin better follows the relationship (13) and (14).

(13)

total antioxidant capacity =+0.015 - 0.53 A + 0.012 B + 0.014 AB - 0.016 A2 - 0.031 B2

(14)

total antioxidant capacity = +0.021 - 0.64 A + 0.016 B + 0.028 AB - 0.033A2 - 0.023 B2

Table 6. ANOVA of total antioxidant activity of protein hydrolysate with pepsin and trypsin

	Sum of squares	df	value P	Estimate the coefficient
				Hydrolysis with pepsin Hydrolysis with trypsin
Model	9.28	5	0.0358	0.53

	0.016	5	0.0412	0.64
A-time	1.85 3.47	1 1	0. 2892 0.1103	-0.015 -0.021
B- E/S	1.08 2.10	1 1	0.0462 0.1918	0.012 0.016
AB	7.29 3.25	1 1	0.4909 0.1197	0.014 0.028
A^2	1.4 6.09	1 1	0.3503 0.0403	-0.016 -0.033
B^2	5. 3 2.94	1 1	0.1006 0.1344	-0.031 -0.023
Lac of fit	6.56 29.61	3 3	0.9510 0.0678	

* A: hydrolysis time B: enzyme to substrate ratio

According to Figure 5A, at fixed values of enzyme concentration by increasing the time of hydrolysis with pepsin enzyme up to 165 minutes, the capacityAntioxidant The total change was not significant. But increasing the hydrolysis time decreased the total antioxidant capacity; So that after 260 minutes of hydrolysis, it decreased from 0.51 to 0.45 (absorbance at 695 nm). On the other hand, in constant values of hydrolysis time, increasing the enzyme concentration up to 1.75% increased the total antioxidant capacity, but further increasing the enzyme concentration had a negative effect. In general, according to the graph of the highest total antioxidant capacity (absorbance at 695 nm, 0.531) in hydrolysis with pepsin in 96.81 minutes, the enzyme concentration was 1.68%. On the other hand, as can be seen in Figure 5B, increasing the hydrolysis time at all constant enzyme concentrations decreased the antioxidant capacity of the total hydrolyzates. On the other hand, at constant times of hydrolysis, changes in enzyme concentration did not have a significant effect on the antioxidant capacity of the total hydrolyzates resulting from trypsin activity. This finding confirms the data shown in Table 6, which indicated that the concentration of the enzyme was not significant on the antioxidant capacity of the total protein hydrolyzed by trypsin. Maximum capacityAntioxidant Total (0.635 absorbance at 695 nm) was obtained in hydrolysis with trypsin enzyme in hydrolysis time of 166.757 minutes and enzyme concentration was 2.39%. Therefore, according to the results, it can be stated that pepsin and trypsin enzymes by hydrolyzing the peptide bonds in the intestinal protein and viscera of Hoover Muscat fish have caused the release of electron-donating peptides, and these peptides have a good ability to convert free radicals into stable compounds, which ultimately increase the capacity. Antioxidants of total hydrolyzed protein have been produced. On the other hand, excessive increase in hydrolysis time or enzyme concentration has caused an inappropriate increase in the hydrolysis of peptides with electron donating properties, as a result of which the total antioxidant capacity has decreased. In general, trypsin showed a better performance in the production of antioxidant peptides. These results were similar to the findings of Mazloumi et al. (2018), Kaveh et al. (2022) and Elvand et al. They reported that an excessive increase in time and enzyme concentration had a negative effect on the antioxidant capacity of the produced hydrolyzates [7, 22, 43].

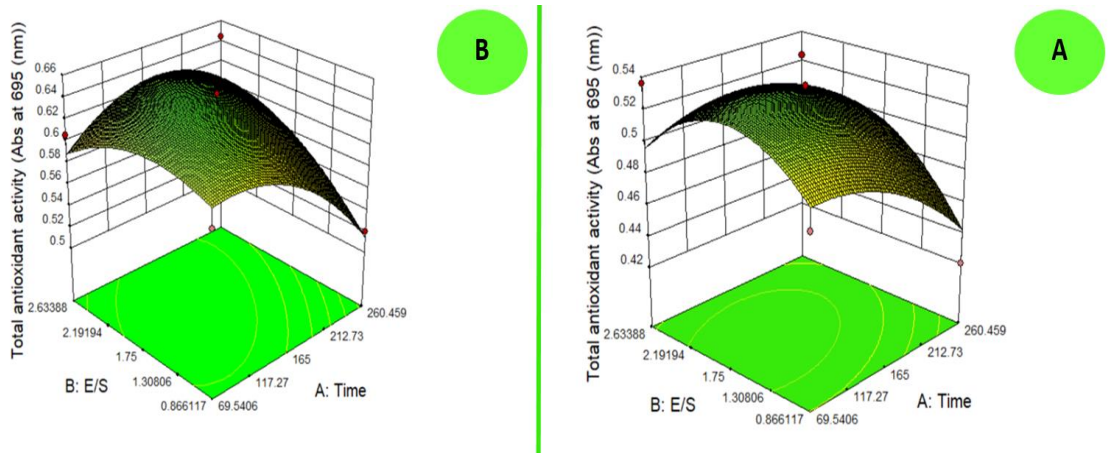

Figure 5- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on total antioxidant activity of protein hydrolysate of skipjack viscera (A: hydrolysis with pepsin, B: hydrolysis with trypsin)

7-3- The effect of time, the ratio of enzyme to substrate and the type of protease Nitric oxide radical scavenging activity

Nitric oxide free radical via enzyme oxidation of arginine to citrullineNitric oxide-Synthetase is produced [44]. Studies have shown that nitric oxide free radical is produced in the reaction of nitrogen with reactive oxygen species and causes dangerous diseases such as osteoporosis, diabetes, cancer and cardiovascular diseases [45].The results of the analysis of variance mentioned in Table 7, all the investigated variables except the hydrolysis time had a significant effect on the nitric oxide radical inhibition activity of the hydrolyzed protein resulting from the activity of pepsin. On the other hand, only the influence of variablesA^2 AndB^2 It was significant on the nitric oxide radical inhibition activity of trypsin hydrolyzates (05/0>p). Nitric oxide radical scavenging activity of hydrolyzates produced by pepsin and trypsin with quadratic reaction parameters and regression coefficient respectively9121/0R^2= And8570/0R^2= Was. The high regression coefficient and non-significance of the misfit factor indicate the appropriate ability of the model to predict the effect of process variables on the desired response.The nitric oxide radical inhibition activity and the variables of the hydrolysis process with pepsin and trypsin follow the relationship (15) and (16) better.

(15)

nitric oxide radical inhibition activity = + 3.23 + 53.52 A + 8.34 B + 11.23 AB – 15.27 A2 – 9.48 B2

(16)

nitric oxide radical inhibition activity = + 9.46 + 64.86 A + 8.28 B + 6.23 AB – 19.19 A2 – 17.90 B2

-

Table 7. ANOVA of NO radical scavenging activity of protein hydrolysate with pepsin and trypsin

	Sum of squares	df	value P	Estimate the coefficient
				Hydrolysis with pepsin Hydrolysis with trypsin
Model	2615.75	5	0.0113	53.52

	4426.90	5	0.0358	64.86
A-time	83.55 715.40	1 1	0.2543 0.0791	3.23 9.46
B- E/S	556.18 548.12	1 1	0.0210 0.1121	8.34 8.28
AB	504.68 155.38	1 1	0.0250 0.3522	11.23 6.23
A^2	1317.46 2079.745	1 1	0.0037 0.0133	-15.27 -19.19
B^2	507.68 1809.04	1 1	0.0247 0.0173	-9.48 -17.90
Lac of fit	251.03 738.13	3 3	0.0691 0.0615	

* A: hydrolysis time B: enzyme to substrate ratio

According to Figure 6A, at constant times of hydrolysis, increasing the enzyme concentration up to 1.75% increased the nitric oxide radical inhibition activity of hydrolyzed pepsin, but further increase in enzyme concentration reduced the ability to inhibit the nitric oxide radical of the resulting hydrolyzates. On the other hand, at a constant concentration of pepsin enzyme, increasing the hydrolysis time up to 165 minutes had a positive effect and further increasing the hydrolysis time had a negative effect on the ability to inhibit nitric oxide radicals. In general, the highest amount of nitric oxide radical inhibition activity (56.72%) of the hydrolyzed products was obtained from the activity of pepsin in the hydrolysis time of 196.54 minutes and the concentration of the enzyme was 2.26%. In the case of the trypsin-derived hydrolysates, as shown in Figure 6B, at constant enzyme concentrations, increasing the hydrolysis time up to 165 minutes, the nitric oxide radical scavenging activity of the produced hydrolysates increased, and a further increase in the hydrolysis time had a negative effect on this ability. had On the other hand, at fixed times of hydrolysis with trypsin, increasing the concentration of the enzyme up to 1.75% increased the nitric oxide radical inhibition activity, and this ability decreased with further increase in the concentration of the enzyme. In general, according to the graph, the highest nitric oxide radical inhibition activity (46.5%) was obtained from trypsin enzyme activity at 1.94% enzyme concentration and 200.037 minutes hydrolysis time.The increase in nitric oxide radical inhibition activity with appropriate increase in hydrolysis time can be due to the increase in hydrolysis and as a result the more hydrophilic properties of the produced peptides, which have been found to have a greater ability to inhibit nitric oxide radicals. In general, the nitric oxide radical inhibition activity of hydrolyzed proteins depends on various factors, including the amino acid composition, the degree of hydrolysis, the type of enzyme used in the hydrolysis process, and the hydrolysis conditions [46]. Similar to the findings of this research, Shariat Alavi et al. (2017) with the hydrolysis of tomato seed protein with alcalase enzyme stated that with the appropriate increase in the hydrolysis time, the nitric oxide radical scavenging activity of the hydrolyzed protein increased, but increasing the concentration of the enzyme had a negative effect on this ability. 47 [. Also, in accordance with these findings, Sadeghi Mahonek and Kaveh (1401) reported with the hydrolysis of pumpkin meal protein that the hydrophilic property of the produced hydrolyzed protein has a positive effect on their nitric oxide radical inhibition activity [46].

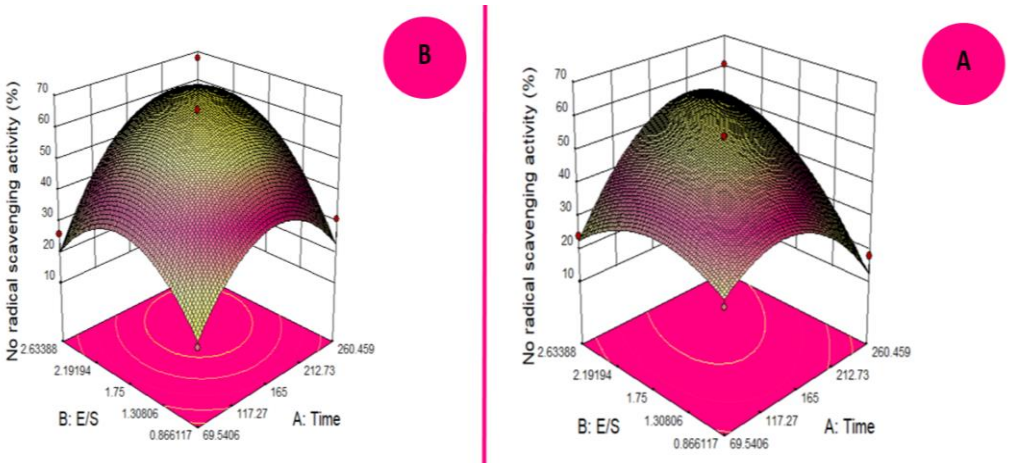

Figure 6- The effect of hydrolysis time, enzyme to substrate ratio and proteases type on NO radical scavenging activity of protein hydrolysate of skipjack viscera (A: hydrolysis with pepsin, B: hydrolysis with trypsin)

8-3- Selection of optimal treatment and validation of the model

After analyzing the data, the software predicted that the highest DPPH radical scavenging activity (35.47%), capacityAntioxidant total (0.509 absorbance at 695 nm), nitric oxide radical inhibition (54.18 percent), iron ion reduction (0.582 absorbance at 700 nm) and iron ion chelation (39.37 percent) with pepsin enzyme in The hydrolysis time is 179.09 minutes and the enzyme concentration is 2.63%. On the other hand, the highest level of DPPH radical inhibition activity (50.71 percent), capacityAntioxidant total (0.640 absorbance at 695 nm), nitric oxide radical inhibition (60.32 percent), iron ion reduction (0.499 absorbance at 700 nm) and iron ion chelation (41.15 percent) with trypsin enzyme in The hydrolysis time was 143.62 minutes and the enzyme concentration was 1.94%. In this situation, the software predicted that the degree of hydrolysis resulting from the activity of trypsin will be higher than that of pepsin, 17.39% and 15.93%, respectively. In order to evaluate the validity of the model, hydrolysis was performed under these conditions. DPPH radical scavenging activity, capacityAntioxidant total, inhibition of nitric oxide radical, iron ion reduction and iron ion chelating activity with the enzyme pepsin beteratib 34.25%, absorption 0.584 at 695 nm, 50.24%, absorption 0.635 at 700 nm and 42.51% and With trypsin enzyme, 52.35%, 0.584 absorbance at 695 nm, 60.35%, 0.523 absorbance at 700 nm and 42.47% were calculated respectively. The degree of hydrolysis resulting from the activity of trypsin was also higher than pepsin and beteratib by 19.25% and 15.18%. These results indicate the very good ability of the investigated model to predict the effect of hydrolysis time variables and enzyme concentration on the hydrolysis process with pepsin and trypsin enzymes.

4 - Conclusion

In this research, the effect of hydrolysis conditions (hydrolysis time and enzyme concentration) and type of protease on the antioxidant properties and the degree of hydrolysis of the hydrolyzed protein obtained from the intestines and viscera of Muscat Hoover fish were evaluated. The results showed that the intestinal and visceral protein of Hoover Muscat fish is rich in antioxidant peptides, which can be released by hydrolysis under appropriate conditions and using pepsin and trypsin enzymes. The antioxidant properties of the produced hydrolyzed protein were affected by the enzyme type, hydrolysis time and

enzyme concentration. Comparing the antioxidant activity of the hydrolyzed protein resulting from the activity of pepsin and trypsin showed that the hydrolyzates resulting from the activity of trypsin had more antioxidant capacity than pepsin. In general, according to the results of this research, the hydrolyzed protein of the intestines and viscera of Hoover Muscati fish obtained from trypsin enzymein hydrolysis time 143.62 minutes and enzyme concentration 1.94%, has a very good antioxidant potential, which can compete with synthetic antioxidant compounds that are widely used in the food industry after conducting clinical studies and evaluating its toxicity, and as a compound with high added value in food formulations to produce useful products and supplements Food for athletes and elderly people.

5- Resources

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بررسی خواص آنتی اکسیدانی پپتیدهای زیست فعال تخلیص شده از ضایعات حاصل از فرآوری ماهی هوور مسقطی(*Katsuwonus pelamis*) با استفاده از آنزیم های گوارشی پپسین و تریپسین

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چکیده

در صنایع فرآوری ماهی‌ها ۷۰-۵۰ ٪ ماهی اولیه به‌عنوان ضایعات تولید می‌شوند درحالیکه منابع غنی از پروتئین و آمینواسیدهای ضروری هستند. استفاده‌ی بهینه از این ضایعات و تولید ترکیبات با ارزش افزوده‌ی بالا که خواص سلامتی بخش قابل توجهی داشته باشند از چالش‌های مهم صنایع فرآوری ماهی‌ها است. در این پژوهش تاثیر شرایط هیدرولیز (زمان: ۳۰۰-۳۰ دقیقه و غلظت آنزیم ۳-۰/۵ درصد) و نوع پروتئاز (پپسین و تریپسین) بر درجه هیدرولیز و ویژگی‌های آنتی اکسیدانی (مهار رادیکال DPPH، شلاته کنندگی یون آهن، مهار رادیکال نیتریک اکسید، ظرفیت ضداکسایشی کل و احیاء کنندگی یون آهن) پروتئین هیدرولیز شده حاصل از امعاء و احشا ماهی هوور مسقطی با استفاده از روش سطح پاسخ ارزیابی شد. نتایج نشان داد که شرایط بهینه برای تولید پروتئین هیدرولیز شده با بیشترین ویژگی‌های آنتی اکسیدانی با آنزیم‌های پپسین و تریپسین به‌ترتیب عبارت بودند از: زمان هیدرولیز ۱۷۹/۰۹ و ۱۴۳/۶۲ دقیقه و غلظت آنزیم ۲/۶۳ و ۱/۹۴ درصد؛ در این شرایط درجه هیدرولیز پروتئین‌های هیدرولیز شده‌ی حاصل از فعالیت تریپسین بیشتر از پپسین محاسبه شد. مقایسه ویژگی‌های آنتی اکسیدانی هیدرولیز شده‌های حاصل از دوآنزیم مورد استفاده نشان داد که پروتئین هیدرولیز شده حاصل از تریپسین از پتانسیل آنتی اکسیدانی قوی‌تری نسبت به پپسین برخوردار بود. بنابراین می‌توان بیان نمود که پروتئین هیدرولیز شده امعاء احشا ماهی هوور مسقطی با استفاده از آنزیم تریپسین به‌عنوان یک ترکیب سلامتی بخش و با ارزش افزوده بالا قابلیت کاربرد در تولید محصولات غذایی فراسودمند و مکمل‌های سلامتی بخش برای ورزشکاران و افراد سالمند را دارد.