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Study Antioxidant and Functional Properties of Protein Hydrolysate Prepared from *Silurus glanis* Skin Using Papain Enzyme

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Abstract: This study aimed to prepare protein hydrolysate from the hydrolysis of silurus *glanis* skin using papain enzyme, and study the functional properties and antioxidative activity. Crud protein extracted from the same fish skin used for comparesion. Fish skin were mixed with phosphate buffer (pH 7.0, 0.2 M) and placed on magnetic stirrer for 3, 6, 9, 12 hour at 45c° after addition of enzyme (1mg / 100gm skin).the crud protein extracted designated as (H) and the obtained hydrolysate as (P1, P2, P3 and P4). The percentages of soluble nitrogen and total nitrogen in skin were (0.14) and (3.15) % respectively. The degree of hydrolysis for the obtained hydrolysates were (33.3) ، (21.1),(1 .51) , (54.28)% for P1, P2 , P3, P4 respectively. The results of functional properties and antioxidative activity showed that the percentages of water holding capacity were (3)% for (H) and (2, 2, 1, 1 %) for P1, P2, P3 and P4 respectively. The solubility values were (12.8, 43.4, 64.7, 75, 92) % for (H, P1, P2, P3 and P4) respectively. The emulsion capacity values were (20 ml) for (H) and (12, 13, 14, 16 ml) for P1, P2, P3 and P4 respectively. It has been noticed that the reducing power values were (0.591 -1.21) , (0.540 – 1.11) for BHT and citric acid respectively, and (0.403 – 0.754) for (H) and (0.350 - 0.570) , (0.444 – 0.992) ,(0.526 – 0.642) , (0.449 – 0.769) for P1, P2 , P3 and P4 respectively .

Keywords: Protein hydrolysate, papain, antioxidante, fish skin.

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Introduction:

Fish are important sources of food for humans and are an important source of animal protein in most countries of the world and in some of them make up 50% of the total protein consumed, and importance of many studies have shown that wastes, which include (bones, viscera, skin) Approximately 50% of the weight of fish is a good source of protein, including enzymes, as well as fat (1-3), and good substrates for the fermentation of lactic acid (4). Large amounts of these residues remain a major environmental problem as they are eliminated without any effort to restore protein (6,5), or benefit from them in different fields. Many researchers have resorted to the possibility of exploiting these wastes and making them of vital value by converting them into protein hydrolysate using protein-modifying enzymes (8,7) or treating them thermally or chemically. The enzymes that are used to produce dietary protein It was of bacterial origin that the living organisms produced by these enzymes should be non-succulent and non-toxic (*Silurus glanis*) is a commercial fish spread in Iraq and other countries, they were selected to take advantage of their skins in protein hydrolysate synthesis By the enzyme papain . The aim of this study is to evaluate the chemical

structure and some functional properties of proteolytic proteins prepared by enzymatic hydrolysase (Water capacity, emulsification, and solubility), as well as the reduced ability of these metabolites

Materials and methods:

Raw protein extract for fish skin:

Silurus glanis skin which was available in the local markets of Baghdad City. The extraction process was done after washing and cleaning the skin of the fish from any impurities stuck and then cut into small pieces ranging in size between 10-15 cm

Enzymatic hydrolyses using papain enzyme:

Mixed 400 g of skin with three times the weight of sodium hydroxide solution (pH 7.0 and 0.2 molar). the mixture was subjected to a process of pasteurization at a temperature of 90 - 95 ° C for 7 - 10 minutes to stop the work of the enzyme and then add the enzyme at a concentration of 1 mg per 100 g skin ,and then added 0.8% chloroform to maintain the decomposition of microbial damage and put in a shaking incubator at 45 ° C and 100 cycles / min for different times (4, 7, 10, 13) With the pH at 7.0 for the duration of the digestion. These parameters were indicated by p1, p2, p3 and p4. After the digestion period, a 90 °C water bath was placed for 20 min to stop enzyme activation. The samples filtered with a soft cloth to remove undigested proteins the fatty layer was removed and dried using vacuum oven at 50 ° C and kept in clean bottles until use.

Total Nitrogen Determination:

Total nitrogen was estimated by the Semi-Micro Kjieldahl method described by Pearson (9) and the protein ratio was calculated by multiplying the nitrogen content by the conversion factor (6.25).

Total dissolved nitrogen:

Total dissolved nitrogen (TSN) was estimated by crushing 10 g of the sample then adding a sufficient amount of KCl (0.5 molar) solution and mixing well. The mixture was then transferred to a 100 mL glass flask and completed to the mark with the same solution. leave the mixture for 30 minutes with a shake from time to time and then centrifuged for 15 minutes at a speed (850 xg). Then the supernatant was taken and nitrogen was dissolved in a Kjieldahl manner. The results were then calculated on the basis of wet weight according to the method described by Kline and Stewart (10) and the degree of degradation of the protein analyzes was estimated as the formula below :
Degree of decomposition (%) = Total dissolved nitrogen / Total nitrogen * 100

Determination of functional properties of protein hydrolysate:

The solubility and emulsification tests were performed according to the method suggested by the Hindi (11).

Solubility:

The percentage of solubility was estimated by mixing 1 g of powdered protein with 100 ml of distilled water and mix for 45 min at 30 ° C. The solution was then filtered and leachate

was extracted. Its content was estimated from total dissolved nitrogen (TSN) and the percentage of solubility of each Ps model was calculated on the percentage of the total protein of the sample (Pt) according to the following equation:

Solubility (%) = TsN/ pt *100

Water-Holding Capacity (WHC):

Water-Holding Capacity was measured according to the method described by Beuchart (12) by taking 1 g of the decomposed powder sample with 100 ml of distilled water and homogenize for 1 min and centrifuged at (1085 xg) for 5 min.

The water Capacity ratio was calculated as follows:

Water capacity (%) = (water weight added to model - water weight after centrifugal) / weight of model * 100

Emulsification:

Take 1 g of decomposed powder sample and mix with 50 ml distilled water and 100 ml corn oil in the electric mixer for 2 minutes and put in cylinder at 25 ° C and set the emulsion stability after 24 hours.

Measurement of Reduction Power:

The reduction of the hydrolytic power was calculated according to Chou *et al*. (13) method, which included mixing 1 ml of the extract with different concentrations ranging from 10 - 50 mg/ ml with 2.5 ml of 1% Potassium Ferricyanide solution and 2.5 mL of phosphate solution 0.2 molar at pH 6.6, followed by 50 ° C incubation for 20 minutes. Then add 2.5 ml of Trichloro acetic acid 10% and then the centrifugation process at 4000 r/m, then mix 2.5 mL of filtrate with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and leave mixture for 30 min. The absorption was then measured at a 700 nm. BHT and citric acid were used as comparison models, and increased uptake of the reaction mix indicated increased reduction.

Results and discussion:

Determination of nitrogen ratios for prepared protein analyzes:

The dissolved nitrogen ratio may be due to the difference in the duration of the degradation and the degradation levels, as the high degradation levels lead to the increase of non-protein nitrogen substances, including free amino acids, which is consistent with what (14) Proteases with a specialized action analyze the peptide bonds of the protein molecule releasing the free amino acids back to the center of the reaction.

As for the degree of decomposition, (Table 1) shows the percentages of dissolved nitrogen and the degradation levels of the tested samples. The percentage of dissolved nitrogen in the crude protein extract (H) was 0.35%. The total nitrogen ratio in the raw skin was 3.15% The decomposers had different percentages of soluble nitrogen (0.665), (1.05), (1.61) and (1.71)% for product (1P), (P2), (P3) and (P4), respectively.

The decomposition of these analyzes was (21.1), (33.3), (51.1) and 54.28% (for product 1P, P2, P3 and P4 respectively). The results showed an increase in the degree of degradation as the time period of the treatments increased, reaching a maximum of 54.28% after 12 hours, while the

decomposition retained acceptable properties.

See *et al.* (15) achieved a degradation rate of 77.03% for salmon skin lesions using alcalase enzyme at 55 ° C and pH 8.3, the optimal conditions for alcalase activity. Bhaskar and Mahendrakar (16) found that the degree of decomposition of the alcatrae algae was 49.6%.

Table (1): The total dissolved nitrogen values and the degree of degradation of the crude protein extract and proteolytic analyzes.

Sample	Total Dissolved Nitrogen TSN%	Degreasing TSN / TN%
Raw Extract (Zero time) (H)	0.35	-
Dissolved (p1) after (3) hours	0.665	21.1
Dissolved (P2) after 6 hours	1.05	33.3
Dissolved (P3) after 9 hours	1.61	51.1
Dissolved (p4) after (12) hours	1.71	54.28

Total Nitrogen in Raw Skin TN% (3.15)

Functional properties of proteolytic proteins under study:

Water capacity:

(Table 2) shows the water capacity of the crude protein extract in this study the protein analyzes prepared (3%) for H, (2, 2, 1 and 1) for P1, P2, P3 and P4 respectively, the completely insoluble proteins increase the absorption potential of dissolved proteins and reduce the amount of absorbed water (17). The results of the present study were similar to those of (13). The water capacity values of the herbivores were estimated to be between 2 - 4% and were very similar to the water capacity of the proteolytic species under study. Diniz and Martin (18) and Wasswa *et al*. (19) reported that the water carrying values of shark protein-protein analyzes were 4-15%. The protein's ability to hold and carry water is of great importance when used in different food products. It may affect the sensory properties of food as proteins vary in their ability to bind water (20).

Table (2): The capacity of the water and solubility values of the crude protein extract to the prepared protein analyzes

Models	Water carrying capacity%	Solubility%
Raw Extract (zero time) (H)	3	12.8
Dissolved (p1) after (3) hours	2	43.4
Dissolved (p2) after (6) hours	2	64.7
Dissolved (P3) after 9 hours	1	75
Dissolved (p4) after (12) hours	1	92

Solubility:

(Table 2) showed that the solubility of the crude protein extract (H) was 12.8 and the prepared protein (P1, P2, P3 and P4) was 43.4, 64.7, 75 and 92% respectively, (22), (21) found that the use of enzymes degradable is necessary to break down the protein into small units and then the amino and carboxylic groups of amino acids become ionized as well as increasing the groups of water The reason for the high solubility of proteolytic proteins is due to the openness of the protein and the availability of water-loving aggregates for water and ionic aggregates, thus increasing solubility, which is an

important indicator of protein conversion. Several studies have shown that increasing the degree of decomposition (DH) increases the proportion of dissolved proteins and thus increases solubility of the analyzes, including silver catfish catheters, salmon (23) and yellow tuna decomposers (24). These results were consistent with the results of the present study. Protein solubility or solubility is an indication of the extent to which it can be used or not for use in a particular food. It therefore gives useful information in the ideal manufacturing methods and thus identifies the thermal parameters that may affect the possibility of using this protein in different diets.

Emulsification property:

(Table 3) shows the size of both the emulsion layer and the water layer of the crude protein extract and the proteinuria under study, which was prepared by mixing 1 g of samples with 50 ml distilled water with 100 ml oil and left for 24 hours. The emulsion layer after 24 hours for crude protein extract (H) (20) and P (P1, P2, P3 and P4) (16, 14, 13 and 12) respectively, showing that the emulsifying stability of proteinuria decreases with increasing degradation. (25), (19) demonstrating that the stability of the emulsifier decreases with the increased degree of degradation of proteolytic analyzes. . This is because the degree of degradation (DH) leads to the formation of smaller peptides, which are less effective in achieving the persistence of emulsions and the reduction of water-related groups and changes in the size of peptide during the process of decomposition (25). Jalili *et al*. (26) Shows.

There are several factors that greatly affect emulsification properties, including temperature during emulsion formation, emulsion type, size of fatty droplets and emulsion viscosity, as well as the movement of emulsion vibration. Li-Chan *et al*. (27) reported that the effect of high temperature on the amplification capacity of proteins separated from fish and observed that a temperature higher than 45 c leads to reduced amplification capacity of separated proteins significantly, and attributed the cause to the mutagenic proteins, the surface of the protein in terms of water-distressing groups.

Table (3): Stabilization of emulsifier for crude protein extract and proteinurized preparations prepared when using 1 g powder + 50 ml distilled water + 100 ml oil

Models \ Duration For emulsification (hour)		0	1/2	1	2	3	4	5	6	24
H	Emulsion layer	56	21	21	21	21	21	20	20	20
	Water layer	0	35	35	35	35	35	36	36	36
P1	Emulsion layer	56	17	17	17	17	16	16	16	16
	Water layer	0	39	39	39	39	40	40	40	40
P2	Emulsion layer	56	15	15	15	15	15	14	14	14
	Water layer	0	41	41	41	41	41	42	42	42
P3	Emulsion layer	56	14	14	14	14	14	14	13	13
	Water layer	0	42	42	42	42	42	42	43	43
P4	Emulsion layer	56	13	13	13	13	13	13	12	12
	Water layer	0	43	43	43	43	43	44	44	44

Measurement of Reducing Power:

(Figure 1) shows the reduced power of samples under experiment when used in different concentrations

Ranged between 10 - 50 mg / ml of sample and the reduction of BHT and citric acid in the same concentrations. It was observed that the relation between the reduction of all the samples was increased with the addition of the concentrations. Reduction of the industrial antioxidant BHT and citric acid in all P3 and P4 concentrations, some studies (28, 29,30) indicated the use of different enzymes in the analysis of fish gelatin and the use of the resulting as antioxidant. The result was increased of these neutralizers to reduction The free radicals are generally characterized by their antioxidant effect according to the degree of degradation, as well as the difference in the quality of the enzyme used in the analysis. This may be due to the fact that the different degrees of degradation give peptides with different amino acids and therefore their potential as different antioxidants. The size, rotation and synthesis of amino acids in the peptide, the enzymatic degradation clearly affects the degradability of the decomposer.

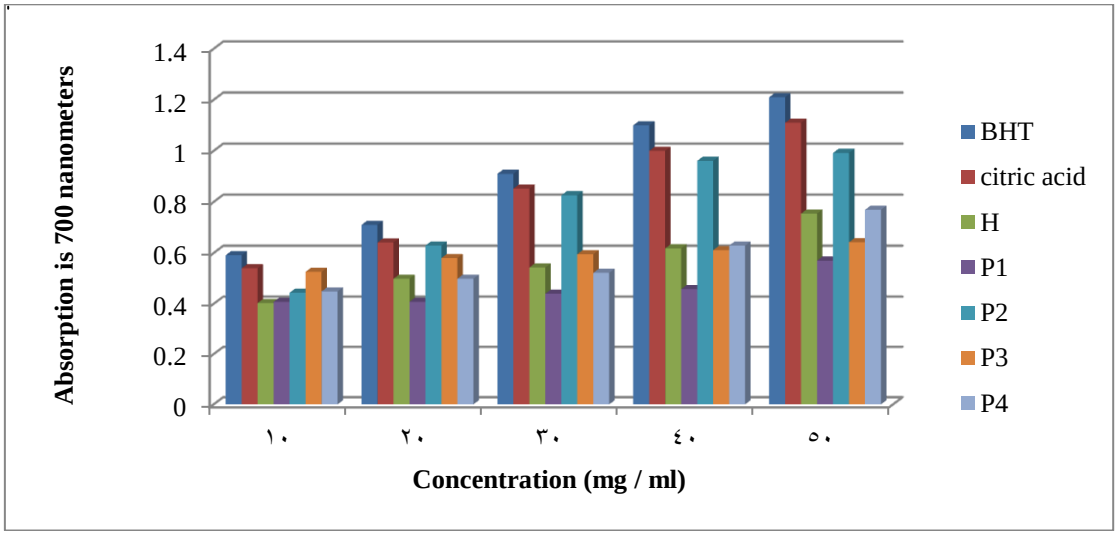

Figure (1): The reduced strength of the crude protein extract and the prepared protein analyzes compared with the industrial antioxidant BHT and citric acid.

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