Enzymatic Protein Hydrolysates of Common Carp Fish: I. Functional Properties and Molecular Weight Distribution

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ABSTRACT

The present work aims to investigate the preparation of a value added product from common carp fish in the form of a protein hydrolysate. Protein hydrolysates were prepared using papain, bacterial protease, and bovine protease. The optimum conditions for proteolytic activity of enzymes were investigated. This was followed by the determination of the rate of enzymatic degradation. The effect of the enzymatic hydrolysis on the molecular weight distribution of the protein was carried out by gel chromatography on Sephadex G-100. The protein hydrolysates were examined with respect to their content of biogenic amines, then analysed for their functional properties. Protein content of fish hydrolysates ranged between 85-89%. Rate of enzymatic degradation showed that papain enzyme resulted in higher degradation of the protein than bacterial and bovine proteases, thus more solubilization of protein was achieved. Gel chromatography of the three hydrolysates demonstrated the degradation of protein with molecular weight 90,000 Dalton to peptides with molecular weight 2,500 Dalton. Amino acid analysis indicated that protein hydrolysates were rich in lysine and sulphur containing amino acids. Biogenic amines were found to be within the permissible levels. Enzymatic hydrolysis generally improved solubility and wettability of proteins.

Key Words: common carp, enzymatic hydrolysis, proteases, functional properties, molecular weight.

INTRODUCTION

Man has eaten fish since early civilization; fish have served as one of the major sources of animal protein and lipids. However, fish have markedly gained popularity in recent years owing to their functional food components such as omega fatty acids and antioxidant properties. Although seafood proteins are important by possessing a well balanced amino acid composition, yet many species of fish remain underutilized. Small size, high bone content, unappealing shape and look, fatty natures are among the drawbacks that make fish underutilized (Shahidi, 1998).

Therefore production of novel protein preparations from these fish species has been attempted. These include protein concentrates, dispersions, hydrolysates, surimi and fish sauce among others (Shahidi, 1998). The application of enzyme technology for converting fish processing waste and underutilized fish species into value added products has attracted considerable interest.

Many investigators reported that proteolytic enzymes could be used for the preparation of fish protein hydrolysates from different fish species. Cheftel *et al*. (1971) studied the enzymatic solubilization of fresh Red Hake to be applied on continuous solubilization process of fish protein concentrate. Pepsin and pronase were found particularly effective. Quaglia & Orban (1990) studied the influence of enzymatic degradation of defatted comminuted sardines by alcalase. Results showed that the emulsifying properties, the surface hydrophobicity and the high molecular weight fraction decreased as degree of hydrolysis increased. Rebeca *et al*. (1991) eviscerated mullet fish and hydrolyzed them with three bacterial proteases. Protein was solubilized faster with pescalase 560, increasing the protease concentration was associated with a quadratic increase in soluble nitrogen. Shahidi *et al*. (1994) used microbial enzymes, alcalase and neutrase to hydrolyze muscle proteins of harp seal. The yield and degree of hydrolysis by alcalase under optimum conditions were 92.75% and 19.5%, respectively, whearas it was 72.85% for neutrase. Gilmartin & Jervis (2002) used alcalase in combination with other commercial enzyme preparations to produce cod muscle hydrolysate. The enzyme preparations containing predominantly protease or endopeptidase activities achieved high degree of hydrolysis and produced a significant amount of peptides below a molecular weight of 3000 Dalton. Sathivel *et al*. (2003) evaluated the functional and nutritional properties of hydrolyzed herring and herring by-products. Protein hydrolysate powders were light yellow and contained 77% to 87% protein. The emulsifying capacity and stability of all

fish protein hydrolysate powders were lower than those of egg albumin and soy protein. Sathivel *et al*. (2005) studied the effect of different proteolytic enzymes and different reaction durations on functional and nutritional properties of red salmon hydrolysate. They found that the degree of hydrolysis was correlated with to the hydrolysate solubility. Maximum emulsifying stability and fat absorption were achieved at lower degrees of hydrolysis. Fish protein hydrolysate was produced from fish soluble concentrate, a by- product from canned fish industry (Nilsang *et al*., 2005). The spray dried fish protein concentrate produced with flavourzyme, contained high protein content and the bitterness was less than 1ppm as caffeine solution.

Common carp fish locally known as "El-Mabrouk" in Egypt is a cheap underutilized fish grown in mixed fish farms; it is not desirably palatable because of its high bone content. The aim of the present study was to prepare a value added product in the form of a fish protein hydrolysate from "El-Mabrouk" fish. Three proteolytic enzymes of different origin were employed, papain (Pa.) of plant origin, a bovine protease of animal origin (Bov.P), and a bacterial protease (Bac.P) of bacterial origin. Relative activity, optimum conditions of enzyme activity and rate of enzymatic hydrolysis as a function of time were determined. Molecular weight of the protein hydrolysates was estimated by means of gel filtration. Analysis of the protein hydrolysates included: amino acid composition, biogenic amines, and functional properties were also studied.

MATERIALS AND METHODS

Materials

Fresh Common Carp fish (*Cyprinus carpo* L.) with average weight 1.43 kg and length 50 cm were obtained from Abbassa Research Centre-Abou Hammad, Sharkeiah. The fish was headed, gutted, skinned then minced. The minced fish was soaked in 2% sodium bicarbonate for 1 hr. to ease the defatting process then the minced fish was washed, defatted using by n-hexane until the oil in the meat did not exceed 1%.

Proteolytic enzymes: Papain, Bovine and Bacterial Protease were obtained from Sigma chemicals Co. (St. Louis. Missouri, USA). Papain, (Papaya Latex, MW 21 kDa) Product of Sri-lanka. Bovine protease from pancreas (MW 27 kDa) product of Germany. Bacterial Protease (Bacillus Licheniformis), (MW 27 kDa) product of Denmark.

Methods:

Relative activity of enzymes

The relative activity of the enzymes was determined according to the method described by Arzu *et al*. (1972). A hundred milliliters of water were added to a minced fish sample (30g) in a 250 ml beaker and was placed in a thermostatic water bath. The mixture was continuously stirred with an electric stirrer. The pH and temperature were adjusted for each enzyme within its optimum range. The pH was 6.2, 7.5, 7.5 and temperature was 25°C, 37°C, 37°C for papain, bovine protease, bacterial protease, respectively. The enzyme was added at the tested concentration and the experiment was carried out while stirring occasionally for 30 min. The reaction was stopped by lowering the pH to 3.0, by adding drops of 0.01 N HCl, and raising the temperature to 80°C. The hydrolysate was filtered through Whatman No. 4 filter paper and the residue was washed with distilled water until an approximate volume of 250 ml was collected. A control experiment was carried out without the addition of the enzyme. Aliquots from the filtrate were analyzed for total nitrogen using the Kjeldahl method and the relative activity was calculated using the following equation:

$$\text{Relative activity} = \frac{\text{Hp-Cp x 100}}{\text{Cp}}$$

Where:
Hp: total protein solubilized in enzyme assay
Cp: total protein in control.

Determination of optimum conditions for proteolytic enzymes

This was accomplished through a series of experiments:

In the first set of experiments, the papain was used together with sodium sulphite (to activate enzyme) using fish as substrate. The relative activity was determined as mentioned previously.

The first investigated criterion was the papain concentration. Concentrations used were 550, 1100, 1650, 2200 and 2705 units (enzyme unit is the amount of enzyme that hydrolyzes 1.0 μmole of N-α-benzoyl-L-arginine ethylester (BAEE) per minute at pH 6.2 at 25°C) and the experiment proceeded as in determination of relative activity.

The pH was the second investigated criterion including pH(s) 6, 6.2, 6.4 and 6.6. The third investigated criterion was the temperature which ranged between 20, 25, 30 and 35°C.

In the second set of experiments, the bovine protease was used. The third investigated criterion was enzyme concentration 3650, 7300, 14600, 21900 and 29200 units [Enzyme unit is the amount of enzyme that hydrolyzes casein to produce colour equivalent to 1.0 µmole (181µg) of tyrosine per minute at pH 7.5 at 37°C (colour by Folin-Ciocalteu reagent)], the pH(s) were 7.3, 7.4, 7.5 and 7.6 and the temperatures were 30, 35, 40 and 45°C.

In the third set of experiments, the bacterial protease was used. The enzyme concentrations were 621.15, 1242.3, 1863.45, 2484.6, and 3726.9 units [enzyme unit is the amount of enzyme that hydrolyzes casein to produce colour equivalent to 1.0 µmole (181µg) of tyrosine per minute at pH 7.5 at 37°C (colour by Folin-Ciocalteu reagent)], the pH(s) and the temperatures were as for bovine protease. Time was fixed for 30 min for the three previous set of experiments.

Enzymatic hydrolysis of fish protein

The hydrolysis process of minced fish is illustrated in Fig. (1), according to Shahidi *et al*. (1994). The hydrolysis experiment was carried out using minced filleted fish (MFF) with the three enzymes under the optimum conditions previously determined. A sample of 500 grams of minced fish was mixed with an equal volume of distilled water and homogenized in a waring blender for 2 min. The mixture was placed in a thermostatic water bath and subjected to continuous stirring. The temperature and pH were adjusted according to the optimum condition previously determined for each enzyme, then the enzyme was added (E/S previously determined) and the mixture was continuously stirred for 3 hr., monitoring the temperature and pH throughout hydrolysis. Then the pH was lowered to 3.0 and temperature was increased to 60°C for 15 min to inactivate the enzymes. Decolourization was carried out with 2% charcoal and constant stirring for 20 min. at 55°C. After decolourization and filtration using whatman no. 4 filter paper, the samples were subjected to freeze drying (-40°C, m bar =1) using (Edwards Modulo Freeze Drier, Germany)

Determination of the rate of enzymatic degradation

Ten ml aliquots were taken during the hydrolysis experiment at 30 minutes intervals and carrying out formal titration. The amount of 0.1N NaOH consumed by the formal titration was plotted against time of the reaction. The milliliters of NaOH represent the amount of –COOH groups present in the 10 ml hydrolysate. The acidity as shown by the titration is an approximate measure of the amino nitrogen present (Taha & Ibrahim, 2002).

Determination of molecular weight distribution of the hydrolysates

The molecular weight distribution of the three fish hydrolysates was carried out by gel chromatography on Sephadex G-100 in a glass column diameter (45×2.5 cm^2) to height ratio of 1:18 that would

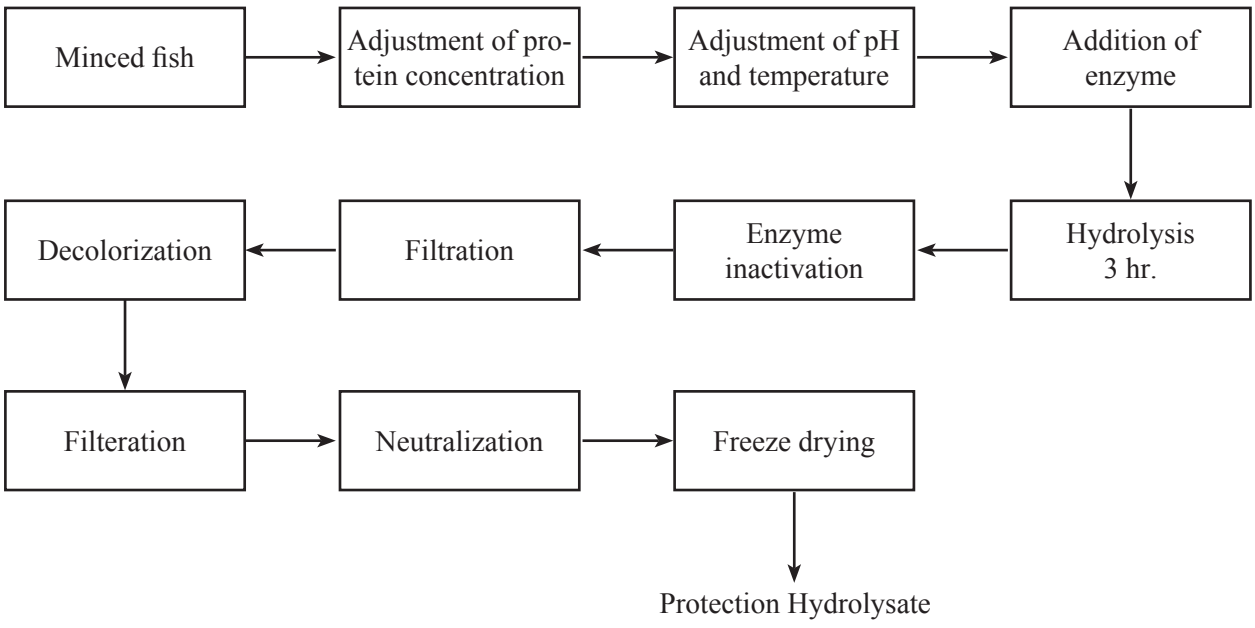

Fig. 1: Schematic representation of the hydrolysis process of minced fish

give a bed volume of 210-260 ml according to Andrews (1964) and Fox & Tarassuk (1968). The first ten milliliters eluted from each sample were collected from the column. This value is considered as the void volume (Vo). The rate of elution was maintained at 5ml / 7 sec. The effluent was monitored at 280 nm by a UV detector connected to a recorder. Egg albumin (MW 42 KDa), hemoglobin (MW 17 KDa) and insulin (MW 6.5 KDa) (Sigma Chemicals Co., st. Louis, Missouri, USA), were used as standard proteins. Fractions of 10 ml were collected and analyzed for the effect of the enzymatic hydrolysis on the molecular weight, by the method of El Tanboly (2001). A control sample of unhydrolysed fish was run for comparison on the Sephadex G-100 column as previously described and used for the enzymatically hydrolyzed samples.

Biogenic amines determination

Histamine, putrescine, cadaverine and tyramine were extracted as follows: Five grams of samples were blended with 25ml 5% trichloro-acetic acid. Filtration was achieved using filter paper Whatman No 1. Five milliliters of the extracts were transferred into a suitable culture tube with 4g NaCl and 1 ml of 50% NaOH then shaked vigorously for 2 min. Centrifugation were carried out for 5 min. at 5000 xg and the upper layer was transferred to 50 ml separating funnel using disposable Pasteur pipette. To the upper layer extract, 15 ml of n-heptane were added and extracted three times with 1 ml portions of 0.2 NHCl. The extracts were collected in a glass stoppered tube and evaporated to dryness using water bath at 95°C with the aid of a gentle current of air. This was followed by the formation of Dansylamines as described by Maijala & Eerola (1993). Biogenic amines were determined in all tested samples according to Ayesh *et al*. (1995). The HPLC analyses was performed with an Agilent 1100 HPLC system (Agilent Technologies Waldbronn, Germany) model G1311A equipped with detector model G1314A set at 254nm wavelength, autosampler model G1329A and VP-ODS shimpack (150×4.6mm) column (Shimadzu, Kyoto, Japan). Data were intergrated and recorded using Chemstation Software Program. The gradient program used for biogenic amines separation is shown in Table (1).

Table 1: Gradient solvent program for the separation of biogenic amines by HPLC

Time (min.)	Flow Rate ml/min	Solvent (%)		
		Acetic acid (0.02N)	Methanol	Acetonitrile
0	1	60	20	20
10	1	20	40	40
15	1	15	35	50
20	1	60	20	20
25	1	60	20	20

Functional Properties:

Nitrogen solubility index (NSI)

Nitrogen solubility index was determined according to Smith & Circle (1997). Five grams sample and 200 ml water were mixed in a 400 ml beaker and the mixture was continuously stirred for 20 min with a magnetic stirrer at 30°C. The mixture was transferred quantitatively to a 250 ml volumetric flask, 2 drops of antifoaming agent were added and diluted to mark with distilled water and mixed thoroughly. It was allowed to stand for a few minutes then 40ml were taken in a 50 ml centrifuge tube and centrifuged for 10 min at 5000 xg and the supernatant was filtered through a funnel containing a plug of glass wool, the clear filtrate was collected in a 100ml beaker and aliquots were taken for nitrogen determination. Water soluble protein was calculated as follows:

$$\text{Water soluble protein \%} = \frac{(S\text{-}B) \times N \times 0.014 \times 100}{\text{Wt of Sample}}$$

Where:
S = ml of alkali back titration of sample
B = ml of alkali back titration of blank
N = normality of alkali

The nitrogen solubility index was calculated as follows:

$$\text{Nitrogen Solubility Index} = \frac{\text{\% water soluble nitrogen} \times 100}{\text{\% total nitrogen}}$$

Emulsifying capacity (EC)

Emulsifying capacity was determined as reported by Shahidi *et al*. (1995). To a sample (3-5 g), 50 ml of distilled water and 50 ml of soybean oil were added. The mixture was then homogenized for 30sec. using a Polytron homogenizer, then centrifuged for 5 min at 2000 xg and the emulsifying capacity was calculated as the ratio of emulsified versus total volume.

Wetting ability (WA)

Wetting ability means the ability of powder to be wetted. It is expressed as the time in seconds of

a certain quantity of powder needs to penetrate into a calm water surface. This is an important criterion for the instant products. The WA was determined according to Taha & Ibrahim (2002). A 400 ml wide mouth beaker was filled with 150 ml water and covered with a glass plate. A plastic hopper was adjusted above the plate with a lower aperture (30mm). The sample (1g) was poured into the hopper and then drawn aside the glass plate so the powder can drop onto the surface of the water. Time is stopped in seconds when all the powder is wetted and WA was calculated.

Flowability (FL)

Flowability was determined as described by Taha & Ibrahim (2002). Flowability signifies the ability of a powder to flow, it is measured in seconds as the time a certain powder requires to leave a funnel with 50° wall inclination and a lower opening 30 mm diameter.

Thermostability (TS)

Thermoatability was determined as described by Taha & Ibrahim (2002). Suspensions of unhydrolysed fish sample and fish hydrolysate samples were agitated on a magnetic stirrer for 15 minutes and two aliquots (0.5 ml) were assayed for protein content by the Kjeldahl method. Another two aliquots (10 ml each) were placed in screw cup test tubes and heated in a boiling water bath for 20 minutes. After rapid cooling to 22°C and centrifugation at 2000 xg for 20 min, the protein content of the supernatant was assayed. Thermal aggregation of enzyme modified hydrolysate was represented by the difference in protein content of the supernatant caused by heating.

Proximate composition

Moisture, crude protein, crude fat and ash were determined according to the methods described in the AOCS (1998).

Amino acid composition

The amino acid composition of lyophilized and powdered samples was determined by digestion with 6N- HCI at 110°C (Blackburn, 1968), the acid was then removed under vacuum and the resultant dried material was reconstituted with a lithium citrate buffer (pH 2.2). The amino acids were then quantified using a Beckman 121MB amino acid analyzer. Cysteine and methionine were subjected to performic acid oxidation prior to their digestion with 6N HCL and were determined as cysteic acid and methionine sulphone, respectively (Blackburn, 1968).

Statistical analysis

The tests were done in triplicate and data were averaged. Standard deviation was also calculated. Data were subjected to analysis of variance (ANOVA) according to McClave & Benson (1991).

RESULTS AND DISCUSSION

Protein hydrolysates were prepared from minced filleted fish (MFF) of common carp using three proteolytic enzymes namely, papain (Pa.), bacterial protease (Bac.P), and bovine protease (Bov.P). The proximate analysis of minced filleted fish (MFF) showed a moisture content (79.30%), crude protein (17.06%), crude fat (1.70 %) and Ash (1.56 %). All values are within the normal values for this fish. Thus, the common carp fish can be considered as a lean fish.

Optimum conditions for the activity of enzymes

In order to establish the optimum conditions for the three investigated enzymes namely, Pa., Bac.P, and Bov.P using the MFF protein as a substrate, the relative activities of the enzymes were determined. The relative activity of each enzyme was determined using different enzyme concentrations, pH values and temperatures, while time was 30 min.

Data in Table (2) reveal that the optimum conditions of Pa.P were E/S 0.03, pH 6.2 and temp. 25°C. Bov.P and Bac.P showed highest relative activity at pH 7.5, whearas, E/S was 0.02 and 0.01, and temperature was 35 and 40°C, respectively.

Rate of enzymatic degradation

Figure (2) is a diagrammatic representation of the rate of enzymatic degradation of MFF when using the three protease enzymes. It can be seen that when using Pa. enzyme the rate of hydrolysis increased gradually reaching maximum hydrolysis after 120 min then the degradation rate decreased sharply. On the other hand, using Bac.P and Bov.P resulted in more or less similar patterns of rate of degradation of the protein, both enzymes showed a sudden increase in the rate of degradation after 30 min which was followed by a smooth increase until 180 min. From the figure, it can be deduced that Pa resulted a more degradation and thus higher solubilization of MFF protein. Only, at the end of the hydrolysis, the rate of degradation and solubilization resulting from Pa. and Bac.P approach one another.

Table 2: Optimum conditions of the activity of the three investigated enzymes using MMF as a substrate

Enzyme		Concentration					pH				Temperature			
Pa.	E/S	0.01	0.02	0.03	0.04	0.05	6	6.2	6.4	6.6	20	25	30	35
	RA	16.69	20.87	24.43	23.37	20.47	80.8	85.17	84.41	74.65	44.5	55.37	50.03	42.83
		±0.37	±0.35	±0.40	±0.12	±0.21	±0.72	±0.31	±0.45	±0.63	±0.49	±0.55	±0.31	±0.46
Bov. P.	E/S	0.01	0.01	0.02	0.03	0.04	7.3	7.4	7.5	7.6	30	35	40	45
	RA	48.44	72.4	78.24	76.67	74.13	65.46	77.04	79.38	70.91	85.85	90.71	81	76.53
		±0.45	±0.45	±0.58	±0.40	±0.57	±0.64	±0.25	±0.46	±0.76	±0.25	±0.37	±0.62	±0.40
Bac.p.	E/S	0.01	0.01	0.02	0.02	0.03	7.3	7.4	7.5	7.6	30	35	40	45
	RA	81.63	96.21	94.4	92.83	76.2	82.13	85.53	89.48	80.43	64.43	78.13	85.07	69.37
		±0.87	±0.62	±0.49	±0.31	±0.79	±0.21	±0.15	±0.50	±0.21	±0.25	±0.40	±0.06	±0.57

RA = Relative activity of enzyme Bov.= bovine P.= protease MFF= minced filleted fish Pa.=Papain
E/S= Enzyme to substrate ratio Bac.= bacterial Results are mean values of three replicates±standard deviation

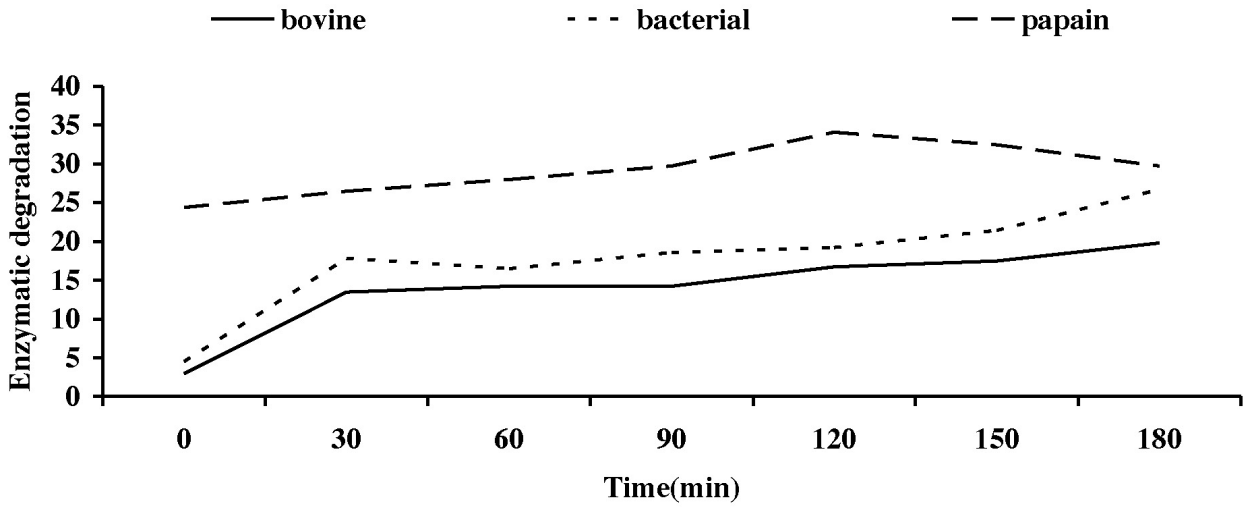

Fig. 2: Rate of enzymatic degradation of fish protein using the three investigated enzymes

Proximate composition of the three freeze dried hydrolysates resulting from hydrolysis of MFF are represented in Table (3). Data indicate that crude protein content ranged between 85 and 89%, crude fat content of all samples were less than 1%, and ash content ranged between 7.7 and 10.2%. The hydrolysates were off-white colour and bland taste. Shahidi *et al*. (1994), reported that the dehydrated seal hydrolysate contained 73% crude protein, 0.74% lipids and 20.67% minerals as sodium chloride. Rebeca *et al*. (1991) used different bacterial proteases to produce fish protein hydrolysate from mullet and reported the following chemical composition for the four prepared hydrolysates: protein 82-86%, lipids 3.3-7.7%, ash 3.3-6.2% and moisture 2.7-4.4%. Sathivel *et al*. (2003) showed the following composition of herring fish hydrolysate as 84.4% protein, 1.2 % lipids, 10.3 % ash and 4.3% moisture, respectively.

Table 3: Proximate composition of the three lyophlized hydrolysates prepared from common carp fish

%	Pa. hydrolysate	Bac. P hydrolysate	Bov. P hydrolysate
Moisture	3.3 ± 0.15	4.3 ± 0.21	3.53 ± 0.31
Protein	89.13 ± 0.57	86.9 ± 0.62	85.03 ± 0.70
Fat	0.76 ± 0.15	0.33 ± 0.04	0.28 ± 0.03
Ash	7.75 ± 0.15	8.57 ± 0.06	10.20 ± 0.54

Pa = papain Bac.P = bacterial protease
Bov.P = bovine protease
Results are mean values of three replicates±standard deviation.

Molecular weight distribution of the fish protein hydrolysates

Figures (3, 4, 5 and 6) show the absorbance (A) at 280 nm of the collected fractions resulting from untreated fish hydrolysate, Pa., Bac.P and Bov.P treated hydrolysates, respectively. It is clear from the figures that there is a change in the molecular weight distribution due to the enzymatic hydrolysis. It was found that the higher molecular weight peptides decreased while the lower molecular weight peptides increased. It is clear from Figs. (3 to 6) that the three enzymatic fish hydrolysates as well as the untreated MFF all started with the protein having MW in the range of 90.000 Dalton. The molecular weight distribution of the hydrolysates showed degradation from 90.000 to 2.500 Dalton. Also, it can be seen from the figures that at the end of the hydrolysis, the three enzyme hydrolyzates showed a similar degradation of protein to small peptides, although the results in Fig. (2) show that the rate of degradation of fish protein using papain was much higher than that of bovine and bacterial proteases. Cheftel *et al*. (1971) performed batch experiments to prepare fish protein hydrolysates using several proteases, gel chromatography showed the molecular weights of the soluble peptides to be less than 2000 Dalton. Quaglia & Orban (1990) studied the influence of enzymatic modification of sardine fish with alcalase. They determined molecular weight distribution by gel chromatography on a super fine Sephadex G50, they found that the molecular weight distribution profile for the hydrolysates of the lowest degree of hydrolysis (5%) and the highest hydrolysis (20%) exhibited the range of molecular weights of 30.000 to 1000 Dalton, respectively. Gilmartin & Jervis (2002) studied the influence of combinations of commercial enzyme preparations on cod hydrolysate peptide size range. They reported that the enzyme preparations containing predominantly protease or endopeptidase produced significant amount of peptides with molecular weight below 3000 Dalton. Alcalase combined with exopeptidase produced hydrolysates rich in low-molecular weight peptides.

Amino acid composition

Data in Table (4) show the essential amino acid composition of the three fish hydrolysates as well as the minced filleted fish (MFF). It is clear that fish protein and fish protein hydrolysates are very rich sources of lysine, other essential amino acids are presented in adequate quantities as compared to FAO requirements for human adults. Tyrosine and phenylalanine are the limiting amino acids in fish protein. Isoleucine is border line in all hydrolysates. Enzymatic hydrolysis of fish protein with Pa enzyme gave a protein hydrolysate with the closest pattern to untreated fish protein with the same limiting amino acids. Treatment with Bac.P resulted in a hydrolysate limiting in leucine, tyrosine and phenylalanine. The hydrolysate treated with Bov.P is limiting in threonine, phenylalanine and tyrosine. Rebeca *et al*. (1991) found that the amino acid composition of fish protein hydrolysates prepared from mullet fish, with different bacterial proteases to be more or less the same. Shahidi *et al* (1994) found that the amino acid composition of harp seal hydrolysate was similar to harp seal meat. Protein hydrolysates prepared from herring and herring by-products using alcalase enzyme were studied by Sathivel *et al*. (2003). They reported that all samples had desirable amino acid profiles.

Biogenic amine content of the fish hydrolysates

Generally, amines in foods are determined for two reasons: First is their potential toxicity. The second is the possibility of using them as food quality markers. Biogenic amines at low concentrations are essential for many physiological functions (Bardocz *et al*. 1995), while ingestion of large amounts may result in health problems (Joosten 1988).

Histamine, putrescine, cadaverine and tyramine were determined in the water extracts of the three fish hydrolysates, and results are shown in Table (5). It can be observed that the concentration of histamine for all hydrolysates was below the permissible level which is 50ppm. Histamine is supposed to be the most toxic amine (Križek *et al*. 2002). The same authors suggested the ranged of 10 to 20 ppm putrescine for acceptable quality, and concluded that putrescine is a good quality marker as its concentration measures with the sensory signals of the samples. Cadaverine content recorded the highest level (16.7 ppm) for the bacterial protease hydrolysates. It can be concluded that all values were within the permissible levels.

Functional Properties

Nitrogen solubility index (NSI)

It is clear from Table (6) that hydrolysis of MFF with the three investigated enzymes resulted in improvement in their NSI. Unhydrolysed MFF had 80.49% NSI, whearas Pa., Bac.P and Bov.P

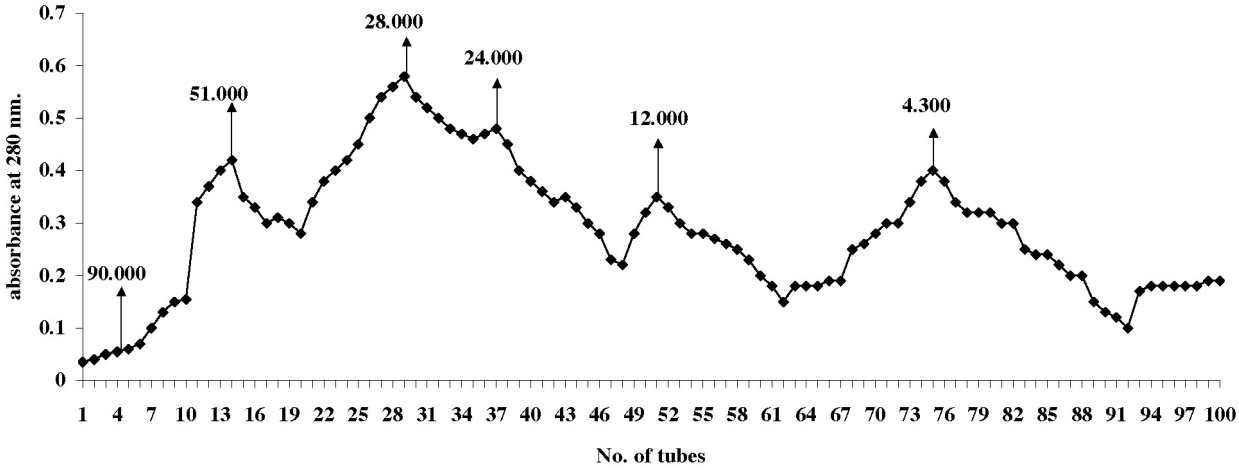

Fig. 3: Molecular weight distribution of fish hyrolysate (Untreated)

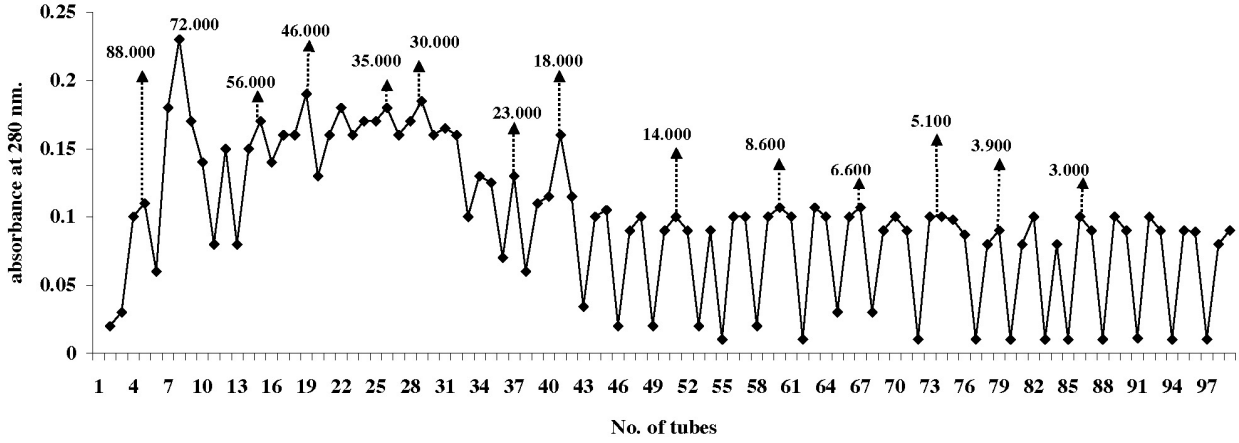

Fig. 4: Molecular weight distribution of fish hydrolysate treated with papain (3%, 2 hrs.)

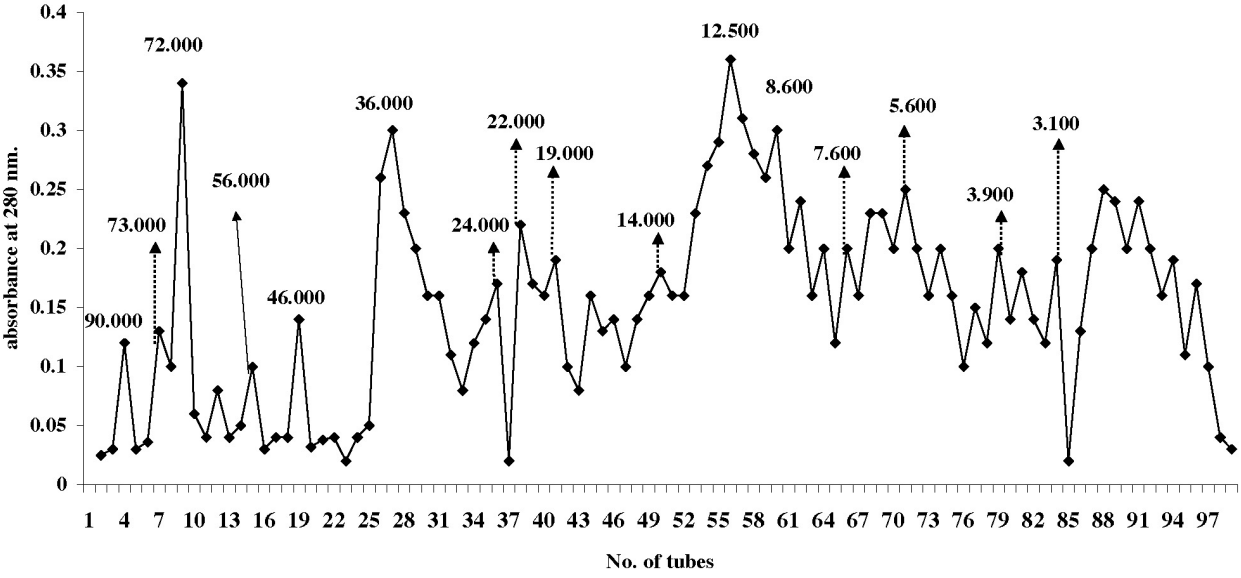

Fig. 5: Molecular weight distribution of fish hydrolysate treated with bacterial protease (3%,2 hrs.)

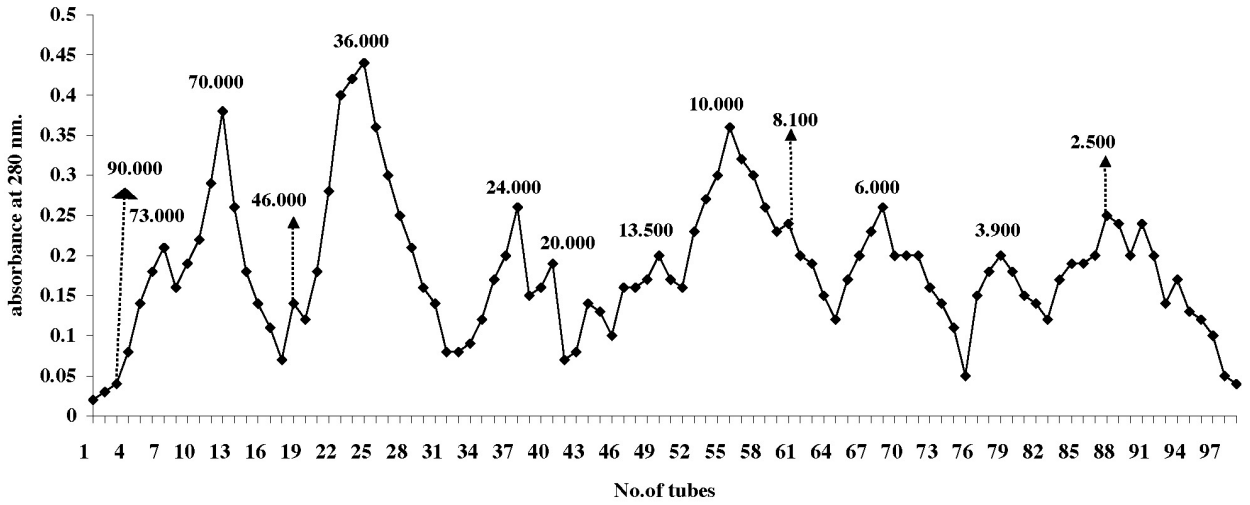

Fig. 6: Molecular weight distribution of fish hydrolysate treated with bovine protease (3%, 2hrs.)

Table 4: Essential amino acids (g/16g N) of minced filleted fish, the three lyophlized hydrolysates, as well as FAO reference

Amino acids	MFF	Pa	Bac.P	Bov.P	FAO
Lysine	8.24	8.73	8.92	9.11	5.8
Valine	5.76	4.62	4.99	5.73	3.5
Threonine	4.43	3.42	3.42	3.01	3.4
Methionine + Cysteine	3.58	3.53	2.46	3.38	2.5
Leucine	7.03	7.68	6.24	6.96	6.6
Isoleucine	2.36	2.7	2.65	2.72	2.8
Phenylalanine + Tyrosine	5.18	4.93	4.36	5.13	6.3

MFF = minced filleted fish
Pa = papain hydrolysate
Bov.P =bovine protease hydrolysate
Bac.P = bacterial protease hydrolysate
FAO/WHO/UN pattern 1991

Table 5: Biogenic amines content of the three lyophlized fish hydrolysates

Hydrolysate	Concentration (ppm)			
	Histamine	Putrescine	Cadaviene	Tyramine
Papain	2.9	1.1	9.4	0.41
Bacterial	3.53	1.14	16.7	1.09
Bovine	6.13	11.5	7.2	0.31

Table 6: Functional Properties of minced filleted fish and the three lyophlized fish hydrolysates

Functional properties	MFF	Pa. hydrol.	Bac. P. hydrol.	Bov. P. hydrol.
Nitrogen solubility index (%)	80.49 ± 0.47^{d}	92.85 ± 0.82^{b}	90.35 ± 0.72^{a}	88.74 ± 0.63^{c}
Emulsifying capacity (ratio)	48.37 ± 0.65^{c}	50.25 ± 0.74^{b}	51.40 ± 0.62^{d}	50.23 ± 0.45^{b}
Thermostability (%)	40.30 ± 0.33^{d}	42.20 ± 0.37^{b}	41.22 ± 0.19^{c}	43.33 ± 0.81^{d}
Wettability (sec)	6	2	2	2
Flowability (sec)	20	20	20	20

MFF = minced filleted fish
Bac. P = bacterial protease
Pa = papain
Bov. P = bovine protease
Means with different letters within each column are significant, means followed by the same alphabetical letter are not significantly different at 5% level and means without letters are not significant.
Results are mean values of three replicates ± standard deviation.

hydrolysates showed 92.85%, 90.35% and 88.74% NSI, respectively. The results in Figure (2) are in agreement with those results which showed that Pa enzyme has achieved the highest degradation thus highest solubility. Increased solubility of diverse proteins through enzymatic hydrolysis has been extensively reported by Adler-Nissen & Olsen (1979), Pour-El (1981) and Mahmoud (1994). The enhanced solubility of the hydrolysates is attributed to the smaller molecular size of the peptides as reported by Chobert *et al*. (1988) and Shahidi *et al*. (1994). Meanwhile, Phillips & Beuchat (1981) reported that the newly exposed ionizable amino and carboxyl groups increase the hydrolysate's hydrophobicity, thus resulting increased solubility.

Emulsifying capacity (EC)

Emulsifying capacity and film forming ability are essential for proteins to perform well in meat systems. Also the protein's ability to form emulsions is critical to their applications in mayonnaise, salad dressing, milk and frozen dessert. Data in Table (6) indicate the effect of hydrolysis on the EC of the hydrolysates. The three hydrolyates under study possessed EC ranged from 50.25 for Pa. to 51.4 for Bac. P., as compared to 48.2 for MFF. Although the EC of the hydrolysates was slightly improved than the unhydrolysed fish yet EC is still low. Controlled enzymatic hydrolysis can improve EC (Adler-Nissen & Olsen 1979, Mahmoud 1994). Phillips & Beuchat (1981), explained the observed improvement in the emulsifying property that it could presumably attributed to exposure of the hydrophobic protein interior which enhances adsorption on the interface, forming a cohesive interfacial film with the hydrophobic residues interacting with oil and hydrophilic residues with water. It was found that as the period of hydrolysis was elongated as the EC decreased (Adler-Nissen 1986). Sathivel *et al*. (2003) reported that EC of herring protein concentrate was less than reference proteins including egg albumin and soy protein concentrate.

Thermostability (TS)

Thermostability is the ability of a protein hydrolysate to remain soluble without aggregation leading to precipitation under elevated processing and sterilization temperature. Upon hydrolysis with the three investigated enzymes, the hydrolysates became slightly more thermostable than the MFF (Table 6).

Wettability (WA)

Protein wettability is the ability of a powder to become wetted and is important in instant products. The results in Table (6) show that the wettability of hydrolysates was markedly improved by hydrolysis. The MFF was wetted after 6 sec whearas the three hydrolysates were wetted after 2 sec.

Flowability (FL)

Data in Table (6) indicate that enzymatic hydrolysis of MFF had no influence on the FL property of the three hydrolysates under study.

CONCLUSION

The use of proteases of different origins did not result in a significant difference between the properties and composition of the three hydrolysates. In general enzymatic hydrolysis of common carp fish resulted hydrolysates with higher protein content, improved solubility and wettability, slightly improved EC and TS. The degradation of the protein exhibited higher degradation by papain, yet molecular weight distribution of the three hydrolysates were more or less the same. The hydrolysates had a bland taste and an off–white colour. The results suggest the use of the common carp fish hydrolysates in instant food formulas and geriatric, and sports nutrition, due to their high solubility and wettability.

REFERENCES:

A.O.C.S. **1998**. "Official Methods and Recommended Practices" of the American Oil Chemists Society, 5th ed. AOCS press, Champaign, IL.

Adler-Nissen, J. **1986**. A Review of food hydrolysis-specific area. In: Enzymatic Hydrolysis of Food Proteins, Adler –Nissen J. (Ed.) Elsevier Applied Science Publishing Co., New York, pp. 57-81.

Adler-Nissen, J. & Olsen, H.S. **1979**. The influence of peptide chain length on taste and functional properties of enzymatically modified soy protein. In: Functionally and Protein Structure, Pour-El (Ed.) ACS Symposium series 92, Washington DC, pp. 120-146.

Andrews, P. **1964**. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J., **91**: 222-233.

Arzu, A., Mayorga, H., Gonzales, J. & Rolz, C. **1972**. Enzymatic hydrolysis of cottonseed protein. J. Agric. Food Chem., **20**: 805-809.

Ayesh, A.M., Amra, H.A., Abu-Arab, A.A.K. & Naguib, K. **1995**. Detection and determination of eight biogenic amines in frozen mackerel and sardine using HPLC. J. Egypt Ger. Soc. Zool., **17**: 121-135.

Bardocz, S., Duguid, T.G., Brown, D.S. & Grant, G. **1995**. The importance of dietary polyamines in cell regeneration and growth. Br. J. Nutr., **73**: 819-828.

Blackburn, S. **1968**. Amino Acids Determination and Techniques. (1st edn.) Marcel & Decker Inc., New York, USA.

Cheftel, C., Ahern, M., Wang, D.I.C. & Tannenbaum, S. R. **1971**. Enzymatic solubilzation of fish protein concentrate: Batch studies applicable to continuous enzyme recycling processes. J. Agric. Food Chem., **19**: 155-161.

El-Tanboly, E. **2001**. The B-galactosidase System of a Novel Plantfrom Durian Seeds (Durio zibethinus). I. Isolation and Partial Characterization. Pakistan Journal of Biological Science, 4, **12**: 1531-1534.

Fox, D.F. & Tarassuk, N.P. **1968**. Bovine milk lipase isolation from skim milk. J. Dairy Sci., **51**: 826.

Gilmartin, L. & Jervis, L. **2002**. Production of cod (Gadus morhua) muscle hydrolysates. Influence of combinations of commercial enzyme preparations on hydrolysate peptide size range. J. Agric. Food Chem., **50**: 5417-5423.

Joosten, H.M.L.G. **1988**. The biogenic amines contents of Dutsch cheese and their toxicological significance. Neth Milk Dairy Science, **42**: 25-42.

Križek, M., Pavlicek, T. & Vacha, F. **2002**. Formation of selected biogenic amines in carp meat. J. Sci. Food Agric., **82**: 1088-1093.

McClave, J.T. & Benson, P.G. **1991**. Statistical Business and Economics. Dellen Publishing Co., USA, pp. 227- 295.

Mahmoud, M. **1994**. Physico-chemical and functional properties of protein hydrolysates in nutritional products. Food Technol., **48**: 89-95.

Maijala, R.L & Eerola, S.H. **1993**. Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. Meat Science, **35**: 387-395.

Nilsang, S., Lertsiri, S. Suphantharika, M. & Assavanig, A. **2005**. Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. J. Food Engineering, **70**: 571-578.

Phillips, R.D. & Beuchat, L.R. **1981**. Enzyme modification of proteins. In: Protein Functionality in Foods, J. P. Cherry (Ed.) ACS Symposium Series, 147, Washington, DC, pp. 275-298.

Pour-El., S. **1981**. Protein functionality: classification, definition and methodology. In: Protein Functionality in Foods, J. P. Cherry (Ed.) ACS symposium, Series 147, Washington, DC., Chapter 6.

Quaglia, G. B. & Orban, E. **1990**. Influence of Enzymatic hydrolysis on structure and emulsifying properties of sardine (Sardina Pilchardus) protein hydrolysates. J. Food Sci., **55**:1571-1573.

Rebeca, B. D.; Pena-vera, M.T. & Diaz-Castaneda, M. **1991**. Production of fish protein hydrolysates with bacterial proteases; yield and nutritional value. J. Food Sci., **56**: 309-314.

Sathivel, S., Bechtel, P. J., Babbitt, J. & Smiley, S. 2003. Biochemical and functional properties of herring (Clupea harengus) by-product hydrolysates. J. Food Sci., 68: 2196-2200.

Sathivel, S., Smiley, S., Prinyawiwatkul, W. & Bechtel, P.J. **2005**. Functional and nutritional properties of red salmon (Oncorhynchus nerka) ebzymatic hydrolysates. J. Food Sci., **70**: 401- 406.

Shahidi, F., Synowiecki, J. & Balejko, J. **1994**. Proteolytic hydrolysis of muscle proteins of harp seal (phoca groenlandica). J. Agric. Food Chem., **42**: 2634-2638.

Shahidi, F., Xiao-Qing, H. & Synowieck J. **1995**. Production and characteristics of protein hydrolysates from capelin (Mallotus villosus). Food Chem., **53**: 285-293.

Shahidi, F. **1998**. Functional sea food lipids and proteins. In: Functional Foods, Biochemical and Processing Aspects., Mazza G. (Ed), Technomic publishing Co. Inc., Lancaster, P.A., Chapter 12.

Smith, A. K. & Circle, S.J. **1997**. Nitrogen solubility index and protein digestibility. In: Soybean Chemistry and Technology. Vol. I., Proteins. Appendix, AVI Publishing Co., pp: 451-454.

Taha, S.F. & Ibrahim, M.A. **2002**. Effect of degree of hydrolysis on functional propeties of som oilseed proteins. Grassas Y Aceites, **53**: 273-281

بروتينات سمك المبروك المتحللة إنزيمياً
١ـ الخواص الوظيفية وتوزيعات الأوزان الجزيئية

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تم إعداد منتج يمثل قيمة مضافة إلى سمك المبروك على هيئة بروتينات متحللة إنزيمياً. تم تجهيز البروتينات المتحللة باستخدام ثلاثة إنزيمات محللة للبروتين وهى الباباين و البروتيز البكتيرى و البروتيز البقرى. تم التعرف على الظروف المثلى لنشاط إنزيمات التحلل البروتينى. ثم تبع ذلك تقدير معدل التحلل الحادث بالإنزيم، وأيضا دراسة تأثير التحلل الإنزيمى على توزيع الأوزان الجزيئية للبروتين الناتج بواسطة جهاز الفصل الكروماتوجرافى باستخدم الجيل (كروماتوجرافيا الغربلة الجزئية على السيفاديكس «G-100») و كذا الكشف عن وجود الأمينات الحيوية الضارة فى البروتين المتحلل، مع دراسة الخواص الوظيفية له.

وجد أن المحتوى البروتينى لنواتج تحلل السمك قد زادت من ١٧٪ فى السمك المفروم قبل المعاملة إلى ما يتروح ما بين ٨٥-٨٩٪. أوضحت النتائج أن معدل تحلل البروتين بإنزيم الباباين كان أعلى من إنزيمى البروتيز البكتيرى والبروتيزالبقرى . أوضحت نتائج الفصل الكروماتوجرافى بالجيل لنواتج البروتينات الثلاثة المحللة بالإنزيمات السابق ذكرها أن نواتج تكسير البروتين نتج عنها ببتيدات ذات أوزان جزيئية تتراوح ما بين ٢,٥٠٠ إلى ٩٠,٠٠٠ دالتون. أوضحت نتائج تحليل الأحماض الأمينية لنواتج التحلل الإنزيمى أنها مصدر جيد لحمض الليسين والأحماض الأمينية الكبريتية. كما وجد أن نواتج التحلل الإنزيمى لم ينتج عنها أمينات حيوية ضارة وعملية التحلل الإنزيمى قد حسنت من خواص ذوبان البروتينات و قدرتها على الاحتفاظ بالماء.