# FUNCTIONAL PROPERTIES OF CATFISH SKIN COLLAGEN HYDROLYSATES

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**ABSTRACT**

**Collagen hydrolysates were obtained from catfish skin collagen hydrolysis using catfish collagenase, pepsin and trypsin individually and mixed for 15-300min. The degree of hydrolysis , antioxidant activity based on, DPPH radical-scavenging activity (RSA), and reducing power (RP) for all obtained hydro lysates were studied , then the collagenase hydrolysate was (CH) was selected to be evaluated for antibacterial activity , functional properties including solubility, emulsification and foaming properties besides the toxicity . The highest values for RSA (72.5%) has been noticed when DH reached (24.30%) after 30 min. of hydrolysis by collagenase (CH). The water and oil holding capacities for this hydro lysate was compared to that for ASC (acid soluble collagen and PSC (pepsin soluble collagen), the results showed that the values recorded by CH were significantly higher than ASC & PSC. The molecular weight of CH peptides ranged from 180 to 11 Da. as analyzed by SDS-PAGE. The toxicity assay result revealed that CH is safe for human consumption. There was no antibacterial function for CH toward *E.coli* and *S. aureus*. At concentration of 2mg/ml. This study suggests that catfish collagen hydro lysate could be a good natural alternative for synthetic antioxidants in food industries.**

**Keywords: antioxidant, antimicrobial, SDS- PAGE, collagenase, toxicity, fish waste**
**Part of Ph.D. Dissertation of the first author.**



## الخصائص الوظيفة لمتحللات كولاجين جلد سمك الجري

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**المستخلص**

**حضرت متحللات الكولاجين باستعمال انزيم كولاجنيز السمكي وأنزيم الببسين والتربسين بشكل مفرد وخليط في تحليل جلد سمك الجري لمدة 15–300 دقيقة. قدرت درجة التحلل والفعالية المضادة للاكسدة باعتماد اختبار DPPH والفعالية الاختزالية لجميع المتحللات المحضرة. وتم اختيار متحلل الكولاجنيز لأكمال بقية الاختبارات في هذه الدراسة. اشارت النتائج ان اعلى قيمة للفعالية المضادة للاكسدة لمتحلل الكولاجينيز كانت 72.5 %** (DPPH) **عندما كانت درجة التحلل 24.30% ب عد30 دقيقة من بدء التحلل. كانت قابيلية حمل الماء وحمل الدهن لمتحلل الكولاجينيز أعلى معنويا بالمقارنة مع الكولاجين الذائب بالحامض والكولاجين الذائب بالببسين، وتراوحت الاوزان الجزيئية للببتيدات في متحلل الكولاجينيز من 180–11 دالتون.واوضح فحص السمية صلاحية استعمال متحلل الكولاجينيز للاستهلاك البشري. كما اظهرت النتائج عدم قابلية المتحلل عند تركيز 2ملغم/مل في تثبيط نمو كل من** *E.coli* and *S. aureus*. **وعليه تقترح هذه الدراسة أمكانية استعمال هذا المتحلل كبديل أمن لمضادات الاكسدة الصناعية.**

**الكلمات المفتاحية: الفعالية المضادة للأكسدة، المضادة للميكروبات، الترحيل الكهربائي، السمية، مخلفات الأسماك**
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## INTRODUCTION

Protein hydrolysis, leading to the cleavage of peptide bonds, may be carried out via enzymatic or chemical processes. Chemical processes, including alkaline or acid hydrolysis, are harmful not only to the environment but also to humans who consume the resulting products. Besides chemical processes, which are difficult to control, yield products containing modified amino acids (39). In contrast, enzymatic hydrolysis may be performed under gentle conditions, thus avoiding extreme environments that are required by chemical treatments. Moreover, the processes neither produce side reactions nor decrease the nutritional value of the protein source (15). Several proteases, including collagenase, Alcalase, pepsin, Protamex, trypsin, and Neutrase, are commonly used to hydrolyze proteins, resulting in various types of protein hydrolysates and peptides with a variety of bio-functional activities. Synthetic antioxidant (BHT) has been used in food industries for inhibiting lipid oxidation (free radical scavenging ) , and most used of these antioxidant, but in food products their usage has been weakening according to the toxicologists and nutritionists who documented, some of these synthetic antioxidants side effects such as, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (13). Their instability, because of a suspected action as promoters of carcinogenesis (32). However, for this reason, there is a growing interest in the study of natural additive. The use of natural peptides like collagen hydrolysates has been widely utilized due to their excellent bio-compatibility, easy biodegradability, and weak antigenicity (26). When the antioxidant defenses are not enough, deterioration of physiological functions may occur and result in diseases. The antioxidant capacity of collagen hydrolysate is mostly due to the presence of hydrophobic amino acids in the peptide (11). Therefore, the antioxidants in human diet are of great interest as possible protective agents to help human body reduce oxidative damage.====The objectives of this study were to investigate the functional properties of catfish skin collagen hydrolysates prepared by enzymatic hydrolysis and to evaluate their antioxidant and antibacterial activities.

## MATERIALS AND METHODS

### Skin sample preparation

Catfish skin was collected from local market, Baghdad, in an icebox, and transported to the laboratory within 2 h. The skin was washed with cold water (0-4°C), drained, and cut into small pieces (0.5 x 0.5 $cm^2$) using a scissor. Then kept at -20°C in polyethylene bags.

### Preparation of pepsin soluble collagen

Catfish skin pepsin soluble collagen (PSC) was prepared according to the method described by (30). Catfish skin was pretreated with 0.1 M NaOH at a ratio of 1:8 (w/v) with stirring to remove the non-collagen proteins and washed with water until a neutral or faintly basic pH was obtained. Then the fat was extracted using 10% (v/v) butyl alcohol at a ratio of 1:10 (w/v) and the skin was washed with cold water. Then the defatted skin was soaked in 0.5 M acetic acid at a ratio of 1:30 (w/v) in the presence of pepsin (1g/100 g of defatted skin) for 72 h with gentle stirring. The mixture centrifuged at 9,000xg for 30 min. at 4C. The supernatant was salted-out by addition of NaCl to a final concentration of 2M in 0.05 M Tris-HCl (pH 7.5) and allowed to stand for 1 h to inactivate pepsin, then the mixture centrifuged at 9,000xg for 30 min. at $4^oC$. The pellet was dissolved in 0.5 M acetic acid and dialyzed against 0.1 M acetic acid in a dialysis bag (MW cut-off 14 kDa USA). Subsequently, the solution was dialyzed with distilled water until a neutral pH of washed water was obtained, the resulting dialysate was lyophilized.

### Preparation of collagen hydrolysate

Skin Collagen hydrolysates with different DHs were produced according to the method of Kittiphattanabawon(21).The hydrolysis reaction was carried out in two separate flasks as follows. Catfish collagenase (1%) was added to PSC (which prepared by dissolving 0.02 g/100ml phosphate buffer (10mM pH 7.8) and kept at 37 ˚ C for 30 min. and collagenase + trypsin (1%) were added to another flask containing PSC held for 90 min.at ( 37 ˚ C) , after that the hydrolysis process was stopped by raising the temperature of reaction mixture to 90 ˚ C for 10 min.,

cooled to 5˚ C , centrifuged at 5000 xg for 30 min. Then the supernatant was concentrated by rotary evaporator. lyophilized and kept in tied container at -18 ˚ C.

**Degree of hydrolysis measurement**

The DH was determined by formaldehyde titration method according to Yue (45), as follows: Five milliliters of hydrolysates supernatant were diluted with 60 ml distilled water, and titrated by NaOH solution (0.05M) (standard titration solution) to pH 8.2 and the volume recorded. Then, 10 ml formaldehyde (14%) was titrated with 0.05 M NaOH to reach pH 9.2, the consumed volume of NaOH was also recorded. The value of DH was calculated according to the following equation

$$DH = \frac{1C \times (V1 - V2) \times V/5}{m \times \%\ \text{protein in raw material}} \times 100$$

C : concentration of standard titration solution of NaOH(0.05M)

V1 : consumed volume of 0.05 M NaOH titrating up to pH 9.2

V2 : consumed volume of 0.05 M NaOH titrating up to pH 8.2

V: total volume of collagen protein hydrolysate

**Antioxidant assay**

**(1) Radical Scavenging Activity (RSA)**

The radical scavenging activity using α, α-diphenyl-β-picrylhydrazyl (DPPH) was measured according to method described by Salman (35). Sample solution (1.5 mL) was added to 1.5 mL of 0.1 mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min. at room temperature in dark place. The absorbance of reaction solution was measured at 517 nm. The lower absorbance (*A*517) represents higher DPPH scavenging activity, which is expressed as:

[(**blank A517— sample A517) / blank A517] × 100%.**

**(2) Reducing power**

The reducing power of collagen hydrolysate was measured according to the method described by choi, (4). One mL of each sample (2 mg/mL) was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50$^{o}$C for 20 min, and then 1 mL of 10% trichloroacetic acid was added. An aliquot of 2 mL from this incubated mixture was mixed with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride. After 10 min, the absorbance of the resulting solution was measured at 700 nm by a spectrophotometer (Selecta Co., Barcelona, Spain) Increase in absorbance reading at 700 nm of the reaction mixture was considered to indicate increased reducing power.

m: mass of the raw material

**Antibacterial activity**

This experiment was adopted according to the method described by Hussein. (17) with some modification, Brain Heart Infusion BHI agar (Oxoid) were prepared and poured in the sterile petri dishes and allowed to solidify for overnight bacterial cultures *Staphylococcus aureus, E. coli*. were swabbed separately on the media using sterile cotton buds, then filter paper disks (0.5 cm diameter), soaked with 40 µl of collagen hydrolysate (2 mg/mL w: v) and put on the plate surface and incubated at (37$^{o}$ C) for 24 h. The antibacterial activity of the collagenase hydrolysates (CH) toward *E.coli* , *staph* . *aureus*. (the inhibition activity) was expressed as the difference between the diameter of the inhibition zone (clear zone) and the diameter of the disk after 24 h. Analyses were performed in duplicate.

**Molecular weight determination**

The collagenase hydrolysate preparation molecular weight was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by the Hames, *et al.* (16). System with an 10% resolving gel, and 4% stacking gel was applied. The molecular weights of ladder proteins determined ranged between 11 to 245 kDa .

**Detection of collagen hydrolysate toxicity**

This experiment conducted due to the method explained by Nair, (29), Fresh human blood (1 ml) was mixed with 20 ml of normal saline solution. Different concentration of collagen hydrolysate (250 ,500,1000) ppm were prepared. Aliquot of each concentration (100 µL ) was mixed individually with 2 ml of blood suspension . And for control sample 100 µL of distilled water was used instead of collagen hydrolysate. All samples Incubated at 37 ° C, and the turbidity was read at 540nm after 10, 30 and 60 minutes of incubation.

**Water holding capacity (WHC)**

The water holding capacity (WHC) of collagen and collagen hydrolysate was measured

following the method described by (8). One milligram collagen and collagen hydrolysate was taken into a 10 mL centrifuge tube and mixed with one ml of water. The mixture was thoroughly vortexed for 10 min at 26 $^{o}$C and centrifuged at 1431 xg for 20 min at room temperature. The water absorbed by collagen and collagen hydrolysate samples were determined according to the following equation:

$$\text{WHC(g/g)} = \frac{\text{(wet sample weight - dry sample weight)}}{\text{Dry sample weight}}$$

## Oil holding capacity (OHC)

Oil holding capacity of the collagen hydro lysates were determined as described by Tang,(38). Skin collagen hydrolysates samples (0.1 g) were mixed with 1mL of sunflower oil. After 10 minutes mixing, the mixture was centrifuged at 1600 xg for 25 min and the oil holding capacity was calculated using the following equation: :

$$\text{OHC(g/g)} = \frac{\text{(wet sample weight - dry sample weight)}}{\text{Dry sample weight}}$$

## Foaming properties

Foaming capacity (FC) and foaming stability (FS) of acid soluble collagen ASC , pepsin soluble collagen PSC and collagenase hydrolysates CH were determined according to the method of Arogundade (1). An aliquot (25 mL) of 0.3% of experimental samples solution (0.3g in100 ml of 10 Mmol/L PBS, pH 7) was homogenized at a speed of 12,000 rpm, to incorporate air for 2 min at room temperature 25 ± 1 ∘ C. Then the whipped sample was immediately transferred into a 50 mL cylinder and the total volume was read after 30 seconds. The FC was calculated according to the following equation:

**FC (%) = ($A_0$ - B) /B x 100 by** (Naczk 28)

Where $A_0$ is the volume after whipping (ml), B is the volume before whipping (mL). The whipped sample was allowed to stand at 25$^{o}$C for 10, 30, 60, 90 and 120 min and the volume of whipped sample was then recorded. Foam stability was calculated as follows:

**FS (%) = (At-B) /B x100**

Where at is the volume after standing (mL), and B is the volume before whipping (mL).

## Emulsifying Properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were calculated based on the method of Jamdar (18) with some minor modifications. Vegetable (sunflower) oil (2 ml) and six mL of 0.1% experimental samples solution were mixed and the pH was adjusted to 2.5, 5.0, 7.5 and 10.0. The mixture was homogenized using homogenizer at a speed of 20,000 rpm for 1 min. An aliquot of the emulsion (50 µL) was pipetted from the bottom of the container at 0 and 10 min after homogenization and mixed with 5 mL of 0.1% sodium dodecyl sulphate (SDS) solution, diluted 100-fold ( 50 µL sample to 5ml SDS) . The absorbance measured (at 500 nm) immediately (A0) and also after 10 min (A10) of emulsion formation, the following equation was used to calculate the emulsifying activity index (EAI) and emulsion stability (ES) as follows:

$EAI(m^2/g)=(2 \times 2.303 \times A_0 \times \text{Dilution})/C \times (1-\phi) \times 10^4$

Φ: oil volume

$ESI\ (min) = A0 \times \Delta t / \Delta A$

where $\Delta A = A0 - A10$ , $\Delta t = 10$ min

## RESULTS AND DISCUSSION

### Degree of hydrolysis

Table 1 shows degree of hydrolysis of catfish skin collagen which was hydrolyzed by collagenase ,trypsin and pepsin individually , the degrees of hydrolysis (DH) were significantly increased with hydrolysis time for all samples. These results were in accordance with Guarard (14) findings, who reported that DH of yellowfin tuna waste protein increased with increasing hydrolysis time. Table 1 also shows the antioxidant activity of the experimental hydro lysates based on DPPH and reducing power assay. Collagenase hydrolyzed collagen samples (CH) gave the highest radical scavenging activity (RSA 72.5 %) and reducing power value (RP 1.12 ) after 30 min. of hydrolysis when the DH reached to (24.37 %) . However trypsin hydrolyzed collagen (THC) showed the highest values for RSA (70.59 %) and RP (0.21) after 120 min. when the DH was (22.33% ). The highest value for RSA was 68.64% and for RP was0.41 after 90 min by using pepsin for collagen hydrolysis (PHC) with DH 19.99. Table 2 shows the DH of collagen hydrolysates using more than one enzyme in collagen hydrolysis being (collagenase + trypsin ,collagenase + pepsin and trypsin + pepsin) .The DH trend

was similar to the previous treatment (single enzyme) there were significant increase ($p \leq 0.05$) in DH% with reaction time. Collagenase- trypsin hydrolyzed collagen reached the highest DH% (37.78%) after 90 min. of hydrolysis, collagenase - pepsin hydrolyzed samples showed the highest DH(31.60) after 120 min. and pepsin + trypsin hydrolyzed sample recorded the highest value (28.20%) after 90 min. . The relatively high initial rate indicated that the maximum cleavage of peptide occurred within 90 min of hydrolysis; this was chosen as the optimum reaction time for further experiments. It has been noticed from table 2, the antioxidant activity of collagenase- trypsin and collagenase –pepsin treated samples were higher (74.30 % and 73.09 %) than that of trypsin- pepsin treated samples (71.2 %). These values were higher than using single enzyme. These results suggested that the experimental collagen hydrolysates from fish skin are a good source as electron donors and could react with free radicals to form more stable products, protein hydrolysates may also inhibit oxidation by their ability to chelate transition metal ions (9). Many researchers were reported that some antioxidant peptides had been identified from some by-product sources for example head (2), dark meat (36), skin and viscera (7).

**Table 1. Degree of hydrolysis and antioxidant activity (based on DPPH, ferric reducing power) for collagen hydrolysates prepared by Collagenase ,pepsin and trypsin assisted hydrolysis**

| Enzymes | | Time (min.) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 |
| Collagenae | DH% | 4.60 ± 0.30 | 24.3 7 ± 0.61 | 29.3 9 ± 1.37 | 33.6 7 ± 0.09 | 36.5 0 ± 0.50 | 40.0 0 ± 0.00 B | 51.5 0 ± 0.50 B | 55.5 0 ± 0.50 B | 57.5 0 ± 0.50 | 60.5 0 ± 0.50 | 64.50 ± 0.50 |
| Trypsin | DPPH % | 13.9 9 ± 0.33 | 72.5 8 ± 0.03 | 69.3 7 ± 1.15 | 58.8 2 ± 0.00 | 59.7 3 ± 0.65 | 64.1 0 ± 0.27 | 52.5 9 ± 0.42 | 43.1 9 ± 0.77 | 41.6 0 ± 0.35 | 37.9 0 ± 0.60 | 26.95 ± 0.68 |
| | Ferric reducing power | 0.29 6 ± 0.02 | 1.12 ± 0.04 | 0.81 7 ± 0.01 | 0.60 6 ± 0.02 | 0.47 6 ± 0.01 | 0.21 8 ± 0.00 | 0.11 9 ± 0.01 | 0.11 3 ± 0.01 | 0.19 5 ± 0.02 | 0.03 6 ± 0.01 | 0.004 1 ± 0.01 |
| | DH% | 3.65 ± 0.55 | 5.25 ± 0.25 | 10.9 0 ± 0.70 B | 19.0 5 ± 0.25 B | 22.3 3 ± 0.53 | 34.5 0 ± 0.50 | 40.5 0 ± 0.50 B | 55.5 0 ± 0.50 B | 58.5 0 ± 0.50 | 62.5 0 ± 0.50 | 68.50 ± 0.50 |
| Pepsin | DPPH % | 42.9 4 ± 0.37 | 59.0 4 ± 0.88 | 65.3 7 ± 0.57 | 69.1 6 ± 0.03 | 70.5 9 ± 0.05 | 52.0 8 ± 0.55 | 44.8 4 ± 0.87 | 38.7 0 ± 0.69 | 36.9 1 ± 0.56 | 32.3 6 ± 0.86 | 26.97 ± 0.08 |
| | Ferric reducing power | 0.04 2 ± 0.00 6 | 0.059 ± 0.006 0.120 ± 0.028 | | 0.16 7 ± 0.01 2 | 0.21 0 ± 0.00 8 | 0.14 6 ± 0.00 4 | 0.12 3 ± 0.00 2 | 0.11 2 ± 0.00 2 | 0.08 0 ± 0.00 6 | 0.04 8 ± 0.00 7 | 0.004 ± 0.001 |
| | DH% | 3.08 ± 0.23 | 5.20 ± 0.20 | 7.33 ± 0.12 | 19.9 9 ± 0.14 | 20.7 7 ± 0.83 | 39.9 5 ± 0.19 B | 69.9 5 ± 0.55 | 77.4 8 ± 1.01 B | 87.0 2 ± 0.98 0 B | 94.5 0 ± 1.50 | 94.50 ± 1.50 |
| | DPPH % | 30.9 9 ± 0.36 | 57.7 3 ± 0.45 | 62.0 1 ± 0.41 | 68.6 4 ± 0.74 | 65.5 0 ± 0.39 | 60.7 3 ± 0.56 | 26.7 2 ± 0.84 | 46.0 3 ± 0.26 | 39.4 9 ± 0.39 | 54.1 1 ± 107 | 26.72 ± 0.84 |
| | Ferric reducing power | 0.02 7 ± 0.00 1 | 0.06 1 ± 0.00 4 | 0.10 2 ± 0.00 8 | 0.41 6 ± 0.00 6 | 0.33 0 ± 0.00 7 | 0.13 7 ± 0.00 1 | 0.10 6 ± 0.00 4 | 0.07 8 ± 0.00 1 | 0.05 9 ± 0.00 2 | 0.03 1 ± 0.00 1 | 0.015 ± 0.005 |

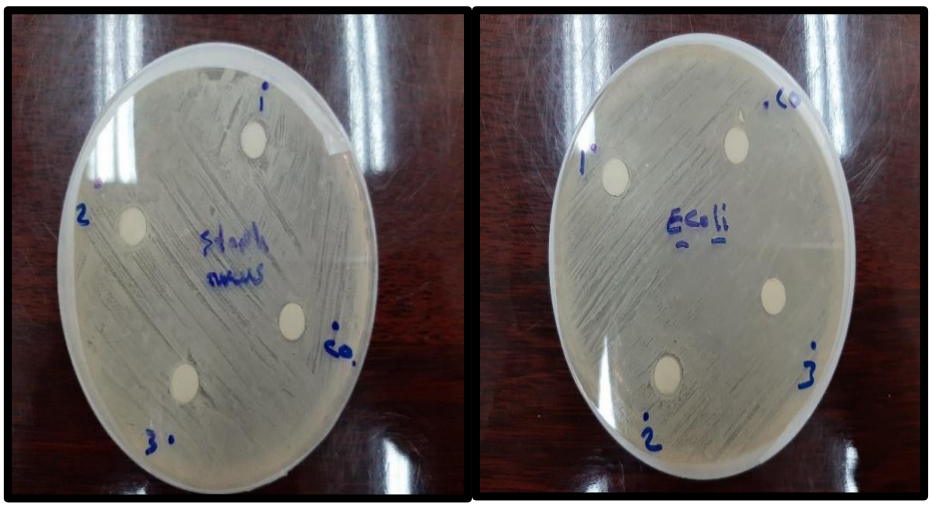
Letter B means Bitterness appearance

**Table 2. Degree of hydrolysis, DPPH, ferric reducing power ,bitterness for collagen hydro lysate prepared by synergetic function of collagenase, pepsin and trypsin**

| Enzymes | | Time (min.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | parameter | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 |
| Collagenase- trypsin | DH % | 12.17±4.84 | 30.29 ± 1.31 | 37.78 ± 0.71 | 44.17 ± 0.52 | 52.79 ± 1.51 | 60.52 ± 0.04 B | 67.18 ± 1.08 B | 73.87 ± 1.05 B | 77.10 ± 0.39 B | 79.65 ± 0.56 B |
| | DPPH% | 71.04 ±0.23 | 72.59 ± 0.06 | 74.31 ± 0.46 | 64.92 ± 1.17 | 58.50 ± 1.33 | 48.19 ± 0.76 | 34.87 ± 0.73 | 30.33 ± 0.21 | 26.41 ± 0.62 | 24.84 ± 0.52 |
| | Ferric reducing | 0.106 ±0.003 | 0.241 ± 0.005 | 0.382 ± 0.002 | 0.205 ± 0.002 | 0.163 ± 0.001 | 0.126 ± 0.002 | 0.071 ± 0.002 | 0.048 ± 0.003 | 0.034 ± 0.003 | 0.012 ± 0.003 |
| Pepsin – Trypsin | DH% | 6.10 ± 0.40 | 9.95 ± 0.21 | 22.86 ± 0.91 | 31.60 ± 0.90 | 37.67 ± 0.96 | 40.81 ± 0.51 | 54.20 ± 0.40 | 62.54 ± 1.16 B | 71.80 ± 1.00 B | 79.41 ± 0.91 B |
| | DPPH % | 33.06 ± 0.09 | 69.07 ± 0.86 | 63.55 ± 0.76 | 71.29 ± 0.49 | 58.09 ± 0.42 | 46.34 ± 0.88 | 44.79 ± 0.58 | 39.12 ± 0.21 | 32.54 ± 0.92 | 24.51 ± 0.69 |
| | Ferric reducing | 0.079 ± 0.002 | 0.168 ± 0.009 | 0.242 ± 0.006 | 0.333 ± 0.004 | 0.284 ± 0.016 | 0.191 ± 0.01 | 0.096 ± 0.009 | 0.139 ± 0.02 | 0.059 ± 0.005 | 0.033 ± 0.003 |
| Collagenase - pepsin | DH% | 22.50 ± 0.90 | 28.20 ± 1.30 | 41.15 ± 0.45 | 44.90 ± 0.59 | 54.54 ± 1.35 B | 63.57 ± 0.12 B | 69.85 ± 0.35 B | 75.92 ± 0.78 B | 88.20 ± 0.20 | 91.24 ± 0.86 |
| | DPPH | 71.26 ± 0.63 | 73.09 ± 0.15 | 70.87 ± 0.24 | 63.06 ± 0.66 | 59.11 ± 0.61 | 53.84 ± 0.17 | 47.91 ± 1.08 | 31.53 ± 0.69 | 23.72 ± 1.02 | 17.88 ± 0.97 |
| | Ferric reducing | 0.152 ± 0.01 | 0.493 ± 0.390 | 0.401 ± 0.01 | 0.261 ± 0.03 | 0.232 ± 0.01 | 0.122 ± 0.01 | 0.113 ± 0.01 | 0.082 ± 0.01 | 0.068 ± 0.01 | 0.035 ± 0.01 |

**Letter B mean Bitterness**

**Antibacterial properties** : Figure (1) shows the result of antibacterial activity of the experimental collagen hydro lysates against to *E.coli and S. aureus*. , both strains were resistant to the hydro lysate which used at concentration of ( 2 mg/ml ) . In another word the experimental hydrolysates were negative to both Gram-positive and Gram-negative bacteria. This might be due to the insufficient concentration of the collagen hydrolysate. Rajendran.(33) found that the hydrolysate of dogfish (*Squalus acanthias*) skins which prepared using three different proteases, (α-chymotrypsin, trypsin and papain) had no inhibition function towared *bacteria E.coli and S. aureus.* The reason has been attributed to either the insensitivity of these bacteria to hydrolysate or absence of peptides that possess antibacterial activity in the hydro lysate, or may be due to insufficient exposure of the bacteria to the hydrolysate . Floris (10) found that the differences existing in membrane composition have implications for the mode of action and the specificity of the antibacterial compounds.




**(a) (b)**

**Figure 1. Antibacterial activity of fish skin collagen hydrolysate by using two strain (a) *Staph. Aureus* , (b) *E.coli.***

**Protein pattern of collagen hydrolysate**

The electrophoretic patterns of the protein hydro lysate on SDS-PAGE is illustrated in Fig. 2. Standard proteins (markers line 2) with (line1 sample) molecular weight range (11-135kD) was used to determine the MW of hydro lysate fractions. During enzymatic hydrolysis, a major structural change will take place, in which the protein is slowly hydrolyzed in to smaller peptide units (22). Some natural food products contained oligopeptides that can reduce the rate of autoxidation in foods. Potatoes and mushrooms contained low-molecular weight peptides (approximately 1,000 Da) which inhibit polyphenol oxidase activity (27). Similarly, a peptide found in honey (approximately 600 Da) inhibited polyphenol oxidase in apple slices, grape juice, and model food system (31). Many related studies reported that several peptides derived from protein hydrolysates possessed antioxidant activities (3; 44) .Wu (44) indicated that the peptide of mackerel protein hydrolysate with molecular weight approximately 1,400 Da showed stronger in vitro antioxidant activity than other peptides 900 and 200 Da peptide. Chen (3) demonstrated that antioxidant activity of the peptides depends on amino acid composition and their sequences. Histidine-containing peptides possessed a higher activity than others, and can be attributed to the chelating and lipid radical trapping abilities of the imidazole ring (3; 41 ).

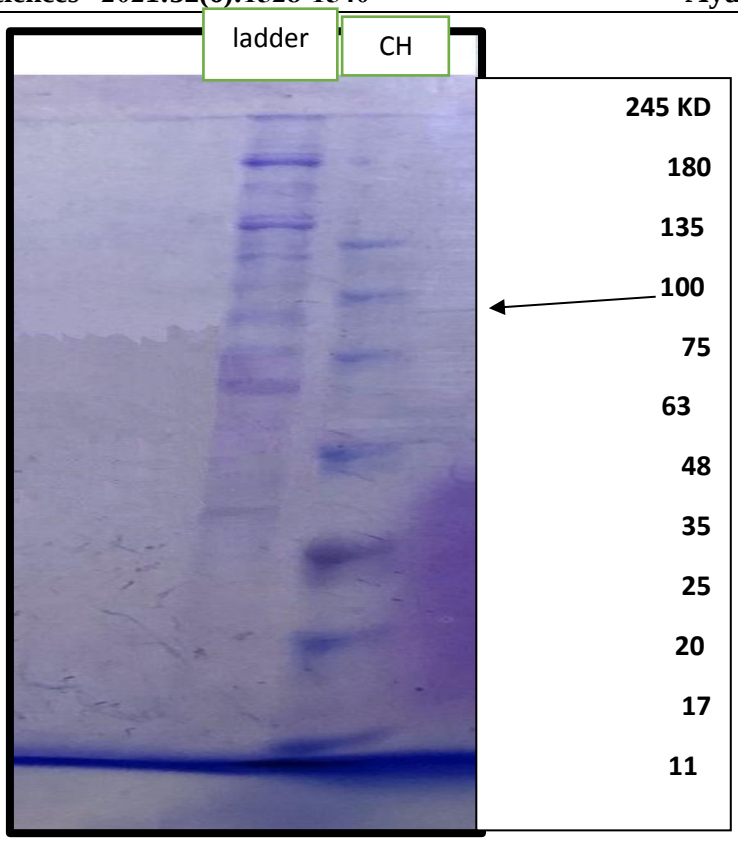
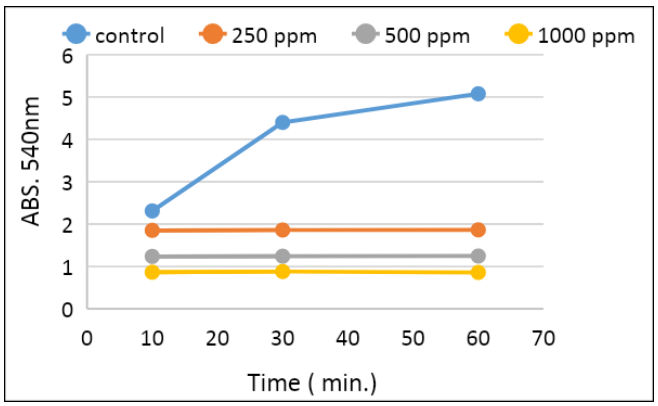


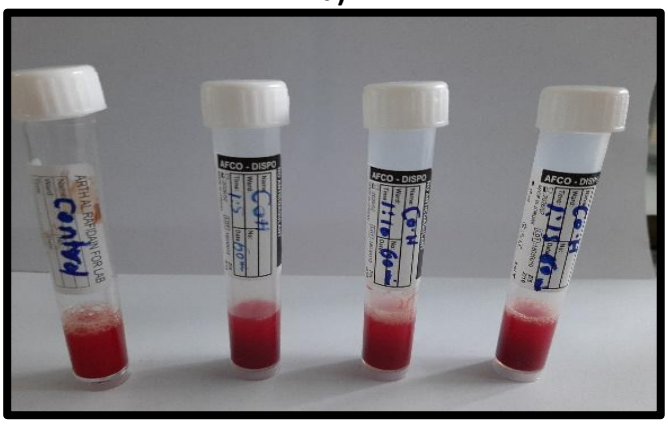

**Figure 2. SDS-PAGE of collagen hydrolysate from collagen skin of catfish**

**Cellular toxicity of collagen hydrolysate**

Figure 3 represent the results of cellular toxicity test for catfish skin collagen hydrolysate. The results revealed that collagenase hydrolysate (CH) which used at different concentration (250 ,500 , 1000 ppm) for different time had no toxicity function on human blood and no increase in absorbency, since there were no coagulation occurred . All tested samples were similar to control sample. This mean that the experimental collagen hydrolysate are safe for human consumption, so it can be used in many application such as medical field and as antioxidants in the food industry. These findings were similar to Liang (25) finding who mentioned that peptides prepared from salmon skin are safe for use in functional foods and medicines. The results of this study were also agreed with many related studies which showed that there were no toxic or side effect for collagen hydrolysate when experimental animals orally ingested with, and even when they were injected under the skin (37). Additionally, Vernon *et al* (42) mentioned that their findings suggest that collagen hydrolysate were a safe product for short and long term use, and that it is among the food products that the US Food and Drug Administration (FDA) considers as being safe products.




a)



b)

**Figure 3. Detection of cellular toxicity of collagen hydrolysate in human blood when used at different concentration (250 ppm ,500 ppm, 1000ppm) a) absorbance at 540nm. , b) blood appearance**

**Water holding capacity (WHC)**

The functional properties of proteins in a food system depend in part on the water protein interaction. Water holding capacity (WHC) refers to the ability of the protein to imbibe water and retain it against a gravitational force within a protein matrix. Water-holding capacity (WHC) and oil-holding capacity (OHC) of the experimental collagen and collagen hydrolysate are illustrated in table 3. It has been seen that both WHC & OHC of CH was significantly* **(P≤0.05)** higher than ASC & PSC. The highest value of WHC of CH after 90 min was 2.8 ± 0.07. This finding is in agreement with those reported by Zoheat (46) for hydrolyzed collagen from jellyfish treated with protamex. High WHC was due to the presence of low molecular weight peptides which have more hydroph-ilic properties as compared to peptides of higher molecular weight (19). The highest value WHC for CH was 2.8± 0.07, while the value of ASC & PSC from catfish skin were 2.39 ± 0.05 and 1.69 ± 0.03 respectively. The OHC of ASC, PSC and CH were 2.67±0.08 ,1.4±0.03 and 3.3±0.11 respectively. The OHC and WHC express the quantity of oil and water absorbed by collagen samples respectively, directly bound by the protein and are of great interest, especially in the meat and confectionary industries (12) . Several studies have shown that fish protein hydrolysates have excellent water holding capacity and can increase the cooking yield when added to minced meat (24) . Skin collagen hydrolysates absorbed more oil than ASC and PSC, increased oil absorption capacity may be attributed to the unfolding of protein structure, as well as exposure of more hydrophobic groups allowing the physical entrapment of oil. The mechanism of oil absorption has been attributed mostly to the physical entrapment of oil, and may also be influenced by hydrophobicity of the protein sample. Kinsella (20). The different skin protein hydrolysate exhibited good oil holding capacities (OHC) and could very well be used in the food industry.

**Table 3. Water holding capacity (WHC) and oil holding capacity (OHC) of (ASC, PSC) and collagen hydrolysate (CH) from skin collagen catfish**

| Functional properties | ASC Collagen | PSC Collagen | Collagen hydrolysate CH | LSD value |
|---|---|---|---|---|
| WHC | 2.39 ± 0.05 | 1.69± 0.03 | 2.8 ± 0.07 | 0.407 |
| OHC | 2.67 ±0.08 | 1.41 ±0.03 | 3.3 ± 0.11 | 0.571 |

**(P≤0.05)**

**Foaming:** The stability of foams is a consequence of the well-ordered orientation of the molecules at the interface, where the polar head is located in the aqueous phase and the hydrophobic chain faces the a polar component (34) .The foam capacity (FC) and foam stability (FS) of the ASC , PSC and collagen hydrolysate CH shown in table4:

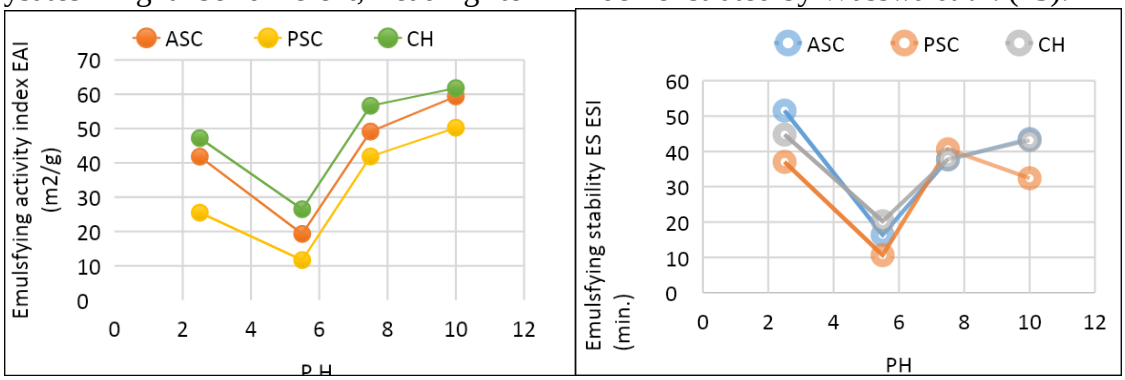
**Table 4. Foaming properties , foaming capacity (%) and foaming stability (%) of ( ASC, PSC) and collagen hydrolysate (CH) from skin collagen catfish**

| Foaming properties | Collagen ASC | Collagen PSC | Collagen hydrolysate CH | LSD value |
|---|---|---|---|---|
| Foaming capacity FC (%) | 120± 5.3 | 95±3.9 | 135 ±7.2 | 9.28 |
| Foaming stability FS (%) | | | | |
| 10min | 90 ±3.8 | 70± 2.6 | 102.5± 5.4 | 7.46* |
| 30 min. | 75± 2.6 | 62± 2.8 | 86.5± 3.6 | 7.08* |
| 60 min. | 55±2.1 | 52.5±2.5 | 71±3.3 | 6.55* |
| 90 min. | 42.5±2.7 | 40±1.9 | 53±2.8 | 5.29* |
| 120 min. | 38±1.8 | 32±1.4 | 43.5±1.7 | 5.87* |

The result indicated that the FC of collagen hydrolysate (135% ± 7.2) was significantly higher than those for ASC and PSC (120 % ± 5.3 _ 95% ± 3.9) respectively . similarly, collagen hydrolysate CH showed higher FS (102.5% ± 5.4 and 86.5% ± 3.6 after 10 and 30 min) than those of ASC (90% ± 3.8 and 75% ± 2.6) and PSC (70 % ± 2.6 and 62% ± 2.8, the hydrolysate with higher MW polypeptides, could be beneficial for formation of a stable film around the gas bubbles, this might be the primary cause of their higher foaming properties. The result suggested that collagen hydrolysates with high MWs peptides had better foaming properties than low MW fractions. However, foaming abilities of collagen hydrolysates with high MWs peptides were rapidly decreased after 10 min and the duration time was shorter than that of common foaming agents. Therefore, more research are needed to make sure whether those collagen hydrolysates can be applied as foaming agents in food processing or not(40).

**Emulsifying:** Figure 4 illustrate emulsion properties (a EAI , b ESI ) for ASC ,PSC ,CH . samples. It is obvious that the emulsion activity for all samples are pH dependent. At pH 2 the EAI value for CH was the highest (47.203) $m^2/g$ as compared to ASC and PSC. The same pattern was shown at pH 10, while remarkable decline were appeared in EAI values at pH 5. In general, the EAI for all samples increased beyond pH 5, and decreased at pH higher than 2 up to 5. The curve for ESI was similar to that for EAI at pH range (2-7), whereas, beyond pH 7 the values started to decline gradually up to pH 10 . The low EAI and ESI found at pH 5, correlates with solubility decrease (72.03%). Since the lowest solubility occurred at pH 5, peptides could not move rapidly to the interface, additionally, the net charge of peptide could be minimized at pH 5. The higher EAI of hydrolysates accompanied their higher solubility. The highest ESI of collagenase treated hydrolysates is due to its molecular weight (figure 2). Dagorn -Scaviner *et al*. (5) reported that the amino acid with amphiphilic character at an oil/water interface was more important than was peptide length for emulsion properties. The flexibility of protein or peptide structure may also be a vital factor governing the emulsifying properties. Hydrolysates with high solubility can rapidly diffuse and adsorb at the interface (6). Hydrolysates that had better EAI were not

necessarily having better ESI for all pH values tested suggesting that the sequence and composition of amino acids in peptide between hydrolysates might be different, leading to varying charge of the resulting peptides at a particular pH. Emulsifying properties were influenced by specificity of enzyme as also demonstrated by Wasswa *et al*. (43).




(a) (b)

**Figure 4. Emulsion properties (a Emulsion activity index (EAI), b) emulsion stability index (ESI) of catfish skin collagen ASC,PSC and collagen hydrolysate**

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