

# Production And Characterization Of Protein Hydrolysate: Effective Utilization Of Trawl Bycatch

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## Research Article

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# Abstract

**Background:** Fishing is an industry that provides livelihood to millions who live along the coast and delicious delicacies for the rest. Hence, improving the profitability of the trade has been of utmost importance and has been the research focus of major fishery industries around the globe. Exploring the potential commercialization of wastes produced by the industry is one way to achieve this goal. In this context, the waste may point towards discarded fishes, fish parts, etc. In this research, we are going to study the possibility to efficiently extract Fish Protein Hydrolysate (FPH) which has a huge commercial value from discarded by-catch fishes. Trawling is a fishing method which when employed often results in the capture of a huge quantity and diversity of non-target species due to lack of selectivity of the trawl net. These non-targeted species often termed as by-catch, are often disposed of. These under-valued species are targeted in this work for producing Fish Protein Hydrolysates (FPHs) using commercially available enzymes such as Papain (FPH<sub>1</sub>), Proteinase K (FPH<sub>2</sub>), and their functional characteristics such as solubility, foaming and emulsifying properties, oil and water-binding capacity are compared. The experimental design is as shown in the graphical abstract below.

**Results:** Among the two, Fish Protein Hydrolysates (FPHs), prepared FPH<sub>1</sub> has comparatively slightly improved functional properties.

**Conclusion:** It offers the potential to be used for various applications in the food industries.

## Introduction

Trawling is one of the methods of fishing in which the trawl net lacks selectivity and as a result, there is a capture of a copious quantity and diversity of non-target species which are commonly called bycatch. The term bycatch denotes the incidental catch (retained catch) of non-target species plus the discarded catch. These bycatches are often disposed of hastily because of the following reasons: these species have little or no commercial value, the cost involved in landing the fish, including sorting, storage, and processing is high compared to their commercial value, there is limited storage capacity in trawlers, as this facility is exclusively used for the target species. This practice of negligent discarding of by-catch produces a deleterious impact on the marine ecosystem (Vidotti et al., 2003.) as the bycatch in the main consists of eggs and juvenile species of commercially valuable species and endangered species.

Proteins derived from fish are nutritionally superior when compared to those of plant sources. They sustain a more nutritional balance of the dietary essential amino acids compared to all other animal protein sources (Ghaly et al., 2013.). So one productive way to exploit these proteinaceous under-valued species, which are a rich source of essential nutrients and bioactive peptides, is to convert them into protein hydrolysates (Aspmo et al., 2005.). Out of many methods which are available to produce protein hydrolysates, enzymatic hydrolysis remains an efficient way to recover the proteins from under-utilized species in a concentrated form without damaging the nutritional value and biological value of the fishes. Besides enzymatic hydrolysis improves the functional characteristics of protein like stability, foaming,

and emulsifying properties so that the hydrolysates can be used for various applications in the food industries (Balti et al., 2010.). During enzymatic hydrolysis, the peptide bonds present in the parent protein are broken down, and peptide chains of small length are produced.

The functionalities of the fish protein hydrolysate depend on the parameters such as protein sources, proteases used, degree of hydrolysis, pH, reaction temperature, and time (Huaixia Yin et al., 2010.). Besides the functional properties of protein hydrolysates also depend on intrinsic factors such as size, shape, amino acid sequence. The extrinsic factors that influence the functional properties of the protein are pH, temperature, and the ions present (Elavarasan et al., 2014.). Among various parameters that influence the hydrolytic process, the degree of hydrolysis is used for monitoring the hydrolysis process. It also acts as an indicator for comparing various protein hydrolysates (Gimenez et al., 2009.). Over the years the protein hydrolysates are reported to have improved functional properties like solubility, water, and oil binding capacities, emulsifying and foaming properties. The increase in solubility of the protein hydrolysates can be anticipated to the loss of secondary and tertiary structure and the release of small peptides (Taheri et al., 2011.), reduction in molecular size, and an increase in the number of polar and hydrophilic groups (Chalamaiah et al., 2014.). Hydrolysates tend to be surface-active materials and promote oil-in-water emulsion as they comprise both hydrophilic and hydrophobic groups. The presence of an increased quantity of larger molecular weight peptides or hydrophobic peptides contributes to the stability of the emulsion (Tanuja et al., 2012.). The protein hydrolysates have low-fat content than the native protein from which they were recovered and therefore they can be exploited as additives to improve the product's stability (Nilsang et al., 2005.). The ability of the hydrolysates to absorb water is apparently because of the increased concentration of polar groups such as COOH and NH<sub>2</sub> groups (Kristinsson and Rasco, 2000).

The emulsifying properties of hydrolyzed compounds are directly related to the effectiveness of hydrolysate in reducing the interfacial tension between the hydrophobic and hydrolytic components in food products (Cho et al., 2008). The foaming properties of protein hydrolysates are related to the transportation, penetration, and rearrangement of molecules at the air-water interface (Naveen Kumar et al., 2014.). Foam is produced when Proteins present in dispersion reduces the surface tension at the water-air interface. The oil-binding capacity is one of the notable functional characteristics of the ingredients used in the meat and confectionery industries because it influences the taste of the product. The mechanism of oil absorption is attributed primarily to bodily entrapment of the oil, and thus higher the bulk density of the protein, the more oil it absorbs. Although the substrate specificity of enzymes performs a crucial role in determining oil-binding capacity (Kristinsson et al., 2000.). This paper deals with the manufacturing and characterization of fish protein hydrolysates from underused catch trawls (dead fish) and offers the potential for various applications (Figure 1) in the food industry.

## **Materials And Methodology**

### **SAMPLE COLLECTION AND OTHER MATERIALS COLLECTION**

Trawl bycatch (dead fish) was collected from Nagapattinam fishing harbor and transported to Chennai in an ice container. The fishes were beheaded, eviscerated, and filleted to obtain the flesh. The flesh was washed with water to remove impurities and was stored in a deep freezer at -20°C until further use. Commercial enzymes Proteinase K and Papain were purchased.

## **PROTEIN HYDROLYSATE PRODUCTION**

Fish protein hydrolysate was produced according to the procedure of Halim and Sarbon (2019). The frozen flesh was thawed before use. 200 g of flesh was minced manually and boiled with twice the amount of water at 85°C for 20 mins to inactivate the endogenous enzymes. The meat was cooled at room temperature and subjected to enzymatic hydrolysis. Proteinase K with enzyme concentration 0.01% and papain of enzyme concentration 0.5% was added to the minced flesh and hydrolysis was carried out immediately. The parameters for enzymatic hydrolysis by Proteinase K are temperature 37°C, pH 7, time 120 minutes. The parameters for enzymatic hydrolysis by Papain are temperature 50°C, pH 7, time 90 m. The hydrolysis process was terminated by heating both mixtures to 85°C for 15 minutes to inactivate the mixtures. The fish protein hydrolysate was then filtered. The filtrate was then centrifuged at 6000 rpm for 20 mins. The soluble fraction was then freeze-dried and stored at -20°C until further use.

## **PROXIMATE ANALYSIS**

Moisture, protein, fat, and ash of both flesh and FPH were determined by following the AOAC (1993) methodologies (sec 950.46, 938.08, 960.39, and 955.04 respectively).

## **FUNCTIONAL PROPERTIES OF THE FISH PROTEIN HYDROLYSATE.**

### **SOLUBILITY**

The solubility of the fish protein hydrolysate at different pH was studied using the method of Jamil et al (2016) with some slight modifications. About 0.2% concentration of FPH was taken in distilled water and was dissolved at 60°C and room temperature until completely dissolved. The hydrolysate solution was adjusted to pH 4, pH 7, pH 10 using 1N HCl, 1N NaOH, 6N NaOH. After adjusting the pH the hydrolysate was centrifuged at 8500 rpm for 10 minutes. The protein content in the supernatant is determined by Lowry's method. The solubility of the fish protein hydrolysate is given by

$$\text{Solubility (\%)} = \frac{\text{protein content in supernatant}}{\text{total protein content in sample}} \times 100$$

### **WATER BINDING CAPACITY**

The water holding capacity of the fish protein hydrolysate was determined by the method described by Halim et al.,(2019) with some slight modification. About 10 mL FPH solution of concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 1.0% (w/v) were taken and mixed for 2 mins. The mixture was kept at room

temperature for 30 mins and then centrifuged at 5000 rpm for 30 mins. The supernatant was filtered through a filter paper and the volume recovered was measured. The water holding capacity of fish protein hydrolysate was calculated as follows

**Water holding capacity (mL/g)**

$$= \frac{\text{The volume of water added (mL)} - \text{Volume of supernatant (mL)}}{\text{Mass of hydrolysate (g)}}$$

## **OIL ABSORBING CAPACITY**

The oil binding capacity of FPH was determined according to a method by Razali et al. (2015) with slight modification. FPH solution with concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 1.0% (w/v) were taken in 10 mL centrifugal tubes and 5 mL of corn oil was added. The sample was thoroughly mixed and kept for 30 mins at 25°C with intermittent mixing every 10 minutes and then centrifuged at 2500 rpm for 25 minutes. The free oil obtained after centrifugation was decanted and the oil binding capacity of the fish protein hydrolysate was calculated by using the following formula

$$\text{Oil binding capacity (mL/g)} = \frac{\text{The volume of oil added (mL)} - \text{Volume of oil decanted (mL)}}{\text{Mass of hydrolysate (g)}}$$

## **EMULSIFYING PROPERTIES OF THE FISH PROTEIN HYDROLYSATE:**

The emulsifying properties were determined according to the method as described by Jamil et al. (2016) with some modification. Approximately 5 ml of vegetable oil was mixed with 5 mL of 1% eel protein hydrolysate (EPH) solution and the pH value was adjusted to pH 4, 7, and 10. Then, the mixture was sonicated. About 50  $\mu$ L aliquot of an emulsion was pipetted from the container at 0 and 10 mins after sonication and mixed with 5 mL of 0.1% sodium dodecyl sulfate (SDS) solution. Using a spectrophotometer, the absorbance of the diluted solution was measured at 500 nm ( $A_{500}$ ). The absorbance was measured immediately at 0 min ( $A_0$ ) and 10 mins ( $A_{10}$ ) after the emulsion formation. The formulas used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) were as follows:

$$\text{Emulsifying activity index (EAI) (m}^2\text{/g)} = (2 \times 2.303 \times A_{500}) / 0.25 \times \text{protein weight (g)}$$

$$\text{Emulsion stability index (ESI) (min)} = (A_0 \times \Delta t) / \Delta A$$

Where  $A_{500}$  is the absorbance at 500 nm,  $\Delta A$  is  $A_0 - A_{10}$  and  $\Delta t$  is the time at 10 mins

## **FOAMING PROPERTIES:**

The foaming properties were determined by using a method of Jamil et al. (2016) with some modification. About 10 mL of 0.5% hydrolysate solution was adjusted to pH 4, 7, and 10, followed by sonication for 10 mins. The whipped sample was immediately transferred into a 25-mL measuring cylinder and the total volume was read after 30 s. The foaming capacity was calculated using the following formula:

$$\text{Foaming capacity (\%)} = [(A-B)/B] \times 100$$

Where A is the volume after whipping (mL) and B is the volume before whipping (mL).

The whipped sample was allowed to stand at 20°C for 3 mins and the volume of the whipped sample was then recorded.

Foam stability was calculated as follows:

$$\text{Foam stability (\%)} = [(A-B)/B] \times 100$$

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

## **Results And Discussion**

### **PREPARATION AND CHARACTERIZATION OF FISH PROTEIN HYDROLYSATES:**

#### **PREPARATION OF FPHs:**

According to Halim and Sarbon (2016), the yield of fish protein hydrolysates (FPH) prepared accounts for around 8.96 percent of the total weight of the fish used. The inclusion of papain and proteinase k during the enzymatic hydrolysis process catalyzed the breakdown of the Fish's complex amino acid chains into multiple units of smaller and shorter amino acid chains, with the number of peptide bonds varying depending on the enzyme concentration, hydrolysis time, mixture pH, and temperature used for hydrolysis (Srichanun et al., 2014; Jamil et al., 2016). During the entire hydrolysis process, the yield of hydrolysate formed is dependent on the number of broken peptide bonds, which is determined by the number of peptide bonds in protein mass (degree of hydrolysis DH) (Hamid et al., 2015; Halim and Sarbon, 2016). This means that a higher DH contributes to a higher yield of protein hydrolysate.

#### **PROXIMATE COMPOSITION:**

The proximate composition of hydrolysates prepared with papain (FPH<sub>1</sub>) and proteinase k (FPH<sub>2</sub>) enzymes is shown in Table 1. The hydrolysate made from papain has a high protein content, which is beneficial for hydrolysis because it releases amino acids that improve the functional properties of the fish protein hydrolysate, allowing it to be used as an important source of protein (Baharuddin et al., 2015). When comparing papain hydrolysate to proteinase k hydrolysate, papain hydrolysate produces more ash.

Both enzymes generated hydrolysates with significantly low fat and moisture content, suggesting that these hydrolysates have the potential to be used in the food industry. The lipid content of both FPHs has dropped. Fish protein hydrolysates obtained by enzymatic hydrolysis were found to have a lower lipid content (Jemil et al., 2014, Taheri et al., 2012). The membranes of muscle cells begin to round up and form insoluble vesicles during the hydrolysis process, resulting in the removal of membrane structured lipids (Kristinsson and Rasco, 2000).

## **FUNCTIONAL PROPERTIES:**

### **SOLUBILITY:**

Figure 2 depicted the solubility of fish protein hydrolysates made with papain and proteinase K at various pH levels. In comparison to the solubility of the selected sample (unprocessed muscle protein), a notable improvement in the solubility of the FPHs can be seen in the graph. For pH 2, the sample demonstrated solubility of 6.2%, whereas the hydrolysate from FPH<sub>1</sub> (papain) reached 75.7%, whereas FPH<sub>2</sub> (proteinase K) showed solubility of 73.4%. At pH 10 the solubility of the hydrolysate produced from papain has a solubility of 93.23%. The protein hydrolysate produced by proteinase K has 89.98%. The charge on the weak acidic and basic side chain groups is affected by pH, so the hydrolysates have low solubility at their isoelectric points. (Nazeer et al., 2013). The protein molecules are broken down into smaller peptide units during the hydrolysis process, which explains the FPHs' high solubility at pH 10. (Yin et al., 2010.). The degree of hydrolysis (DH) of the protein hydrolysate is related to its solubility. The peptide bonds are broken during the hydrolysis process, exposing the protein's hydrophilic site (Jamil et al., 2016.). Hydrolysates with exposed hydrophilic sites have a higher solubility since they can form hydrogen bonds with water (Milewski, 2001).

FPH extracted with papain has a higher solubility at pH 10 than hydrolysate from pink perch muscle (*Nemipterus japonicas*) (Naqash and Nazeer, 2013). As a result, both of these FPHs have a lot of potential as a key component in human and animal food production.

### **FOAMING PROPERTIES:**

Figures 3a and 3b show the foaming capacity and stability of protein hydrolysates made from papain and proteinase K compared to untreated muscle protein (sample). Both hydrolysates have a higher foaming capacity than the sample. The protein hydrolysate made from papain has a slightly higher foaming capacity than the protein hydrolysate made from proteinase K. Foaming capacity was found to be higher at pH 2 and decreases as pH increases. An effective foaming agent demonstrates a high ability to rapidly migrate protein to the air-water interface. The ionic repulsions of peptides at the air-water interface may be responsible for the reduced foaming capacity in high alkaline environments (Klompong et al., 2007). The transportation, penetration, and rearrangement of molecules at the air-water interface are all linked to the foaming properties of protein hydrolysates (Elavarasan et al., 2014). When protein is dispersed in low water tension at the air-water interface, foam is formed (Tanuja et al., 2012). Furthermore, the low molecular weight (MW) of the hydrolysate affects foaming properties, as low MW

hydrolysates are unable to sustain the well-ordered, interface orientation of the molecule (Nalinanon et al., 2011). Figure 3b shows that FPH's foaming stability was lower than its foaming capability at all pH levels. The results of this study are close to those of Naqash and Nazeer (2013), who noticed that the foaming properties of pink perch muscle hydrolysate decreased at pH 4 and increased as the pH increased. The results, on the other hand, were higher than Chi et al (2014) recorded foaming ability of Spanish mackerel hydrolysate as 65%.

As shown in fig.3b, both hydrolysates have good foaming stability over a pH range. FPH1 (papain) foams up more consistently than FPH2 (Proteinase kinase). Protein molecules should form continuous intermolecular polymers that envelope the air bubbles because intermolecular cohesiveness and elasticity are needed for producing stable foams. The low surfactant activity of short peptide chains may explain the decrease in foam stability in hydrolysates (Mutilangi et al., 1996). The results of this study are close to those of Naqash and Nazeer (2013), who found that the foaming properties of pink perch muscle hydrolysate decreased at pH 4 and increased as the pH increased.

### **WATER BINDING CAPACITY:**

The water holding capacity of Fish protein hydrolysates FPH 1(papain) and FPH 2(proteinase k) significantly decreased ( $p < 0.05$ ) with increasing hydrolysate concentration from 0.2% to 1.0% (figure 4). The result showed that at 1.0% of both the FPHs had almost the highest surface area to mass ratio, resulting in the highest water holding capacity. In contrast, FPHs with a concentration of 1.0% consist of the least exposed surface that can be imbibed with water, therefore decreasing their water holding capacity. During the hydrolysis process, the complex protein is broken down into shorter amino acid chains, typically exposing the N-terminal (polar groups) which is ready to preferentially bind with the H-bond of water.

The ability of a protein to imbibe water and maintain it against gravity within a protein matrix is referred to as its water-holding ability (Foh et al., 2010). A higher water holding capacity in hydrolysate indicates a higher surface area to the mass ratio in hydrolysate that can interact with water's H-bond (Slizyte et al., 2009). The water-holding ability of hydrolysate was improved due to the low molecular weight of the hydrolysate and the increased concentrations of polar groups exposed during the breakdown of amino acid chains (Taheri et al., 2013.). FPHs can contain hydrophilic polar group amino acids such as serine, threonine, asparagine, and glutamine, which may have a positive impact on the hydrolysates' water-holding capability, which is critical for increasing cooking yield (Halim and Sarbon, 2016). FPH<sub>1</sub> has a higher water holding capacity than those of hydrolysates produced from bluewing searobin (3.75 mL/g), tilapia (1.77 – 2.10 mL/g), and zebra blenny (6.10 mL/g) muscles (dos Santos et al., 2011; Foh et al., 2011; Jemil et al., 2014).

### **OIL BINDING CAPACITY OF FISH PROTEIN HYDROLYSATE:**

Figure 5 shows the oil binding capacity of fish protein hydrolysates FPH<sub>1</sub> (Papain) and FPH<sub>2</sub> (Proteinase K) at different concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1.0%). The FPH 1(papain) at 1.0% showed the

highest oil binding capacity, followed by FPHs at 0.6%, 0.8%, 0.2%, and 0.4%. However, the oil binding capacity of FPHs displayed no significant differences ( $p>0.05$ ) at different hydrolysate concentrations.

Both FPHs have a lower oil binding ability than water-binding capacity. The higher amount of polar than non-polar groups at the N-terminal of amino acid chains can explain this. As a result, FPHs showed lower oil binding than water binding. Oil binding potential is normally correlated to the hydrophobicity of the protein surface, according to dos Santos et al. (2011). Based on these main findings, FPHs at various concentrations usually exhibited similar surface hydrophobic properties, allowing them to naturally bond with oil. The oil binding potential of protein hydrolysates is also influenced by the bulk density of peptides and enzyme/substrate specificity (Cho et al., 2008). The ability of hydrolysate to absorb oil is essential for influencing the taste and functional characteristics of products such as meat, salad dressings, bakery products, and confectionaries (Cho et al., 2008; dos Santos et al., 2011). As a result, both FPHs have been shown to have the potential to bind oil in food products.

FPHs, on the other hand, had an oil binding potential of less than 1.00 mL/g at all concentrations, which was lower than that of striped catfish (1.35 mL/g) and dagaa (3.50 mL/g) muscle hydrolysates (Tanuja et al., 2012; Betty et al., 2014).

#### **EMULSIFYING ACTIVITY:**

The emulsifying activity index (EAI) and emulsifying stability index (ESI) showed no significant differences ( $p>0.05$ ) of both FPHs but FPH<sub>1</sub> (papain) shows higher EAI and ESI at all pH levels than FPH<sub>2</sub> except for pH 4 because of low solubility at pH 4. the highest EAI of FPH1 at pH 10 (83.89 m<sup>2</sup>/g) followed by EAI of FPH<sub>1</sub> at pH 2 (81.9- m<sup>2</sup>/g), EAI at pH 8 (78.85 m<sup>2</sup>/g) and pH 6 (65.02 m<sup>2</sup>/g), while the highest ESI was at pH 10 (81.11min) followed by ESI at pH 2 (72.49 min), pH 8 (71.87 min) as presented in figure 6a and figure 6b.

Since they are less effective at reducing interfacial stress than larger peptides due to the lack of unfolding and reorientation at the interface, small peptides spread to and absorb quickly at the interface (Gbogouri et al., 2004). Polypeptides undergo structural unfolding as a result of the negative charges formed at high pH (pH 10). This peptide unfolding can induce repulsion while also improving interface orientation. This could lead to better exposure of hydrophilic and hydrophobic residues in peptides, which would promote interaction at the oil-in-water interface (Finger & Mangino, 1991). The effectiveness of the hydrolysate compound in reducing the interfacial stress between the hydrophobic and hydrolytic components in food products is directly related to the emulsifying properties of hydrolyzed compounds (Cho et al., 2008). By altering the surface hydrophobicity and charge of the protective layer surrounding the lipid globules, the pH of the environment may have a major impact on the emulsifying properties of hydrolysate (Taheri et al., 2013). The amino acid composition of the eel protein hydrolysate (EPH) also contributes to its emulsifying properties, in addition to the pH level. The presence of hydrophobic amino acids in hydrolysates may affect their emulsifying properties (Cho et al., 2008). According to studies, the EAI of

fish protein hydrolysates was highest at pH 6–10, and lowest at pH 4 (Pacheco-Aguilar et al., 2008; Naqash and Nazeer, 2013), which was consistent with the findings of this research.

## Conclusion

The functional properties of the fish protein hydrolysate like solubility, emulsifying activity index, and foaming capacities varied with the enzymes used. Overall the FPH<sub>1</sub> produced by papain exhibited better functional properties than FPH<sub>2</sub> produced by proteinase K. The functional properties of the hydrolysates were also affected by pH. Therefore the hydrolysates produced from trawl bycatch have the potential to be used in food industries as food emulsifiers and stabilizers to improve the quality of processed foods. However further research is essential to use them as additives in the food industry

## Declarations

**Ethics approval and consent to participate:** Not Applicable

**Consent for publication:** All authors gave the consented for publication..

**Availability of data and materials:**

The datasets generated and analysed during the current study are available in the Science Data Bank repository (**Science Data Bank**. <http://www.dx.doi.org/10.11922/sciencedb.00764>) and also the datasets used and analysed during the current study are available from the corresponding author on reasonable request.

**Conflict of interest**

The authors declare that they have no conflict of interest for this study.

**Funding support**

The authors declare that they have no funding support for this study.

**Author's contributions**

All authors collected samples from the fishing port of Nagapattinam (Trawl bycatch - dead fish) and brought them in ice containers to Chennai. All the authors have been researching the Vel Tech Engg College and Addis Ababa science and technology university, biotechnology laboratory. The final confirmatory enzyme and revisions to the manuscript are contributed to by all authors. A final version of the manuscript was approved by all the authors and the content therein was held accountable.

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## Table

**TABLE1:** Proximate analysis of Protein hydrolysates produced from papain and Proteinase K. values represent mean  $\pm$  SD of three replicates

| PROXIMATE COMPOSITION % | FPH FROM PAPAIN  | FPH FROM PROTEINASAE K |
|-------------------------|------------------|------------------------|
| MOISTURE                | 5.95 $\pm$ 0.48  | 4.86 $\pm$ 1.22        |
| PROTEIN                 | 92.14 $\pm$ 0.59 | 89.24 $\pm$ 3.63       |
| FAT                     | 0.60 $\pm$ 0.002 | 1.02 $\pm$ 0.73        |
| ASH                     | 6.23 $\pm$ 0.014 | 4.79 $\pm$ 0.33        |

## Figures

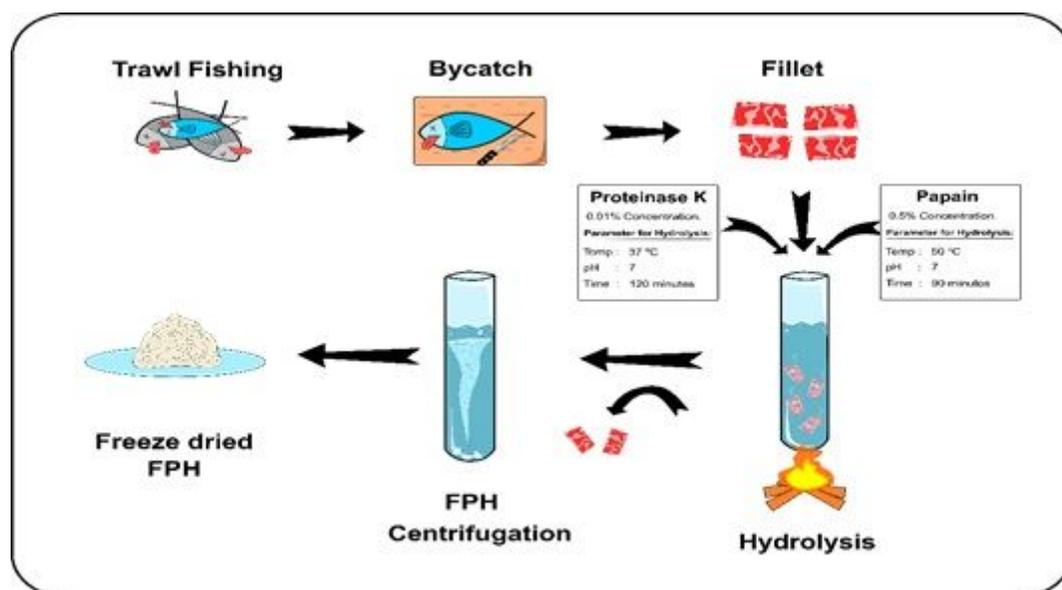
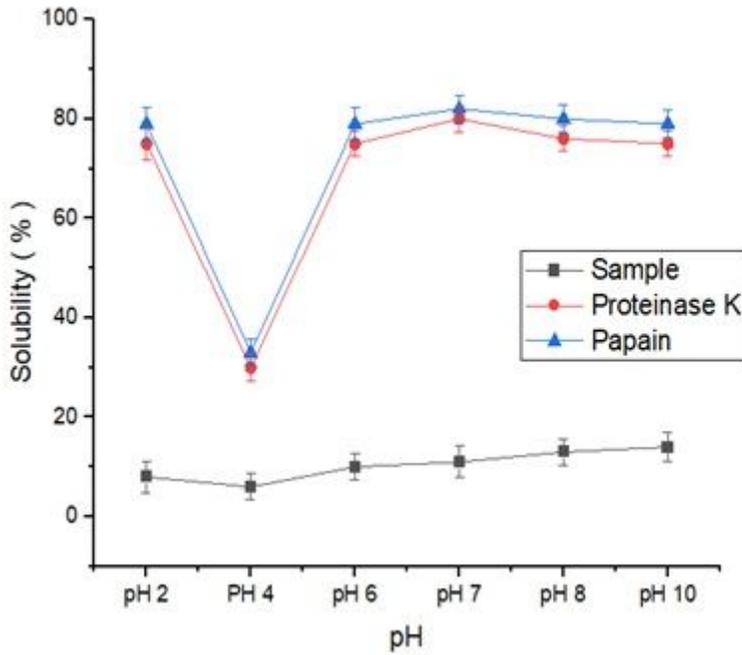


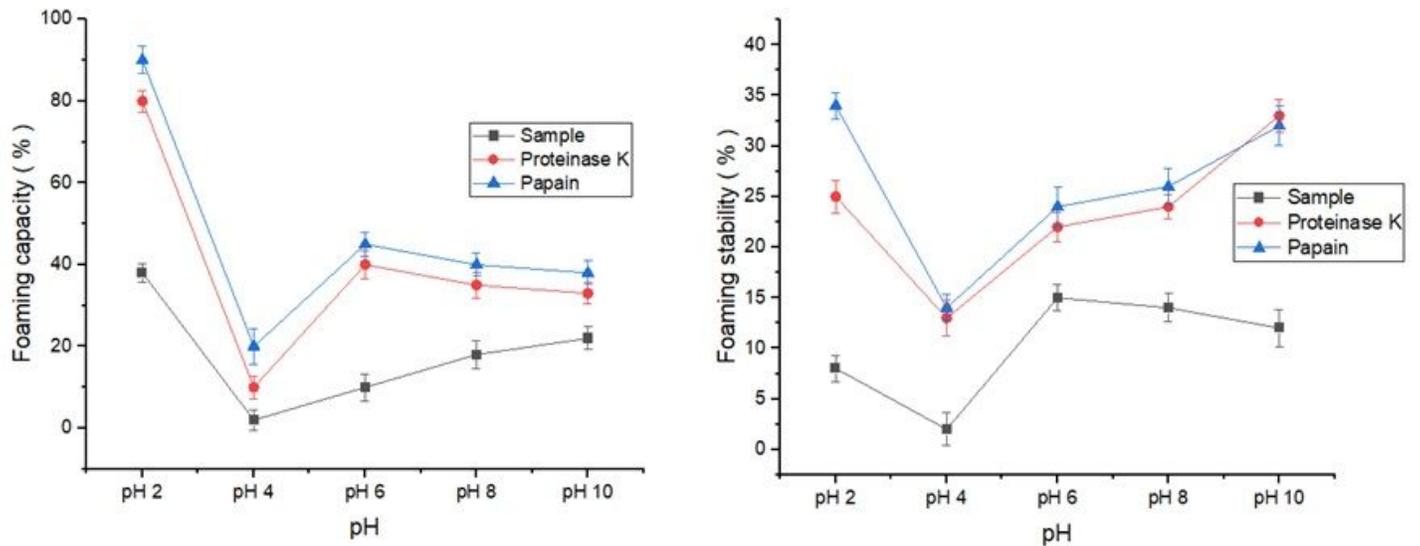
Figure 1

## Graphical abstract



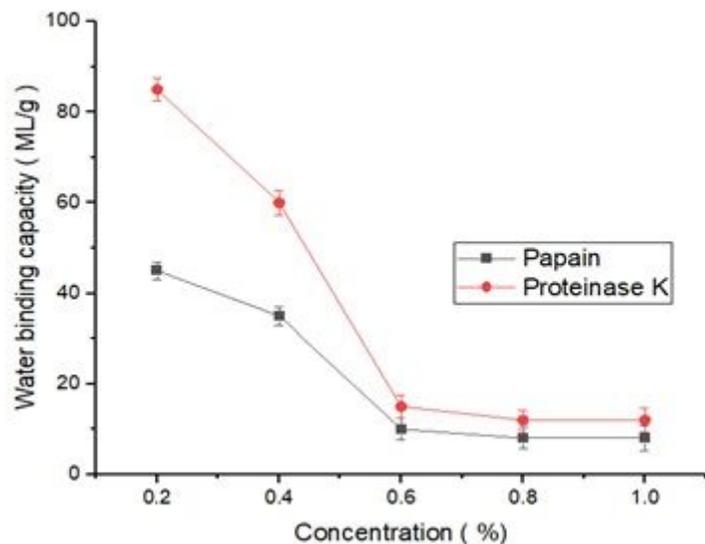
**Figure 2**

The solubility of the protein hydrolysates FPH1 (papain), FPH2 (proteinase K), and the sample at different pH.



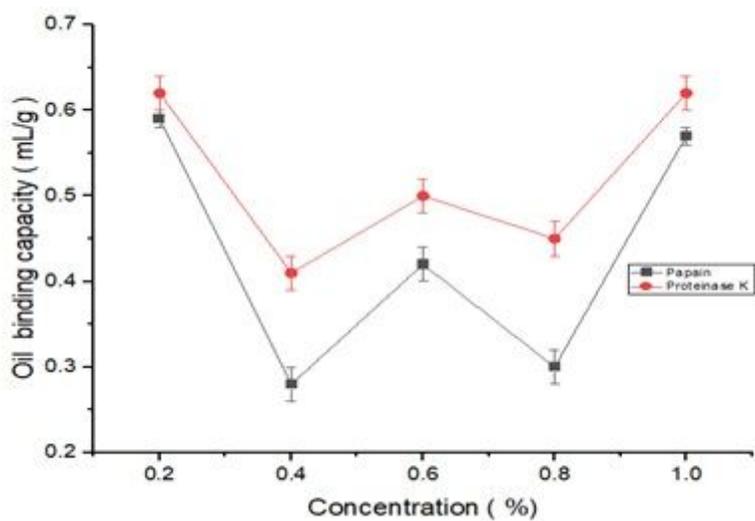
**Figure 3**

a. Foaming Capacity of the protein hydrolysates FPH1 (papain), FPH2 (proteinase k), and sample at different pH. b. Foaming stability of the protein hydrolysates FPH1 (papain), FPH2 (proteinase k) and sample at different pH.



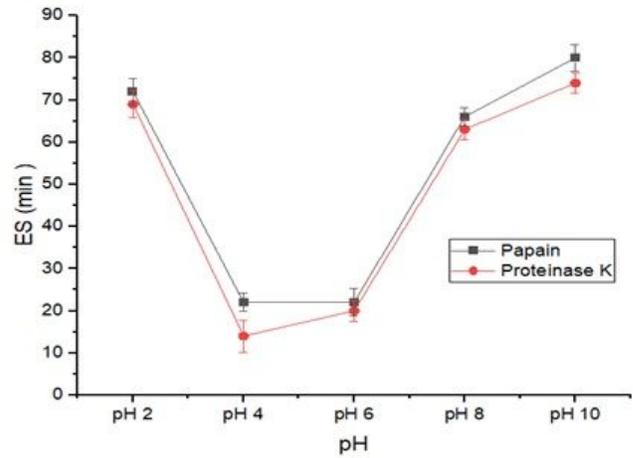
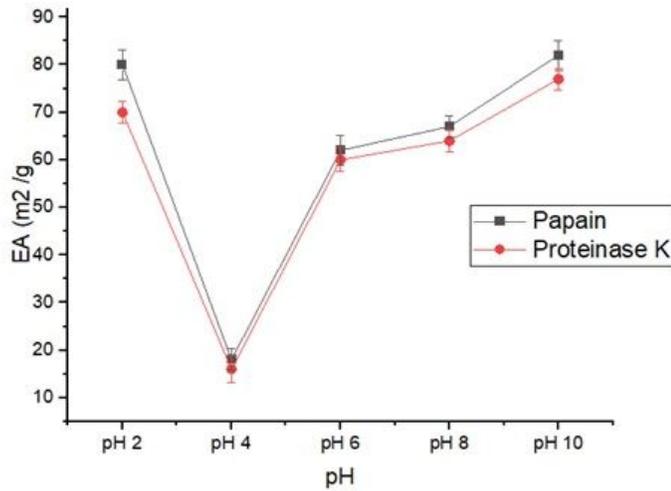
**Figure 4**

Water binding capacity of the protein hydrolysates FPH1 (papain) and FPH2 (proteinase K) at different concentrations.



**Figure 5**

Oil binding capacity of protein hydrolysates FPH1 (papain) and FPH2 (proteinase K) at different concentrations.



**Figure 6**

a. Emulsifying Activity Index (EAI) of protein hydrolysates FPH1 (papain) and FPH2 (proteinase K) at different pH. b. Emulsifying Stability Index (ESI) of the protein hydrolysates FPH1 (papain) and FPH2 (proteinase k) at different pH.

## Supplementary Files

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