

---

## Comparison the functional properties of protein Hydrolysates from poultry byproducts and rainbow trout (*Onchorhynchus mykiss*) viscera

Taheri, A.<sup>1\*</sup>, Anvar, S.A.A.<sup>2</sup>, Ahari H.<sup>3</sup>, Fogliano, V.<sup>4</sup>

Received: September 2011    Accepted: July 2012

### Abstract

Poultry by-products and rainbow trout (*Onchorhynchus mykiss*) viscera are abundant and underutilized resources that can be used as a unique protein source to make protein hydrolysates. In this study protein hydrolysate were made from these two different sources with Alcalase 2.4L. The functional properties of Fish viscera protein hydrolysate (FPH) compared to poultry by-products protein hydrolysate (PPH) were studied: Solubility, water holding capacity (WHC), oil absorption capacity (OAC), colour, emulsifying and foaming properties. Furthermore, the products were characterized by analyzing their amino acid composition. WHC, emulsifying activity, emulsifying stability and foaming properties and color of the FPH was significantly ( $P < 0.05$ ) higher than PPH, while OAC was not significantly ( $P > 0.05$ ) different. Methionine and histidine in both protein hydrolysates were the limiting amino acids and FPH had more hydrophobic residue. The differences in the amino acid composition between PPH and FPH may also be responsible for their different behaviours at various pH.

**Keywords:** Protein hydrolysate, Rainbow trout viscera, Poultry by-products, Functional properties, Alcalase

---

1-Faculty of Marine Sciences, Chabahar Maritime and Marine Sciences University, Chabahar, Iran,  
P.O. Box: 99717-56499.

2-Department of Food Hygien, Science and Research Branch, Islamic Azad University, Tehran, Iran.  
P.O. Box: 1954845554.

3-Young Researchers Club, Science and Research Branch, Islamic Azad University, Tehran, Iran.  
P.O. Box:1954845554.

4-Centro Interdipartimentale di Ricerche per la Gestione delle Risorse Idrobiologiche e per l'Acquacoltura  
Università i Napoli "Federico II" Parco Gussone Edificio 84,80055 Portici , Napoli, Italy.

\*Corresponding author's email: taheri@cmu.ac.ir

## Introduction

The use of fish waste has been of increasing interest in past years. It is considered to be a safe, high-protein material with many nutritional benefits, and a good pattern of essential amino acids (Guerard et al., 2001). Rainbow trout is the major aquaculture cold freshwater fish in Iran, with 73,642 tons being harvested annually, leaving a negotiable amount of viscera in related industries (FAO, 2011). Traditionally, fish wastes have been partly used to prepare fish feeds.

Also Iran produces about 1.6% of 73,402,695 tons indigenous poultry meat, annually produced worldwide (FAO, 2009). By-products of the poultry industry, which include viscera, bone, blood, head, feet, and feathers, constitute 28–30% of the total weight (Ockerman and Hansen, 2000). These by-products are the most agricultural wastes and rich in both protein and proteolytic enzymes. Notwithstanding reports on utilizing poultry viscera as a source of protein in animal feed (Ibid), no report on poultry by-products have been recorded to date.

Many of these protein-rich by-products have a range of dynamic properties that can potentially be used in food systems as binders, emulsifiers, and gelling agents (Balti et al., 2010). Improving the functional properties of these proteins, including solubility, water holding, oil holding, emulsifying, and foaming characteristics are a major challenge for food science. One alternative is to produce a form of concentrated protein product that may be used as food

ingredients due to the capability of their functional properties (Liceaga-Gesualdo and Li-Chan, 1999). Enzymatic hydrolysis is a good way to protein recovery from by products and produce value added products from wastes (Gildberg et al., 2002; Šližytė et al., 2009). Unlike mechanical or chemical treatments that often damage the product and reduce product nutrition, enzymatic proteolysis is mild, fast and controllable (Kristinsson and Rasco, 2000). Use of proteolytic enzymes is often an attractive means for improving functional properties of food proteins, without losing their nutritional value. Produced hydrolysates by enzymatic treatment are containing well defined peptide profiles and there is an extensive review on the application of enzymatic protein hydrolysates in human nutrition (Clemente, 2000). The application of enzyme technology to recover and modify fish proteins may produce a broad spectrum of food ingredients and industrial products for a wide range of applications. The enzymatic treatment of proteins generates peptides and amino acids, which can modify the biological and functional characteristics of the proteins and improve their quality and offers interesting opportunities for food applications (Balti et al., 2010).

Under controlled conditions, enzymatic hydrolysis influences the molecular size, hydrophobicity, and polar groups of the hydrolysate (Adler-Nissen, 1986; Kristinsson and Rasco, 2000). The characteristics of the hydrolysate directly

affect its functional properties (Kristinsson and Rasco, 2000). Protein hydrolysates have excellent solubility with a high degree of hydrolysis (Klompong et al., 2007). The high solubility of fish protein hydrolysate over a wide pH range is a useful characteristic for many food applications. Furthermore, it influences other functional properties, such as emulsifying and foaming properties (Kristinsson and Rasco, 2000; Gbogouri et al., 2004). Conversely, a very high degree of hydrolysis has adverse effects on the functional properties (Kristinsson and Rasco, 2000). Different industrial enzymes have been used for this purpose where Alcalase has been shown to give good functional properties (Kristinsson and Rasco, 2000).

Several underutilized aquatic sources have been investigated for the production of functional protein hydrolysates; these include *Clupea harengus* (Hoyle and Merritt, 1994; Sathivel et al., 2003), *Selaroides leptolepis* (Klompong et al., 2007), *Mallotus villosus* (Shahidi et al., 1995), and *Merluccius productus* (Benjakul and Morrissey, 1997) *Cirrhinus mrigala* (Chalamaiah et al., 2010). It seems recovered proteins from fish viscera such as rainbow trout may be modified to improve their quality and functional characteristics by enzymatic hydrolysis, which has been developed to convert under-utilized fish and their by-products into marketable and acceptable forms. Also this procedure may be able to use for the treatment of poultry by-products (Taheri et al., 2011). The objective of this study was to establish the feasibility of enzymatic hydrolysis using Alcalase to

increase the potential commercial value of most underutilized by-products (rainbow trout viscera and poultry by-products) by yielding value-added products with improved functional properties.

### Materials and methods

Poultry by-products (head and leg) were prepared from Tehran Slaughter House (Iran), and rainbow trout viscera were prepared fresh from fish market (Tehran, Iran), and then stored at -20°C before analysis. Alcalase (declared activity of 2.4 AU/g and density of 1.18 g/ml) was provided by the Iranian branch of the Danish company, Novozyme. All chemical reagents used for experiments were of analytical grade.

#### *Preparation of protein hydrolysates*

To establish the parameters that would provide protein hydrolysate with the same degree of hydrolysis, a pilot study by response surface methodology (RSM) was conducted (data not shown). The rainbow trout viscera and poultry by-products (head and leg) were first minced in a blender (Hootkhash Co., Iran) then heated at 85 °C in a water bath for 20 minutes (Guerard et al., 2002). The samples were mixed with distilled water 1:2 (w:v) and homogenized for 2 minutes. Alcalase was added to the substrate (0.07 AU/g protein for poultry, and 0.06 AU/g protein for trout viscera). All reactions were performed in a shaking incubator with constant agitation (200 rpm) at the optimum temperatures (52.51°C for poultry by-products and 50.32 °C for trout viscera based on RSM results). Following treatment, the reaction was terminated by heating the solution at

95 °C for 20 minutes (Guerard et al., 2002). The hydrolysates were then centrifuged at  $6700 \times g$  for 20 minutes (Hermle labortechnik GmbH, Z 206A, Korea). Degree of hydrolysis was estimated using the methods of Hoyle and Merritt (1994). The supernatant was then freeze-dried, ground into a fine powder and stored at 4°C in a dessicator for the next analysis.

#### *Proximate composition*

Proximate analysis of the raw materials and the protein hydrolysates were performed according to the procedures outlined by the Association of Official Analytical Chemists for moisture, ash, and protein, (AOAC) (1995). The total fat was extracted with a mixture of chloroform and methanol by following the methods of Bligh and Dyer (1959).

#### *Solubility*

In 20 ml of deionized water, 200 mg of protein hydrolysate were dispersed, and the mixture's pH was adjusted to between 2-12. Each mixture was stirred at room temperature for 30 minutes and centrifuged at  $7500 \times g$  for 15 minutes. The protein content of each supernatant was determined using the Biuret method; the total protein content was determined following solubilization of the sample in 0.5 N NaOH (Robinson and Hodgen, 1940). Protein solubility was calculated as

$$\text{Solubility} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100$$

follows:

#### *Water holding capacity (WHC)*

The water holding capacity (WHC) was determined using the method described by Rodriguez-Ambriz et al. (2005). Protein

samples (100 mg) were mixed with 1000 µl of distilled water using a stirrer. The protein suspension was then centrifuged at  $1800 \times g$  for 20 minutes at 22 °C. The supernatant was decanted, and the tube was drained at a 45° angle for 10 minutes.

#### *Oil absorption capacity (OAC)*

OAC was determined using the method described by Lin and Zayas (1987); 100 mg of protein sample was vortex with 1000 µl of sunflower oil for 30 sec. The resulting emulsion was incubated at room temperature for 30 minutes, and then centrifuged at  $13600 \times g$  for 10 minutes at 25 °C. The supernatant was decanted and drained at a 45° angle for 20 minutes. The volume of oil absorbed equals the sample's fat absorption capacity.

#### *Emulsifying properties*

The emulsifying activity index (EAI) and the emulsion stability index (ESI) were determined using the method described by Klompong et al. (2007). Three hundred milligrams of protein samples were dissolved in 30 ml of deionized water. This protein solution was mixed with 10 ml of sunflower oil, and the pH was adjusted to 2, 4, 6, 8 and 10. The mixture was homogenized at a speed of  $14000 \times g$  for 1 minute.

Aliquot of the emulsion was homogenized and 15 µl were pipetted from the bottom of the container at 0 and 10 min after homogenization. Afterward the sample mixed with 5 ml of 0.1% sodium dodecyl sulphate solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (Jenway, 6305, UK). This was used to calculate EAI and ESI using the method suggested by Pearce and Kinsella (1978):

$$\text{Emulsifying activity index (EAI)} (m^2 / g) = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{protein weight (g)}}$$

$$\text{Emulsion stability index (ESI)} (min) = \frac{A_{10} \times Dt}{DA}$$

Where  $A_0$  is the absorbance at 0 minutes following homogenization;  $A_{10}$  is the absorbance at 10 minutes following homogenization;  $Dt = 10$  min; and

$$DA = A_0 - A_{10}.$$

### *Foaming properties*

Foaming capacity and stability were determined according to the method of Sze-Tao and Sathe (2000): 250 mg of each

$$\text{Foam capacity (FC)} (\%) = \frac{\text{Volume after whipping} - \text{volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

$$\text{Foam stability (FS)} (\%) = \frac{\text{Volume after standing} - \text{volume before whipping (ml)}}{\text{Volume before whipping (ml)}} \times 100$$

### *Colour measurements*

The colour of the hydrolysate powders was evaluated using the Hunter Lab colorimeter (model Miniscan XE), working with D65 (day light), and a measure cell with an opening of 30 mm, being used the CIELab colour parameters:  $L^*$ ; from black (0) to white (100);  $a^*$ ; from green (-) to red (+); and  $b^*$ ; from blue (-) to yellow (+) (Kunte et al., 1997). Chroma and hue angle (degree) were calculated as follows (Hunt, 1977):

$$\text{chroma} = \sqrt{a^{*2} + b^{*2}}$$

$$H^\circ = \tan^{-1}(b^*/a^*)$$

### *Amino acid composition*

Dry hydrolysates were dissolved in distilled water at 1 mg/ml. Fifty microliters of each sample were dried and hydrolyzed in vacuum-sealed glass tubes at 110 °C for

protein sample were dissolved in 250 ml of distilled water, and the pH was adjusted to 2, 4, 6, 8 and 10. This protein solution was whipped for 3 minutes and poured into a 100 ml graduated cylinder. The total sample volume was taken at the zero minutes for foam capacity, and up to 60 minutes for foam stability. Foam capacity and stability were then calculated using the following equations:

24 h in the presence of constantly boiling 6 N HCl containing 0.1% phenol and using norleucine (Sigma–Aldrich, Inc., St. Louis, Mo., USA) as the internal standard. Then samples were vacuum dried, dissolved in the application buffer, and injected into a Biochrom 20 amino acid analyzer (Pharmacia, Spain).

### *Statistical analysis*

In this study *t*-test was performed using the computer program Graphpad Prism 5 for Windows; the confidence level was set at  $P \leq 0.05$ .

## **Results**

### *Proximate analysis and degree of hydrolysis*

In this study, hydrolysis was carried out to the similar degree for two different sources, allowing for a reliable comparison between the two products. The DH of PPH and FPH was  $15.42 \pm 0.8\%$  and  $15.4 \pm 0.3\%$ ,

respectively. Proximate composition of raw material and freeze-dried protein hydrolysates are displayed in Table 1.

Protein, ash, fat, and moisture of raw materials and protein hydrolysates from both sources was statistically different ( $p < 0.05$ ); the moisture, fat and ash in raw

materials were higher than those for the protein hydrolysates, while both hydrolysates demonstrate more protein content than do the corresponding raw materials. The ash content of the PPH was higher than FPH.

**Table 1: Proximate composition (%) of the freeze dried protein hydrolysate and the raw materials (n = 3)**

	Moisture	Fat	Protein	Ash
Poultry by-product	66.90± 1.65 <sup>a</sup>	7.86±0.46 <sup>a</sup>	20.85±0.76 <sup>b</sup>	10.62±0.88 <sup>a</sup>
PPH	3.78±0.04 <sup>b</sup>	0.7±0.1 <sup>b</sup>	84.66±0.09 <sup>a</sup>	4.70±0.34 <sup>b</sup>
Rainbow trout viscera	71.65± 0.89 <sup>a</sup>	13±0.76 <sup>a</sup>	15±0.063 <sup>b</sup>	2.73±0.89 <sup>a</sup>
FPH	3.45± 0.02 <sup>b</sup>	0.8±0.6 <sup>b</sup>	88.32±0.07 <sup>a</sup>	1.14±0.88 <sup>b</sup>

Results reported are means of triplicate samples ± standard deviation. Values in the same column with different superscripts are significant different at  $P < 0.05$

### Amino Acid Profile

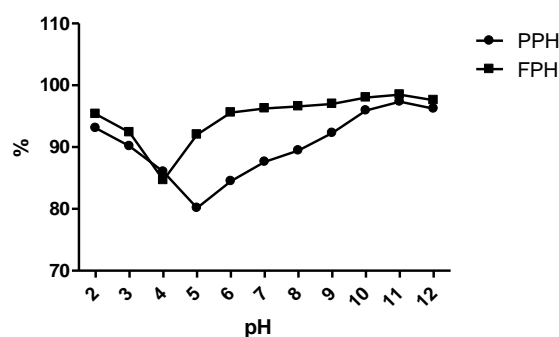
The amino acid composition of both protein hydrolysates, expressed as residues per 1000 residues, is shown in Table 2; the most abundant amino acid was Glycine (>29%). The total amino acid content of both FPH and PPH was 9.5% and 16.6%, respectively. PPH had more Hyp and Hyl content versus the FPH. Instead FPH had the more hydrophobic amino acids. Methionine and histidine in both protein hydrolysates were the first and second

limiting amino acids in comparison with the reference proteins.

### Functional properties

#### Solubility

The solubility of PPH and FPH in the pH range of 2–12 is shown in Figure 1; the maximum solubility of the two hydrolysates was over 96%, and FPH was more soluble than PPH. The least solubility of FPH and PPH was in pH 4 and 5, respectively.



**Figure 1: Solubility of poultry by-products protein hydrolysates (PPH) and rainbow trout protein hydrolysate (FPH) prepared by Alcalase as influenced by pHs.**

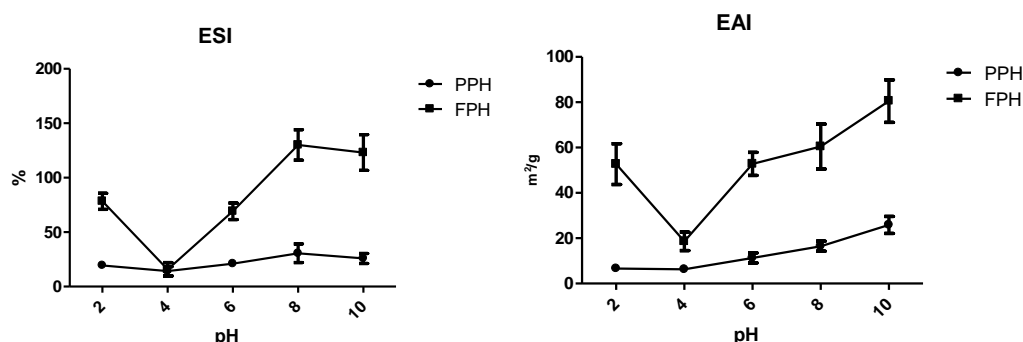
### Water Holding Capacity and Oil

#### Absorption Capacity

In this study, FPH had a significantly higher WHC ( $5.1 \pm 0.2$  ml /g hydrolysate) than PPH ( $2.8 \pm 0.2$  ml /g hydrolysate) ( $p < 0.05$ ). Also FPH and PPH showed similar OAC ( $3.1 \pm 0.12$  ml and  $2.8 \pm 0.10$  ml /g hydrolysate, respectively).

#### Emulsifying Properties

Proteins have the ability to stabilize food emulsions. The emulsifying activity index (EAI) and the emulsion stability index (ESI) of both hydrolysates are shown in Figure 2; FPH has higher emulsifying activity than does PPH at different pHs ( $p < 0.05$ ).



**Figure 2:** Emulsifying activity and stability of rainbow trout viscera protein hydrolysates (FPH) and poultry by-products protein hydrolysate (PPH) prepared by Alcalase at different pHs.

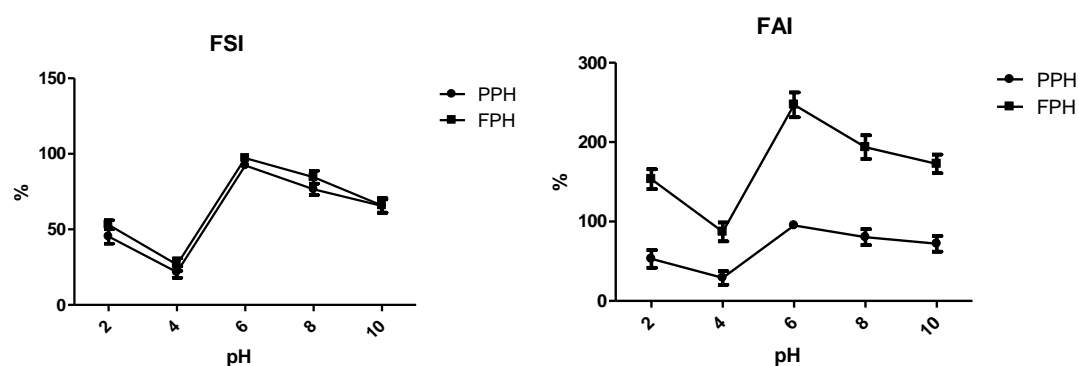
The maximum and minimum EAI was at pH 10 and 4 for both hydrolysates and FPH showed a higher ESI than PPH.

#### Foaming Properties

Results for PPH and FPH foaming capacity and stability are shown in Figure 3. FPH shows a significantly ( $p < 0.05$ ) higher foaming activity index (FAI) than the PPH, but the foaming stability index (FSI) of both protein hydrolysates was the same. In this study, the highest foam stability was found at pH=6, while stability decreased at both an acidic or basic pH. Also Low foam stability in acidic pH was related to poor solubility at pH=4.

#### Colour Measurement

In order to evaluate how hydrolysis influences the color of the hydrolysates, lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) of the powders were measured. The experimental  $L^*$ ,  $a^*$  and  $b^*$  mean values have been shown in Table 2. PPH had higher lightness value than FPH ( $p < 0.05$ ), with lower  $a^*$  and  $b^*$  values ( $p < 0.05$ ). Color data showed that FPH protein hydrolysate has a more yellowish color and is darker than PPH. PPH powder had a white appearance with minimal poultry odor and taste.



**Figure 3: Foaming capacity and stability of rainbow trout viscera protein hydrolysates (FPH) and poultry by-products protein hydrolysate (PPH) prepared by alcalase at different pHs.**

**Table 2: Hunter lab color parameter values of rainbow trout viscera protein hydrolysate (FPH) and poultry by-products protein hydrolysate (PPH)**

Sample	Hunter color parameters				
	L*	a*	b*	H°	chroma
FPH	68.9 ± 0.9 <sup>b</sup>	- 3.73 ± 1.2 <sup>a</sup>	18.4 ± 0.7 <sup>a</sup>	-78.54	18.77
PPH	78.8 ± 0.7 <sup>a</sup>	- 4.71 ± 0.9 <sup>b</sup>	11.1 ± 0.7 <sup>b</sup>	-67	12/06

## Discussions

In the present study functional properties of protein Hydrolysates from poultry by-products and rainbow trout was compared. The ash content of the PPH was higher than FPH, most likely due to the use of head and legs for the production of the protein hydrolysate. These sources contain a negotiable amount of bone, which is the major source of minerals in the hydrolysate. The low fat content of PPH and FPH demonstrate that centrifugation efficiently separates the fat moiety that is released from enzymatic hydrolysis. Nilsang et al. (2005) report the protein hydrolysate has a low fat content which can enhance the product's stability. With respect to protein content, similar results were reported by other authors for different fish and degrees of hydrolysate (Kristinsson and Rasco, 2000; Gbogouri et al., 2004).

The increased solubility of protein hydrolysates compared to that of the original protein is due to a loss of secondary and tertiary protein structure and to the release of small peptides (Chobert et al., 1988). Proteins and protein hydrolysates have the lowest solubility at the isoelectric point (pI) (Kristinsson and Rasco, 2000). The least solubility of FPH and PPH suggesting PPH has a different isoelectric point than does FPH. Solubility variations could be attributed to both the net charge of peptides that increase as pH moves away from pI and surface hydrophobicity, which promotes aggregation via hydrophobic interaction (Sorgentini and Wagner, 2002). The high solubility of both hydrolysates over a wide range of pH is due to the low molecular weight of the peptides, which are also quite rich in hydrophilic amino acids.



Imino acids are abundantly present in connective tissue and skin that contains collagen (TaHERi et al., 2011). The higher levels of glycine, hydroxyproline, and proline in PPH indicate that higher amounts of connective tissues were present in the raw material during the production of this protein hydrolysate. During the enzymatic hydrolyzing process, shaking introduced oxygen into the water and protein oxidation may have taken place. Furthermore, the heat treatment performed at the end of the procedure to denature the proteases may have caused the partial decomposition of these amino acids.

Fish protein hydrolysates have an excellent water holding capacity (WHC) and can increase the cooking yield (Kristinsson and Rasco, 2000). The increased concentration of polar groups such as COOH and NH<sub>2</sub> that is caused by enzymatic hydrolysis has a substantial effect on the amount of adsorbed water (Kristinsson and Rasco, 2000). In agreement with our results about of WHC,

a similar trend was observed for shark protein hydrolysate in water absorption from 5 to 8 ml/g of sample in different DHs: 6.5, 13.0 and 18.8% (Diniz and Martin, 1996). The WHC of minced cod was reported to be 12% for FPH obtained from frozen backbones and 16% for FPH that was obtained from fresh backbones and also in cuttlefish protein hydrolysate reported from 2.5 to 5.5 ml/g of sample (in different DHs: 5.0, 10.0 and 13.5%) (Šližytė et al., 2009; Balti et al., 2010). The obtained results indicate that FPH, having more hydrophilic polar side chains, can absorb more water in comparison to PPH. FPH contains more glutamic and aspartic acids (Table 3) than PPH, and these residues can bind almost 3 times more water than non-ionizable polar groups (Deeslie and Cheryan, 1988). These results also suggest that the hydrolysates could be used as an additive in intermediate-moisture (IM) foods to bind water and improve texture (Chiang et al., 1999).

**Table 3: Amino acid composition of poultry by-products hydrolysate (PPH) and rainbow trout viscera hydrolysate (FPH).**

Amino acids	Number of residues/1000 residues	
	PPH	FPH
Asx	34	65
Thr	16	22
Ser	45	36
Glx	45	67
Pro	71	66
Gly	340	229
Ala	64	63
Cys	8	6
Val	43	57
Met	22	29
Ile	18	51
Leu	34	64
Tyr	30	63
Phe	14	23
His	16	18
Lys	33	46
Arg	41	55
Hyl	31	12
Hyp	95	28
Total	1000	1000
Imino Acids	166	95

Determinations were performed in triplicate and data correspond to mean values. Asx = Asp + Asn; Glx = Glu + Gln.

OAC showed the quantity of oil is bound by the protein and it is an important functional characteristic for the meat and confectionary industries (Gbogouri et al., 2004). Hydroxy proline content affects OAC, and a powder containing higher amounts of charged amino acids, such as aspartic acid, glutamic acid, lysine and arginine is able to absorb more fat (Šližyte et al., 2009). The OAC values of this study are similar to those found for grass carp skin hydrolysates, which were from 3.6 to 2.4 ml oil/g hydrolysate (Wasswa et al., 2007),

but lower than those reported for red salmon head (Sathivel et al., 2005).

In this study, the emulsifying properties of two different protein hydrolysates at the same DH have been compared. When each enzyme needs a specific side chain on protein for action, the same DH is not equivalent to the same peptide length. It is concluded that differences in the EAI and ESI values from two different protein hydrolysates are derived from the different nature of peptides that are produced during hydrolysis. Based on the research of Chobert et al. (1988), Peptides with low molecular weight may not be amphiphilic enough to exhibit good emulsifying properties. However, Kristinsson and Rasco (2000) mentioned that there is no clear connection between peptide size and emulsification, suggesting that the physical and chemical characteristics of the peptides may play an important role in functional properties.

It is reported that amino acid composition, sequence of the polypeptide and its amphiphilic character is more important than the peptide length in emulsion properties (Rahali et al., 2000).

Poultry by-products contain skin collagen and this could affect on the amino acid profile of produced PPH (Table 2). Skin collagen contains a noticeable amount of Imino acids such as hydroxyproline (Taheri et al., 2009). The amino acid profiles showed hydroxyproline and hydroxylysine in PPH was higher than FPH. This may cause a higher degree of protein-protein interaction via hydroxyl groups from Hyl and Hyp. Furthermore, FPH has higher protein content in hydrophobic residues. These may lead to a more hydrophilic/hydrophobic distribution of

the amino acids which, as discussed above, has been reported to be more relevant to the length of the peptides that affect emulsifying properties (Rahali et al., 2000). Giménez et al. (2008) report the same result for squid skin hydrolysate: due to the higher content of hydroxylated amino acid.

The maximum and minimum EAI was at pH 10 and 4 for both hydrolysates. The tendency was similar to that of protein solubility. A significant increase in EAI at pH=10 may be due to higher quantities of soluble proteins generated by hydrolysis under alkaline conditions ( $P<0.05$ ). Factors such as blending speed, protein source, temperature, pH, type of oil added, and water content can influence emulsion capacity (Linder et al., 1996). Environmental pH also affects emulsifying properties by changing the solubility and surface hydrophobicity of proteins, as well as the charge of the protective layer surrounding the lipid globules. Ions alter the electrostatic interactions, conformation, solubility of the proteins, and hydrophilic-lipophilic balance (Sikorski, 2002). At highly alkaline pH, polypeptides can be unfolded due to negative charges. Repulsion could be resulted from this change and allowing for better orientation at the interface (Pacheco-Aguilar et al., 2008). This could result in a more efficient exposure of hydrophilic and hydrophobic residues in these peptides, promoting a major interaction at the oil-water (O:W) interface. Since the lowest solubility occurred at pH 4 and 5 for FPH and PPH, respectively, peptides could not move rapidly to the interface. Additionally, the net charge of the peptide will be minimized at these pH values. The higher EAI of the hydrolysates accompanied their increased solubility.

Hydrolysates with high solubility can rapidly diffuse and adsorb at the interface, as noted by Klompong et al. (2007) study on yellow-striped trevally protein hydrolysate.

In this study FPH showed a higher ESI than PPH, which means that FPH cannot produce a stable emulsion. An increase in ESI with the increase in pH values after the isoelectric point had been attributed to the formation of charged layers around fat globules, causing mutual repulsion and forming a hydrated layer around the interfacial material (Aluko and Yada, 1995). Results suggest that peptides of the poultry by-products and rainbow trout hydrolysate have different amino acid composition and leading to a varying charge at a particular pH, and so different EAI, and ESI values.

Foam formation is governed by three different factors: the transportation, penetration and reorganization of molecules at the air–water interface (Wilde and Clark, 1996). A protein that can produce good foaming properties must be capable of rapidly migrating to the air–water interface, thereby lowering the surface tension, rapidly unfolding and reorganizing its structure (Martin et al., 2002). It should be noted that the adsorption rate to the air–water interface may be influenced by the molecular size, protein structure and hydrophobicity of the hydrolysates (Martin et al., 2002). These are highly dependent on the parent protein from which they are obtained and the hydrolysis procedure. The hydrolysis of protein produces a range of peptides that possess altered hydrophobicity, net charge, and conformation in comparison to the native molecule. Their reduced molecular weight makes them more flexible, form a stable interfacial layer and increase the rate of

diffusion to the interface, which in turn improves foaming ability (Wilde and Clark, 1996).

FPH peptides that were produced in this study were efficiently absorbed and denatured in order to sufficiently reduce the interfacial tension and form the viscoelastic film that is required for an effective foaming agent. Conversely, PPH has demonstrated weak foaming capacity. Our data about the highest foam stability suggests that pH has a major effect on foam stability when using protein hydrolysate. The decreased foam stability at very acidic or alkaline pHs may be due to the repulsion of peptides via ionic repulsion (Klompong et al., 2007).

Foam stability is enhanced by flexible protein domains that enhance the viscosity of the aqueous phase, protein concentration and film thickness (Phillips et al., 1994). However, according to Damodaran (1996), foaming capacity and stability are influenced by two different sets of molecular properties of protein/peptides that are often antagonistic. While the first property is affected by absorption rate, flexibility and hydrophobicity, the other depends on the viscoelastic nature of the film.

A protein may have excellent foam ability, but it may not necessarily produce stable foam, and vice versa (Wilde and Clark, 1996). In the current study, PPH demonstrates a weak foaming capacity in comparison with FPH, but good foam stability. Forming strong films and more stable foam could be resulted by reorganization the tertiary structure of proteins at the interface and maintain an extensive intermolecular network (protein-protein interactions) (Liceaga-Gesualdo and Li-Chan, 1999). Good foam stabilization of

PPH may be due to its amino acid composition. As mentioned above, it is high in hydroxyproline and hydroxylysine. Pro and Lys hydroxylation, which increases the number of hydrogen bonds, results in a dense protein network that favors foam stabilization (Giménez et al., 2008). In this study, peptide preparations are able to provide stable foam. Foaming properties were mostly affected by the peptide structure and amino acid composition, net charge of molecules, distribution of this charge and hydrophobicity (Adler-Nissen, 1986).

Colour influences the overall acceptability of food products and is affected by several factors such as species, processing, fat content, moisture, light, temperature, haemoglobin, myoglobin, and new protein ingredients in food formulations (Bueno-Solano et al., 2008).

Color data showed that FPH protein hydrolysate has a more yellowish color and is darker than PPH. PPH powder had a white appearance with minimal poultry odor and taste, but FPH powder was yellowish and had a pronounced fishy odor and taste. Trout viscera have different sections of digestive tract that contain high colorant pigments. The darker, more yellowish color of FPH may be due to the higher levels of hemoglobin, myoglobin, and other pigments that are found in the digestive tract that maintain soluble after centrifugation.

### Conclusions

The results of the current study show that rainbow trout viscera protein hydrolysate has better functional properties than do poultry by-products protein hydrolysate. Enzymatic treatment permits the production of functional hydrolysates from poultry by-products with low commercial value. The

technology of enzymatic hydrolysing makes it possible to increase direct human consumption of vastly underutilized protein sources.

### Acknowledgements

Authors wish to thank Mr. Asadollah Sardarzehi and papercheck for editing the English text and Chabahar Maritime University for their support of this research.

### References

- Adler-Nissen, J., 1986.** Enzymatic hydrolysis of food proteins. London: Elsevier Applied Science. 57–109.
- Aluko, R. E. and Yada, R.Y., 1995.** Structure–function relationships of cowpea (*Vigna unguiculata*) globulin isolate: influence of pH and NaCl on physicochemical and functional properties. *Food Chemistry*, 53, 259–265.
- Association of Official Analytical Chemists, 1995.** Official methods of analysis. 16<sup>th</sup> ed. Washington, DC.
- Balti, R., Bougatef, A., El-Hadj Ali, N., Zekri, D., Barkia, A. and Nasri, M., 2010.** Influence of degree of hydrolysis on functional properties and angiotensin I-converting enzyme inhibitory activity of protein hydrolysates from cuttlefish (*Sepia officinalis*) by-products. *Journal of Science of Food and Agriculture*, 90, 2006–2014.
- Benjakul, S. and Morrissey, M., 1997.** Protein hydrolysates from Pacific whiting solid wastes. *Journal of Agricultural and Food Chemistry*, 45, 3423–3430.
- Bligh, E. G. and Dyer, W. J., 1959.** A rapid method of total lipid extraction and purification. *Canadian Journal of*

- Biochemistry and Physiology*,37,911–917.
- Bueno-Solano, C., López-Cervantes, J., Campas-Baypoli, O. N., Lauterio-García, R., Adan-Bante, N. P. and Sánchez-Machado, D. I., 2008.** Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. *Food chemistry*, 112, 671-675.
- Chiang, W. D., Shih, C. J. and Chu, Y. H., 1999.** Functional properties of soy protein hydrolysate produced from a continuous membrane reactor system. *Food Chemistry*,65,189-194.
- Chobert, J. M., Bertrand-Harb, C. and Nicolas, M. G., 1988.** Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *Journal of Agriculture and Food Chemistry*, 36, 883–886.
- Clemente, A., 2000.** Enzymatic protein hydrolysates in human nutrition. *Trends in Food Science and Technology*, 11, 254–262.
- Damodaran, S., 1996.** Amino acids, peptides and proteins. In O. R. Fennema (Ed.), *Food chem.* Marcel Dekker, Inc., New York, 321–429.
- Deeslie, W. D. and Cheryan, M., 1988.** Functional properties of soy protein hydrolysates from a continuous ultrafiltration Reactor. *Journal of Agriculture and Food Chemistry*,36, 26-31.
- Diniz, A. M. and Martin, A. M., 1997.** Optimization of nitrogen recovery in the enzymatic hydrolysis of dogfish (*Squalus acanthias*) protein: Composition of the hydrolysates. *International Journal of Food Science and Nutrition*, 48, 191-200.
- FAO STAT-Agriculture, 2011.** Available at <http://faostat.fao.org> ,[internet],
- FAO/WHO., 1985.** Energy and protein requirements. Report of Joint FAO/WHO/UNU Expert Consultation Technical Report. FAO/WHO and United Nations University, Geneva, Series No. 724, 116–129.
- Gbogouri, G. A., Linder, M., Fanni, J. and Parmentier, M., 2004.** Influence of hydrolysis degree on the functional properties of salmon byproduct hydrolysates. *Journal of Food Science*, 69, 615–622.
- Giménez, B., Gómez-Estaca, J., Alemán, A., Gómez-Guillén, M. C. and Montero, P., 2008.** Physico-chemical and film forming properties of giant squid (*Dosidicus gigas*) gelatin.*FoodHydrocolloid*,23,585-92.
- Guerard, F., Duffose, L., De La Broise, D. and Binet, A., 2001.** Enzymatic hydrolysis of proteins from yellowfin tuna (*Thunnus albacares*) wastes using alcalase. *Journal of Molecular CatalysisB:Enzymatic*,11,1051-1059.
- Guérard, F., Guimas, L. and Binet, A., 2002.** Production of tuna waste hydrolysates by a commercial neutral protease preparation. *Journal of Molecular Catalysis B: Enzymatic*, 19-20, 489-498.
- Hoyle, N. T. and Merritt, J. H., 1994.** Quality of fish protein hydrolysate from Herring (*Clupea harengus*). *Journal of Food Science*, 59, 76–79.
- Hunt, R. W. G., 1977.** The specification of colour appearance. Concepts and terms.

- Color Research and Application, 2, 55-68.
- Klompong, V., Benjakul, S., Kantachote, D. and Shahidi, F., 2007.** Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influence by the degree of hydrolysis and enzyme type. *Food Chemistry*, 102, 1317-1327.
- Kristinsson, H. G. and Rasco, B. A., 2000.** Fish protein hydrolysates: production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition*, 40, 43-81.
- Kunte, L. A., Gennadios, A., Cuppett, S. L., Hanna, M. A. and Weller, C. L., 1997.** Cast films from soy protein isolates and fractions. *Cereal Chemistry*, 74, 115-118.
- Liceaga-Gesualdo, A. M. and Li-Chan, E. C. Y., 1999.** Functional Properties of Fish Protein Hydrolysate from Herring (*Clupea harengus*). *Journal of Food Science*, 64(6), 1000-1004.
- Lin, C. S. and Zayas, J. F., 1987.** Functional of defatted corn germ proteins in a model system: Fat binding and water retention. *Journal of Food Science*, 52, 1308-1311.
- Linder, M., Fanni, J. and Parmentier, M., 1996.** Functional properties of veal bone hydrolysates. *Journal of Food Science*, 61, 712-6.
- Martin, A. H., Grolle, K., Bos, M. A., Stuart, M. A. C. and VanVliet, T., 2002.** Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability. *Journal of Colloid and Interface Science*, 254, 175-183.
- Nilsang, S., Lertsiri, S., Supphantharika, M. and Assavanig, A., 2005.** Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. *Journal of Food Engineering*, 70, 571-578.
- Ockerman, H. W. and Hansen, C. L., 2000.** Poultry by-products. Ockerman, H. W., Hansen, C. L. (Eds.), *Animal by Product Processing and Utilization*. CRC press, New York, pp. 439-455.
- Pacheco-Aguilar, R., Mazorra-Manzano, M. A. and Ramírez-Suárez, J. C., 2008.** Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease. *Food Chemistry*, 109, 782-789.
- Pearce, K. N. and Kinsella, J. E., 1978.** Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agriculture and Food Chemistry*, 26, 716-723.
- Phillips, L. G., Whitehead, D. M. and Kinsella, J. E., 1994.** Protein stabilized foams. In L. G. Phillips, D. M. Whitehead, & J. E. Kinsella (Eds.), *Structure-function of food proteins*. New York, Academic Press. 131-152.
- Rahali, V., Chobert, J. M., Haertlé, T. and Guéguen, J., 2000.** Emulsification of chemical and enzymatic hydrolysates of b-lactoglobulin: Characterization of the peptides adsorbed at the interface. *Nahrung/Food*, 44, 89-95.
- Robinson, H. W. and Hodgen, C. G., 1940.** The biuret reaction in the determination of serum protein, A study of the condition necessary for the production of the stable color which bears a quantitative relationship to the protein

- concentration. *The Journal of Biological Chemistry*, 135, 707–725.
- Rodríguez-Ambriz, S. L., Martínez-Ayala, A. L., Millán, F. and Dávila-Ortiz, G., 2005.** Composition and functional properties of *Lupinus campestris* protein isolates. *Plant Foods for Human Nutrition*, 60, 99–107.
- Sathivel, S., Bechtel, P. J., Babbitt, J., Smiley, S., Crapo, C., Reppond, K. D., et al., 2003.** Biochemical and functional properties of Herring (*Clupea harengus*) byproduct hydrolysates. *Journal of Food Science*, 68, 2196–2200.
- Sathivel, S., Smiley, S., Prinyawiwatkul, W. and Bechtel, P. J., 2005.** Functional and nutritional properties of red salmon (*Oncorhynchus nerka*) enzymatic hydrolysates. *Journal of Food Science*, 70, 401–406.
- Shahidi, F., Han, X. Q. and Synowiecki, J., 1995.** Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). *Food Chemistry*, 53, 285–293.
- Sikorski, Z. E., 2002.** Chemical and Functional Properties of Food Components. Second Edition. CRC Press LLC.
- Sinha, R., Radha, C., Prakash, J. and Kaul, P., 2007.** Whey protein hydrolysate: Functional properties, nutritional quality and utilization in beverage formulation. *Food Chemistry*, 101, 1484–1491.
- Šližytė, R., Mozuraitytė, R., Martínez-Alvarez, O., Falch, E., Fouchereau-Peron, M. and Rustad, T., 2009.** Functional, Bioactive and Antioxidative Properties of Hydrolysates Obtained from Cod (*Gadus morhua*) Backbones. *Process Biochemistry*, 44, 668–677.
- Sorgentini, D. A. and Wagner, J. R., 2002.** Comparative study of foaming properties of whey and isolate soy bean proteins. *Food Research International*, 35, 721–729.
- Sze-Tao, K. W. C. and Sathe, S. K., 2000.** Functional properties and in vitro digestibility of almond (*Prunus dulcis* L.) protein isolate. *Food Chemistry*, 69, 153–160.
- Taheri, A., Abedian Kenari, A. M., Gildberg, A. and Behnam, S., 2009.** Extraction and physico-chemical characterisation of Greater lizardfish (*Saurida tumbil*) skin and bone gelatine. *Journal of Food Science*, 74, 160–165.
- Taheri, A., Abedian Kenari, A., Motamedzadegan, A. and Habibi-Rezaei, M., 2011.** Poultry By-Products Enzymatic Hydrolysis: Optimization by response Surface Methodology Using Alcalase®2.4L. *International journal of Food Engineering*, 7(5), 1–17.
- Vieira, C., Biasutti, E., Capobianco, M., Afonso, W. and Silvestre, M., 2006.** Effect of salt on the solubility and emulsifying properties of casein and its tryptic hydrolysates. *Ars Pharmaceutica*, 47(3), 281–292.
- Wasswa, J., Tang, J., Gu, X. H. and Yuan, X. Q., 2007.** Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp skin. *Food Chemistry*, 104, 1698–1704.
- Wilde, P. J. and Clark, D. C., 1996.** The competitive displacement of blactoglobulin by Tween 20 from oil–water and air–water interfaces. *Journal of Colloid and Interface Science*, 155, 48–54.