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Effect of Enzymatic Hydrolysis on the Nutritional and Functional Properties of Nile Tilapia (*Oreochromis niloticus*) Proteins

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ABSTRACT

In this study, we examined the effects of enzymatic hydrolysis on the functional and nutritional properties of Nile tilapia (*Oreochromis niloticus*) proteins. Nile tilapia was enzymatically hydrolyzed by several commercially available proteases (Alcalase 2.4 L, Neutrase and Flavourzyme), with protein recovery of 89.86, 81.92 and 73.12%, respectively. The hydrolysates were prepared with 1% enzymes/substrate ratio (E/S). Essential amino acids were above the recommended amount by Food and Agricultural Organization/World Health Organization for humans. Lower molecular weights were more predominant in Hot Water Dip Hydrolysates (HWDH) whose peaks ranged from 328- 1876 Da. Furthermore, the Hot Water Dip Concentrates (HWDC) were mainly composed of higher molecular weight (214-19,576 Da). HWDH and HWDC have varied solubilities above 80% at pH 12.0. Hydrophobicities of 168.01 and 200.28, water-binding capacity was in the range of 1.77 and 2.43 mL g^{-1} while oil absorption capacity ranged between 2.23 and 3.36 g mL^{-1}, bulk density of 0.53 and 0.36 mL g^{-1} and emulsifying capacity of 21.40 and 20.40 mL 0.5 g^{-1} for both HWDH and HWDC, respectively. Foam capacity and foam stability ranged from 124.53 to 37.25 mL g^{-1} for HWDH and from 80.3 to 45.57 mL g^{-1} for HWDC. The hydrolysate was more easily digestible than the concentrate with a significant difference ($p<0.05$). All the estimated nutritional parameters based on amino acids composition suggested that Nile tilapia protein hydrolysates and concentrates have good nutritional quality and could be used as protein ingredient in food industries.

Key words: Surface hydrophobicity, molecular weight, amino acids, solubility

INTRODUCTION

Fish have been used for food, religious functions as well as a medium of exchange since time immemorial. More than half of world's population depends on fish as a principal source of animal protein (Jhingram, 1987). Tilapia is an important food fish in many tropical areas of Africa, America and Asia. Many species of tilapia have been cultured in developing countries, where animal protein is lacking. Tilapias are considered suitable for culture, because of their high tolerance to adverse environmental conditions, their relatively fast growth and the ease with which they can breed good utilization of artificial diets, resistance to disease, excellent quality of its firmly

textured flesh and finely appetizing fish to consumers (Jhingram, 1987). Tilapias are among the most important warm-water fishes used for aquaculture. They originated from tropical and subtropical Africa but are now farmed throughout the world. Nile tilapias inhabit a variety of fresh water habitats. Traditionally they have been of major importance in small scale commercial or subsistence fishing worldwide, especially Africa and Asia. It is the third most widely cultured fish, after carp and salmonids (El-Sayed, 2006). The global production has been greatly influenced by rapid expansion of Nile tilapia (*Oreochromis niloticus*) and Mossambique tilapia (*Oreochromis mossambicus*), cultured in China, the Phillipines and Egypt (Foh *et al*., 2010). Tilapia fish is nutritious and forms a healthy part of a balanced diet that is high in protein (16-25%), low in fat (0.5-3.0%) and substitutes well in any seafood recipe.

Protein functional properties are determined to a large extent by a protein's physicochemical and structural properties (Diniz and Martin, 1997). Protein solubility is an important prerequisite for food protein functional properties and it is a good index of potential applications of proteins (Sathivel *et al*., 2003). Researchers have reported that protein solubility has a close relationship with emulsifying properties (Quaglia and Orban, 1990) and foaming properties (Quaglia and Orban, 1987, 1990). Bulk density is an important parameter that determines the packaging requirement of a product (Kamara *et al*., 2009a). Proteins isolates are the basic functional components of various high protein processed food products and thus determine the textural and nutritional properties of the foods (Quaglia and Orban, 1990; Kamara *et al*., 2009b), digestibility of the nutrients must be known in order to evaluate fully the significance of nutrient concentration (Kamara *et al*., 2009b).

Modification of a protein is usually realized by physical, chemical, or enzymatic treatments, which change its structure and consequently its physicochemical and functional properties (Chobert *et al*., 1988; Adler-Nissen, 1986). Enzymatic treatment is a particularly attractive technique to modify proteins due to the milder process conditions required, the relative ease to control the reaction and minimal formation of by-products (Mannheim and Cheryan, 1992; Kamara *et al*., 2011). Enzymatic hydrolysis has been widely used to improve the functional properties of proteins, such as solubility, emulsification, gelation, water and fat-holding capacities and foaming ability and to tailor the functionality of certain proteins to meet specific needs (Kim *et al*., 2007; Panyam and Kilara, 1996; Kamara *et al*., 2011). However, extensive hydrolysis could have a negative impact on the functional properties (Kristinsson and Rasco, 2000; Wasswa *et al*., 2008). The objective of this study was to evaluate the functional properties of protein hydrolysates from Nile tilapia hydrolysed by alcalase through amino acid analysis, molecular weight distribution, nitrogen solubility, surface hydrophobicity, *in vitro* digestibility foam capacity and stability, emulsifying capacity, water and oil holding and bulk density compared to its concentrates.

MATERIALS AND METHODS

The Tilapia fish (*Oreochromis niloticus*) was purchased from a local fresh water products market in Wuxi, China, on the 8th January 2010 and were transported within 24 h in ice boxes to the School of Food Science and Technology (SFST) laboratory of Jiangnan University, Wuxi, Jiangsu, People's Republic of China. The fish (450-580 g fish^{-1} with length range of 25-30 cm fish^{-1}) were prepared using the handling method; disemboweled, beheaded and skin removed before thoroughly washing with clean water to remove contaminants or unwanted particles. Fish muscle retrieved with care, separating the bones from the meat, chopped into pieces about 0.25 cm. Hot Water Dip (HWD) sample was obtained by sinking a portion of the chopped meat in hot water

Table 1: Optimum conditions for hydrolysis of Nile Tilapia (*Oreochromis niloticus*) with different proteases

Enzyme	Form	pH	T (°C)
Alcalase 2.4 L (AU g^{-1})*	Liquid/grain	8.0	55
Flavourzyme (500 L APU g^{-1})†	Powder	7.0	50
Neutrase (1.5 AU g^{-1})	Liquid/grain	7.0	45

*AU (Anson units) is the amount of enzyme that under standard conditions digests hemoglobin at an initial rate that produces an amount of trichloroacetic acid-soluble product which gives the same color with the Filon reagent as one milliequivalent of tyrosine released per minute. †LAPU (Leucine aminopeptidase unit) is the amount of enzyme that hydrolyzes 1 µmol of leucine-p-nitroanilide per minute

95±5°C and maintained for 15 min (HWD), hence endogenous enzyme was inactivated and furthers impurities and some oil removed. It was allowed to cool at room temperature, eventually vacuum packed in polyethylene bags. The sample was kept frozen at -20°C for subsequent analysis.

Alcalase 2.4 L is a bacterial endoproteinase from a strain of *Bacillus licheniformis* was obtained from Novozymes China Inc. and stored at 4°C for subsequent analysis. Prior to the hydrolysis process, the sample was thawed overnight in a refrigerator, 4±1°C. All chemical reagents used in the experiments were of analytical grade. The experiment was carried out in the SFST laboratory from January to April 2010.

Preparation of fish protein hydrolysates and concentrates: HWD sample was hydrolyzed with three different enzymes, under the conditions given in Table 1, based on optimum hydrolysis conditions. One hundred grams of samples were weighed into a vessel immersed in a water bath maintained at appropriate temperature and 700 mL of distilled water was added to make a suspension. The suspension was, for each enzyme, adjusted to appropriate pH and preheated to appropriate temperature; then (1%) enzyme substrate ratio (w/w) was used for all samples with continuous stirring. Hydrolysis was carried out for 5 h. Seventy five millliliter aliquots were taken after 30, 60, 90, 120, 160 and 300 min. After hydrolysis, the enzymes were inactivated by placing in boiling water for 15 min. The hydrolysate was allowed to cool down and centrifuged at 7,500× g for 15 min at 4°C with a D-3756 Osterode am Harz model 4515 centrifuge (Sigma, Hamburg, Germany). The tilapia Fish Protein Hydrolysates (FPH) and the raw samples were lyophilized (fish protein concentrate-FPC) and stored at -20±2°C until used. All experiments were performed in triplicate and the results are the average of the three values.

Amino acid analysis: The dried samples were digested with HCl (6 M) at 110°C for 24 hr under nitrogen atmosphere. Reversed phase high performance liquid chromatography (RP-HPLC) analysis was carried out in an Agilent 1100 (Agilent Technologies, Palo Alto, CA, USA) assembly system after precolumn derivatization with o-phthaldialdehyde (OPA). Each sample (1 µL) was injected on a Zorbax 80 A C18 column (i.d. 4.6×180 mm, Agilent Technologies, Palo Alto, CA, USA) at 40°C with detection at 338 nm. Mobile phase A was 7.35 mM L^{-1} sodium acetate/triethylamine/ tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mM L^{-1} sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g of amino acid per 100 g of protein.

Nutritional parameters: The nutritional parameters of Nile tilapia protein hydrolysates and the concentrates were calculated using their amino acid composition including:

- Proportion of essential amino acids (E) to the total amino acids (T) of the protein
- Amino Acid Score (AAS) = (mg of amino acid g^{-1} of test protein/mg of amino acid g^{-1} of FAO/WHO/UNU standard pattern)×100

The FAO/WHO reference pattern of essential amino acid requirements (g/100 g of protein) (FAO, 2007) was used as the standard.

- Predicted Protein Efficiency Ratio (PER) values. The predicted PER values of HWDH and HWDC were estimated by three regression equations developed by Chavan *et al.* (2001).

$$PER = -0.684 + 0.456\ (Leu) - 0.047\ (Pro) \quad (1)$$

$$PER = -0.468 + 0.454\ (Leu) - 0.105\ (Tyr) \quad (2)$$

$$PER = -1.816 + 0.435\ (Met) + 0.780\ (Leu) + 0.211\ (His) - 0.944\ (Tyr) \quad (3)$$

Determination of molecular weight: The samples were determined using a Waters™ 600E Advanced Protein Purification System (Waters Corporation, Milford, MA, USA). A TSK gel, 2000SWXL (7.8×300 mm) column was used with 10% acetonitrile + 0.1% TFA in HPLC grade water as the mobile phase. The calibration curve was obtained by running bovine carbonic anhydrase (29,000 Da), horse heart cytochrome C (12,400 Da), bovine insulin (5800 kDa), bacitracin (1450 Da), Gly-Gly-Tyr-Arg (451 kDa) and Gly-Gly-Gly (189 Da). The total surface area of the chromatograms was integrated and separated into eight ranges, expressed as a percentage of the total area.

Determination of surface hydrophobicity: Surface hydrophobicity of HWDH and HWDC were determined by using the fluorescence 1-anilino-8-naphthalene sulfonate (ANS) binding method (Hayakawa and Nakai, 1985). HWDH and HWDC solutions (0.0015, 0.003, 0.006, 0.012, 0.015%, w/v) were prepared in 0.01 M phosphate buffer (pH 7.0) and vortexed homogeneously. Ten microliter of 8 mM ANS in 0.01 M phosphate buffer (pH 7.0) was added into each of 4.0 mL of the protein solutions, then mixed well by vortexing for 10 sec. Fluorescence intensity of these solutions were measured at 390 nm of excitation and 484 nm emmision using a Kontron Spectrofluorometer (model SFM23/B; Kontron Ltd., Zurich, Switzerland). The surface hydrophobicity plotted as the slope of fluorescence intensity against protein concentration and was calculated by linear regression.

Nitrogen Solubility (NS): Nitrogen solubility was determined according to the procedure of Diniz and Martin (1997), with slight modifications. Samples were dispersed in distilled water (10 g L^{-1}) and pH of the mixture was adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 with either 0.5 N HCL or 0.5 N NaOH while continually shaking (Lab-Line Environ-Shaker; Lab-Line Instrument, Inc., Melrose Park, IL, USA) at room temperature for 35 min. A 25 mL aliquot was then centrifuged at 4000× g for 35 min. A 15 mL aliquot of the supernatant was analyzed for nitrogen (N) content by the Kjeldahl method and the NS was calculated according to equation:

$$\text{Nitrogen solubility (\%)} = \left(\frac{\text{supernatant (N) concentration}}{\text{sample (N) concentration}}\right) \times 100 \quad (4)$$

Oil-Holding Capacity (OHC): Oil-Holding Capacity (OHC) of tilapia FPH was determined as the volume of edible oil held by 0.5 g of material according to the method of Shahidi *et al.* (1995). A

0.5 g sample of each FPH was added to 10 mL soybean oil (Gold Ingots Brand, QS310002012787, Suzhou, People's Republic of China) in a 50 mL centrifuge tube and vortexed for 30 sec in triplicate. The oil dispersion was centrifuged at 3000× g for 25 min. The free oil was decanted and the OHC was determined by weight difference.

Water-Holding Capacity (WHC): To determine the Water Holding Capacity (WHC) of tilapia FPH, the method outlined by Diniz and Martin (1997), with slight modifications. Triplicate samples (0.5 g) of samples were dissolved with 10 mL of distilled water in centrifuge tubes and vortexed for 30 sec. The dispersions were allowed to stand at room temperature for 30 min, centrifuged at 3000× g for 25 min. The supernatant was filtered with Whatman No.1 filter paper and the volume retrieved was accurately measured. The difference between initial volumes of distilled water added to the protein sample and the volume retrieved. The results were reported as mL of water absorbed per gram of protein sample.

Emulsifying Capacity (EC): Emulsifying capacity was measured using the procedure described by Rakesh and Metz (1973), with modification. A 0.5 g of each freeze-dried sample was transferred into a 250 mL beaker and dissolved in 50 mL of 0.5 N NaCl and then 50 mL of soybean oil (Gold Ingots Brand, QS310002012787, Suzhou, P.R. China) was added. The homogenizer equipped with a motorized stirrer driven by a rheostat Ultra-T18 homogenizer (Shanghai, China) was immersed in the mixture and operated for 120 sec at 10,000 rpm to make an emulsion. The mixture was transferred to centrifuge tubes, maintained in water-bath at 90°C for 10 min and then centrifuged at 3000× g for 20 min. Emulsifying capacity was calculated as in equation:

$$EC = \frac{V_A - V_R}{W_S} \quad (5)$$

where, V_A is the volume of oil added to form an emulsion, V_R is the volume of oil released after centrifugation and W_S is the weight of the sample.

Foaming Capacity (FC) and Foam Stability (FS): Estimation of foaming capacity was done following the method of Bernard-Don *et al.* (1991) with minor modifications. Thirty milliliter of 30 g L^{-1} aqueous dispersion was mixed thoroughly using an Ultra-Turrax 25 homogenizer at 9,500 rpm for 3 min in a 250 mL graduated cylinder. The total volume of the protein dispersion was measured immediately after 30 sec. The difference in volume was expressed as the volume of the foam. Foam stability was determined by measuring the fall in volume of the foam after 60 min.

Bulk Density (BD): Bulk density of freeze-dried tilapia hydrolysates was estimated with approximately 3 g of each sample packed into 25 mL graduated cylinders by gently tapping on the lab bench 10 times. The volume was recorded and bulk density was reported as g mL^{-1} of the sample.

***In vitro* Protein Digestibility (IVPD):** *In vitro* Protein Digestibility (IVPD) was carried out according to the method described by Elkhalil *et al.* (2001), with slight modifications. Twenty mg of tilapia FPH (HWDPC and HWDPH) samples were digested in triplicate in 10 mL of trypsin (0.2 mg mL^{-1} in 100 mM Tris-HCl buffer, pH 7.6). The suspension was incubated at 37°C for 2 h.

Hydrolysis was stopped by adding 5 mL 50% trichloroacetic acid (TCA). The mixture was allowed to stand for 30-35 min at 4°C and was then centrifuged at 10,000× g for 25 min using a D-3756 Osterode AM Harz Model 4515 Centrifuge (Sigma, Hamburg, Germany). The resultant precipitate was dissolved in 5 mL of NaOH and protein concentrate was measured using the Kjeldahl method. Digestibility was calculated as follows:

$$\text{Protein digestibility (\%)} = \frac{(A - B)}{A} \times 100 \quad (6)$$

where, A is total protein content (mg) in the sample and B is total protein content (mg) in the TCA precipitate.

Statistical analysis: Data are result of at list three determinations. One way Analysis of Variance (ANOVA) was used to determine the statistical difference at ($p<0.05$), using Origin Pro Version 8.0.

RESULTS AND DISCUSSION

Enzymatic hydrolysis with different proteases: The enzymatic hydrolysis of Nile Tilapia (*Oreochromis niloticus*) with Alcalase 2.4 L showed the highest increase in protein content of HWDH during the first 240 min of hydrolysis 89.86%. The amount of proteins released decreased slightly to 86.64% (Fig. 1). Neutrase solubilized 81.92% of protein during the first 240 min of hydrolysis and the amount solubilized increased to 82.54% with the remaining hydrolyzing period. Flavourzyme showed an increase in protein solubilization during the first 240 min of hydrolysis with 73.12% of the protein being solubilized (Fig. 1). The results of our study exhibited a behavior that is, similar to Adler-Nissen (1986) and Panyam and Kilara (1996). The high efficiency of Alcalase 2.4 L may be a result of a high frequency of potential cleavable sites in HWD which may have contributed to the high degree of solubilization. Alcalase 2.4 L was selected for the current study because of its high yield under optimum conditions, cost effectiveness and readily available.

Amino acid analysis: HWDH contains all the essential amino acids in good proportion as compared to HWDC. The results in Table 2 indicated that the amino acid composition of HWDH and HWDC closely resembles each other. The predominant amino acids amongst the non essential

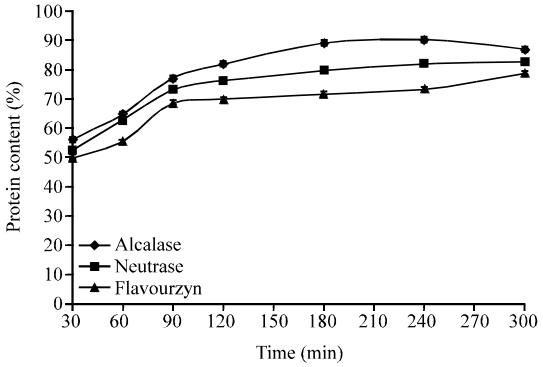

Fig. 1: Amount of protein solubilized by enzymatic hydrolysis of Nile tilapia (*Oreochromis niloticus*) by different proteases. Value represent the Mean±SD of n = 3 duplicate assays

Table 2: Comparative amino acid profiles of Nile tilapia *(Oreochromis niloticus)* protein hydrolysates and its concentrates g/100 g of protein

			FAO/WHO/UNU[a]	
Items	HWDH	HWDC	Child	Adult
Essential amino acids				
Isoleucine (Ile)	3.22±0.10	4.16±0.06	3.00	3.0
Leucine (Leu)	7.81±0.08	8.11±0.04	6.00	5.9
Lysine (lys)	9.58±0.03	9.34±0.03	4.80	4.5
Methionine (Met)	2.53±0.04	3.07±0.05		
Met + Cys	3.17±0.07	3.50±0.04	2.30[b]	1.6[b]
Phenylalanine (Phe)	3.07±0.05	4.14±0.06		
Phe + Tyr	5.14±0.06	7.19±0.06	4.10[c]	3.8[c]
Threonine (Thr)	4.17±0.04	4.32±0.03	2.50	2.3
Valine (Val)	3.96±0.12	4.57±0.05	2.90	3.9
Histidine (His)	2.01±0.04	2.38±0.03	1.60	1.5
Tryptophan (Trp)	0.28±0.03	0.35±0.04	0.66	0.6
Nonessential amino acids				
Alanine (Ala)	3.22±0.10	4.16±0.06		
Arginine (Arg)	7.81±0.08	8.11±0.04		
Aspartic acid (Asp)[d]	9.58±0.03	9.34±0.03		
Cysteine (Cys)[e]	2.53±0.04	3.07±0.05		
Glutamic Acid Glu)[f]	3.17±0.07	3.50±0.04		
Glycine (Gly)	3.07±0.05	4.14±0.06		
Serine (Ser)	5.14±0.06	7.19±0.06		
Tyrosine (Tyr)	4.17±0.04	4.32±0.03		
Proline (Pro)	3.96±0.12	4.57±0.05		

The data are means and standard deviations of triplicate [A]FAO/WHO/UNU energy and protein requirements (2007); [B]Requirements for methionine + cysteine; [C]Requirements for phenylalanine + tyrosine; [D]Aspartic acid + asparagines; [E]Cysteine + cysteine; [F]Glutamic acid + glutamine

amino acids were aspartic acid, glutamic acid and alanine; those amongst the essential amino acids were lysine, threonine and leucine (Table 2). Both samples have a well-balanced amino acid composition. Moreover, Most of the essential amino acids of their proteins were at a higher level than the Food and Agricultural Organization/World Health Organization reference. These values are generally in accordance with previous publications (Usydus *et al*., 2009; Vidotti *et al*., 2003). However, Tryptophan and cystine were much less in HWDC compared with that of HWDH.

Nutritional parameters: Protein is one of the essential nutrients in the human diet. Both the amount and quality of protein provided by a food are important. The protein quality, also known as the nutritional or nutritive value of a food, depends on its amino acid content and on the physiological utilization of specific amino acids after digestion, absorption and minimal obligatory rates of oxidation. Because direct assessment of protein nutritional value in human subjects is impractical for regulatory purposes, methods based on *in vitro* (chemical) and *in vivo* bioassays for assessment of protein quality have been developed. Herein, a case is made for the use of amino acid data as a basis for estimation of nutritional quality of fish proteins. The ratio of essential to total amino acids, amino acid score and PER of HWDH and HWDC are shown in Table 3. HWDH and HWDC have a higher ratio of essential to total amino acids than the pattern recommended by FAO/WHOUNU. HWDC had the highest ratio of 49.05% compared to HDWH with a ratio 44.52%.

Table 3: Nutritional parameters of Nile tilapia (*Oreochromis niloticus)* protein hydrolysates and its concentrates

Parameters	HWDH	HWDC
E/T%	44.52	49.05
Estimates of PER		
1	3.06±0.03	3.11±0.04
11	3.36±0.06	3.55±0.05
111	3.25±0.06	3.35±0.04
Amino acid scores		
Leucine	130.17±0.03	135.17±0.04
Histidine	144.67±0.06	158.67±0.03
Threonine	166.8±0.04	172.80.03
Valine	141.72±0.03	157.59±0.05
Met + Cys	137.83±0.07	152.17±0.04
Isoleucine	107.33±0.10	138.67±0.06
Phe + Tyr	125.36±0.06	175.37±0.06
Lysine	199.58±0.03	194.58±0.03
Tryptophan	87.88±0.11	83.330.04

The data are means and standard deviations of triplicate; E/T, proportion of essential amino acids (E) to total amino acids (T); PER, predicted protein efficiency ratio

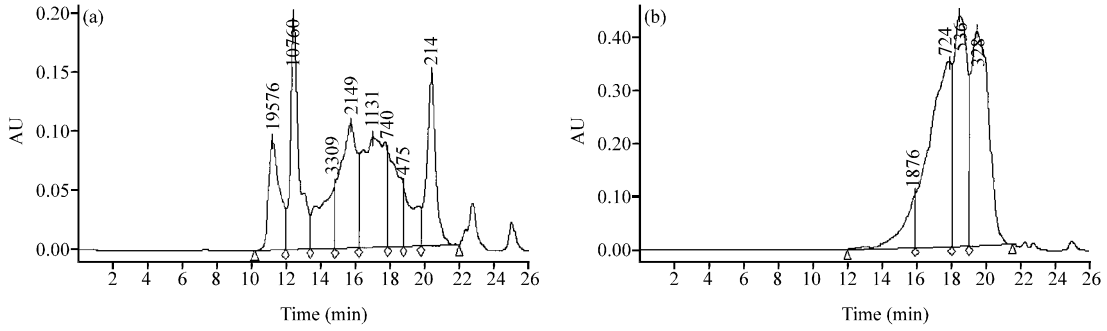

Fig. 2: Molecular weight distribution of Nile tilapia (*Oreochromis niloticus*) proteins. (a) HWDC and (b) HWDH

Predicted PER values of HWDH and HWDC all exceeded 2.00, which describes a protein of good and high quality (Friedman, 1996). HWDC have the highest PER values (3.11, 3.55 and 3.35%) for PER I, II and III respectively. The PER values of HWDH and HWDC were rather satisfactory when compared with the standard casein PER of 2.5 (Friedman, 1996). However, total essential amino acid scores for HWDH and HWDC reached the FAO/WHO requirement (2007) for the essential amino acids for children (Table 3).

Molecular weight distribution: The Gel Permeation Chromatography (GPC) using an HPLC system was used to study molecular weight distribution profiles of HWDH and HWDC. Figure 2a, and b show the molecular size distribution profiles of HWDH and HWDC. The chromatographic data indicated that the HWDH composed of lower molecular weight peptides whose peaks ranged from 328-1876 Da (Fig. 2). However, HWDC composed of much higher molecular weight polypeptides whose peaks ranged from 214-19,576 Da. In this study, results revealed that HWDH has lower molecular weight distribution; this is probably associated with higher functional

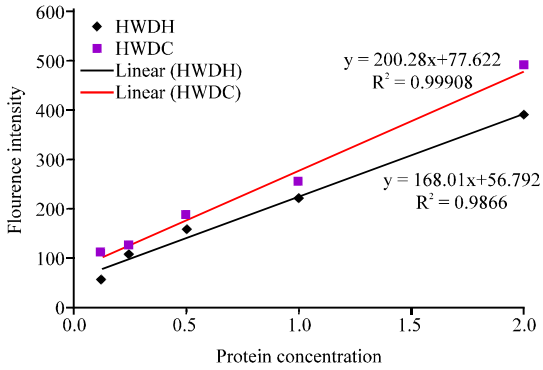

Fig. 3: Fluorescence intensity of Nile tilapia (*Oreochromis niloticus*) proteins

attribute. These findings are in agreement with observations from other studies and support the fact that functional properties are highly influenced by molecular weight distribution (Wang *et al*., 2006; Kim *et al*., 2007).

Surface hydrophobicity of proteins: The surface hydrophobicity value is an indicator of the number of hydrophobic groups on the surface of a protein in contact with the polar aqueous environment. The surface hydrophobicity, is an index of the protein's capacity for intermolecular interaction and hence its functionality. The surface hydrophobicities (So) of HDWH (168.01) and HDWC (200.28), respectively and the linear relationships between protein concentration and fluorescence intensity are shown in Fig. 3. Present result follow similar trend of Achouri and Zhang (2001). The surface hydrophobicity of a protein is an index of the number of hydrophobic groups on its surface in contact with the polar aqueous environment. Changes in surface hydrophobicity as result of proteolysis; influences the functional properties especially the interfacial properties of the hydrolysates. There was a significant difference in surface hydrophobicity between HDWH and HWDC. In the native proteins, the hydrophobic amino acids are buried in the central core of the protein molecule. This feature is lost when protein is denatured or hydrolyzed into shorter peptides (Wang *et al*., 1999).

Protein solubility: Solubility is one of the most important characteristics of proteins because it is not only important by itself, but also influences other functional properties. Good solubility of proteins is required in many functional applications, especially for emulsions, foams and gel, because soluble proteins provide a homogenous dispersibility of the molecules in colloidal systems and enhanced the interfacial properties (Zayas, 1997).

The solubilities of HDWH and HWDC at pH 2.0 to pH 12.0 are presented in Fig. 4. At pH 4.0 and 5.0, near the isoelectric point at which the net charge of the original protein is minimized (83.27 and 82.23%) and consequently more protein-protein interactions and fewer protein-water interactions occur (Adler-Nissen, 1976; Chobert *et al*., 1988). Above pH 6.0, the nitrogen solubilities increased rapidly with an increase in pH up to 12.0. The solubility of HDWH reached 95.23%, while solubility for HWDC was 89.25% at pH 12.0 (Fig. 4). These trends in solubilities are in agreement with (Choi *et al*., 2009; Sathivel *et al*., 2009). An increase in the extent of enzymatic hydrolysis corresponded to a considerable increase in the nitrogen solubility, over the pH range studied, indicating a positive relationship (Fig. 4). It has been suggested that an increase in the solubility

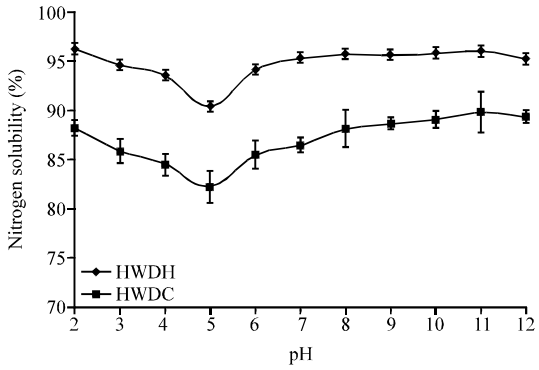

Fig. 4: Effect of pH on nitrogen solubility of Nile tilapia (*Oreochromis niloticus*) proteins. Value represent the Mean±SD of n = 3 duplicate assays

Table 4: Functional properties of Nile tilapia (*Oreochromis niloticus)* protein hydrolysates and concentrates

Functionality	HWDH	HWDC
In vitro protein digestibility (%)	93.20±0.20*	87.60±1.13*
Water holding capacity (mL g^{-1})	1.77±0.06**	2.43±0.47**
Oil holding capacity (mL g^{-1})	2.23±0.25*	3.30±0.44*
Emulsion capacity (mL/0.5 g)	21.40±0.36*	27.40±0.53*
Bulk density (grams mL^{-1})	0.53±0.06**	0.36±0.02**
Foaming capacity (% vol. increase)	124.50±0.30*	80.83±0.29I*

Values are Means±SD of three determinations. * indicate significant difference and ** insignificant difference at ($p<0.05$)

of protein hydrolysates over that of the original protein is due to the reduction of its secondary structure and also to the enzymatic release of smaller polypeptide units from the protein (Adler-Nissen, 1986; Chobert *et al*., 1988).

Water/oil holding capacity (WHC/OHC): The functional properties of proteins in a food system depend in part on the water-protein interaction. WHC refers to the ability of the protein to imbibe water and retain it against gravitational force within a protein matrix, such as protein gels, beef and fish muscle, it is positively correlated with water-binding capacity (Foh *et al*., 2010). The WHC of HWDH was 1.77 and 2.43 mL g^{-1}, respectively, with an insignificant difference ($p<0.05$) (Table 4). Interactions of water and oil with proteins are very important in the food systems because of their effects on the flavor and texture of foods. Intrinsic factors affecting water binding of food protein include amino acids composition, protein conformation and surface hydrophobicity/polarity (Kamara *et al*., 2009b; Barbut, 1999).

For oil holding capacity, HWDC was higher (3.30 mL g^{-1}) while, HWDH was (2.23 mL g^{-1}), with a significant difference ($p<0.05$) (Table 4). Present results corroborated to other fish proteins studied (Diniz and Martin, 1997). Further more, high oil absorption is essential in the formulation of food systems like sausages, cake, batters, mayonnaise and salad dressings.

Emulsifying Capacity (EC): The EC is a measure of the effectiveness of proteinaceous emulsifiers (Pearce and Kinsella, 1978). The ability of proteins to form stable emulsions is important owing to the interactions between proteins and lipids in many food systems. An increase in the number of peptide molecules and exposed hydrophobic amino acid residues due to hydrolysis of

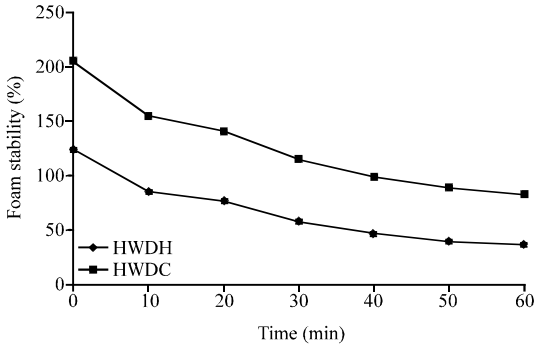

Fig. 5: Foam stability of Nile tilapia (*Oreochromis niloticus*) proteins. Value represent the Mean±SD of n = 3 duplicate assays

proteins would contribute to an improvement in the formation of emulsions. From the results, HWDH (21.40 mL 0.5 g $^{-1}$) shows an appreciable EC than HWDC (20.40 mL 0.5 g $^{-}$)[1]with a significant difference ($p<0.05$) (Table 4). Present results were similar to Wasswa *et al.* (2008) and Abdul-Hamid *et al.* (2002).

Foam capacity and stability (FC and FS): Proteins are good foaming agents, since they can rapidly diffuse to the air-water interface and they form a strong cohesive and elastic film by partial unfolding. Foaming properties are correlated with amount of hydrophobic amino acids exposed at the surface of the protein molecule (Wang *et al.*, 1999). Dispersed proteins lower the surface tension at the water-air interface, thus creating foaming capacity (Turgeon *et al.*, 1992).

To have foam stability, protein molecules should form continuous intermolecular polymers enveloping the air bubbles, since intermolecular cohesiveness and elasticity are important to produce stable foams (Kamara *et al.*, 2009a). A significant increase was observed in the foaming capacity of HWDH (124.5 g mL^{-1}) compared to HWDC (80.3 g mL^{-1}) with a significant difference ($p<0.05$) (Table 4). An improvement in foaming capacity for enzymatically modified food proteins is reported by Adler-Nissen (1986). The foam stability of the HWDC was found to be less than that of HWDH. Enzymatic hydrolysis of Nile tilapia proteins caused an increase in the foam volume initially and then a decrease with time. The foam stability values ranged from 124.5 to 37.2 and 80.3 to 32.33 g mL^{-1} for HWDH and HWDC respectively (Fig. 5). Present results were similar to (Wasswa *et al.*, 2008; Abdul-Hamid *et al.*, 2002).

Bulk density: Bulk density is a measure of heaviness of the powder. Moreover, bulk density is an important parameter that determines the packaging requirement of a product. Further more; Bulk density signifies the behavior of a product in dry mixes. Also, it varies with the fineness of the particles. HDWH and HWDC had varying bulk densities of 0.53 and 0.35 g mL^{-1}, respectively with an insignificant difference ($p<0.05$) (Table 4). Present results obtained for HDWH and HWDC were similar compared to reported values (Wasswa *et al.*, 2007). The low bulk density of HWDH and HWDC was due to its lower particle density and the large particle size. High bulk density is disadvantageous for the formulation of weaning foods, where low density is required (Kamara *et al.*, 2009a).

***In vitro* Protein Digestibility (IVPD):** The *in vitro* protein digestibility of HWDH and HWDC were significantly different ($p<0.05$). The *in vitro* protein digestibilities of both samples were evaluated by the release of TCA-soluble nitrogen, after incubation time of 120 min at 37°C. Table 4 shows that all the protein samples exhibited very good trypsin digestibility. Nonetheless, HWDH had higher digestibility value (93.20%) while HWDC was lower (87.60%). This probably resulted from pre-hydrolysis processing, which led to the existence of fewer attack sites being available to the enzymes in the digestibility assay. Prsent results are within the values reported by Abdul-Hamid *et al.* (2002).

CONCLUSION

Conclusively, understanding change of Nile tilapia complex during enzymatic hydrolysis can be useful for producing modified proteins with the desired nutritional parameters and functionality. From the results presented here, it is proposed that excellent solubility of the protein hydrolysates could be attributed to reduce size of the polypeptides obtained after proteolysis. The results indicated close relationships between functional properties and molecular size of the modified Nile tilapia. The amino acids of both samples were higher than FAO/WHO requirement for both infants and adults. All the estimated nutritional parameters based on amino acids composition showed that Nile tilapia protein hydrolysates and concentrates have good nutritional quality. Furthermore, the hydrolysates that are obtained also have an effect on improving functionality such as solubility, foaming properties and other important properties of proteins than the concentrate. Not only these hydrolysates can be used as food additives to improve the functionality but also improving the nutritional profile by incorporating them in selected foods. Nile tilapia protein hydrolysates could excellent applications for future product development by virtue of their nutritional and functional properties.

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REFERENCES

Abdul-Hamid, A., J. Bakar and G.H. Bee, 2002. Nutritional quality of spray dried protein hydrolysate from black tilapia (*Oreochromis mossambicus*). Food Chem., 78: 69-74.

Achouri, A. and W. Zhang, 2001. Effect of succinylation on the physicochemical properties of soy protein hydrolysates. Food Res. Int., 34: 507-514.

Adler-Nissen, J., 1976. Enzymatic hydrolysis of proteins for increased solubility. J. Agric. Food Chem., 24: 1090-1093.

Adler-Nissen, J., 1986. Enzymatic Hydrolysis of Food Proteins. Elsevier Applied Science Publishers Ltd., London, UK., pp: 116-124.

Barbut, S., 1999. Determining Water and Fat Holding. In: Methods of Testing Protein Functionality, Hall, G.M. (Eds.). Blackie Academic and Professional, New York, ISBN-13: 978-0751400533, pp: 186-225.

Bernard-Don, L.S., A.M.R. Pilosof and G.B. Bartholomai, 1991. Enzymatic modification of soy protein concentrates by fungal and bacterial proteases. J. Am. Oil Chem. Soc., 68: 102-105.

Chavan, U.D., D.B. Mckenzie and F. Shahidi, 2001. Functional properties of protein isolates from beach pea (*Lathyrus maritius* L.). Food Chem., 74: 177-187.

Chobert, J.M., C. Bertrand-Harb and M.G. Nicolas, 1988. Solubility and emulsifying properties of caseins and whey proteins modi?ed enzymatically by trypsin. J. Agric. Food Chem., 36: 883-886.

Choi, Y.J., S. Hur, B.D.Choi, K. Konno and J.W. Park, 2009. Enzymatic hydrolysis of recovered protein from frozen small croaker and functional properties of its hydrolysates. J. Food Sci., 74: 17-24.

Diniz, F.M. and A.M. Martin, 1997. Effects of the extent of enzymatic hydrolysis on the functional properties of shark protein hydrolysate. Lebensmittel-Wissenschaft und-Technologie, 30: 266-272.

El-Sayed, A.F.M., 2006. Tilapia Culture. CAB International, Wallingford, UK., pp: 304.

Elkhalil, E.A.J., A.H. El-Tinay, B.E. Mohamed and E.A.E. El-Shseikh, 2001. Effect of malt pretreatment on phytic acid and *in vitro* protein digestibility of sorghum flour. Food Chem., 72: 29-32.

FAO, 2007. Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation. Geneva, Switzerland: WHO Technical Report Series, No. 935.http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=10&codcch=935.

Foh, M.B.K., I. Amadou, B.M. Foh, M.T. Kamara and W.S. Xia, 2010. Functionality and antioxidant properties of tilapia (*Oreochromis niloticus*) as influenced by the degree of hydrolysis. Int. J. Mol. Sci., 11: 1851-1869.

Friedman, M., 1996. Nutritional value of proteins from different food sources. A review. J. Agric. Food Chem., 44: 6-29.

Hayakawa, S. and S. Nakai, 1985. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. J. Food Sci., 50: 486-491.

Jhingram, V.G., 1987. Introduction to aquaculture. RAF/82/009. Port Harcourt, Nigeria: UNDP/FAO/Nigerian Institute for Oceanography and Matine Research Project.

Kamara, M.T., K. Zhu, I. Amadou, F. Tarawalie and H. Zhou, 2009a. Functionality, *in vitro* digestibility and physicochemical properties of two varieties of defatted foxtail millet protein concentrates. Int. J. Mol. Sci., 10: 5224-5238.

Kamara, M.T., Z. Huiming, Z. Kexue, I. Amadou and F. Tarawalie, 2009b. Comparative study of chemical composition and physicochemical properties of two varieties of defatted foxtail millet flour grown in China. Am. J. Food Technol., 4: 255-267.

Kamara, M.T., I. Amadou, K. Zhu, M.B.K. Foh and H.M. Zhou, 2011. The Influence of debittering and desalting on defatted foxtail millet (*Setaria italica* L) protein hydrolysate. Am. J. Biochem. Mol. Bio., http://scialert.net/abstract/?doi=ajbmb.0000.18521.18521

Kim, S.Y., J.Y. Je and S.K. Kim, 2007. Purification and characterization of antioxidant peptide from hoki (*Johnius belengerii*) frame protein by gastrointestinal digestion. J. Nutr. Biochem., 18: 31-38.

Kristinsson, H.G. and B.A. Rasco, 2000. Biochemical and functional properties of atlantic salmon *Salmo salar* muscle hydrolyzed with various alkaline proteases. J. Agric. Food Chem., 48: 657-666.

Mannheim, A. and M. Cheryan, 1992. Enzyme modi?ed proteins from corn gluten meal: Preparation and functional properties. J. Am. Oil Chem. Soc., 69: 1163-1169.

Panyam, D. and A. Kilara, 1996. Enhancing the functionality of food proteins by enzymatic modification. Trends Food Sci. Technol., 7: 120-125.

Pearce, K.W. and J.E. Kinsella, 1978. Emulsifying properties of proteins: Evaluation of turbidimetric techniques. J. Agric. Food Chem., 26: 716-723.

Quaglia, G.B. and E. Orban, 1987. Influence of the degree of hydrolysis on the solubility of the protein hydrolysates from sardine (*Sardina pilchardus*). J. Sci. Food Agric., 38: 271-276.

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

Rakesh, J. and A. Metz, 1973. Acid precipitated ?sh protein isolate exhibits good functional properties. Food Prod. Dev., 7: 18-24.

Sathivel, S., P.J. Bechtel, J. Babbitt, S. Smiley, C. Crapo, K.D. Reppond and W. Prinyawiwatkul, 2003. Biochemical and functional properties of herring (*Clupea harengus*) byproduct hydrolysates. J. Food Sci., 68: 2196-2200.

Sathivel, S., H.X. Yin, J.P. Bechtel and J.M. King, 2009. Physical and nutritional properties of catfish roe spray dried protein powder and its application in an emulsion system. J. Food Eng., 95: 76-81.

Shahidi, F., X.Q. Han and J. Synowiecki, 1995. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem., 53: 285-293.

Turgeon, S.L., S.F. Gauthier and P. Paquin, 1992. Emulsifying property of whey peptide fractions as a function of pH and ionic strength. J. Food Sci., 57: 601-604.

Usydus, Z., J. Szlinder-Richert and M. Adamczyk, 2009. Protein quality and amino acid profiles of fish products available in Poland. Food Chem., 112: 139-145.

Vidotti, R.M., E.M.M. Viegas and D.J. Carneiro, 2003. Amino acid composition of processed fish silage using different raw materials. Anim. Feed Sci. Technol., 105: 199-204.

Wang, J., M. Zhao, X. Yang and Y. Jiang, 2006. Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. J. Cereal Sci., 44: 93-100.

Wang, M., N.S. Hettiarachchy, M. Qi, W. Burks and T.J. Siebenmorgen, 1999. Preparation and functional properties of rice bran protein isolate. J. Agric. Food Chem., 47: 411-416.

Wasswa, J., T. Jian and G. Xiao-Hong, 2007. Desalting fish skin protein hydrolysates using macroporous adsorption resin. Am. J. Food Technol., 2: 406-413.

Wasswa, J., J. Tang and X. Gu, 2008. Functional properties of grass carp (*Ctenopharyngodonidella*), Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) skin hydrolysates. Int. J. Food Properties, 11: 339-350.

Zayas, J.F., 1997. Solubility of Proteins: Functionality of Proteins in Food. Springer-Verlag, Berlin, pp: 6-22.