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Chemical and Physicochemical Properties of Tilapia (*Oreochromis niloticus*) Fish Protein Hydrolysate and Concentrate

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ABSTRACT

In this study, we examined the chemical and physicochemical properties of tilapia (*Oreochromis niloticus*) fish protein hydrolysate and concentrate. Fresh Minced Meat Hydrolysate (FMMH) and Hot Water Dip Hydrolysate (HWDH) were hydrolyzed by using commercial protease Alcalase 2.4 L, with an estimated Degree of Hydrolysis (DH) 23.40 and 25.43%, respectively, exhibiting superior physico-functional properties over concentrates ($p<0.05$). Maximum solubility of 90 and 82% at pH 11.0 above, with a U shaped solubility curves. Furthermore, HWDH has the highest bulk density (0.53 g mL^{-1}). *In vitro* protein digestibility (93.20%) and a good foaming stability. The hydrolysates were light yellow in color as influenced by the hydrolysis process and Scanning Electron Microscopy (SEM) showed a smoother microstructure than the concentrate. The molecular weight ranged below 8,000 and 8,000 Da above and the essential amino acids were above the amounts recommended by the Food and Agricultural Organization/World Health Organization (2007) for humans. However, Fish Protein Concentrates (FPC) had higher mineral elements and ash content than fish protein hydrolyzates (FPH) ($p<0.05$). The HWDC possessed the highest differential scanning calorimetry result (peak temperature of 59.13°C, delta H = 195.564 J g^{-1}), while FMMC had the lowest (peak temperature 52.84°C, delta H = 11.0480 J g^{-1}), respectively.

Key words: Fish proteins, amino acid, molecular weight, minerals, functional properties

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a fresh water Fish that is hardy, prolific, fast growing tropical fish that is farmed mainly in Africa and Asia. Tilapia fish are beneficial to human beings as they make up a major part of the human diet and provide humans with as much of needed proteins as in meat (Ghorbani and Mirakabad, 2010). The concentration of dietary essential amino acids is a major factor determining the nutritional value of food protein. Fish muscle contains an excellent amino acid composition and is an excellent source of nutritive and easily digestible proteins (Venugopal *et al*., 1996; Yanez *et al*., 1976). Common fresh water fish harvested include Carps, Tilapia, Catfish, Gourami, Buffalo fish, Crayfish, Whitefish etc.

Physicochemical and functional properties of fish protein play a fundamental role in the food industry and its end products. Fish Protein Concentrate (FPC) processing preceded the field of enzyme hydrolysis of fish proteins. Proteolytic modification of food proteins to improve palatability and storage stability of the available protein resources is an early technology (Adler-Nissen, 1986).

One major advantage and goal of enzymatically hydrolyzed fish proteins is to modify and improve their functional properties. 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). Enzymes have 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.*, 1990; Panyam and Kilara, 1996). Protein modification is usually realized by physical, chemical, or enzymatic treatments, which change its structure and consequently its physicochemical and functional properties (Chobert *et al.*, 1988). Alcalase 2.4 L assisted reactions have been repeatedly favoured for fish protein hydrolysis, due to the high degree of hydrolysis that can be achieved in a relatively shorter period under moderate pH condition (Aspmo *et al.*, 2005). Protein hydrolysis produces peptides with functional, bioactive and sensory properties that are better than those of native proteins from which they are obtained (Cheison and Wang, 2003). Freeze drying is an alternative method of converting hydrolysates to powder form that has the advantage of stability and ease of handling. Nevertheless, modification in structure, texture and color manifestation depends on the treated samples, temperature and protein concentration. The uniqueness of fish proteins make them heat sensitive, with a greater tendency to denature at elevated temperatures (Sikorski, 1994). Earlier study in fish protein hydrolysates were directed towards the use of fish protein for non-dietary purposes (Sripathy *et al.*, 1962) and for animal feed (Keyes and Meinke, 1966) rather than for human nutrition that has an outstanding advantage of intestinal absorption in nutrition. Hence the objectives of this study have been designed to investigate the influence of hydrolysis on the chemical and physicochemical properties of tilapia fish (*Oreochromis niloticus*).

MATERIALS AND METHODS

The Tilapia fish *(Oreochromis niloticus)* were purchased from a local fresh water products market in Wuxi, China 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 (480-600 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. A portion of the fresh fish meat was minced using a meat mincer and the pulverized fish meat (homogenate) was vacuum packed in polyethylene bags (100-250 g per unit) and was labeled as Fresh Minced Meat (FMM) sample. Hot Water Dip (HWD) sample was obtained by sinking a portion of the chopped meat in hot water 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. All samples were kept frozen at -20°C till when needed for the experiment. 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, samples were thawed overnight in a refrigerator, 4±1°C. All chemical reagents used in the experiments were of analytical grade. The research was conducted in the School of Food Science and Technology and State Key Laboratory of Jiangnan University, Wuxi, China, from December 2009 to February 2010.

Table 1: Characteristics used in preparation of samples for hydrolysis

Enzyme	Form	pH	T (°C)
Alcalase 2.4 L (AU g^{-1})	Liquid/grain	8.0	55

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

Preparation of Fish Protein Hydrolysates (FPH) and Concentrates (FPC): The HWD and FMM were hydrolyzed with Alcalase 2.4 L, under the optimum conditions (pH 8.0 and Temperature 55°C) of hydrolysis (Table 1). One hundred grams of tilapia fish were weighed into a vessel immersed in a water bath maintained at an appropriate temperature and 300 mL of distilled water was added to make a suspension. The suspension was adjusted to an optimal pH condition and pre heated for 15 min to the appropriate temperature. An enzymes substrate ratio (1.5%) was added with continuous stirring, the hydrolysis process was monitored for 120 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.

Degree of Hydrolysis (DH) determination: The degree of hydrolysis is defined as the percent ratio of the number of peptide bonds broken (h) to the total number of bonds per unit weight (h_{tot}). Reactions were monitored by measuring the extent of proteolytic degradation by means of the DH according to the pH-stat method described by Adler-Nissen (1986). In each case, was calculated from the amount of base consumed as described by Van der Plancken *et al.* (2003), as given below:

$$DH(\%) = \frac{V_b \times N_b}{\alpha \times mP \times h_{tot}} \times 100 \quad (1)$$

where, V_b is base consumption in mL; N_b is normality of the base; α is average degree of dissociation of the α-NH_2 groups; mP is mass of protein (N×6.25) in g and h_{tot} is total number of peptide bonds in the protein substrate. All the experiments were performed in triplicate and the results are the average of three values.

Proximate analysis: The proximate analysis of tilapia fish (*Oreochromis niloticus*) protein hydrolysates (Alcalase 2.4 L), concentrates and raw samples of Fresh Minced Meat (FMM) and Hot Water Dip (HWD) were determined according to AOAC (2005). The moisture content was determined by drying in a forced-air convection oven at 105°C until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at 550°C for 8-12 h. Total crude protein (N×6.25) content was determined using the Kjeldahl method. The extraction and determination of total lipids in samples was determined by Soxhlet extraction.

Amino acid analysis: The dried samples were digested with HCl (6 M) at 110°C for 24 h 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.

Mineral analysis: The mineral content of tilapia fish protein hydrolysates (FMMH and HWDH) and concentrate (FMMC and HWDC) samples were determined in triplicate by the acid digestion method involving microwave technology (CEM microwave, MDS-2000, CEM Corp., Matthews, N.C., USA). A 0.5 g sample was placed in a vessel and 6 mL HNO_3 was added. The sealed vessel was heated until digestion was completed. The sample was cooled for 5 min. The inductively coupled argon plasma machine (Model CIROS, SPECTRO Analytical Instruments, Kleve, Germany) was used to analyze the mineral content.

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 Da), bacitracin (1450 Da), Gly-Gly-Tyr-Arg (451 Da) 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 Differential Scanning Calorimetry (DSC): Thermal denaturation of FMMC and HWDC samples were examined with a Perkin-Elmer differential scanning calorimeter. Lyophilized samples (1 mg each) weighed in aluminum pans and 10 μL of distilled water added, using an empty pan as a reference. The scanning temperatures were from 30 to 120°C at a heating rate of 10°C min^{-1}. Indium standards were used for temperature and energy calibrations. Thermal denaturation temperature (Td) and denaturation enthalpy (ΔH) were calculated from thermograms.

Color measurements: Sample color was evaluated using the Hunter Lab colorimeter (WSC-S Color Difference Meter, USA) and reported as L*, a* and b* values, in which L* is a measure of lightness, a* represents the chromatic scale from green to red and b* represents the chromatic scale from blue to yellow. All the experiments were performed in triplicate and the results are the average of three values.

Scanning Electron Microscopy (SEM): Microstructure of tilapia fish protein hydrolysates (FMMH and HWDH) and concentrate (FMMC and HWDC) samples was determined by using Mastersizer 2000 (MALVERN Instruments Ltd., Worcestershire WR 14 1XZ UK) for laser light scattering and Quanta-200 for scanning electron microscope.

Nitrogen Solubility (NS): Nitrogen solubility was determined according to the procedure of Diniz and Martin (1997), with slight modification. 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 and 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 and 25 mL aliquot was centrifuged at 2800×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:

$$\text{Nitrogen solubility (\%)} = \left(\frac{\text{Supernatant (N) concentration}}{\text{Sample (N) concentration}}\right) \times 100 \quad (2)$$

Water-Holding Capacity (WHC): To determine Water Holding Capacity (WHC) of tilapia fish protein hydrolysates and concentrates, the method outlined by Diniz and Martin (1997), with slight modification was applied. Triplicate samples (0.5 g) of hydrolysates 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 2800×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.

Oil-Holding Capacity (OHC): Oil-Holding Capacity (OHC) of tilapia FPH and FPC were 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 of each sample 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 2800×g for 25 min. The free oil was decanted and the OHC was determined by weight difference.

Foaming Capacity (FC) and Foam Stability (FS): Estimation of foaming capacity was done following the method of Bernard-Don *et al.* (1991), with minor modification. Thirty milliliter of 30 g L^{-1} aqueous dispersion was mixed thoroughly using an Ultra-turrax 25 homogenizer at 9500×g 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.

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, 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×g 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 2800×g for 20 min. Emulsifying capacity was calculated as in equation.

$$EC = \frac{V_A - V_R}{W_S} \quad (3)$$

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.

***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 modification. Twenty milligram of sample (FMMH, HWDH and FMMC, HWDC) 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:

$$\text{Protein digestibility (\%)} = \frac{(A - B)}{A} \times 100 \quad (4)$$

Where:
A = Total protein content (mg) in the sample
B = Total protein content (mg) in TCA precipitate

Bulk Density (BD): Bulk density of freeze-dried tilapia FPH 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.

Statistical analysis: Data analysis was carried out with SPSS Inc. software (version 13.0) (SPSS, 2009). One-way analysis of variance (ANOVA) was used to determine significant differences between means, with the significance level taken at a = 0.05. Tukey's HSD test was used to perform multiple comparisons between means.

RESULTS AND DISCUSSION

Degree of Hydrolysis (DH): The enzymatic hydrolysis of FMM and HWD processed with Alcalase 2.4 L (Fig. 1), exhibited a behavior that is similar to Adler-Nissen (1986). Hydrolysis with proteases developed rapidly in early reaction stage, as shown in the rise in DH. The reaction was asymptotic in 60 min after hydrolysis began (Fig. 1). In the first 80 min it reached DH of 23.03 and 23.53%

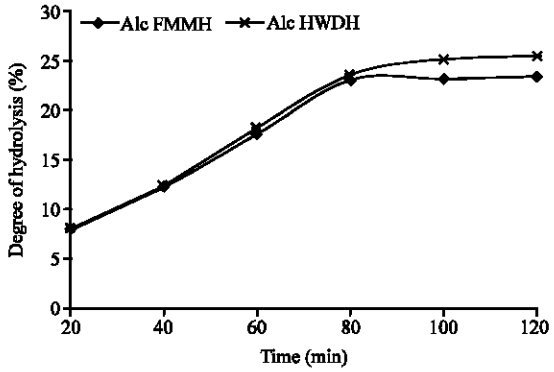

Fig. 1: Effect of time on the degree of hydrolysis (DH) of tilapia fish protein hydrolysates (TFPH) (*Oreochromis niloticus*). FMMH: Fresh minced meat hydrolysate, HWDH: Hot water dip hydrolysate. Value represent the Mean±SD of n = 3 duplicate assays

Table 2: Proximate composition of Fresh Minced Meat (FMM) and Hot Water Deep (HWD) protein hydrolysates (Alcalase 2.4 L), concentrates and raw samples of Tilapia fish (*Oreochromis niloticus*)

Composition	FMMH	HWDH	FMMC	HWDC	FMM	HWD
Protein	97.57±0.12f	85.40±0.62e	82.39±0.55d	74.50±0.46c	19.04±1.39a	21.28±0.91b
Lipid	0.67±0.04a	1.24±0.05b	1.81±0.12d	2.03±0.18d	3.73±0.19c	4.13±0.11c
Moisture	1.22±0.02a	3.17±0.04ab	3.81±0.18ab	4.81±0.63b	77.81±2.12c	75.45±0.96c
Ash	2.25±0.13b	9.85±0.14d	8.89±0.25c	12.04±0.16e	1.04±0.03a	1.05±0.04a

The data are means and standard deviations of triplicate. Column with different letter(s) indicate statistical differences ($p<0.05$). FMMH: Fresh minced meat hydrolysate, HWDH: Hot water dip hydrolysate, FMMC: Fresh minced meat protein concentrate and HWDC: Hot water dip protein concentrate

for FMMH and HWDH respectively, indicating that enzymatic hydrolysis reacted rapidly; though hydrolysis increased gradually during the remaining reaction and eventually reaching DH of 23.40 and 25.43% for FMMH and HWDH respectively. Similar behavior was observed by Guerard *et al.* (2002) and Sathivel *et al.* (2003).

Proximate composition: The protein contents of FMMH and HWDH were 97.57 and 85.40%. FMMC and HWDC have protein contents of 82.39 and 74.50%, while the protein contents for raw samples (FMM and HWD) were 19.04 and 21.28%, respectively (Table 2). There was a significant difference ($p<0.05$) in the crude protein content amongst the samples and our results are within data reported by Wasswa *et al.* (2008) and Murphy *et al.* (1991). Protein composition in muscles varies by muscle type (Suzuki, 1981) as the white meat is generally more abundant (18 to 23% of protein) depending on the species and time of harvesting, contains less lipids than the dark meat and is the most widely consumed type of muscle tissue. The lipid content of FMMH and HWDH were 0.67 and 1.24%, FMMC and HWDC were 1.81 and 2.03% and for the raw samples FMM and HWD were 3.73 and 4.13%, respectively. Nonetheless our results corroborated the results reported for fish protein hydrolysates (Wasswa *et al.*, 2008). The decreasing lipid content in the protein hydrolysates might significantly increase stability towards lipid oxidation, which may also enhance product stability (Diniz and Martin, 1997; Kristinsson and Rasco, 2000; Shahidi *et al.*, 1995). Moisture content was higher in the raw samples (FMM 77.81% and HWD 75.45%) than in the concentrates (FMMC 3.81% and HWDC 4.81%). The moisture content of hydrolysates were the lowest (FMMH 1.22% and HWDH 3.17%) respectively. HWDC has the highest ash content value of 12.03% and FMM with the lowest ash content of 1.04% with a significance difference ($p<0.05$) (Table 2). The results from this study were within the range reported for other fish proteins studied (Yanez *et al.*, 1976; Wasswa *et al.*, 2008).

Amino acid analysis: The total amino acid composition of Tilapia FPH (FMMH and HWDH) and FPC (FMMC and HWDC) are shown in Table 3, along with the recommendations made by FAO/WHO/UNU (2007) for essential amino acid composition. It is clear that tilapia fish protein contains all the essential amino acids in good proportion as reported by Sathivel *et al.* (2003). The results in Table 3 shows that the essential amino acid composition of FPH (FMMH and HWDH) is lower in value compared to FPC (FMMC and HWDC) with a significant difference ($p<0.05$). On the other hand, the non-essential amino acid values for both samples closely resemble though, aspartic acid, alanine and glutamic acid, were found to be more in FPH than FPC. Both FPH and FPC of FMM and HWD have a well-balanced amino acid composition with higher level than FAO/WHO/UNU (2007). The values are generally in accordance with previous studies (Shahidi *et al.*, 1995; Gbogouri *et al.*, 2004).

Table 3: Amino acid composition of Tilapia fish protein hydrolysates and concentrates, Fresh Minced Meat (FMM) and Hot Water Dip (HWD) (g 100 g^{-1} protein)

					FAO/WHO/UNU[A]	
Amino acid composition	HWDH	FMMH	FMMC	HWDC	Child	Adult
Essential amino acid						
Isoleucine	3.22±0.10a	3.59±0.06b	4.07±0.03c	4.16±0.06c	3.00	3.0
Leucine	7.81±0.08ab	7.67±0.08a	7.92±0.12bc	8.11±0.04c	6.00	5.9
Lysine	9.58±0.03c	8.65±0.03a	9.27±0.04b	9.34±0.03b	4.80	4.5
Methionine	2.53±0.04a	2.87±0.12b	3.14±0.04c	3.07±0.05c	2.30	1.6
Met+Cys[B]	3.17±0.07a	3.46±0.11b	3.47±0.15b	3.50±0.04b		
Phenylalanine	3.07±0.05a	3.63±0.02b	3.93±0.12c	4.14±0.06d	4.10	3.8
Phe+Tyr[C]	5.14±0.06a	6.39±0.02b	7.25±0.06c	7.19±0.06c		
Threonine	4.17±0.04a	4.37±0.04b	4.32±0.03b	4.32±0.03b	2.50	2.3
Valine	4.11±0.03a	3.96±0.12a	4.58±0.03b	4.57±0.05b	2.90	3.9
Histidine	2.17±0.06a	2.01±0.04b	2.37±0.06c	2.38±0.03c	1.60	1.5
Tryptophan	0.58±0.11a	0.28±0.03b	0.32±0.03a	0.35±0.04a	0.66	0.6
Nonessential amino acid						
Alanine	6.18±0.05d	6.41±0.07c	5.37±0.05a	5.56±0.05b		
Arginine	5.57±0.06ab	5.71±0.05a	5.86±0.07b	5.73±0.10ab		
Aspartic acid[D]	9.55±0.10c	9.65±0.02c	8.75±0.11a	9.07±0.03b		
Cysteine[E]	0.61±0.04ab	0.56±0.05b	0.53±0.03ab	0.48±0.04a		
Glutamic acid[F]	15.65±0.18c	17.48±0.04b	14.87±0.11a	15.08±0.05a		
Glycine	4.82±0.03b	4.44±0.03c	4.05±0.04a	4.12±0.04a		
Serine	3.86±0.07a	3.87±0.05a	3.85±0.06a	3.80±0.03a		
Tyrosine	2.73±0.06a	2.05±0.04b	3.26±0.06c	3.17±0.06c		
Proline	4.21±0.03d	5.35±0.04c	2.51±0.02b	2.08±0.04a		

The data are means and standard deviations of triplicate. Column with different letters indicate statistical differences. ($p<0.05$). [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

Table 4: Mineral composition (µg g^{-1}) of Tilapia fish protein hydrolysates and concentrates of Fresh Minced Meat (FMM) and Hot Water Dip (HWD)

Mineral composition	HWDH	FMMH	HWDC	FMMC
Zn (µg g^{-1})	17.88±0.16b	16.48±0.09a	32.29±0.15d	30.69±0.28c
Fe (µg g^{-1})	26.09±0.09c	21.24±0.06b	27.42±0.10d	17.05±0.07a
Cu (µg g^{-1})	8.17±0.25c	3.61±0.02b	2.59±0.04a	3.88±0.12b
Mn (µg g^{-1})	1.87±0.05c	0.71±0.03a	12.80±0.18d	1.04±0.07b
K (µg g^{-1})	17228.23±0.68c	29681.33±0.58d	11052.00±1.00b	10254.53±0.50a
Na (µg g^{-1})	37504.07±0.81c	38369.00±1.00d	961.67±0.58b	835.43±0.51a
Mg (µg g^{-1})	1079.83±0.38b	903.00±0.20a	1198.30±0.61c	1418.27±0.64d
Ca (µg g^{-1})	511.53±0.50b	402.07±0.21a	1346.93±0.12d	702.00±0.10c
P (mg g^{-1})	0.02±0.00a	0.02±0.00a	0.02±0.00a	0.02±0.00a

The data are means and standard deviations of triplicate. Column with different letters indicate statistical differences ($p<0.05$). FMMH: Fresh minced meat hydrolysate, HWDH: Hot water dip hydrolysate, FMMC: Fresh minced meat protein concentrate, HWDC: Hot water dip protein concentrate

Mineral composition: There are differences in the contents of zinc (Zn), manganese (Mn), sodium (Na) and calcium (Ca) in the FPH (FMMH and HWDH) and FPC (FMMC and HWDC) samples (Table 4). The sodium level in the FPH samples (FMMH; 38369.00 ug g^{-1} and HWDH; 37504.07 µg g^{-1}) is higher than in FPC (FMMC; 835.43 µg g^{-1} and HWDC; 961.67g g^{-1}) with a

Table 5: Molecular weight distribution of Tilapia fish protein hydrolysates and concentrate

	Area (%)			
Molecular weight (Da)	FMMH	HWDH	FMMC	HWDC
>8000	–	–	16.12	12.44
3000-8000	–	5.68	11.25	16.64
2000-3000	4.98	–	–	–
1000-2000	–	34.63	29.82	32.62
600-1000	32.47	–	15.31	22.75
300-600	27.87	26.9	20.82	11.54
200-300	19.54	–	3.65	4.72
<200	15.11	32.79	9.95	4.48

FMMH: Fresh minced meat hydrolysate; HWDH: Hot water dip hydrolysate, FMMC: Fresh minced meat protein concentrate and HWDC: Hot water dip protein concentrate

significant difference ($p<0.05$). This is indicating that the hydrolysis process could have influenced the increase in the sodium ion. The calcium content in the samples (FMMC; 702.00 μg g^{-1} and HWDC; 1346.93 μg g^{-1}) is also higher with a significant difference ($p<0.05$) compared to FPH samples (FMMH; 402.07 μg g^{-1} and HWDH; 511.53 μg g^{-1}). However, among FPH and FPC powders, the highest levels of Zn, (32.29 μg g^{-1}), Fe (27.42 μg g^{-1}), Mn (12.80 μg g^{-1}) and Ca (1346.93) were found in HWDC. The results from this study were within the range reported for other fish proteins studied (Sathivel *et al*., 2003).

Molecular weight analysis: Molecular weight analysis of tilapia FPH (FMMH and HWDH) and FPC (FMMC and HWDC), are shown in Table 5 and were determined by SE-HPLC, by a TSK gel, 2000SWXL (7.8×300 mm) column. The molecular weights for all samples were calculated according to the equation:

$$\text{Log Mol Wt} = 6.70 - 0.214T \text{ with } R^2 = 0.9953 \quad (5)$$

The rising level of DH inversely corresponds to lower molecular weight distributions. The result in Table 5 shows that hydrolysates (FMMH and HWDH) have lower molecular weights than protein concentrates (FMMC and HWDC). However, present results corroborated the results reported for fish protein hydrolysates (Wasswa *et al*., 2008). This result also indicated that cleavage of peptide bonds by the protease had taken place. The FPC (FMMC and HWDC) were characterized by a higher percentage of peptides with molecular weights above 8,000 Da.

Differential Scanning Calorimeter (DSC): The Poikilothermic characteristics of fish proteins make them more heat sensitive than mammalian muscle proteins, with a greater tendency to denature at elevated temperatures (Sikorski, 1994). Denaturation temperatures are normally referred to as measures of the thermal stability of tilapia fish powder (FMM and HWD). However, these denaturation temperatures are influenced by heating rate and sample concentration. According to the results, the samples have varied denaturation temperatures (52.84 and 59.13°C) for FMM and HWD respectively. The enthalpy differs among both varieties. The enthalpies of the various samples as stated above were 11.0480 and 195.5644 J g^{-1}, respectively (Fig. 2a, b). In this study, the various protein concentrate where less denatured than similar product (Chevalier *et al*., 2001).

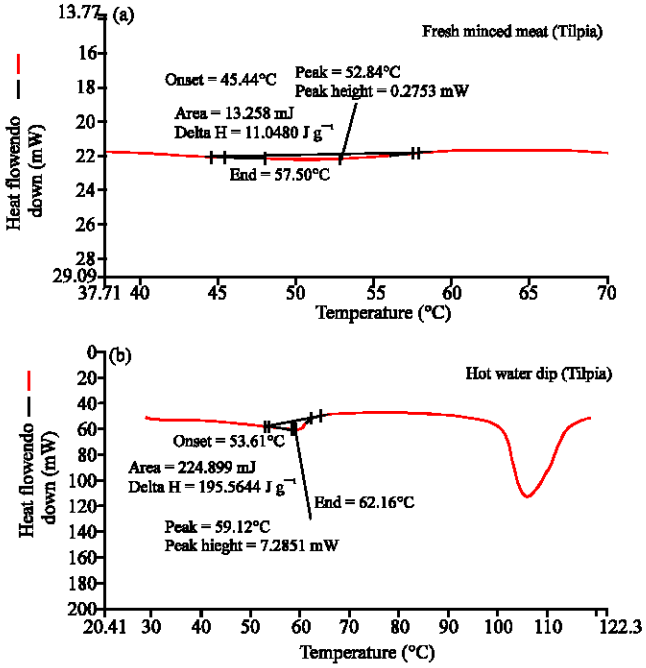

Fig. 2: (a) Differential Scanning Calorimetry (DSC) profile of Fresh minced meat of tilapia fish (FMM) and (b) Differential Scanning Calorimetry (DSC) profiles of tilapia fish, Hot water dip (HWD)

Table 6: Hunter color parameter values of Tilapia fish protein hydrolysates and concentrates of (Fresh Minced Meat (FMM) and Hot Water Dip (HWD)

Sample	L*	a*	b*
FMMH	92.45±2.51b	0.45±0.18a	10.72±3.19a
HWDH	90.62±0.48b	0.62±0.12a	13.41±1.46ab
FMMC	71.85±4.56a	2.19±0.19b	17.22±0.99bc
HWDC	68.74±1.18a	2.04±0.25b	19.09±1.02c

The data are means and standard deviations of triplicate. Row with different letter(s) indicate statistical differences ($p<0.05$). FMMH: Fresh minced meat hydrolysate, HWDH: Hot water dip hydrolysate, FMMC: Fresh minced meat protein concentrate and HWDC: Hot water dip protein concentrate

Color measurement: Color influences the overall acceptability of food products. The fish protein hydrolysate powders were light yellow in color (Table 6). The HWDC was the darkest ($p<0.05$); (L* = 68.74) and most yellowish (b* = 19.09). Whereas, FMMH was the lightest (L* = 92.45) and least yellowish (b* = 10.72). FMM and HWD hydrolysates were lighter (L* = 92.45; 90.62) and less yellow (b* = 10.72; 13.41) compared with FMM and HWD tilapia fish protein concentrates that were darker with values (L* = 71.85; 68.74) and more yellow (b* = 17.22; and 19.09), respectively. These results appear to indicate that color of protein hydrolysates is affirmatively influenced by enzymatic (Alcalase 2.4 L) treatment though not ruling out the possibility of freeze drying having contributed to the increase in L* value obtained. The FMMH and HWDH samples had significant ($p<0.05$) lightness L* value than FMMC and HWDC. The values are generally in accordance with previous studies reported by Diniz and Martin (1997) and Wasswa *et al.* (2007). However, a slight fish odor and taste were apparent in the samples although sensory properties of tilapia FPH and FPC were not evaluated in this study.

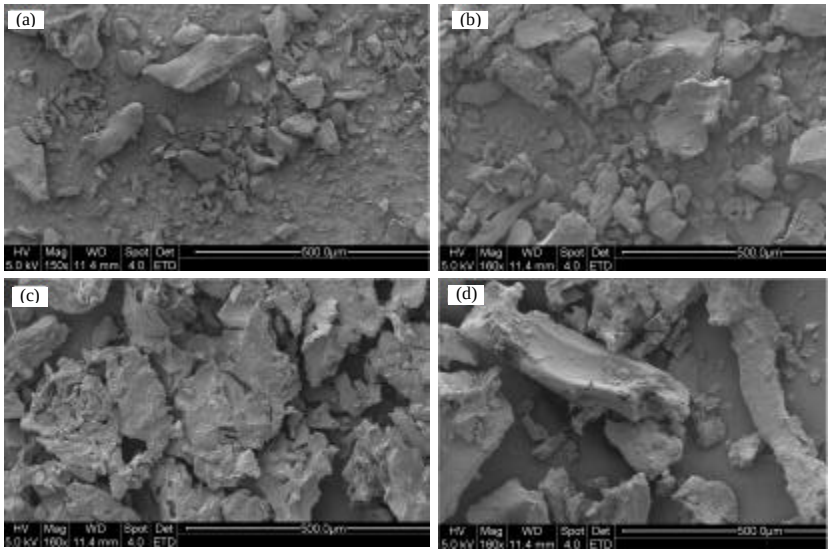

Fig. 3: Scanning electron micrograph of Tilapia fish protein hydrolysates and concentrates powders. (a) FMMH: Fresh minced meat hydrolysate, (b) HWDH: Hot water dip hydrolysate, (c) FMMC: Fresh minced meat protein concentrate and (d) HWDC: Hot water dip protein concentrate. (HV = 5.0 KV; Mag = 160x; WD = 12 mm; Spot = 4.0)

Scanning Electron Micrographs (SEM): The microstructure of Tilapia fish protein hydrolysates and concentrates (FMMH, HWDH and FMMC and HWDC) were analyzed using Scanning Electron Microscope (SEM). The general microstructure of the samples obtained from (FMM and HWD) under the same parameters (HV = 5.0 KV; Mag = 160x; WD = 12 mm; Spot = 4.0) were observed with difference. FMMH and HWDH displayed a smoother matrix compared to FMMC and HWDC (Fig. 3), respectively, showing aggregates of packed flake-like structures. Present results were similar to data reported by Rawdkuen *et al.* (2008). The data further shows that FMMH and HWDH samples resulted to reduced particle size products. The differences in particle size could be attributed to the processing methods of the samples although normally SEM results are empirical.

Nitrogen solubility: Nitrogen solubility is one of the most important physicochemical and functional properties of protein hydrolysates (Kinsella and Melachouris, 1976; Mahmoud *et al.*, 1992). An increase in the extent of enzymatic hydrolysis corresponded to a considerable increase in the nitrogen solubility over the pH range studied. Figure 4 shows nitrogen solubility values for FMMH, HWDH, FMMC and HWDC. Between pH 4.5 and 5.5 near the isoelectric point (pI) at which the net charge of the original proteins are minimized and consequently, more protein-protein interactions and fewer protein-water interaction occur. Tilapia FPH and FPC share solubility profiles; exhibiting a U shaped curve in which FMMH and HWDH have higher solubility values at both alkaline and acidic pH levels. In acidic condition, all proteins had solubility above 80%. At pH 6.0, nitrogen solubility increased rapidly with an increase in pH up to 12.0. These trends in solubility are in agreement with Gbogouri *et al.* (2004), Diniz and Martin (1997) and Sathivel *et al.* (2003). At pH 11.0, the solubility of FMMH and HWDH were 96.93 and 93.23% and the solubility for FMMC and HWDC were less than 92%, respectively.

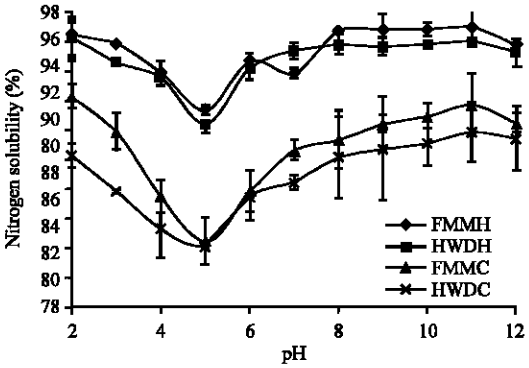

Fig. 4: Nitrogen solubility of Nile tilapia fish (*Oreochromis niloticus*) protein hydrolysates (Alcalase 2.4 L) and protein concentrates. FMMH: Fresh minced meat hydrolysate, HWDH: Hot water dip hydrolysate. FMMC: Fresh minced meat protein concentrate, HWDC: Hot water dip protein concentrate. Value represent the Mean±SD of n = 3 duplicate assays

Table 7: Functional properties of Tilapia fish protein hydrolysates and protein concentrates of Fresh Minced Meat (FMM) and Hot Water Dip (HWD)

	FMMH	HWDH	FMMC	HWDC
In vitro protein digestibility (%)	92.73±0.76b	93.20±0.20b	89.37±0.67a	87.60±1.13a
Water holding capacity (mL g^{-1})	2.10±0.10a	1.77±0.06a	2.47±0.57a	2.43±0.47a
Oil holding capacity (mL g^{-1})	2.27±0.06a	2.23±0.25a	2.43±0.21a	3.30±0.44b
Emulsion capacity (mL 0.5 g^{-1})	22.33±0.58a	21.40±0.36a	19.40±0.17b	20.40±0.53b
Bulk density (g mL^{-1})	0.45±0.01b	0.53±0.06b	0.34±0.02a	0.36±0.02a
Foaming capacity (% volume)	125.50±1.06c	124.50±0.30c	90.17±0.76b	80.83±0.29a

The data are means and standard deviations of triplicate. Column with different letter(s) indicate statistical differences ($p<0.05$)

Protein solubility at various pH values may serve as a useful indicator of how well FPH and FPC will perform when they are incorporated into food systems. The solubility curve is typical to that of most fish protein hydrolysates. Enzymatic protein hydrolysis leads to smaller peptides, consequently, to more soluble products. Since many functional properties of proteins depend upon their capacity to go into solution initially, the excellent solubility of the FPH suggests that they may have many potential applications in formulated food systems.

Foam capacity and stability (FC and FS): From our studies, it was observed that foam capacity and stability is concomitant with nitrogen solubility. A Significant ($p<0.05$) increase was observed in the foaming capacity of FMMH and HWDH (125.5 and 124.5%) compared to FMMC and HWDC (90.3 and 80.1%), respectively (Table 7). The values are generally in accordance with previous studies reported by Diniz and Martin (1997) and Wasswa *et al.* (2008). The results imply an increase in surface activity, probably due to the initial greater number of polypeptide chains that arose from partial proteolysis, allowing more air to be incorporated. Related FC has been reported in previous studies (Kuehler and Stine, 1974; Klompong *et al.*, 2007). The formation of protein-based foams involves the diffusion of soluble proteins toward the air-water interface and rapid conformational change and rearrangement at the interface; the Foam stability requires formation of a thick, cohesive and viscoelastic film around each gas bubble (Klompong *et al.*, 2007) hence foam ability is a function of the configuration of protein molecules. To have foam stability, protein

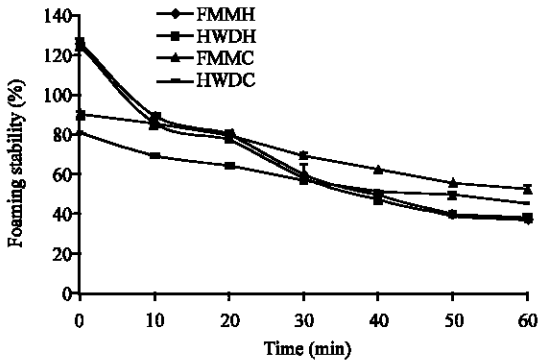

Fig. 5: Foaming stability of tilapia fish protein hydrolysates (Alcalase 2.4 L) and protein concentrates. FMMH: Fresh minced meat hydrolysate, HWDH: Hot water dip hydrolysate, FMMC: Fresh minced meat protein concentrate and HWDC: Hot water dip protein concentrate. Value represent the Mean±SD of n = 3 duplicate assays

molecules should form continuous intermolecular polymers enveloping the air bubbles, since intermolecular cohesiveness and elasticity are important to produce stable foams. Foam stability values ranged from 125.5 to 38.2, 124.53 to 37.25%, for FMMH and HWDH; also from 90.17 to 52.63, 80.83 to 45.57% for FMMC and HWDC, respectively, with (Fig. 5). Present results were similar to data reported by Diniz and Martin (1997) and Wasswa *et al.* (2008). Further hydrolysis could reduce the foaming stability since the more microscopic peptides do not have the strength needed to maintain stable foam (Shahidi *et al.*, 1995). These foaming properties suggest that tilapia fish protein hydrolysate and the protein concentrate powders could serve as better foaming mediator in protein foods.

Oil/water holding capacity (OHC/WHC): An important functionality that influences taste of the product that is required in the food (meat and confectionary) industry is the ability of FPH and FPC powders to absorb oil. As shown in Table 7, The OHC for FMMH and HWDH; 2.27 and 2.23 mL g^{-1} and for FMMC and HWDC; 2.43 and 3.30 mL g^{-1}, respectively were reported with significant difference ($p<0.05$). However, our results are in agreement with Wasswa *et al.* (2008).

On the other hand, functional properties of proteins in food system broadly depend on the water-protein interaction. The ability of protein to imbibe water and retain it against a gravitational force within a protein matrix is WHC. The WHC for FMMH and HWDH is 2.10 and 1.77 mL g^{-1} and for FMMC and HWDC; 2.47 and 2.43 mL g^{-1}, respectively, with significant difference ($p<0.05$) Table 7. FPH had lower oil absorbing capacity compared to Diniz and Martin (1997).

Emulsifying Capacity (EC): 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 proteins would contribute to an improvement in the formation of emulsions. From the results (Table 7), FMMH and HWDH shows an appreciable EC ($p<0.05$) than FMMC and HWDC. The lowest EC of 19.40 mL 0.5 g^{-1} was manifested in FMMC whereas, the highest EC was found in FMMH (22.33 mL 0.5 g^{-1}). The hydrolysates manifested a good EC than protein concentrate ($p<0.05$).

Although our result corroborated with Gbogouri *et al.* (2004), an extensive protein hydrolysis may result in a marked loss of emulsion properties.

Bulk Density (BD): Bulk density varied among the various samples studied (Table 7). The lowest BD is reported in FMMC (0.34 g mL^{-1}) and HWDH scored the highest (0.53 g mL^{-1}) BD in our experiment. Overall, it was observed that FPH (FMMH and HWDH) has a better BD than FPC (FMMC and HWDC). Furthermore, the BD of tilapia FPH was viewed lower when compared to tilapia skin protein hydrolysate (Wasswa *et al.*, 2008). Bulk density signifies the behavior of a product in dry mixes and is an important parameter that can determine the packaging requirement of a product. Also, it varies with the fineness of particles. High bulk density is unfavorable for the formulation of weaning foods, where low bulk density is required (Kamara *et al.*, 2009).

***In vitro* protein digestibility:** *In vitro* protein digestibility of the samples reported in Table 7 was evaluated by the release of TCA-soluble nitrogen, after incubation time of 120 min at 37°C. The result in Table 7 shows that all protein samples exhibited trypsin digestibility above 85%. FMMH and HWDH have digestibility values with trypsin of 92.73 and 93.20%, whereas FMMC and HWDC with digestibility values of 89.37 and 87.6%, respectively. Present results are within the values reported by Abdul-Hamid *et al.* (2002). The pretreatment undergone by the samples during the cause of hydrolysis improved digestibility of protein and may be attributed to the increase in protein solubility, or structural unfolding of protein molecules (Van der Plancken *et al.*, 2003).

CONCLUSION

The study revealed the practical relevance of tilapia (*Oreochromis niloticus*) FPH and FPC as a good source of desirable quality essential amino acids and mineral source in the food industry. The physicochemical and other functional properties exhibited significant differences between FPH, having superior functionality over FPC. The FPH had desirable micro structure, color, solubility, protein digestibility, emulsion, fat absorption and bulk density. The FPH can prospectively compete with hydrolysates and protein concentrates available in the market. Although, the physicochemical and functional properties of FPC exhibited inferior qualities, it is however portraying characteristic lower sodium content than FPH. This difference in their chemical composition is vital in the areas of general health and nutrition.

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