

**PJN**

ISSN 1680-5194

PAKISTAN JOURNAL OF  
**NUTRITION**

**ANSI***net*

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## Study on the Effects of Enzymatic Hydrolysis on the Physical, Functional and Chemical Properties of Peanut Protein Isolates Extracted from Defatted Heat Pressed Peanut Meal Flour (*Arachis hypogaea* L.)

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**Abstract:** Physical, chemical and functional properties of peanut protein isolates (HPI) and hydrolysis (HPH) extracted from peanut meal obtained from heat treated peanut oil extraction method were analyzed. HPH exhibited superior functional properties such as whipping, emulsification, fat and water absorption properties. Thermal denaturation profiles indicate that there is no significant difference ( $p \leq 0.05$ ) between HPI and HPH at both onset and transition temperatures. However, the conclusion temperatures were found to be significantly different ( $p \leq 0.05$ ). Protein solubility was pH dependent with HPH been more soluble at all pH levels. However, solubility was low at the isoelectric pH range of 4.5-5.5. Electrophoretic patterns obtained from SDS-PAGE show that HPH registered a lower molecular weight ( $< 31$ KDa) compared to HPI ( $\approx 66$  KDa). HPH had relatively smaller particle sizes ( $\pm 0.1 \mu\text{m}$ ) compared to HPI ( $\pm 100 \mu\text{m}$ ). Protein isolates extracted from peanut meal obtained from cold pressed peanut oil extraction method were used as control in analyzing functional parameters but HPH still exhibited superior qualities. Both HPH and HPI were found to be nutritionally rich in most essential amino acids with respect to the recommended FAO/WHO amino acid requirement patterns for both adults and children.

**Key words:** Peanut meal, heat treated method, functional properties, protein isolate and hydrolysates, nutrition, essential amino acids

### INTRODUCTION

Plant proteins, especially oilseed protein sources, are expected to play an invaluable nutritional role in meeting the world's current and future needs for edible and functional proteins to replace the currently decreasing or economically inaccessible animal protein sources. In addition to the fact that animal protein sources are gradually becoming scarce and expensive, studies have shown that they can lead to higher levels of cholesterol and fats in the blood which are considered as risk factors of cardiovascular diseases (Kritchevsky, 1995; Lichtenstein, 1998). Oilseed proteins have potential food uses as they supply desirable functional properties such as whipping capacity and viscosity, emulsification and water and oil holding capacities (Khalil *et al.*, 1985). Peanuts rank in the world as the fifth largest source of vegetable oil, indicative of the fact that a large proportion of peanut produced are used for peanut oil production. Peanut oil has a variety of uses which includes cooking, paints, vanishes, fuel, to mention but a few (Lack *et al.*, 2003). Peanut residue left from oil extraction is referred to as oilcake/meal. Research findings have shown that the oil-free meal obtained after oil extraction contains nearly 50-60% proteins of good nutritional quality (Monteiro and Prakash, 1994; Kain *et al.*, 2009).

Enzymatic hydrolysis of food proteins is carried out for various reasons including improvement of nutritional characteristics, retarding deterioration and modification of functional properties such as solubility, emulsification, foaming and the removal of toxic or inhibitory ingredients (Puski, 1975; Panyam and Kilara, 1996; Aluko and Monu, 2003; Spellman *et al.*, 2005). Solubility of protein hydrolysates improves due to breakdown of the oligomeric structure of 7S globulin which causes alteration of charged particles. Further scientific investigations have shown that hydrolysed protein foodstuffs may have advantages over non-hydrolysed protein foodstuffs in a number of areas of health care. For example, it has been reported that enzymatically hydrolysed proteins are less allergenic, more rapidly digested and absorbed than whole proteins-hence foodstuffs containing hydrolysed proteins are usefully employed in the alimentation of hospital patients with digestive diseases (Adler-Nissen, 1978; Webb, 1990). Basically two methods are employed in peanut oil extraction-cold pressed and heat treated methods (Kain and Chen, 2008). The meal/cake obtained from the two extraction methods are used in this study although the latter forms the principal research material. The principal objectives of this study were to develop protein isolates from defatted peanut meal flour obtained from cold

pressed and heat treated peanut oil extraction methods and conduct a comparative analysis of their physical, chemical and functional properties as indicators of their potential use by the food industry.

## MATERIALS AND METHODS

Cold Pressed Peanut Meal (CPM) and Heat Treated Peanut Meal (HPM) obtained as by-products from two peanut oil extraction methods were from Qingdao Kerry Peanut Oil Co., Ltd (Shandong province, China). The enzyme A.S.1.398 (origin-Bacillus subtilis; type-proteinase; cellulase activity =  $1 \times 10^5$   $\mu$ /g) was obtained from Genecor (Wuxi) Bio-Products Co. Ltd., P.R. China. Molecular Weight calibration kit and its protein components were purchased from Shanghai Institute of Biochemistry, Shanghai, PR China. All other chemicals used were either obtained from the chemical store of Jiang Nan University or from other laboratories.

**Preparation of defatted peanut meal:** CPM and HPM were defatted with petroleum ether at 30 ~ 60°C using the Soxhlet method for 8 h. The Defatted samples were air dried, milled into fine powder using a NIRON blender, sieved to pass through a 60 mesh sieve, oven dried for 3 h and referred to as defatted cold pressed and heat treated peanut meal-DCPM and DHPM, respectively. Biochemical analysis of DCPM indicated the following:

~ 52.10% crude protein (Kjeldahl Nx5.46) ~ 7.13% moisture, ~ 0.91% fat, ~ 7.84% ash, ~ 22.80% carbohydrates and ~ 9.93% crude fiber; while those for DHPM were ~ 50.81% crude protein (Kjeldahl Nx5.46), ~ 6.73% moisture, ~ 0.77% fat, ~ 8.87% ash, ~ 22.80% carbohydrates and ~ 9.50% crude fiber.

**Preparation of Peanut Protein Isolate (PPI) from DCPM and DHPM:** PPI was extracted from DCPM and DHPM according to the procedure described by Kim *et al.* (1990) with some modifications. DCPM/DHPM flour was dispersed in water (w/v = 1:10) and stirred for 30 min at 40°C and then pH was adjusted to 9.0 by using 1.0 M NaOH. After stirring for 60 min, the suspension was centrifuged at 3000 rpm for 20 min. The supernatant was collected and pH adjusted to 4.5 with 1.0 M HCl to precipitate the proteins and centrifuged again at 3000 rpm for 20 min at ambient temperature. The precipitates were washed three times with de-ionized water (pH 4.5), dispersed in a small amount of de-ionized water and pH adjusted to 7.0 by using 0.1 M NaOH, then freeze-dried. The protein isolates extracted from DCPM and DHPM are referred to in this work as CPI and HPI, respectively.

**Chemical analysis of CPI and HPI:** Fat, moisture and ash were determined using standard AOAC (1990) methods 932.06, 925.09 and 923.03, respectively (AOAC, 1990). Total crude protein ( $N \times 6.25$ ) content of each sample was determined using the Kjeldahl

method (AOAC, 1995). Carbohydrate contents were determined by phenol-sulfuric acid method (Dubois *et al.*, 1956).

**Hydrolysis of HPI with proteolytic enzymes:** Hydrolysis of HPI was done according to the method of Achouri *et al.* (1998) with slight modification. Prior to the addition of enzyme, 10 g PPI was dispersed in 250 ml water and heated for 30 min. then proteolysis was carried out using microbial neutral proteinase A.S.1.398 at 45°C and pH 7.

The process was controlled by monitoring Degree of Hydrolysis (DH) using pH-start technique as outlined by Adler-Nissen (1976). Different samples were prepared with DH of 4, 6, 8 and 10, respectively. When the appropriate DH was attained, the enzyme was inactivated by heating at 65°C for 30 min and the supernatant was separated from the precipitate and treated with 0.5% (w/v) activated carbon at 50-55°C for 30 min for the removal of bitterness and off-flavour. The protein hydrolysates were freeze-dried and stored for further analysis. The hydrolysed peanut protein will hereafter be referred to as Heat Pressed Peanut Protein Hydrolysates (HPH).

## Analysis of physical, functional and chemical properties of HPI and HPH

**Particle size determination and distribution:** Particle size distribution of HPI and HPH were carried out by using Mastersizer 2000 (MALVERN Instruments Ltd, Worcestershire WR 14 1XZ UK).

**Determination of thermal characteristics of HPI and HPH:** Thermal characteristics of protein samples (HPI and HPH) were determined with a Perkin-Elmer Differential Scanning Calorimeter (DSC) using a modified form of the method described by Meng and Ma (2001).

Lyophilized samples (1mg each) were directly weighed into coated aluminum pans and 10  $\mu$ l of water was added. The aluminum pans were hermetically sealed and heated from 30-120°C at a rate of 10°C/min.

A sealed empty pan was used as reference. Thermal Denaturation Temperature (Td) and denaturation enthalpy ( $\Delta H$ ) were calculated from thermograms. All experiments were conducted in triplicate.

**Protein solubility:** The solubility of CPI, HPI and HPH were estimated at varying pH levels by using a modified form of the method described by Wu *et al.* (1998). Samples were mixed with water in the ratio of 0.75 g/15 ml (w/v) and pH of the mixture was adjusted to 2.0-10.0 with 0.1 N NaOH and 0.1 N HCl.

The suspension of the samples was let to stir at room temperature for 1 h and then centrifuged at 3000 rpm for 15 min. Protein concentration in original sample and in

supernatant (soluble protein) were determined by Kjadhah method (AOAC, 1995) using 6.25 as conversion factor. Protein solubility, for each sample, was then calculated using the following equation (Were *et al.*, 1997):

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

**Fat Absorption Properties (FAP) and Water Absorption Properties (WAP):** Oil absorption property of each of the samples (CPI, HPI and HPH) was determined using the method of Chakraborty (1986). One gram ( $W_0$ ) of protein isolates were weighed into pre-weighed 15-mL centrifuge tubes and thoroughly mixed with 10 ml ( $V_1$ ) of soybean oil (Gold Ingots Brand, QS310002012787, Suzhou, P.R. China) using a Vortex mixer. Samples were allowed to stand for 30 min. The flour-oil mixture was centrifuged at 3000 rpm for 20 min. Immediately after centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder and volume recorded ( $V_2$ ). Fat absorption capacity (milliliter of oil per gram of flour) was calculated as

$$\text{FAC} = \frac{V_1 - V_2}{W_0}$$

Triplicate samples were analyzed for each sample. Water Absorption Capacity (WAC) was determined using the method outlined by Chun-He *et al.* (2006), with some modifications. One gram of each sample was weighed into pre-weighed 15-mL centrifuge tubes. For each sample, 10 ml of distilled water was added and mixed using a vortex mixer at the highest speed for 2 min. After the mixture was thoroughly wetted, samples were allowed to stand at room temperature for 30 min, centrifuged at 3000 rpm for 20 min. The supernatant was decanted and the centrifuge tube containing sediment was weighed. Water absorption capacity (grams of water per gram of flour) was calculated as:

$$\text{WAC} = \frac{W_2 - W_1}{W_0}$$

where  $W_0$  = weight of dry sample (g);  $W_1$  = weight of tube plus dry sample (g) and  $W_2$  = weight of tube plus sediment (g). Triplicate samples were analyzed for each peanut protein isolates.

**Emulsifying Capacity (EC) and Emulsion Stability (ES):** Emulsifying activity and stability indices were determined using the method of Neto *et al.* (2001). Five milliliter portions of (HPI and HPH) solutions were homogenized with 5 ml soybean oil (Gold Ingots Brand, QS310002012787, Suzhou, P.R. China). Emulsions

were centrifuged (NSKC-1, Nanjing, PR China) at 1100 rpm for 5 min. The height of emulsified layer and that of total contents in tube were measured. EC was calculated as:

$$\text{EC (\%)} = \frac{\text{height of emulsified layer in tube}}{\text{height of total content in tube}} \times 100$$

ESI was determined by heating each of the emulsions above at 55°C before centrifuging at 2000 rpm for 5 min. ES was calculated as follows:

$$\text{ES (\%)} = \frac{\text{final height of emulsified layer after heating}}{\text{initial height of emulsified layer before heating}} \times 100$$

**Whipping properties:** Whipping properties of 3% dispersions of HPI and HPH were determined in triplicate using a modified form of the method described by Lin *et al.* (1974). Samples (10 g) were dispersed in distilled water (250 ml) and pH adjusted to 7.0 using 0.1 M NaOH and 0.1 M HCl. Suspensions were thereafter homogenized for 1 min at maximum speed level using an HR 2839 model Philip Blender and pH checked and adjusted when necessary. Suspensions were then whipped, using maximum speed in a Kenwood Chef Food mixer for 10 min with the wire whip attachment. The resulting foam was immediately poured into a liter-measuring cylinder and foam height was measured at intervals. Percent foam expansion was calculated as follows:

$$\% \text{ Volume Increase} = \frac{A - B}{B} \times 100$$

where A = volume after whipping; B = volume before whipping. Foam volume as percentage was calculated taking the foam volume at zero time as 100%. Leakage was calculated as follows:

$$\text{Leakage} = \frac{C}{D} \times 100$$

where C= Volume of liquid collected; D= volume of liquid before whipping.

**Amino acid determination:** Amino Acids were determined with a Hitachi 835-50G automatic amino acid analyzer (Hitachi Ltd., Tokyo, Japan). Hydrolysis of peanut protein samples was done in a sealed ampoule for 24 h at 110°C using 1 ml of 6 N HCl under vacuum. Hydrolysates were evaporated and then the dried residue dissolved in 0.02 N HCl. Sample was filtered through a 0.45 µm nylon filter before being injected into amino acid analyzer for determination of both amino acids and free amino acids.

**Molecular weight distribution of HPI and HPH:** Non-reduced and reduced SDS-PAGE of samples were carried out using discontinuous system described by Laemmli (1970) with 4% stacking and 12% separating gel. Separating gel was run at a constant current of 20 mA for about 3 h. The gel was stained in coomassie brilliant blue R-250.

Subunit Molecular Weight (MW) was estimated using low MW calibration kit (Shanghai Institute of Biochemistry, Shanghai, China) consisting of the following proteins: phosphorylase (97,400), bovine serum albumin (66,200), rabbit actin (43,000), bovine carbonic anhydrase (31,000), trypsin inhibitor (20,100) and hen egg white lysozyme (14,400).

**Biochemical scores:** Nitrogen contents of both HPI and HPH were determined by micro-Kjeldahl method (AOAC, 1995). A factor of 5.46 was used to convert percentage nitrogen to protein content. Carbohydrate contents were determined by phenol-sulfuric acid method (Dubois *et al.*, 1956). Fat, moisture and ash were determined using standard AOAC methods 932.06, 925.09 and 923.03, respectively (AOAC, 1990).

## RESULTS

**Particle size determination and distribution:** Particle size distribution of HPI and HPH as determined by Mastersizer 2000 are presented in Fig. 1. HPH had smaller particle sizes ( $\pm 0.1 \mu\text{m}$ ) compared to HPI ( $\pm 100 \mu\text{m}$ ).

**Protein denaturation profile with differential scanning calorimetry:** Summary of results calculated from transition peaks are represented in Table 1. No significant difference ( $p \leq 0.05$ ) between HPH and HPI in terms of their onset and transition temperatures. The only significant difference was recorded for conclusion temperatures for HPH and HPI.

**Functional properties:** At all pH levels HPH was more soluble than both CPI and HPI (Fig. 2) All samples demonstrated low solubility at the precipitation pH range of pH 4.5 to pH 5.5. Emulsification properties are presented in Table 2. HPH exhibited superior emulsification properties (EC, ES, FAC, WAC and whipping properties) compared to CPI and HPI.

**Amino acid composition:** Amino acid composition of HPH and HPI are presented in Table 3. HPH and HPI recorded high essential amino acid values that can meet the nutritional requirements of children and adults, although supplementation is required for the latter as some amino acids are limited. In both samples Tryptophan was not detected.

**Molecular weight distribution:** Results obtained from SDS-PAGE are presented in Fig. 3. HPH recorded lower

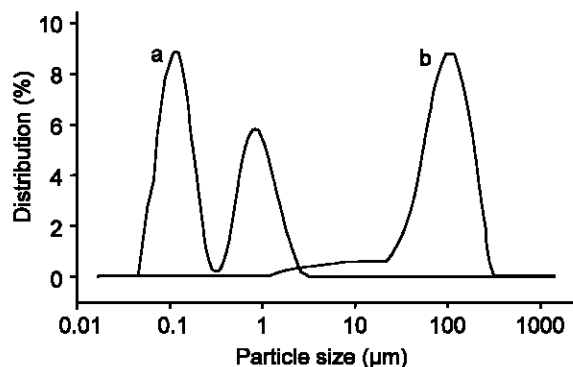


Fig. 1: Particle size distribution of HPH (a) and HPI (b)

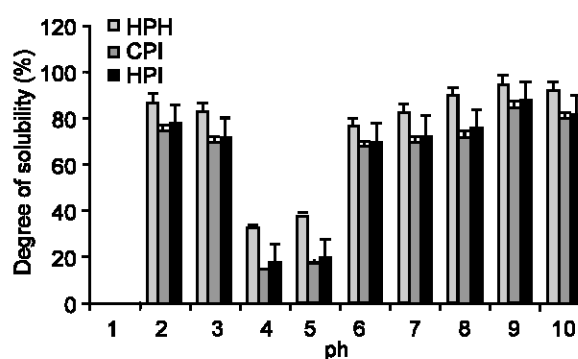


Fig. 2: Protein solubility of HPI, HPH and CPI at different pH levels. Error bars: Standard deviations results are means of triplicate determinations

molecular weight values ( $< 31,000\text{Da}$ ), while HPI recorded higher molecular weight values ( $\approx 66,000\text{Da}$ ).

**Biochemical scores:** Biochemical scores for HPI and HPH are presented in Table 4. HPH recorded higher protein ( $\approx 96\%$ ) and lower carbohydrate ( $\approx 1\%$ ) values compared to HPI ( $p \leq 0.05$ ).

## DISCUSSION

Results, as shown in Fig. 1, indicate that hydrolysis can alter particle size distribution of proteins. In HPI a greater percentage of particle sizes are within  $100 \mu\text{m}$  range whereas in hydrolysates a greater percentage of particle sizes are distributed within the  $0.1$  and  $1.0 \mu\text{m}$  range. This indicates that hydrolysis results to degradation of proteins into smaller fragments. This is in agreement with the findings reported by Radha *et al.* (2008). Protein denaturation profiles for both HPH and HPI are shown in Table 1. (DSC) Denaturation temperatures, which are influenced by heating rate and protein concentration, are normally referred to as measures of thermal stability of proteins. Table 1 indicates that there was no significant difference ( $p \leq 0.05$ ) between HPI and HPH at both onset and transition temperatures.

Table 1: Protein denaturation profile with Differential Scanning Calorimetry (DSC)

Phase transition parameters <sup>a</sup>				
Samples	T <sub>0</sub> (°C)	T <sub>P</sub> (°C)	T <sub>C</sub> (°C)	ΔH (J/g)
HPI	61.00±0.07 <sup>A</sup>	71.65±0.13 <sup>A</sup>	82.91±0.15 <sup>A</sup>	4.15±0.11 <sup>A</sup>
HPH	60.84±0.08 <sup>A</sup>	71.49±0.09 <sup>A</sup>	84.98±0.10 <sup>B</sup>	4.10±0.13 <sup>A</sup>

<sup>a</sup>Onset temperature (T<sub>0</sub>), Transition Temperature Peak (T<sub>P</sub>), conclusion temperature (T<sub>C</sub>); Data are the means±SD, n = 3. Samples means with different superscript letters in the same column are significantly different at p≤0.05.

Table 2: Some functional properties of CPI, HPI and HPH

Functional Properties	Value(± SD)		
	CPI	HPI	HPH
Emulsifying capacity (%)	60.46 (±1.53) <sup>A</sup>	57.28(±1.47) <sup>B</sup>	64.33(±0.31) <sup>C</sup>
Emulsifying stability (%)	66.11(±0.02) <sup>A</sup>	65.87 (±0.30) <sup>A</sup>	70.57(±0.01) <sup>B</sup>
Water absorption capacity (%)	52.44(±0.46) <sup>A</sup>	50.32(±1.21) <sup>B</sup>	55.21(±0.13) <sup>C</sup>
Fat absorption capacity (%)	54.47(±0.11) <sup>A</sup>	52.33(±0.14) <sup>B</sup>	56.48(±0.12) <sup>C</sup>
Foam expansion (%)	77.58(±0.37) <sup>A</sup>	79.71(±0.27) <sup>B</sup>	82.55(±1.20) <sup>C</sup>
Foam leakage after 60 min (%)	47.22(±0.33) <sup>A</sup>	47.27(±0.13) <sup>A</sup>	50.57.16(±0.33) <sup>B</sup>

Each value in the table was the mean of three replications ± standard deviation. Samples means with different superscript letters in the same row are significantly different at p≤0.05.

However, the conclusion temperatures were significantly difference (p≤0.05). The ΔH values calculated from area under transition peaks for HPI and HPH had no significant difference (p≤0.05).

Experiments were conducted in triplicate and results followed the same trend. These results, especially ΔH values, may have been influenced by oil extraction method. The ΔH value is actually a value from a combination of endothermic reactions (disruption of hydrogen bonds determined as 1.7 kcal per mole of hydrogen bond) and exothermic processes (protein aggregation and the breakup of hydrophobic interactions). A detailed analysis of this relationship is required to be able to advance appropriate reasons for differences in ΔH values.

Functional characterization of protein can be generalized as hydration, emulsification, textural and rheological. Those characteristics can be measured through their nitrogen solubility, water absorption, viscosity, fat absorption, foaming, whipping, etc. Solubility is one aspect of hydration, which serves as the most important characteristic in evaluating protein quality since many functional properties of proteins depend upon their capacity to go into solution initially. Protein solubility is affected by many factors, such as pH. The effect of pH on solubility of CPI, HPI and HPH were studied and results are presented in Fig. 2. Solubility profiles shown in Fig. 2 are pH dependent with minimum solubility in all samples observed between pH 4.5 and pH 5.5 which is expected as it falls within the precipitation pH range. At all pH levels HPH demonstrated higher solubility (p≤0.05) than CPI and HPI. This indicates that enzymatic hydrolysis results in increased protein solubility probably due to breakdown of the oligomeric structure of 7S globulin which caused alteration of charged particles. Similar findings have been reported by Prakash

Table 3: Amino acid profiles for HPI and HPM

Amino acid	FAO/WHO (1990) <sup>a</sup> Requirement Pattern			HPI (g/100g sample)
	HPH (g/100g sample)	Children	Adults	
Isoleucine	3.98	2.80	1.30	3.97
Leucine	3.40	6.60	1.90	6.58
Valine	4.37	3.50	1.30	4.84
Histidine	2.04	1.90	1.60	2.17
Lysine	7.54	5.80	1.60	2.45
Methionine	2.53	2.50	1.70	0.52
Phenylalanine	3.93	6.30	1.90	6.02
Threonine	3.40	3.40	0.90	2.28
Tryptophan	ND <sup>b</sup>	1.50	0.50	ND <sup>b</sup>
Arginine	5.14			10.95
Cysteine	0.44			0.22
Tyrosine	3.13			4.50
Alanine	6.21			2.78
Aspartic acid	9.77			12.58
Glutamic acid	15.12			22.83
Glycine	6.37			4.18
Proline	5.80			3.70
Serine	4.19			3.94

<sup>a</sup>FAO/WHO (1990) Essential amino acid requirement patterns as quoted by Zhu *et al.* (2006); ND<sup>b</sup> Not detected.

and Rao (1986); Kim *et al.* (1990) and Chabanon *et al.* (2007). Particle size differences may have also contributed to differences in solubility index of HPH, CPI and HPI because solubility increases with decreasing size of solute particle due to additional surface energy. This effect is generally expected to be small unless particles become very small, typically smaller than 1 μm, which is essentially the case with HPH. Emulsification properties of proteins include fat absorption, foaming and whipping abilities. It could be seen from Table 2 that HPH exhibited superior Emulsifying capacity (p≤0.05.) compared to CPI and HPI.

Table 4: Biochemical scores of HPI and HPH(%)

Samples	Protein (N×5.46)	Ash	Moisture	Fat	Carbohydrate
HPI	84.20 ± 0.07 <sup>a</sup>	4.02 ± 0.32 <sup>a</sup>	3.06 ± 0.02 <sup>a</sup>	0.44 ± 0.11 <sup>a</sup>	4.11 ± 0.01 <sup>a</sup>
HPH	96.16 ± 0.05 <sup>b</sup>	0.60 ± 0.31 <sup>b</sup>	3.73 ± 0.03 <sup>b</sup>	0.28 ± 0.12 <sup>b</sup>	-

Values are shown as mean±SD of triple determinations. Means followed by the same letters in the same column are not significantly different ( $p \leq 0.05$ ).

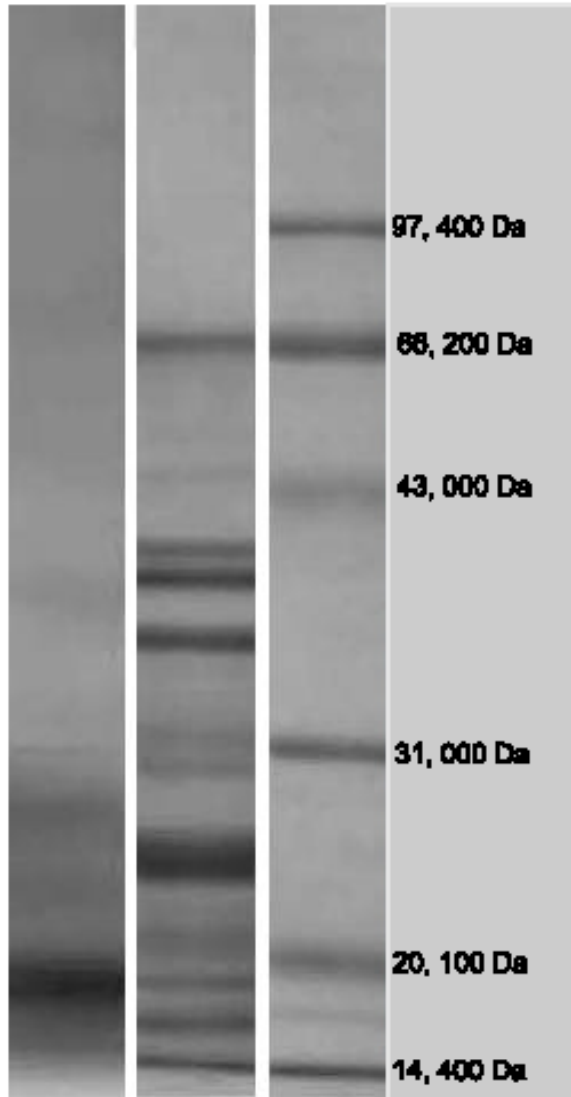


Fig. 3: Electrophoretic patterns of HPH and HPI, HPH= protein hydrolysate extracted from heat treated oil extraction method; HPI=protein isolate extracted from heat treated oil extraction method; ST = standard molecular weight marker

Hydrolysis may have increased the occurrence of protein diffusion and orientation at oil-water interface, leading to orientation of hydrophilic groups towards water phase and hydrophobic groups towards oil phase. This is reflective on the higher oil and water absorption capacities exhibited by HPH compared to CPI and HPI.

This may have also caused the higher emulsion stability values ( $p \leq 0.05$ ) of HPH compared to CPI and HPI (Table 2). Interestingly, CPI and HPI were not significantly different ( $p \leq 0.05$ ) with respect to their emulsion stability. This means that oil extraction method did not affect the emulsion stability properties of CPI and HPI. This agrees with findings of Barbut (1999). Foaming properties are very important in improving texture, consistency and appearance of food, such as baked and confectionery goods. HPH had higher values ( $p \leq 0.05$ ) for whipping properties (foam expansion and leakage) than CPI and HPI (Table 2).

Amino acid composition is important to nutritional and functional quality of protein. Amino acids also contribute to essential amino acid content of protein, which determines its nutritional value, or to total charge and disulfide bonds within subunit or between subunits to form polymer with flexible or rigid structure. Both HPI and HPH can be used as functional protein sources as they compose of essential amino acids in adequate quantities, with respect to the FAO/WHO (1990) amino acid patterns (Table 3).

Electrophoretic patterns of HPI and HPH are shown in Fig. 3. As shown in Fig. 3 HPH has a lower molecular weight compared to HPI. The molecular weight ranges between 66,200 Da to 14,400 Da. This result was expected because reduction in molecular weight of proteins occurs as a consequence of proteolysis. This perfectly agrees with previous findings that hydrolysis results in a decrease in the molecular weight of the hydrolysates (Parado *et al.*, 1993).

Biochemical scores for HPI and HPH are presented in Table 4. HPH recorded higher protein ( $\approx 96\%$ ) and lower carbohydrate ( $\approx 1\%$ ) values compared to HPI ( $p \leq 0.05$ ). This indicates that hydrolysis leads to protein concentration, although both HPI and HPH contains significant quantities of proteins that could be functionally employed in different food formulations.

**Conclusion:** An increase of health and nutrition awareness in modern society has meant that people choose more plant protein sources that are low in fat, cholesterol and sodium. While in other parts of the world where malnutrition is still rampant, food high in nutrient density especially affordable food protein is needed. The concerns encourage more research in improving quality of conventional protein sources and developing new unconventional ones. Peanut proteins are increasingly used by food industries to provide nutritional quality and a variety of functional properties. Hence the need to

extract and analyze the physico-functional and chemical properties of protein isolates and hydrolysates extracted from by-product, a potential waste, from peanut oil extraction. This study has shown that protein extracted from these potential waste products has potential nutritional and functional food properties. It could also be environmentally friendly as it aims at diverting a potentially waste product from being an agent of pollution to a functional food component. If successfully implemented it could contribute to controlling protein-energy-malnutrition that is prevalent in third world countries.

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