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Comparative Analysis of the Effects of Enzymatic Hydrolysis on the Physico-chemical and Functional Characteristics of Peanut Protein Isolates Extracted from Defatted Cold Pressed Peanut Meal Flour (*Arachishypogaea* L.)

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ABSTRACT--- ***Peanut protein was isolated from cold pressed peanut meal, hydrolyzed using neutral proteinase.Comparative analysis of the physico-chemical and functional properties of the isolates and hydrolysates were carried out. Scanning electron micrographs indicated that the hydrolysates had smaller particle sizes. Protein solubility was pH dependent with the hydrolysates exhibiting higher solubility at almost all pH levels. The isolates also demonstrated superior emulsifying and foaming characteristics. The extent of proteolysis on individual amino acids was dependent on their hydropathy characteristics. Arginine, lysine and glutamic acid with hydropathy indices of -4.5, -3.9 and -3.5 respectively, were found to have undergone effective proteolysis. The hydropathy characteristics, to some extent, influenced the rate of generation of free amino acids. As expected, the hydrolysates recorded lower molecular weights than the isolates. The thermal denaturation temperature for the hydrolysates was lower than the denaturation temperature for the isolate.***

Keywords--- Peanut meal cake, hydrolysates, isolates, hydropathy characteristics, molecular weight

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1. INTRODUCTION

Animal protein, our traditional and principal protein source, is becoming expensive and unaffordable in most developing countries. Besides, the emergence of fatal animal diseases has increased the need for alternative and functional food protein sources. Animal proteins, despite being expensive and gradually becoming scarce, can also lead to higher levels of cholesterol and fats in the blood, which are risk factors of cardiovascular diseases [1; 2; 3; 4; 5].Legumes are not only rich protein sources but also rich in other essential nutrients such as carbohydrates [6]. Most peanuts are used for oil, making peanuts the fifth largest source of vegetable oil in the world. Besides cooking, peanut oil has a wide range of uses which include leather dressing, furniture polishes, paints, varnishes, lubricants, insecticides, soaps and cosmetics and fuel [7].

Peanut residue left over from oil manufacture is referred to as oilcake meal. It is principally used asLivestock-feed as well as the production of artificial textile fiber. Research findings have shown that the oil-free meal obtained after oil extraction contains nearly 50%-60% proteins of good nutritional quality [8]. Considering the fact that Peanuts are nature's powerhouses, providing about 12% of the daily-recommended protein allowance per serve, the protein content of the oilcake meal can serve as a potential source of protein, specifically for the third world countries. 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 [9; 10; 11; 12; 13; 14; 15]. Protein hydrolysates have also been reported to have a wide range of functional applications varying from ingredients in the preparation of formulated food as source of nitrogen in the preparation of diets suitable for enteral products for hospitals, hypoallergenic infant formulae, dietetic food or sport drinks [16; 17;18; 19]. Further scientificinvestigations have shown that hydrolyzed protein foodstuffs may have advantagesover non-hydrolyzed protein foodstuffs in a number of areas of health care. Forexample, it has been reported that enzymatically hydrolyzed proteins are less allergenic, more rapidly digested and absorbed than whole

proteins – hence foodstuffs containing hydrolyzed proteins are usefully employed in the alimentation of hospitalpatients with digestive diseases [20; 21; 22]. Basically two methods are employed in peanut oil extraction – coldpressed and heat treated methods [23]. The meal/cake obtained from the former method isused as the principal material for protein extraction and characterization. In thepresent investigation, the effect of enzymatic modification of peanut protein isolatewith proteinase A.S.1.398 was carried out in order to obtain information on the effectof modification on the chemical, physical and functional characteristics. Thestructural changes of isolates due to enzymatic modifications were followed by size exclusion high performance liquid chromatography. The physico-chemical and functional differences between protein isolates and hydrolysates extracted from defatted cold pressed peanut meal cake and their applications in the food industry will be evaluated. The extraction,hydrolysis, physico-chemical and functional characterization of protein extractedfrom the meal cake is bound to serve as a cost-effective protein source which is critically needed in developing countries where protein-energy malnutrition isprevalent. It can also be considered as environmentally friendly since it aims atconverting a potential waste product into a useful food ingredient. Therefore, the principal objective of this study is to extract protein from defatted cold-pressed peanut meal and investigate the physico-chemical and functional characteristics of both hydrolyzed and unhydrolyzed forms of the extracted protein.

2. MATERIALS AND METHODS

Materials

Cold pressed peanut meal (CPM) and heat treated peanut meal (HPM) obtained as by-products from two peanut oil processing methods were obtained from Qingdao Kerry Peanut Oil Co., Ltd (Shandong province, China). Neutral proteinase A.S.1.398 (origin - Bacillus subtilis; cellulase activity $\geq 1 \times 10^5$μ/g) was obtained from Genecor (Wuxi) Bio-Products Co. Ltd., P. R. China. The molecular weight standards used for the determination of molecular weight distribution by Size Exclusion-High Performance Liquid Chromatography were obtained from the Shanghai branch of Sigma Company, PR China. All other chemicals and reagents used were of analytical grade and obtained from the chemical department of Jiang Nan University, Wuxi, P.R China.

Preparation of Defatted Peanut Meal

CPM was defatted with petroleum ether at 30 ~ 60^{o}C using the Soxhlet method for 8h. The Defatted sample was air dried, milled into fine powder using a NIRON blender, sieved to pass through a 60 mesh sieve, oven dried for 3h and referred to as defatted cold pressed peanut meal.(DCPM). The fat content was less than 1%. The chemical analysis of DCPM indicated the following composition: ≈52.1% crude protein (Kjeldahlx5.46), ≈7.1% moisture, ≈0.9% fat, ≈7.8% ash, ≈23.8% carbohydrates and≈9.9% crude fiber.

Isolation of Peanut Protein

Preparation of Peanut Protein Isolate (PPI) from DCPM

PPI was extracted from DCPM according to the procedure described by Kim et al (1990)[24] with some modifications. DCPM flour was dispersed in de-ionized 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 (Figure 1).

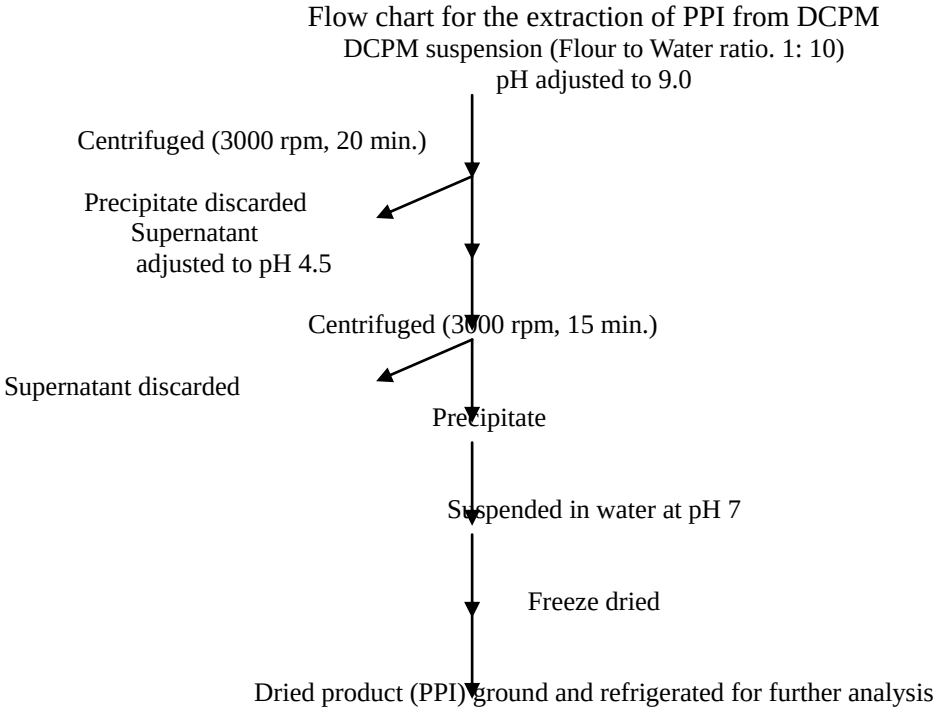


Figure 1: Flow chart showing the extraction of PPI from DCPM

Chemical Analysis of PPI

Moisture content was determined by placing approximately 2 g of sample into a pre-weighed aluminum dish. The samples were then dried in a forced-air convection oven at 105 °C until a constant weight was reached [25]. Ash content was estimated by charring in a crucible at 600 °C until the ash had a white appearance [25]. The total crude protein ($N \times 6.25$) content of the samples was determined using the Kjeldahl method [25]. The extraction and determination of total lipids from the samples was carried out by using the method described by Bligh and Dyer, (1959)[26].

Hydrolysis of Protein Isolates with Proteolytic Enzymes:

Hydrolysis of protein isolates was done according to the method of Achouri*et al.*(1998)[27]with slight modification. Prior to the addition of enzyme,10g PPI was dispersed in 250 ml water and heated for 30 min. and proteolysis was carried out using microbial neutral proteinase A.S.1.398 at 45°C and pH 7. The process was controlled by monitoring the degree of hydrolysis (DH) using the pH-start technique outlined by Adler-Nissen, (1986)[28]. 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 native/unhydrolysed peanut protein (PPI) and the hydrolysed peanut protein will hereafter be referred to as PP_N and PP_H, respectively.

Physico-functional parameters of PP_N and PP_H

Particle size determination and distribution

Scanning electron microscopic (SEM) studies of PP_N and PP_Hwere carried out using the Quanta-200 Scanning Electron Microscope.

Nitrogen Solubility of PP_N and PP_H vs. pH

The solubility of samples was evaluated by the nitrogen solubility index (NSI) according to the method described by Ponnapalam*et al*. (1987)[29]with minor modification as outlined by Guan *et al.* (2007)[30]. Samples (100µg each) were suspended in 15 ml distilled water and the pH of the system was adjusted to the desired values using either 0.1 M HCl or 0.1M NaOH. The suspensions were stirred magnetically for 30 min at room temperature and centrifuged at 4000*rpm* for 30min. The supernatants were analyzed for nitrogen contents and the percentage of soluble nitrogen was calculated at each pH value (2 – 10) using the following equation:

$$\text{NS (\%)} = \frac{\text{nitrogen content in supernatant}}{\text{total nitrogen content in sample}} \times 100;$$

where NS = nitrogen solubility

Foam Properties

Foam properties were determined by the method of Fernandez and Macarulla (1997)[31] with minor modifications. Portions of 40 ml sample solutions (20mg/ml) of varying pH (2.5, 4.5, 7.5, and 10.0) were mixed thoroughly using a homogenizer (IKA EUROSTAR basic, IKA, WERKE) at 10,000 rpm for 3 min. Foaming ability (FA) was calculated as the percent increase in volume of the protein dispersion upon mixing. Foam stability (FS) was estimated as the percentage of foam remaining after 15, 30, and 60 min.

Emulsifying activity (EA) and stability (ES)

Emulsifying activity and stability were determined using the method of Neto*et al*. (2001)[32]. Five milliliter portions of PP_N and PP_H solutions were homogenized with 5 ml Soya Bean Oil (Gold Ingots Brand, QS310002012787, Suzhou, P.R.China). The emulsions were centrifuged (NSKC-1, Nanjing, PR China) at 1100 rpm for 5 min. The height of emulsified layer and that of the total contents in the tube were measured. EA was calculated as: EA (%) = $\frac{\text{height of emulsified layer in tube}}{\text{height of total content in tube}} \times 100$. ES was determined by heating each of the emulsions above at 40°C and 80°C before centrifuging at 1100 rpm for 5 min. ES was calculated as follows:

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

Differential scanning calorimetry (DSC)

The thermal characteristics of protein samples (PP_N and PP_H) were determined with a Perkin-Elmer differential scanning calorimeter (DSC) using a modified form of the method described by Meng and Ma (2001)[33]. Lyophilized samples (1mg each) were directly weighed into coated aluminum pans and 10µl of water was added. The aluminum pans were hermetically sealed and heated from 30 to 120 °C at a rate of 10 °C/min. A sealed empty pan was used as a reference. Thermal denaturation temperature (T_d) and denaturation entalphy (ΔH) were calculated from the thermogram. All experiments were conducted in duplicate or triplicate

Chemico-functional parameters of PP_N and PP_H

Bio-chemical scores

Nitrogen contents of both PP_N and PP_H were determined by the micro-Kjeldahl method [25]. A factor of 5.46 was used to convert percent nitrogen to protein content. Carbohydrate contents were determined by phenol-sulfuric acid method [34].

Determination of Amino Acids and Free Amino Acids

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

Molecular weight distribution of PP_N and PP_H

Molecular weight distributions of PP_N and PP_H were determined by gel permeation chromatography (GPC) using a Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC) system (waters 600, USA). A TSK gel2000 SW_{XL} column (7.8 i.d. × 300mm, Tosoh, Tokyo, Japan) was equilibrated with 45% acetonitrile (v/v) in the presence of 0.1% trifluoroacetic acid. The hydrolysates (100µg/µL) were applied to the column and eluted at a flow rate of 0.5mL/min and monitored at 220nm at room temperature. A molecular weight calibration curve was prepared from the average retention time of the following standards (obtained from Sigma Co., St Louis, MO, USA): cytochrome C (12500 Da), aprotinin (6500 Da), bacitracin (1450 Da), and tripeptide GGG.

Mineral Analysis

Samples were digested in100 ml micro-Kjeldahl flask with $HNO_3/HClO_4$ until the solution became colourless. The sample was cooled and diluted in a 25 ml volumetric flask with 0.1 M HCl. Sodium, potassium, calcium, magnesium, iron, zinc, manganese and copper were measured by atomic absorption Spectrophotometry[35] using a Varian spectra atomic absorption spectrophotometer (Varian SpectrAA220, Varian, Palo Alto, CA). Phosphorus contents of PP_N and PP_Hwere estimated by the procedure of Taussky&Shorr (1953) [36]

3. RESULTS AND DISCUSSION

Physico-functional properties

Particle size distribution and determination of *PP_N and PP_H*

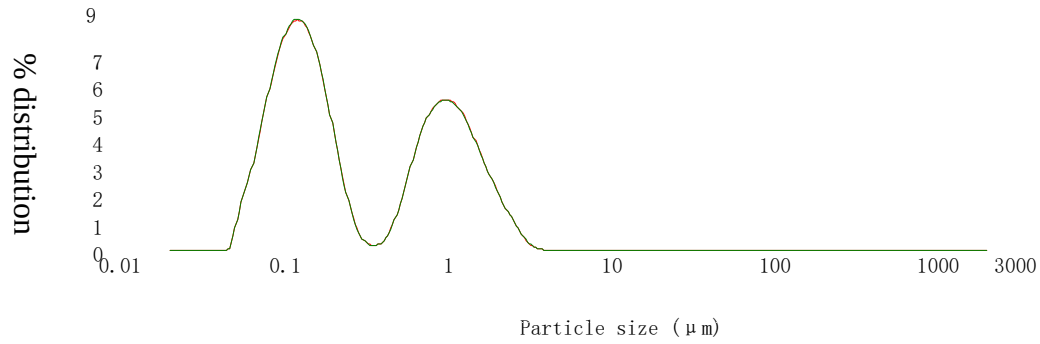


Figure 2: Particle size distribution of PP_H

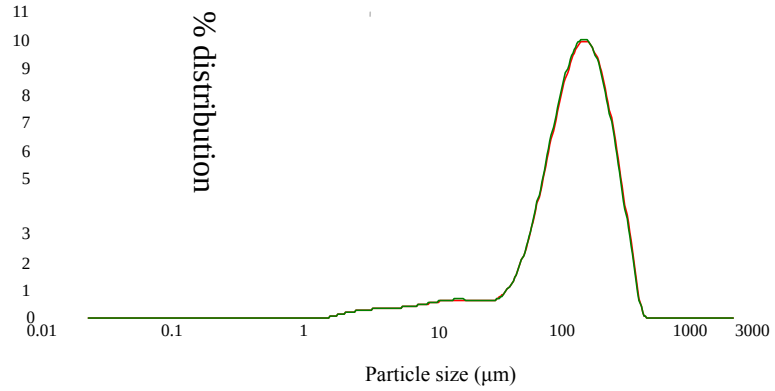
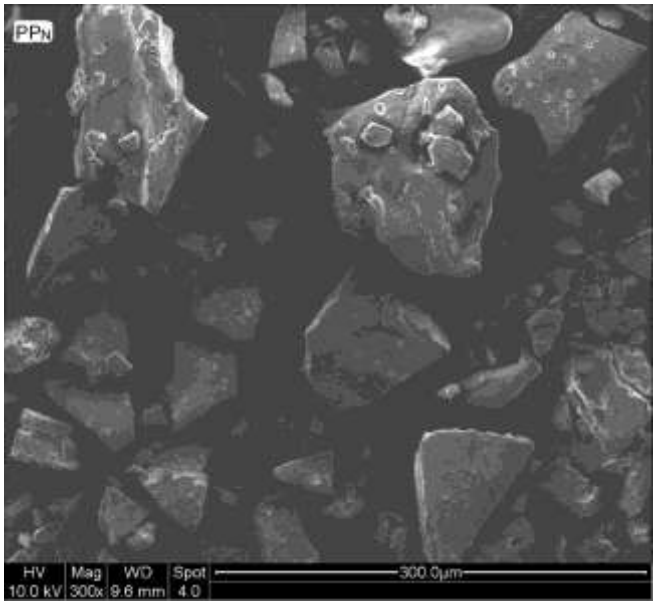


Figure 3: Particle size distribution of PP_N

Particle size determination and distribution of *PP_N and PP_H*

The particle size distribution of PP_N and PP_H as determined by Mastersizer 2000 are presented in Figures 2 and 3, respectively. Results, as shown in Figures 2 and 3, indicate that hydrolysis can alter the particle size distribution of proteins. In PP_N a greater percentage of the particle sizes are within the 100μm range whereas in the hydrolysates greater percentage of the particle sizes are distributed at 0.1μm and 1.0μm. This indicatesthat hydrolysis results in the degradation of proteins into small fragments. This is in agreement with the findings reported by Radha, et al, (2008)[37].The scanning electron micrographs shown in Figures 4a and 4b are in agreement with the particle size distributions shown in Figures 2 and 3. This shows that hydrolysis decreases protein aggregates, thereby inducing the difference in particle size between PP_H and PP_N

(a)



(b)

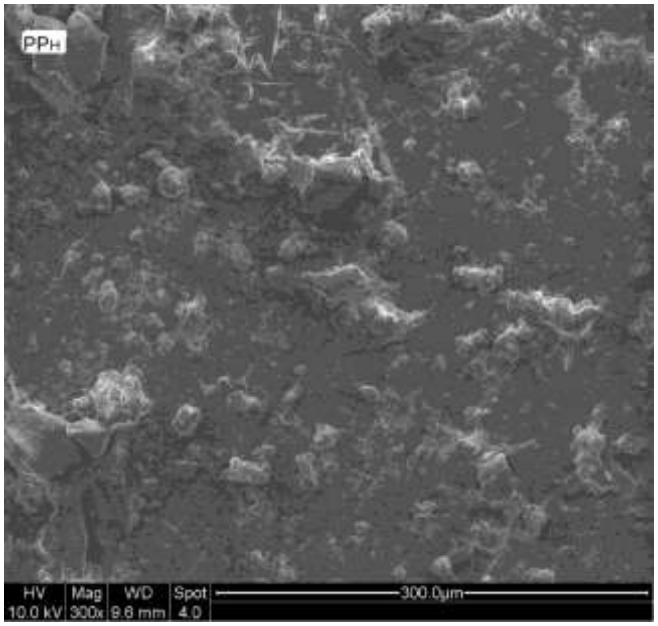


Figure 4: Scanning electron micrographs of (a) PP_H and (b) PP_N (HV = 10.0 KV; mag = 300x; WD =9.6mm)

Protein solubility

The solubility of protein hydrolysates is of immense importance in liquid protein supplements, in which the appearance of insoluble sediment is undesirable. Hence, an important characteristic of protein isolates used for some food applications is solubility at an appropriate pH to the food orbeverage. The influence of enzymatic hydrolysis on solubility characteristics of PP_N is shown in Figure 5. Results indicate that hydrolyzed proteins (PP_H) are dissolved more easily than unhydrolyzed proteins (PP_N).The solubility profiles shown in Figure 5 are pH dependent with minimum solubility in both samples observed between pH 4.5 and pH 5.5 which is expected as it falls within the precipitation pH range. Similar observations have previously been reported by Chabanon*et al*. (2007)[38]. Both PP_N and PP_H recorded highest solubility at pH levels of 10 and above. Enzymatic hydrolysis of peanut protein with DH=8 led to a marked increase in solubility at

all pH values. It is also comparatively more soluble than the isolate at the isoelectric point. Particle size differences may have also contributed to the differences in the solubility index of PP_N and PP_H 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 1μm, which is essentially thecase with PP_H

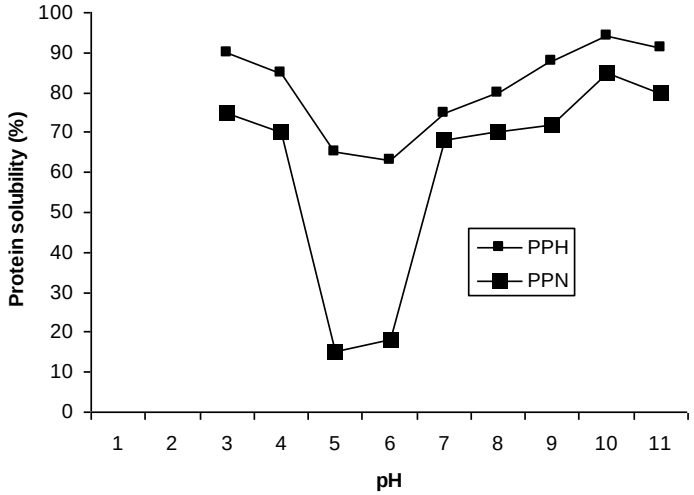


Figure 5 Nitrogen solubility profiles of PP_N and PP_H in de-ionized water as a function of pH (ranging from 2.0 to 10.0). DH of PP_H = 8.0

Emulsifying and foaming properties of PP_N and PP_H

Proteins are often employed as surfactants in emulsion-type processed foods. Protein hydrolysis is bound to increase the number of polar groups and hydrophilicity, decrease molecular weight, alter globular structure of proteins, and expose previously buried hydrophobic regions. These changes consequently affect emulsifying capacity and stability of proteins. This is clearly shown in Table 1 as both EC and ES of PP_H are higher than those of PP_N. Similar findings have been reported by Mahajan &Dua (1998)[39].Foam capacity, measured in terms of percent increase in foam volume, is pH dependent for both PP_N and PP_H.

Table 1: Emulsion capacity (EC) and stability (ES), foam capacity (FC) and stability (FS) of PP_N and PP_H.

Sample	EC (ml/g)	ES (%)		Foam capacity (% volume increase pH levels				Mean foam stability (%)		
		40℃	80℃	2.5	4.5	7.5	10	15 min	30 min	60 min
PP_N	101.3±0.9	**34**±0.1	**30**±0.4	**52**±0.6	**22**±1.1	**70**±0.2	**80**±0.5	12±0.11	5±0.14	4±0.16
PP_H	105.6±2.0	**48**±0.3	**46**±0.7	**50**±0.5	**29**±0.1	**77**±0.4	**88**±1.3	14±0.16	7±0.22	6±0.08

Data are the means ±SD, n = 3.

High foam volumes are obtained at pH 2 and at pH levels above 7, whereas low foam volumes are recorded near the isoelectric pH. This shows that the degree of protein solubility is related to its foam capacity. The high foaming capacity at pH 2 and 10 may be attributed to an increase in the net charge of the protein/peptide molecules, which is bound to weaken hydrophobic interactions and increase protein flexibility. This consequently allows them to spread to the air water interface more quickly thus encapsulating air particles and increasing foam formation. This agrees with the findings of Lawal*et al* (2007)[40]. However, at pH 2,PP_N records higher foam capacity than PP_H, although at all other pH levels PP_H exhibits higher foam capacities than PP_N. Findings also reveals that at all experimental levels/conditions PP_H exhibits more foam stability than PP_N. This result can be attributed to the high degree of solubility of PP_H thus leading to enhanced whipping ability and the formation of stable cohesive films around the air vacuoles. On the whole, PP_H demonstrates superior functional properties than PP_N. Similar observations have been reported by Lawal et al (2007)[40], Chabanon et al (2007)[38] and Lawal (2004)[41].

Protein denaturation profile with differential scanning calorimetry (DSC)

The protein denaturation profiles for both PP_H and PP_N are shown in Table 2. Denaturation temperatures are normally referred to as measures of the thermal stability of proteins. However, these denaturation temperatures are influenced by

heating rate and protein concentration. It could be seen from Table 2 that PP_H demonstrated an early onset temperature compared to PP_N. This indicates that PP_H started undergoing denaturation earlier than PP_N.

Table 2 Protein denaturation profile with differential scanning calorimetry (DSC)

Samples	Phase transition parameters [a]			
	T_0 (°C)	T_P (°C)	T_C (°C)	ΔH (J/g)
PP_N	72.71±0.13 ^{Ab}	78.42±0.10^{A}	81.76±0.12^{A}	0.26±0.03^{A}
PP_H	61.07±0.12^{B}	71.84±0.07^{B}	85.09±0.06^{B}	4.10±0.13^{B}

[a]Onset temperature (To), transition temperature peak (Tp), conclusion temperature (Tc);
[b]Samples means with different superscript letters in the same column are significantly different at $P \leq 0.05$.

The ΔH value calculated from the area under the transition peak is more favorable for PP_N compared to PP_H as the former recorded significantly ($P \leq 0.05$) lower ΔH value (0.26±0.03 J/g) than the latter. This experiment was conducted in triplicate and the results followed the same trend. The reason why PP_N appears to be thermodynamically more favorable than PP_H needs further studies. 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 why PP_H recorded higher ΔH values than PP_N.

Chemico-functional Properties

Table 3: Bio-chemical Scores of PP_N and PP_H

Samples	Protein (N×5.46)	Ash	Moisture	Fat	Carbohydrate
PP_N	88.00 ± 0.07a	3.02 ± 0.32a	5.05 ± 0.02a	0.31 ± 0.11a	4.11 ± 0.01
PP_H	96.16 ± 0.05b	0.60 ± 0.31b	2.32 ± 0.03b	0.26 ± 0.12a	1.41 ± 0.06

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

PP_N and PP_H contain approximately 88% and 96% protein, respectively. Lower contents of ash, moisture, and carbohydrate are found in PP_H compared to PP_N although the fat contents of both samples are not significantly different (Table 3). To achieve high extraction and hydrolytic efficiency optimum protein extraction and hydrolysis parameters are used. The results obtained from the various protein extraction and hydrolysis parameters (such as protein: water ratio, pH, temperature, extraction time, degree of hydrolysis, enzyme concentration, etc.) are not presented in this paper. However, optimum extraction conditions are indicated in Figure 1.

Composition of amino acids and free amino acids

Table 4: Amino acid profiles forPP_N and PP_H and free amino acid profile for PP_H

Indispensable amino acids	Hydropathy index[1]	PP_N (g/100gsample)	FAO/WHO (1990) [2] Requirement Pattern		PP_H	
			Child	Adult	g/100g sample	FAA[3] (mg/g)
Isoleucine	4.5	2.24	2.8	1.3	2.49	0.057
Leucine	3.8	6.02	6.6	1.9	5.30	0.025
Valine	4.2	3.11	3.5	1.3	3.28	N[4]
Histidine	-3.2	1.62	1.9	1.6	1.92	N[4]
Lysine	-3.9	1.61	5.8	1.6	2.47	0.071
Methionine	1.9	1.14	2.5	1.7	0.53	0.078
Phenylalanine	2.8	2.06	6.3	1.9	4.14	0.629
Threonine	-0.7	1.96	3.4	0.9	1.81	0.020
Tryptophan	-0.9	N	1.1	0.5	N	N
Conditionally dispensable amino acids						
Arginine	-4.5	1.61			10.66	0.241
Cysteine	2.5	0.61			N	N
Tyrosine	-3.1	0.96			3.18	0.450
Dispensable amino acids						
Alanine	1.8	7.61			3.03	0.061
Aspartic acid	-3.5	4.02			9.96	0.020
Glutamic acid	-3.5	10.10			18.89	0.330
Glutamine	-3.5	N			N	N
Glycine	-0.4	3.70			3.29	0.210
Proline	-1.6	8.96			2.55	0.028
Serine	-0.8	2.98			3.82	0.010
Total		60.33			77.61	2.240

[1]Kyte & Doolittle (1982) [42]; [2]FAO/WHO (1990) amino acid requirement patterns as quoted by Zhu et al (2006) [43][3]Free Amino Acids (FAA) composition of PP_H; [4] = Negligible quantity; N = not detected

From Table 4 it could be seen that the quantity of dispensable, polar and glucogenic amino acids, glycine (3.70g/100g sample) and alanine (7.61 g/100g sample), in the native protein are more than the quantities found in the hydrolysates. This loss in quantity during hydrolysis may have been due to their non-polar nature. On the other hand, the quantity of the conditionally dispensable, polar and glucogenic amino acid, arginine (10.66g/100g sample), in the hydrolysates shows an increase of about ten folds compared to the quantity recorded for the native protein. This excessive increase may have occurred due to the polar nature of arginine. Also the free amino acids (FAA) generated from arginine during hydrolysis is about 11% of the total FAA. Enzymatic hydrolysis of proteins produces FAA and small peptides [44].The quantity of lysine, an indispensable, polar and ketogenic amino acid, in the hydrolysates is comparatively higher than the quantity found in the native protein. Interestingly, phenylalanine, a non-polar ketogenic amino acid is found in a relatively higher quantity in the hydrolysates than the native protein and generated about 28% of the total free amino acids. This is contrary to leucine, an indispensable non-polar ketogenic amino acid, which is found in higher quantities in the native protein compared to the hydrolysates. Tyrosine, a conditionally dispensable, polar and ketogenic amino acid increases after hydrolysis and generates about 21% of the total free amino acids. Glutamic acid, a polar amino acid, is the highest quantity of amino acids in PP_N and PP_H accounting for about 17 and 24% of total amino acids in PP_N and PP_H, respectively. The general trend observed is that the most hydrophilic amino acids arginine, lysine and glutamic acid with hydropathy indices of -4.5, -3.9 and -3.5 respectively, are found to have increased in quantity during hydrolysis. This is in agreement with findings reported by Kyte& Doolittle (1982)[42].

Molecular weight distribution of PP_N and PP_H

Table 5: Molecular weights of PP_N and PP_H

Peak No.	Molecular weight distribution (Da)	
	PP_N	PP_H
1	16038	12907
2	8137	2386
3	4251	395
4	2950	324
5	2219	209
6	1253	124
7	844	93

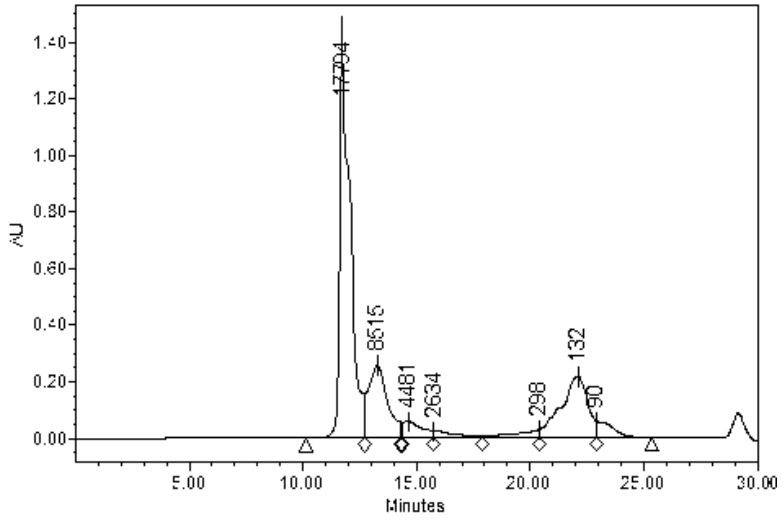


Figure 6: Molecular weight distribution of PP_N

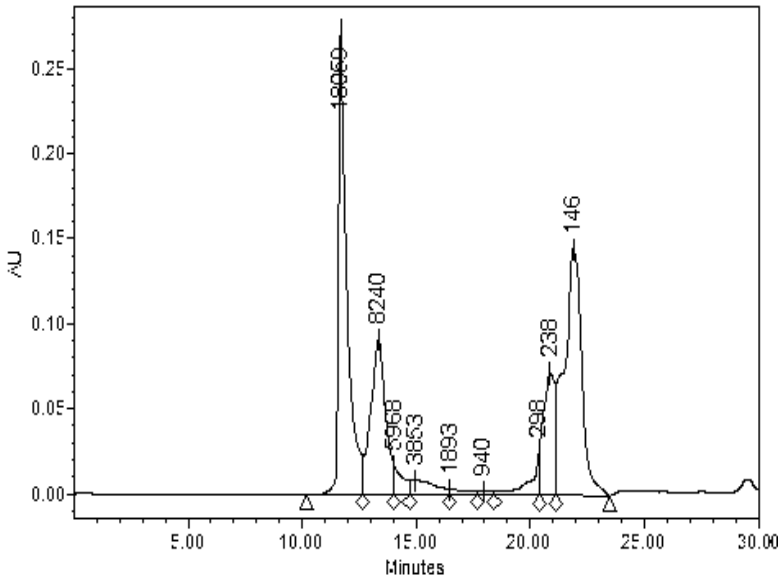


Figure 7: Molecular weight distribution of PP_H

The molecular weight distributions of PP_N and PP_Hwere determined by SE-HPLC
and are shown in Figures 6 and 7 and Table 5. The molecular weights for all samples were calculated according to the standard equation below:
Log MolWt = 6.65e+000 - 2.05e-001 T^1 (R^2 = 0.9986)
Results show that PP_N had a higher molecular weight distribution than PP_H .The molecular weight distributions of the first three peaks for PP_N are 16.04, 8.14, and 4.25 KDa, while those of PP_H are 12.91, 2.39, and 0.40 KDa, respectively. This result was expected because a reduction in molecular weights 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 [45].

Mineral composition

Table 6: Mineral composition of PP_N and PP_H (µg/g; P = %)

Mineral	PP_N	PP_H
P	**0.24**	**0.15**
Fe	**26.2**	**17.5**
Mn	**5.33**	**3.10**
Cu	**5.86**	**15.9**
Zn	**5.73**	**3.04**
K	**489**	**307**
Na	**1015**	**7097**
Mg	**52.9**	**29.6**
Ca	**79.4**	**155**

It could be seen from Table 6 that hydrolysis of the native protein results to a decrease in some minerals such as iron, manganese, zinc, potassium and magnesium. However, the contents of some other minerals in PP_H are higher than PP_N. Minerals such as copper, sodium and calcium seem to have increased in PP_H compared to PP_N.

4. CONCLUSION

This paper looks at the comparative analysis of the physical, chemical and functional attributes of protein isolates and hydrolysates extracted from cold pressed peanut meal cake – a by-product obtained from the extraction of peanut oil using the cold pressed method. The bio-chemical scores obtained from both isolates and hydrolysates show that they can be employed as functional food ingredients, specifically in third world countries. Protein hydrolysates, obtained from such waste products, could be incorporated in the preparation of diets suitable for enteral products for hospitals, hypoallergenic infant formulae, dietetic food or sport drinks. The hydrolyzed proteins (PP_H) exhibit better functional properties (solubility, emulsification and foaming) than the Isolates (PP_N). With respect to the hydrolysis process, the extent of protein degradation is dependent on the hydropathy characteristics of the individual amino acids. Some amino acid contents meet the FAO/WHO requirement patterns for children/adults. This further underscores the need for incorporating hydrolyzed proteins in the preparation of functional foods. Depending on the protein levels, most nutritionists/food scientists believe that diets deficient in amino acids have to be supplemented with free amino acids to meet the amino acid requirements for individuals. This piece of work therefore, serves as a cheap and affordable source of proteins to combat the protein-energy malnutrition that is prevalent in third world countries.

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