

Analysis of functional properties of peanut protein isolates as affected by oil extraction methods

TAMBA S. SONDA AND SANPHA KALLON

The physico-chemical and functional characteristics of peanut protein isolates extracted from peanut cakes obtained as by-products from two different peanut oil extraction methods (cold pressed and heat pressed) were studied. Isolates from cold pressed method (CPI) exhibited superior physical properties such as particle size (as seen from scanning electron micrographs), and thermal denaturation profiles as obtained from differential scanning calorimeter, compared to isolates obtained from heat pressed method (HPI). Solubility profiles obtained for CPI and HPI were pH dependent with CPI been more soluble at all pH levels. Solubility around the isoelectric pH range of 4.5 – 5.5 was low for both samples. Other functional properties such as emulsification, whipping, fat and water absorption were better exhibited by CPI than HPI. From the SE-HPLC, HPI recorded a higher molecular weight (16.64KDa) than CPI (14.91KDa), while CPI had higher biochemical components (protein, carbohydrates) than HPI.

Key Words : Peanut protein isolate, Physical, Functional, Chemical properties

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INTRODUCTION

Both developed and developing nations are today faced with continuous threats posed by the emergence of contagious animal diseases that have had and may continue to have serious health consequences on the world population. Considering the fact that animals are our traditional protein sources and that they are becoming more and more expensive, it is important for scientists to intensify research into alternative and affordable protein sources. Peanut is a rich and underutilized protein source that is cultivated worldwide. However, most

peanuts grown are principally used for oil production, confectionaries and peanut butter (Tate *et al.*, 1990 and Lawal *et al.*, 2007). According to Basha and Pancholy (1982) the extraction of vegetable oil from peanut yields partially defatted peanut flour (PDPF) which is essentially a protein-rich, inexpensive and underutilized by-product of the peanut industry. PDPF comprises 47-55 per cent of high quality protein with high essential amino acid content which could be usefully employed in a variety of food applications (Prinyawiwatkul *et al.*, 1993).

The direct utilization of plant proteins in human foods is limited because these proteins lack certain requisite functional behaviour in fabricated foods. Therefore, for enhanced utilization of plant proteins in fabricated foods, these proteins must possess important functional properties such as solubility, foaming, emulsifying and gelling properties. These properties are affected by the intrinsic factors of protein such as

MEMBERS OF RESEARCH FORUM

Author for correspondence :

TAMBA S. SONDA, Institute of Food Technology, Nutrition and Consumers Studies, Schools of Agriculture, Njala University, SIERRA LEONE (WEST AFRICA)

Email : tssonda@njala.edu.sl/sondat@hotmail.com

Associate Authors' :

SANPHA KALLON, Department of Animal Science, School of Agriculture, Njala University, SIERRA LEONE (WEST AFRICA)

Email : Kallonsanpha@yahoo.com

molecular structure and size, and many extrinsic factors including the method of protein separation/production, pH, ionic strength, and the presence of other components in the food system (Moure *et al.*, 2006 and Yu *et al.*, 2007).

The extraction and characterisation of peanut protein/peanut protein isolate from peanut cake obtained from various oil extraction methods is bound to serve as a potential and affordable protein source. Proteins are being used as ingredients in man-made food products because they contribute to one or more of the desired characteristics of food products. These characteristics might be consumer related, such as texture, mouth feel, appearance or taste, as well as technology related. The latter includes both storage (shelf life and palatability) and processing, such as mixing behaviour, foam, emulsion or gel formation. Proteins contribute to one or more of these characteristics because of their functional properties; physico-chemical properties that govern the performance and behaviour of a protein in food systems during preparation, processing, storage and consumption.

Functional properties of peanut protein have been the subject of limited studies that focused mainly on peanut flour (Prinyawiwatkul *et al.*, 1993) and limited information is available in the literature on the development and functionality of peanut protein isolate (PPI) as affected by oil extraction method. Therefore, the objectives of this study were to develop a protein isolate from defatted peanut meal flour obtained from two different oil extraction methods and study the functional properties of the peanut protein isolates as indicators of their potential use by the food industry; and evaluate the effects of oil extraction methods on the functionality of peanut protein isolate.

METHODOLOGY

Cold pressed peanut meal cake and heat treated peanut meal cake were purchased from Qingdao Kerry Peanut Oil Co., Ltd. (Shandong province-China). Glucose and molecular weight standards were obtained from the Shanghai Branch of Sigma Co., Shanghai, PR China. All other chemicals used, were of reagent grade and obtained from the chemical department of Jiang Nan University, Wuxi, P. R. China.

Extraction of peanut protein isolate :

Cold pressed and heat pressed defatted peanut meal

flours were extracted in 10 per cent (W:V) suspension of water at pH 9 for 1h and used as starting materials to develop peanut protein isolates using isoelectric precipitation and centrifugation separation methods.

Protein recovery tests were conducted at different water/flour ratio to determine the conditions for optimum protein recovery. Flours obtained from both cold pressed and heated treated peanut cakes were mixed with water at varying flour to water ratios of 1/3, 1/7, 1/10, 1/12 and 1/15. The pH of each suspension was adjusted to pH 9, based on the solubility profile of protein in peanut flour, using 1.0 N NaOH and 1.0 N HCl, and stirred for 1 h at room temperature. Suspensions were centrifuged and protein concentration in each supernatant was determined by Kjadhil Nitrogen Analyzer (AOAC) using 6.25 as conversion factor.

The optimum peanut protein recovery was achieved at flour/water ratio of 1/10 and a solubilization pH of 9. These conditions were used in subsequent production of peanut protein isolates (PPI). To produce PPI for experimental analysis, defatted peanut flour (cold pressed and heat pressed) were mixed with water in the ratio of 1/10 (w/v), and pH of the mixture was adjusted to 9.0 with 1.0 N NaOH. The peanut flour suspension was let to stir at room temperature for 1 h, centrifuged at 3000 g for 15 min. The supernatant was collected and adjusted to pH 4.5 with 1.0 N HCl. The precipitates obtained were freeze dried, grind in to powder and stored in refrigerator until use for experimental analysis. The PPI obtained from the cold pressed and heat treated methods are referred to in this work as CPI and HPI, respectively.

Determination of physical, functional and chemical properties of CPI and HPI :

Particle size determination and distribution :

Particle size determination of CPI and HPI was carried out by using laser light scattering and Quanta-200 for scanning electron microscope.

Determination of thermal characteristics of CPI and HPI :

Thermal characteristics of protein samples (CPI and HPI) 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 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 enthalpy (ΔH) were calculated from thermograms. All experiments were conducted in triplicate

Protein solubility:

The solubility of both CPI and HPI were estimated at varying pH levels by using a modified form of the method described by Wu *et al.* (1998). Peanut protein isolates were mixed with water in the ratio of 0.75g/15ml (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 peanut protein isolates was let to stir at room temperature for 1 h, and then centrifuged at 3000 rpm for 15 min. Protein concentration in the original sample and in the supernatant (soluble protein) were determined by Kjadhah (AOAC method) using 6.25 as conversion factor. Protein solubility was then calculated using the following equation:

$$\text{Solubility (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100 \quad (\text{Were } et \text{ al., 1997}).$$

Water absorption properties (WAP) and fat absorption properties (FAP) :

Water absorption properties (WAP) were determined using the method outlined by Tang (2007), with some modifications. One g 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 properties (g of water per g of flour) were calculated as

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

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.

Oil absorption property 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 vegetable oil (soybean oil) using a Vortex mixer. Samples were allowed to stand for 30 min. The flour–oil mixture was centrifuged at 3000rpm for 20 min. Immediately after centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder, and the volume was recorded (V_2). Fat absorption property (milliliter of oil per g of flour) was calculated as

$$\text{FAP} = \frac{V_1 - V_2}{W_0} . \text{ Triplicate samples were analyzed for each sample.}$$

Emulsifying activity index (EAI) and emulsion stability index (ESI) :

Emulsifying activity and stability indices were determined using the method of Neto *et al.* (2001). Five milliliter portions of CPI and HPI solutions were homogenized with 5 ml Oil (pure Maize oil produced by East Ocean oil Grains Industires, Zhang Jiagang, Jiangsu Province, 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. EAI was calculated as:

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

ESI was determined by heating each of the emulsions above at 55°C before centrifuging at 1100 rpm for 5 min. ESI was calculated as follows:

$$\text{ESI (\%)} = \frac{\text{Final height of emulsified layer after heating}}{\text{Initial height of emulsified layer before heating}} \times 100$$

Whipping properties:

Whipping properties of 3 per cent dispersions of peanut protein isolates (HPI and CPI) and commercial soy protein isolate were determined in triplicate using a modified form of the method described by Lin *et al.* (1974). Samples (10g) were dispersed in distilled water (250ml) and pH adjusted to 7.0 using 0.1M NaOH and 0.1 M HCl. The suspensions were thereafter homogenized for 1min 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 the foam height was measured at intervals. The per cent foam expansion was calculated as follows per cent vol.

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

where A = vol. after whipping; B = vol. before whipping.

Foam volume as percentage was calculated taking the foam volume at zero time as 100 per cent. Leakage was calculated as follows:

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

where C = Vol. of liquid collected; D = vol. of liquid before whipping

Molecular weight distribution of HPI and CPI :

Molecular weight distributions of HPI and CPI 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 per cent acetonitrile (v/v) in the presence of 0.1 per cent trifluoroacetic acid. The hydrolysates (100µg/µl) were applied to the column and eluted at a flow rate of 0.5ml/min and monitored at 220 nm at room temperature. A molecular weight calibration curve was prepared from the average retention time of the following standards:

cytochrome C (12500 Da), aprotinin (6500 Da), bacitracin (1450 Da), and tripeptide GGG.

Proximate composition analysis:

Nitrogen contents of both HPI and CPI were determined by the micro-Kjeldahl method (AOAC, 1995). A factor of 5.46 was used to convert the 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).

Data analysis:

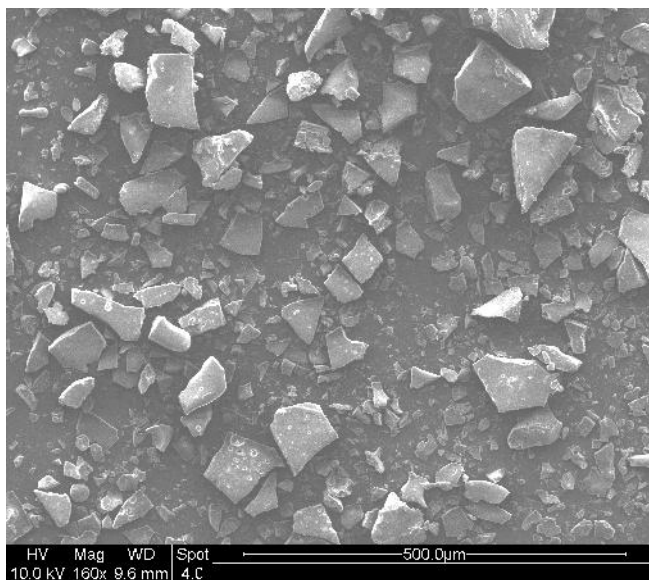
Data were analyzed using PASW Statistics, Version 18 (formerly called SPSS).

OBSERVATIONS AND ASSESSMENT

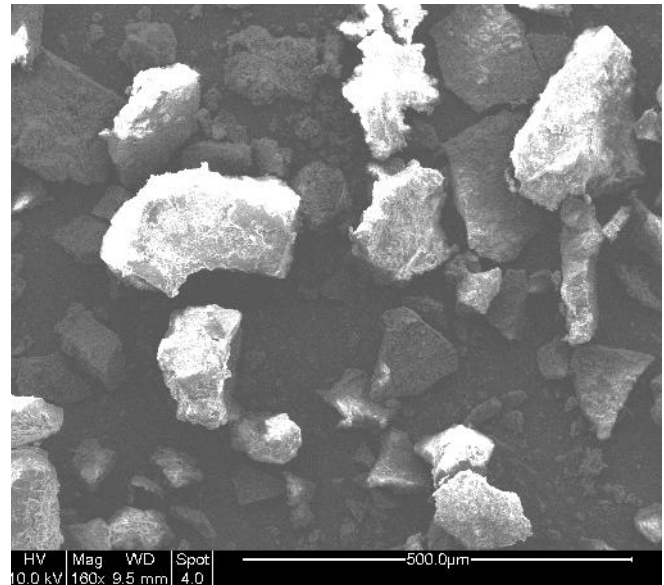
The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Particle size determination of CPI and HPI :

Scanning Electron Microscopy was used to examine the micro structural changes of protein isolates as affected by oil extraction methods. The scanning electron micrographs for CPI and HPI are shown in Fig. 1. The micrographs show that oil extraction method contributed



CPI



HPI

Fig. 1 : Scanning electron micrographs of CPI and HPI; HV = 10.0KV; Mag = 160x; WD = 9.6 mm

to differences in particle sizes of CPI and HPI. Isolates obtained from heat treated method had relatively larger particle sizes compared to those obtained from the cold-pressed method. This difference may have occurred due to protein denaturation and/or the formation of a protein-polysaccharide conjugate during heat treatment. This is in agreement with the findings reported by Radha *et al.* (2007)

Protein denaturation profile with differential scanning calorimetry (DSC) :

Protein denaturation profiles for both CPI and HPI are shown in Table 1. 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 1 that HPI demonstrated an early onset temperature compared to CPI. This indicates that HPI started undergoing denaturation earlier than CPI. The ΔH value calculated from the area under the transition peak is more favorable for CPI than CPH as the former recorded significantly ($P \leq 0.05$) lower ΔH value (0.36 ± 0.02 J/g) than the latter. This experiment was conducted in triplicate and the results followed the same trend. The difference in ΔH value may have been influenced by the different oil extraction methods. 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 the differences in ΔH values.

Protein solubility as influenced by pH :

Protein solubility serves as an important parameter in determining the functionality of proteins as it has a remarkable influence on other functional properties. In general, protein solubility is pH dependent because at both high and low pH levels solubility improves as the

protein is deprotonated and protonated, respectively. However, protein tends to aggregate at isoelectric point (PI) thereby reducing its solubility. The pH dependent solubility study was carried out on both CPI and HPI and the results are presented in Fig. 2. At all pH levels CPI was slightly more soluble than HPI. This is in agreement with the findings of Cherry and McWatters (1975) who reported that heating full fat peanut seed in water at 100–120° C for 15 min decreased protein solubility. This decrease can be attributed to the effect of heating which resulted in an increase in surface hydrophobicity of protein due to unfolding of molecules upon the application of heat and molecular size through hydrophobic interactions and disulfide formation. Minimum solubilities in both samples were observed between pH 4.5 and pH 5.5 which is expected as it falls within the precipitation pH range. Similar findings have been reported by Khalid *et al.* (2003); Ragab *et al.* (2004) and Mashair *et al.* (2006).

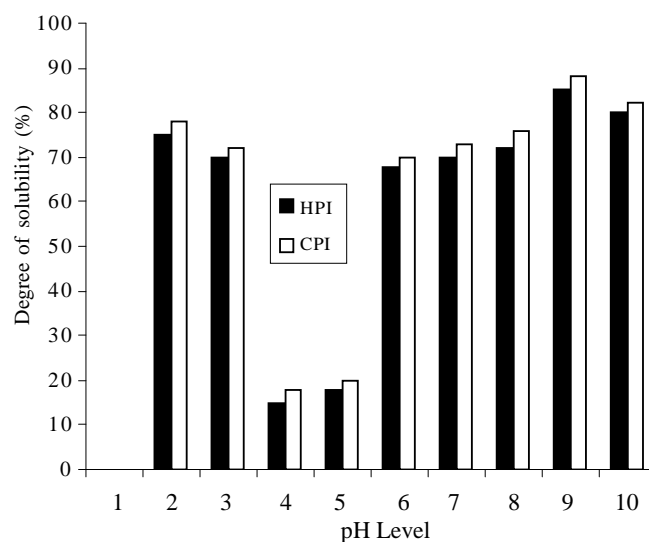


Fig. 2 : Protein solubility of CPI and HPI at different pH levels

Emulsifying properties:

Foam expansion (FE), Foam volume (FV), leakage, fat absorption capacity (FAP), water absorption capacity

Table 1: Protein denaturation profile with differential scanning calorimetry (DSC)

Samples	Phase transition parameters ^a			
	T ₀ (°C)	T _p (°C)	T _c (°C)	H (J/g)
CPI	72.71±0.13 ^A	78.42±0.10 ^A	81.76±0.12 ^A	0.36±0.02 ^A
HPI	61.00±0.07 ^B	71.65±0.13 ^B	82.91±0.15 ^B	4.15±0.11 ^B

^a Onset temperature (To), transition temperature peak (Tp), conclusion temperature (Tc);

Data are the means ±SD, n = 3. Samples means with different superscript letters in the same column are significantly different at $P \leq 0.05$.

(WAP), emulsion activity index (EAI) and emulsion stability index (ESI) of CPI, HPI and SPI. Data are the means \pm SD, n = 3. Samples means with different superscript letters in the same column are significantly different at $P \leq 0.05$.

Food emulsions are thermodynamically unstable mixtures of immiscible liquids (water and oil). The formation and stability of emulsion is very important in food systems such as salad dressing. Proteins are composed of charged amino acids, non-charged polar amino acids and non-polar amino acids, which make protein a suitable emulsifier, the surfactant possessing both hydrophilic and hydrophobic properties and can interact with both water and oil in food systems. CPI demonstrated better emulsifying properties (both EAI and ESI) than HPI (Table 2). The emulsifying properties of HPI and the commercial soy protein isolate were not significantly different ($P \leq 0.05$). Emulsifying properties of CPI and HPI reflected their solubility patterns. This is in agreement with the findings reported earlier by

Parakash and Narasimha Rao (1986) and Khalid *et al.* (2003).

The formation of foam is analogous to the formation of emulsion. In the case of foam, water molecules surround air droplets, and air is the non-polar phase. Theoretically, the amphipathic character of protein makes them good foaming agents that work at air-water interface to prevent bubble coalescence. CPI demonstrated better whipping properties than HPI (Table 2). However, in terms of foam leakage no significant difference ($P \leq 0.05$) was observed between CPI and HPI.

Interactions of water and oil with proteins are very important in food systems because of their effects on the flavour and texture of foods. Intrinsic factors affecting water binding properties of food proteins include amino acid composition, protein conformation, surface polarity/hydrophobicity (Barbut, 1999). However, food processing methods have important impacts on the protein conformation and hydrophobicity. Data obtained in this study show that heat pressed oil extraction method

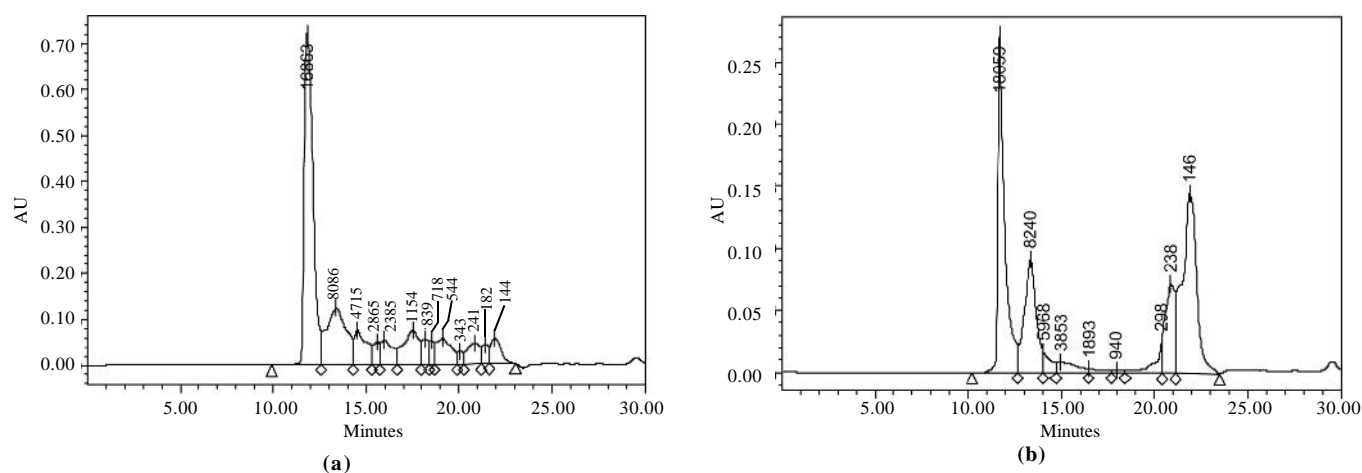


Fig. 3 : Molecular weight distribution of CPI (a) and HPI (b)

Table 2 : Functional parameters of CPI, HPI and commercial SPI

Sample	FE (%)	FV (%)			% leakage (60 min)	FAP (%)	WAP (%)	EAI (ml/g)	ESI (%)
		Over time (min)							
		1	30	60	60				
HPI	121.01 \pm 1.1 ^A	81.03 \pm 0.3 ^A	51.33 \pm 0.3 ^A	19.10 \pm 0.2 ^A	76.43 \pm 0.4 ^A	47.02 \pm 1.1 ^A	66.50 \pm 0.2 ^A	61.32 \pm 0.3 ^B	15.22 \pm 0.4 ^B
CPI	130.43 \pm 0.1 ^B	93.11 \pm 1.0 ^B	55.61 \pm 0.2 ^B	21.33 \pm 0.2 ^B	77.09 \pm 0.1 ^A	59.44 \pm 0.1 ^B	83.17 \pm 0.1 ^B	70.61 \pm 0.4 ^A	22.74 \pm 0.1 ^A
SPI	150.21 \pm 0.3 ^C	98.33 \pm 0.4 ^C	58.11 \pm 0.3 ^C	23.55 \pm 0.1 ^C	74.00 \pm 0.3 ^B	55.32 \pm 0.3 ^C	80.22 \pm 0.3 ^B	58.46 \pm 1.3 ^B	14.58 \pm 1.4 ^B

Table 3 : Proximate chemical composition of PPN and PPH (%)

Samples	Protein (N \times 5.46)	Ash	Moisture	Fat	Carbohydrate
HPI	84.20 \pm 0.07 ^A	3.02 \pm 0.32 ^a	3.06 \pm 0.02 ^A	0.44 \pm 0.11 ^A	5.11 \pm 0.01 ^A
CPI	92.40 \pm 0.05 ^B	0.60 \pm 0.31 ^b	4.56 \pm 0.03 ^B	0.36 \pm 0.12 ^A	3.41 \pm 0.06 ^B

reduced both water and oil absorption properties of HPI (Table 2). The decreased water and oil absorption properties of HPI could be due to irreversible denaturation caused by heating which might have destroyed both hydrophilic and hydrophobic groups of peanut protein, thus reducing both water and oil absorption properties.

Molecular weight distribution :

The molecular weight distributions of CPI and HPI were determined by SE-HPLC and are shown in Fig. 3 (a and b). The molecular weights for samples were calculated according to the standard equation below:

$$\text{Log Mol Wt} = 6.65e+000 - 2.05e-001 T^{\wedge}1 \quad (R^2 = 0.9986)$$

Results show that HPI had a higher molecular weight distribution than CPI. The molecular weight distributions of the first two peaks for HPI are 16.64KDa and 8.36KDa, while those of CPI are 14.91KDa and 6.39KDa, respectively. Heat treatment breaks intermolecular disulfide bonds in proteins and allows the proteins to unfold. Then the unfolded bonds interact and form intermolecular disulfide and hydrophobic bonds. This may polymerize the protein thereby resulting in the formation of high molecular weight aggregates.

Proximate chemical composition of CPI and HPI :

Biochemical scores obtained for CPI and HPI are presented in Table 3.

CPI recorded higher protein ($\approx 90\%$) and carbohydrate ($\approx 5\%$) values than HPI. No

Values are shown as mean \pm SD of triple determinations. Means followed by the same letters in the same column are not significantly different ($p \leq 0.05$) significant differences were recorded for the fat contents of CPI and HPI ($p \leq 0.05$). This further indicates that oil extraction method, to some extent, can contribute to different in the biochemical components of the by-product (peanut cake).

Conclusion :

Peanut Protein Isolates extracted from the two oil extraction methods demonstrated relatively good solubility potentials in both acidic and alkaline pH regions, which can serve as important characteristics for food formulations. CPI showed better functional properties (such as EAI, ESI, FAP, WAP, solubility, etc.) than HPI. On the whole, protein isolates extracted from

peanut cakes, an inexpensive by-product of peanut oil industries, can serve as an economic advantage to peanut oil industries, as they have the potential of adding value to the peanut industries by providing food processors with affordable source of plant proteins with unique flavour and functional characteristics.

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