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Physicochemical Characterisation of Heat and Cold Pressed Peanut Meal Flours

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Abstract: The functional and chemical properties of peanut meal flours obtained as by-products from both cold and heat pressed peanut oil extraction methods were studied. Peanut meal flour was found to consist mostly of protein (≈51%) with lower starch content (≈24%). The processing methods were found to have influenced functional properties such as water and oil absorption, emulsification, foam formation and stability. The neutral sugar components were mostly glucose, galactose and mannose with minute quantities of xylose, arabinose and rhamnose. High pressing temperatures, during the extraction process, resulted to the decomposition of some polysaccharides into other neutral sugar components; hence the significantly high percentage of xylose and arabinose in the polysaccharides extracted from the heat pressed peanut flour. Extraction methods also had profound influence on the fatty acid and amino acid composition of peanut flours obtained. However, flours obtained from the different methods exhibited adequate functional properties that could be usefully employed in different food formulations.

Key words: Physico-functional, cold pressed, heat pressed, peanut flour, neutral sugar, molecular weight distribution, sugar decomposition

INTRODUCTION

Peanuts are a rich source of protein and monounsaturated fat. Many research on peanuts and nuts in general have identified anti-oxidants and other chemicals that may provide health benefits. Peanuts are a significant source of resveratrol, a chemical studied for potential anti-aging effects (Yu *et al*., 2005; Basha and Pancholy, 1982). Peanuts have also found many uses besides serving directly as food. They are used as a source of cooking oil, animal feed and by-products used as shaving cream, metal polish, bleach, ink, soap, shampoo, explosives, paint, rubber, axle grease, paper, wallboard, fireplace logs, cat litter and medicine (Abulude *et al*., 2006). Vegetable oil extraction from peanut yields Partially Defatted Peanut Flour (PDPF). PDPF is a protein-rich, inexpensive and underutilized by-product of the peanut industry that offers the same health and dietary benefits of peanut with less fat. PDPF contains 47-55% high quality protein with high essential amino acid content and lends itself being used in many food applications (Ahmed and Schmidt, 1979; Yu *et al*., 2007). The peanut cake expelled during peanut oil production contains functional food ingredients that are of vital importance to the food industry, especially in producing functional foods (Yu *et al*., 2007). The development of peanut flour from defatted peanut cake can also provide the food industry with a new cost effective and high protein food ingredient for product formulation. This is critically needed in many developing countries where protein deficiencies remain a major health problem, especially among children. Interactions of

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polysaccharides with each other, ions, proteins and lipids affect water holding, gelling, film formation, viscosity and stabilization of foams and lipid emulsions. Studies have shown that processing methods can go a long way in influencing the physico-functional and chemical attributes of food items (Olawale *et al*., 2006; Yu *et al*., 2007). Davis *et al*. (2007) reported the importance of polysaccharides in enhancing the functional properties of high-protein flours. This study was therefore undertaken to investigate the effects of the cold pressed and heat pressed methods, used in peanut oil extraction, on the physico-functional and chemical characteristic of flours obtained from peanut cakes.

MATERIALS AND METHODS

Cold pressed and heat pressed peanut meal cakes were purchased from Qingdao Kerry Peanut Oil Co., Ltd. (Shandong province, P.R. China). The enzyme AS1398 (origin-Bacillus subtilis; type-proteinase; cellulase activity $=1\times10^5$ μ g^{-1}) was obtained from Genecor (Wuxi Branch) Bio-Products Co. Ltd., P.R. China. Some of the dextran standards obtained from ICN Biochemicals were given to us as a gift. The sources of other chemicals/reagents and materials obtained as a gift from other laboratories are stated accordingly in the text. All other chemicals used were of reagent grades and obtained either from other laboratories or from the chemical department of Southern Yangtze University, Wuxi, P.R. China.

Pre-Treatment of Samples

Both Cold Pressed Peanut Cake (CPC) and Heat Pressed Peanut Cake (HPC) were defatted using petroleum ether at 30 ~ 60°C using the Soxhlet method for 8 h. Defatted samples were air dried, milled into fine powder using an HR2839 model Philip blender, sieved to pass through a 70 mesh, oven dried for 3 h at 40°C and stored at 4°C in refrigerator until ready for analysis. The milled and oven dried CPC and HPC are referred to in this study as Cold Pressed Peanut Flour (CPF) and heat pressed peanut flour (HPF), respectively.

Oil Binding Capacities

Oil binding capacity was determined using a modified form of the method described by Chakraborty (1986). Estimations were carried out in triplicates.

Water Absorption

Water holding capacity was determined using the method outlined by Beuchat (1977) while bulk density was determined using the method described by Wang and Kinsella (1976). Estimations were performed in triplicates.

Whipping Properties

The whipping properties of 3% dispersions of CPF and HPF were determined using a modified form of the method described by Lin *et al*. (1974). The percentage foam expansion was calculated according to the method described by Lawhon *et al*. (1972). Foam volume as percentage was calculated taking the foam volume at zero time as 100%. Leakage was calculated as volume of liquid collected over volume of liquid before whipping x100.

Emulsifying Properties

The Emulsifying Properties (EP) and Emulsion Stability (ES) were determined according to the methods of Pearce and Kinsella (1978) and Matusdomi *et al*. (1994).

Chemical Methods of Analysis

Crude protein, fat and ash contents were measured by AOAC (1984) methods. Total sugar was determined by the phenol-sulfuric acid method as described by Dubois *et al*. (1956), using glucose as a standard. Uronic acid was measured by the method of Blumenkratz and Asboe-Hansen (1973), using galacturonic acid as a standard.

Determination of the Neutral Sugar Composition of CPF and HPF (Sample Preparation)

Using the Soxhlet method, CPF and HPF were further de-fatted with petroleum ether at 30 ~ 60°C for 8 h. The de-fatted samples were extracted twice with 8 times the volume of distilled water at 50°C for 1 h after the pH had been adjusted to 7.0 with 2.0 N NaOH. The residue was obtained as a precipitate after centrifugation (5000 x g for 20 min). The remaining protein was hydrolyzed twice with AS1398 Enzyme according to the method described by Sonda *et al*. (2002). The polysaccharides extracted from CPF and HPF are referred to in this study as polysaccharides extracted from Cold Pressed Peanut Flour (PCPF) and polysaccharides extracted from Heat Pressed Peanut Flour (PHPF), respectively. The neutral sugar composition and total sugar, crude protein, ash contents and uronic acid of PCPF and PHPF were determined.

Neutral Sugar Analysis

Monosaccharide components and their ratios were determined by absolute hydrolysis. With this method, each of the samples (PCPF and PHPF) was hydrolyzed with 1.0 M H_2SO_4 at 90°C for 8 h and the hydrolysate was then neutralized with $CaCO_3$. The resulting solution was centrifuged, evaporated for dryness and then followed by the acetylation treatment with Ac_2O- Pyridine at 90°C for 30 min. The pre-treated samples were then analyzed for their neutral sugar compositions according to the method of Guentas *et al*. (2001). Alditol acetates of the reduced sugars and their standards (glucose, mannose, galactose, xylose, rhamnose and arabinose) were used with inositol as the internal standard. The derivatives were analyzed by gas chromatography (Shimadzu GC-2010, Japan) equipped with OV1701 capillary column (30 m×0.32 mm i.d.) and a Flame-Inoization Detector (FID). The operation was done using the following conditions: H_2: 47 mL min^{-1}; air: 400 mL min^{-1}; N_2: 10 mL min^{-1}; Temperature was programmed: 120-190°C (10°C min^{-1}) to 240°C (3°C min^{-1}). High-purity helium was used as the carrier gas. The products were identified by their characteristic retention times. The percentage of monosaccharide in the sample was calculated from the peak areas using response factors.

Amino Acids Analysis

Amino Acids were determined with a Hitachi 835-50G automatic amino acid analyzer (Hitachi Ltd., Tokyo, Japan). The hydrolysis of CPF and HPF extracts was done in a sealed ampoule for 24 h at 110°C using 1 mL of 6.0 M HCl solution under vacuum. The hydrolysate for each sample was 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.

Fatty Acid Profile

The fatty acid analysis was based on the methods described by Hinds (1995) and Holaday and Pearson (1974). Twenty to thirty milligram of the oil sample (about 1 drop) was weighed into a 50 mL screw capped tube. One milliliter of 0.5 M methanolic (Fisher Cat No. A-452-4) potassium hydroxide solution (Fisher Cat No. P-250) was added for the saponification of glycerides.

The tube was then heated for 5 min in a water bath at 80°C and continued for ten minutes after the addition of 1 mL of boron trifluoride (Sigma, Cat No. B1252) in 14% methanolic solution. 1.0 mL of water and 1.0 mL of hexane (Optima grade, Fisher Cat No. H302-4) were added after the tubes cooled slightly. Each solution was vortexed for 30 sec and then allowed to settle into two phases at room temperature. An aliquot of the hexane layer was transferred into a vial and a small amount of

anhydrous sodium sulphate (Sigma cat No. 238597) was added. The prepared hexane solution was used for direct injection into a Gas Chromatograph (GC). The fatty acid methyl ester standards, Kel-Fim FAME-5 Standard (Matreya Inc., Pleasant Gap, PA, Cat No. 4210) and GLC-21 Standard (Nu-Check Standards) were used for identification and comparison of fatty acids present in the test samples. The Fatty Acid Methyl Esters (FAME) were analyzed with a Perkin Elmer Autosampler XL system (Perkin Elmer Instruments, Norwalk, CN) equipped with a Flame Ionization Detector (FID) and a capillary column containing 70% cyanopropyl polysilphenylene-siloxane as the stationary phase (30 m length, 0.25 mm i.d., 0.25 μm film thickness). Helium at 20 psi (1.85 mL min^{-1}) was used as the carrier gas. Hydrogen flow was set to 45 mL min^{-1} and the airflow was fixed at 450 mL min^{-1}. The split flow ratio was 76.9 mL min^{-1}. The temperature of both the injector and the detector was 265°C. A temperature program was used with an initial oven temperature of 60°C held for 2 min, which was increased to 180°C at 10°C min^{-1} and then programmed to a final temperature of 235°C at 4°C min^{-1}. The amount of sample injected was 1.0 μL. The total amount of myristic acid (C14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosenoic, (20:1), behenic (22:0) and lignoceric (24:0) acids were calculated by determining the percent area of each FAME. The mean value of three replicates was used to determine the concentration of each fatty acid. Peaks in ascending retention time order were as follows: C14:0, C16:0, C18:0, C18: 1, C18: 2, C18: 3, C20:0, C20: 1, C22:0, C24:0.).

Colour Analysis

Color was measured so as to determine the effect of oil extraction methods on the colour of Peanut meal flours. The color determination was carried out by measuring the Hunter L-value (Lightness and Darkness), using a Hunter Colorimeter. The Hunter L-value (lightness) was calculated by the formula:

$$L = 10\sqrt{Y}$$

Where, L = 0 is considered black and L = 100 is considered white

The instrument was standardized against a white ceramic tile and measurements of samples followed immediately after calibration (X = 94.84, Y = 99.99, Z = 105.37) Each sample CPF, HPF and CSF were placed in a 3-inch diameter petri-dish and color measurements were taken in triplicate for each sample after remixing the sample in a petri-dish (Fig. 1).

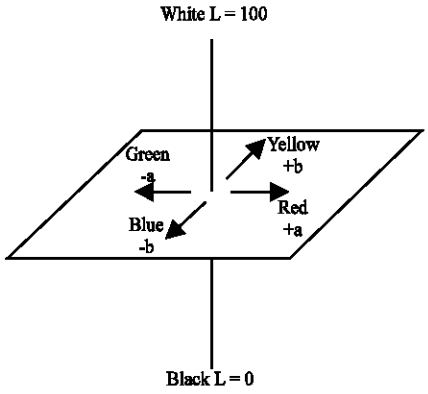

Fig. 1: Hunter lab color system

RESULTS AND DISCUSSION

Proximate Compositions of HPF and CPF

Results in Table 1 show that processing methods affected protein contents of CPF and HPF with the former having higher protein content than the latter. Similar results have been reported by Ahmed and Schmidt (1979). The difference in protein content between CPF and HPF can be attributed to the fact that during the processing of HPF the high temperature employed may have resulted into protein denaturation (Prinyawiwatkul *et al*., 1993). The result indicates that protein forms a major intrinsic component of peanut flour which is in accordance with the findings of Ahmed and Schmidt (1979), Kim *et al*. (1992) and Yu *et al*. (2007).

Comparative Analysis of Some Functional Properties of HPF, CPF and CSF

The properties of high-protein flours which affect functionality include hydrophilicity/ hydrophobicity ratio, secondary structure contents and their distribution, tertiary and quaternary arrangements of the polypeptide segments, inter- and intra-subunit cross-links and the rigidity/ flexibility of the protein to external conditions (Cherry and McWatters, 1975; Abulude *et al*., 2006). Interactions of water and oil with the protein contents of high protein flours are very important in food systems because of their effects on the flavor and texture of foods. Intrinsic factors affecting water binding properties of high protein. Flours include amino acid composition, protein conformation and surface polarity/hydrophobicity. However, food processing methods are bound to have significant impact on flour conformation and hydrophobicity. Studies have also shown that hydrophobicity of amino acid side chains within polypeptides are dependent upon the chemical microenvironment of the interface established between the solute, solvent and the hydrocarbonaceous ligand. Furthermore, the polarity or non-polarity of amino acids represents an important biological consequence of the hydrophobic effect and application in food science. Results obtained in this study show that heating affected both water and oil absorption capacities of HPF. During oil extraction, using the heat pressed method, proteins in HPF were likely denatured by high temperature and longer pressing times, thereby exposing more hydrophobic sites, which explain the relatively reduced water retention capacity of HPF. This agrees with the findings of Voutsina *et al*. (1983). The decrease in oil absorption capacity of HPF could be attributed to a likely occurrence of an irreversible denaturation caused by heating at high temperatures which might have destroyed both hydrophilic and hydrophobic groups of proteins in HPF, thus reducing both water and oil absorption properties (Table 2). Also the amino acid profiles obtained for CPF and HPF show that HPF contained high quantities of isoleucine, threonine and glycine while CPF contains high quantities of methionine, arginine, aspartic acid and glutamic acid Considering the low solubility of non-polar molecules in water, the results in Table 2 could be fully understood. This is consistent with the findings of Basha and Pancholy (1982). Bulk density is important for determining packaging requirements, material handling and application in wet processing

Table 1: Biochemical scores of HPF and CPF (%)

Samples	Protein	Moisture	Fat	Carbohydrate	Ash	Crude fiber
HPF	49.8	5.9	0.9	24.8	8.6	8.0
CPF	52.1	7.1	1.6	23.5	7.6	9.7

Table 2: Comparative analysis of some functional properties of HPF, CPF and CSF

	Functional attribute[§]		
Samples	Fat absorption (%)	Water absorption (%)	Bulk density (g mL^{-1})
CPF	84.01±0.30	87.01±0.10	0.41±0.03
HPF	81.80±0.10	83.40±0.71	0.40±0.04
CSF[1]	83.33±0.04	88.55±0.33	0.41±0.02

[1]: Commercial soy flour; [§]: All experiments were conducted in triplicates

Table 3: Whipping properties of HPF, CPF and CSF

Samples	Foam expansion (%)	Foam volume (%) over time (min)					Leakage (%)	
		1	10	30	60	90	30	90
CPF	76.10±0.44	74.3±0.30	71.7±0.10	58.1±0.21	30.4±0.02	22.5±0.33	22.02±0.01	46.03±0.10
HPF	88.32±0.02	80.4±0.03	76.1±0.11	66.3±0.13	30.3±0.31	16.1±0.41	17.20±0.10	47.22±0.11
CSF[1]	86.41±0.30	78.2±0.33	76.5±0.31	68.1±0.23	35.1±0.01	24.4±0.13	20.04±0.13	46.04±0.30

[1]: Commercial soy flour

in the food industry. Bulk density is generally affected by particle size. Since the same blender and blending speed were used to mill all samples, bulk densities are bound to be almost the same as shown in Table 2. This means that the milling method did not have any significant effect on the bulk density of peanut flours evaluated.

Whipping Properties of HPF, CPF and CSF

Foams are defined as a gas (discontinuous phase) dispersed into a liquid or solid (continuous phase) and are similar to emulsions in that both have hydrophobic fluid dispersed into a hydrophilic liquid. Theoretically, the amphipathic character of proteins makes them good foaming agents that work at air–water interface to prevent bubble coalescence (McWatters and Cherry, 1975, 1977). HPF demonstrated better foam formation and stability than CPF and CSF (Table 3). This is in accordance with the conclusion made by McWatters *et al*. (1976) and McWatters and Holmes (1979) with respect to the positive effect of heat on foam capacity and stability. According to McWatters and Cherry (1975), foam capacity for peanut paste was highest with moist heat treatment at 100°C compared to other moist heat treatment levels of 50 and 75°C. Therefore, HPF recovered from heat pressing method with temperatures between 120-140°C and relatively shorter pressing times may be suitable in food systems that require foaming such as cake and ice cream. On the other hand, flours obtained at high pressing temperatures (>140°C) and longer extraction times may have relatively higher foaming capacity.

Emulsifying Properties

Emulsifying properties are usually attributed to the flexibility of solutes (i.e., the ability to go into solution and adsorb into interfaces) and exposure of hydrophobic domains. Food emulsions are thermodynamically unstable mixtures of immiscible liquids (water and oil). The formation and stability of emulsion are very important in food systems such as salad dressing. Proteins in flours are composed of charged amino acids, non-charged polar amino acids and non-polar amino acids, which make high protein-flours potential choice of emulsifiers, the surfactant possessing both hydrophilic and hydrophobic properties and have the characteristic to interact with both water and oil in food system (Tulstoguzov, 1988). CPF demonstrated higher Emulsifying Properties (EP) compared to HPF and gum acacia (Fig. 2). This difference in EP may have been due to the different processing methods. The effect of high temperatures and longer heating times on the emulsifying capacity of the peanut flour might be balanced by the increase in surface hydrophobicity and decrease in solubility of peanut proteins. Hence the relatively low EP demonstrated by HPF. Results obtained also show that CPF, HPF and gum acacia exhibited thermodynamically unstable characteristics when exposed to varying temperatures of 40, 60 and 80°C (Fig. 3a-c). However, at all temperature levels, as seen in Fig. 3a-c, HPF was slightly more stable than CPF and gum acacia.

Biochemical Scores of PCPF and PHPF

The hydrolysis method was effective in extracting the protein contents of both samples because in the iso-propanol and water mixture, polysaccharides remain insoluble while most of the proteins dissolve in the system thereby creating a suitable medium for enzymatic hydrolysis to take place (Table 4; Sonda *et al*., 2002). Higher uronic acid contents of 14.7 and 17.5% were found in PCPF and PHPF, respectively.

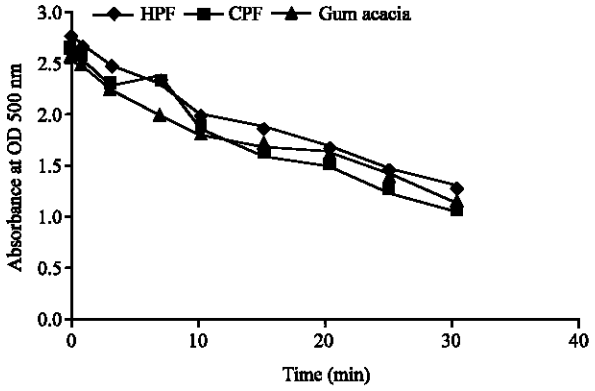

Fig. 2: Emulsion properties of CPF, HPF and Gum Acacia Powder

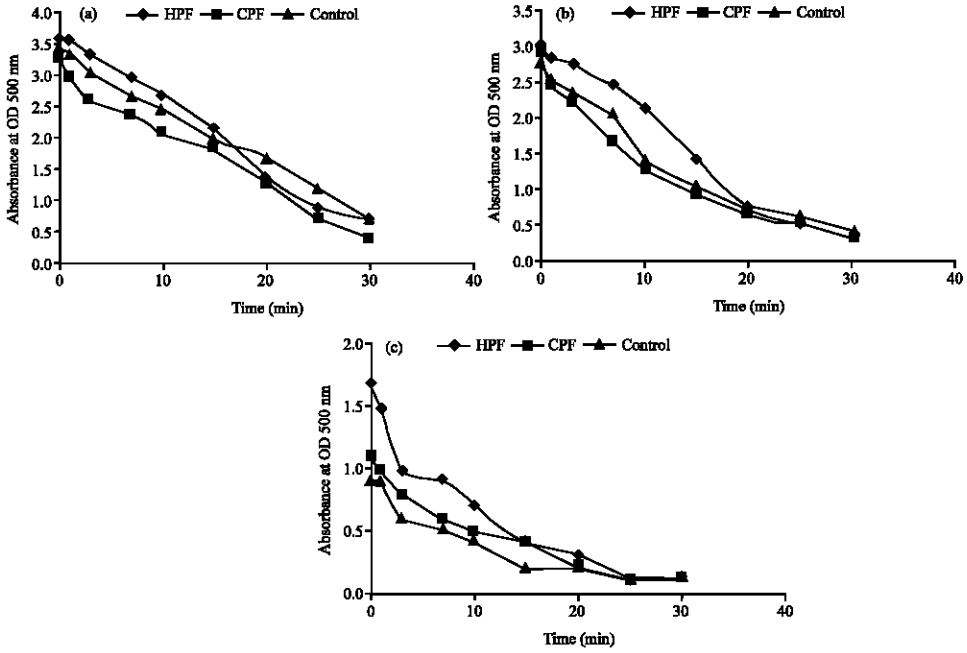

Fig. 3: Heat stability of CPF, HPF and Control (Gum Acacia powder) emulsions stabilized by 0.1% SDS solutions and heated at (a) 40°C, (b) 60°C and (c) 80°C

Table 4 shows that the hydrolysis method was effective in the extraction and purification of PCPF and PHPF.

Many foods contain substantial amounts of glycoproteins, that is, proteins linked to different monosaccharides or oligosaccharide moieties via O-glycosidic or N-glycosidic bonds. The saccharide moieties may participate in covalent interchain cross-links and affect many functional properties of glycoproteins, such as their tensile strength, solubility, viscosity in solutions, ability of interactions with other food components and biological activity. Interactions of polysaccharides with each other, ions, proteins and lipids affect water holding, gelling, film formation, viscosity and stabilization of foams and lipid emulsions. Based on the above, the total and neutral sugar compositions of PCPF and

Table 4: Proximate composition of PCPF and PHPF

Biochemical component	PCPF (%)	PHPF (%)
Protein	3.4	2.1
Ash	1.6	1.1
Total sugar	80.1	79.6
Fat	0.6	0.4
Uronic acid	14.7	17.5

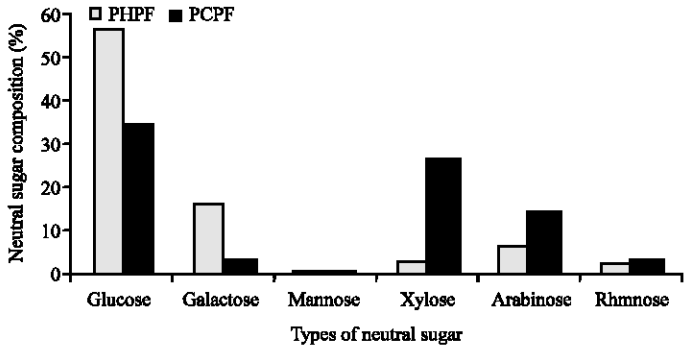

Fig. 4: Neutral sugar composition of PHPF and PCPF

PHPF were investigated so as to properly understand the intrinsic mechanisms that enhance the physico-functional and chemical characteristics of both samples. Comparative analysis of the neutral sugar composition of PCPF and PHPF are shown in Fig. 4. The dominant neutral sugars found in the two samples are glucose, galactose and mannose. It could be seen in Fig. 4 that significantly high quantities of xylose and arabinose were found in PHPF. This difference may have occurred due to the fact that during the extraction process of HPC some polysaccharides decomposed into other sugar components as a result of the high processing temperature thereby leading to an increase in the composition of xylose and arabinose in PHPF.

Amino Acid Analysis of CPF and HPF

Processing methods resulted into an uneven distribution of both glucogenic and ketogenic amino acids in CPF and HPF. It could be shown from Table 5 that sulphur containing amino acids, cysteine and methionine, generally considered non-polar and hydrophobic were minimally affected by the processing methods. On the other hand, hydroxyl amino acids, serine, threonine and tyrosine, which are generally considered polar, hydrophilic and uncharged at physiological pH were affected by heat pressed method.

Fatty Acid Analysis

Table 6 shows that the processing method affected the fatty acid composition of CPF and HPF with the former having more saturated fatty acids than the latter. However, more polyunsaturated fatty acids were detected in HPF than in CPF. Saturated fatty acids with 20 or more carbon atoms were not detected in HPF.

Effect of Oil Extraction Method on the Color of CPF and HPF

The colour of CPF and HPF as determined by the Hunter colorimeter in respect of the L-value are shown in Table 7. CPF recorded the highest L-value compared to HPF and CSF. Based on the Hunter lab color system (Fig. 1) CPF had a lighter colour than HPF indicative of the fact that the heat pressing method had effect on the colour of HPF.

Table 5: Amino acid composition of CPF and HPF

Types of amino acid	Mean amino acid composition (g/100 g sample)	
	CPF	HPF
Essential amino acids		
Isoleucine (Ile)	2.1	6.6
Leucine (leu)	3.7	1.5
Lysine (Lys)	0.8	0.6
Methionine (Met)	1.0	0.8
Phenylalanine (Phe)	3.1	3.8
Threonine (Thr)	8.1	1.2
Valine (Val)	2.3	1.1
Histidine (His)	1.3	2.2
Tryptophan (Trp)	-	-
Non essential amino acids		
Alanine (Ala)	2.1	3.6
Arginine (Arg)	7.0	1.9
Aspartic acid (Asp)	6.7	3.5
Cysteine Cys)	0.5	0.4
Glutamic acid (Gul)	12.7	1.1
Glycine (Gly)	3.1	8.0
Serine (Ser)	2.7	1.5
Tyrosine (Tyr)	2.0	1.5
Proline (Pro)	2.3	3.7

Table 6: Fatty acid composition of CPF and HPF

Types of fatty acids	Fatty acid composition (g/100 g oil)	
	CPF	HPF
C14:0	ND	9.61
C16:0	7.67	12.06
C18:0	9.36	15.17
C20:0	11.34	ND*
C22:0	14.03	ND*
C24:0	18.49	ND*
Total saturated	60.90	36.84
C16:1	7.84	12.35
C18:1	9.60	15.57
C20:1	11.57	ND*
Total monounsaturated	29.03	27.92
C18:2	10.07	16.40
C18:3	ND	18.83
Total polyunsaturated	10.07	35.23

ND: Not Detected

Table 7: Hunter L-values of CPF, HPF and CSF

Samples	L-values
CPF	87.6
HPF	37.8
CSF	84.3

CONCLUSION

The functional and chemical characteristics of CPF and HPF were thoroughly investigated. Results show that processing methods affected the functional (water and fat absorption capacity, emulsification, foamability, whippeability, colour etc.) and chemical attributes (protein, total and neutral sugar components, amino acids, fatty acids etc.) of both CPF and HPF. This confirms other research findings on the effect of processing methods on the intrinsic and extrinsic components of food items. However, most of the bulk of the meal obtained during peanut oil processing comprises protein. CPF and HPF demonstrated high capacity to absorb fat and water and therefore suitable for

application in certain food preparations that require high fat and/or water content. The allergenicity of the protein components of peanut flour was not studied but it is assumed that processing methods, such as heat treatment, biotechnological and enzymatic processes, significantly reduce allergenicity. This study is recommended if CPF and HPF should be employed in different food formulation. Nevertheless, this study is environmentally friendly as it aims at converting a waste product into a functional food ingredient.

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