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Relation Between Binding of Proteins with Phenolics at Different pH Values in Peanut Meal

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Abstract: Proteins and phenolic compounds are essential for the maintenance of good health. Peanuts are rich sources of both proteins and phenolic compounds. Unfortunately several factors cause the binding of phenolic compounds to proteins, thus forming complexes which affect the availability of proteins and the phenolic compounds rendering them unavailable for the human body. The aim of this work is to study the solubility of both the proteins and the phenolic compounds at a wide range of pH in order to determine the pH at which the binding takes place, also the optimum pH for phenolic extraction. In this study the focus will be on the effect of pH on the solubility of proteins, amino acids, soluble and insoluble phenolic compounds present in peanut meal (PM). The results revealed the least solubility of the PM protein is at its isoelectric point between pH 4-5 where only 6.3-5.43% of the protein is solubilized and highest amount of protein remains precipitated 50.7-51.57%, respectively. Going towards alkaline pHs the protein solubility increases, until it reaches complete solubility at pHs 11-12 revealing 57% soluble protein (SP). Highest solubility for the free phenolic compounds (SPC) is at high alkaline pH reaching 3.02 g/100g at pH 12, but the corresponding pH for the precipitate shows that 2.18 g/ 100g meal was still present. The 2.18 correspond to the (EPP) in the precipitate which was extracted sequentially with both methanol: water (50:50 v:v) and acetone: water (70:30 v:v). Meanwhile, the NEPP or the bound phenolics which were extracted after hydrolysis with conc. HCl in 1- butanol (50 ml/L) in a water bath at 100°C showed that highest binding of phenolic compounds took place at or near the IEP of the protein while at alkaline pHs the binding of the phenolics was at its minimum 0.72, 0.36, 0.08 g/100g meal at pH 10, 11 and 12, respectively. It can be concluded that extracting all the phenolic compounds can be achieved at pHs 3, 4, 5 and 6 where about 6g phenolic compounds /100g meal are extracted. It is worthy to mention that at these pH values very little protein is extracted or lost at these pH values. It is clear from the results that both lysine and sulphur amino acids are limiting in PM protein. Yet by looking at the amino acid analysis it can be seen that the supernatant resulting from extraction at pH values 8, 9 and 10 contain more lysine 1.26, 1.37 and 1.26 g/100g meal, respectively, compared to other pH values, PM contain 1.74 g/100g meal. While, the FAO daily requirements for lysine is 5.5 g/100g. Methionine content was highest at pH values 8, 9 and 10 leveling to 0.45, 0.5 and 0.45 g/100g meal, respectively, compared to other pH values, PM contain 0.55g/100g meal. FAO daily requirements for methionine and cysteine are 3.5g/100g meal. The results also indicate that glutamic acid, aspartic acid and arginine are the predominant amino acids. In conclusion this study revealed that highest solubility of free phenloic compounds in peanut meal occurred at alkaline pHs, while least solubility is at and the IEP of the protein. Highest binding of proteins with phenolics takes place at or near the IEP of the protein.

Key words: Amino acid composition · Peanut · Phenolic compounds · Phenolic/protein · Protein · pH values

INTRODUCTION

Both proteins and phenolic compounds are essential for the maintenance of good health. Peanuts are rich sources of both proteins and phenolic compounds. Protein is the building block of any human body. Muscles, ligaments, cartilage, skin, hair are constructed from protein molecules. In addition to these large scale structures that hold us together, smaller protein molecules play a vital role in keeping our body working properly.

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Hemoglobin, hormones, antibodies and enzymes are examples of these protein molecules. Protein also keeps the brain working, supports the cells and acts as a last reserve of energy when carbohydrates and fats are not available [1]. Phytochemicals are not essential; their absence from the diet will not result in death. However they are important to our body especially at times when our exposure to free radical producers is great and with such a stressful life as the one encountered nowadays. Among these phytochemicals phenolic compounds are the most common in nature. Phenolic compounds exhibit a wide range of physiological properties, such as antioxidant, anti-allergenic, antiarthero-genic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects [2-5]. Phenolic compounds have attracted much interest recently because in vitro and in vivo studies suggest that they have a variety of beneficial biological properties which may play an important role in the maintenance of human health. Besides these beneficial effects the presence of phenolic compounds in the food has some disadvantages. The harmful effects of phenolic compounds in nutrition are their ability to bind and precipitate proteins [6]. The ability of polyphenols to form insoluble complexes with proteins interferes with the utilization of dietary proteins and reduces their digestibility. The complexation of polyphenols, as well as their enzymatic and nonenzymatic oxidation products with the protein in seed, meals or flours has been reputed to reduce nutritional properties of proteins from these sources. These products may react with the å-NH_2 group of lysine and CH_2S group of methionine of enzymes and other proteins to form complexes, thus rendering them nutritionally unavailable to monogastric animals [7]. Among the harmful effects of phenolic compounds is the sequestration of dietary iron and inhibition of digestive enzymes [8]. Peanut skin and kernels are rich sources of phenolic compounds including: *p*- hydroxy- benzoic acid, chlorogenic acid, *p*-coumaric acid, flavanols (quercitin and kaempferol), stilbene and resveratrol [9]. Other phenolics reported include ferulic, caffeic, sinapic, vanillic, gentisic andmainly coumaric acids [10]. Peanut kernels are also a rich source of plant protein [11]. Factors affecting the protein-phenolic interactions such as temperature, pH, phenolic structure, protein size, amino acid composition and solvent characteristics in the medium have been reported by Serafini *et al.* [12].

The aim of this work was to study the binding of proteins with the phenolic compounds from peanut meal at different pH values. Also the optimum pH at which the phenolic should be extracted.

MATERIALS AND METHODS

Peanut (*Arachis hypogea* L.) was brought from the local market. Peanuts were deshelled but the red skin was kept. The kernels with the skin were then ground using a pulverizer and then defatted in a Soxhlet apparatus using n-hexane as defatting solvent. The defatted kernel (meal) was spread to dry and designated peanut meal (PM).

Analytical Methods: Moisture, oil, protein, ash and crude fiber contents were determined in peanut meal according to A.O.A.C. [13]. Phenolic compounds of different crude phenolic extracts resulting from extraction of peanut meal at different pH values were determined according to Hung *et al.* [14].

Extraction of Proteins and Phenolic Compounds at Different pH Values: 5g meal were extracted with 50 ml water at pHs from 1-12 by stirring with a magnetic stirrer and adjusting the pH to the desired value using 6N HCl or 1N NaOH for 15 min until the pH is stable then placing the solution (meal + water) in an Ultrasound water bath (Crest-Ultrasonics) for 30 min. the extract was then centrifuged at 3000 xg for 20 min. The supernatant was used for determination of soluble protein (SP), soluble phenolic compounds (SPC) and soluble amino acids (SAA). The precipitate was taken for determination of extractable polyphenols (EPP) and non extractable polyphenols (NEPP) or condensed tannins.

Determination of Phenolic Compounds in Precipitate: precipitate was extracted sequentially with 400ml of methanol : water (50:50 v:v) in an ultrasound water bath for 1hr, centrifuged, then the precipitate re- extracted with 400 ml of acetone: water (70:30 v:v) in an ultrasound water bath for 1hr, centrifuged at 2500 xg for 15 min. Combined extracts gives the extractable polyphenols. (EPP) which were determined by Folin Ciocalteu according to Hung *et al.* [14] using Gallic acid as standard. The residue after the second centrifugation was treated with 100 ml conc. HCl in 1- butanol (50 ml/L) in a water bath at 100°C for 3 hr, with occasional shaking, then centrifuged at 2000 xg, the supernatant was made up to 100 ml with the same solvent and considered as non-extractable polyphenols and designated (NEPP); absorbance of (NEPP) extracts was measured at 555 nm using tannin as standard. Total phenolic compound extracted (TPE) was calculated as follows:

Total phenolic compound extracted (TPE) = EPP +NEPP

The results are presented as average ± standard deviation (SD).

RESULTS AND DISCUSSION

Plant proteins are a valuable source of unconventional proteins and the binding of phenolic compounds with proteins is well documented [8, 15-17]. Meanwhile, the protein/phenolic complex causes changes in the physico-chemical properties and nutritional properties of the proteins [18-20]. The conditions that have an effect on the formation of the phenolic-protein complex include: temperature, pH, phenolic structure, protein size, amino acid composition and solvent characteristics in the medium Serafini, et al. [12]. In this study the focus will be on the effect of pH on the solubility of protein, amino acids, soluble and insoluble phenolic compounds present in peanut meal (PM).

Peanut meal comprised the defatted kernels together with the red skin was analyzed for its chemical composition. Table 1 shows clearly that peanut meal is a rich source of protein (57%) as reported by Abbasy *et al.* [11] and Wu *et al.* [21] and phenolic compounds 6.25g/100g meal by Win *et al.* [9]. As expected the least solubility of the PM protein is at its isoelectric point between pH 4-5 where only 6.3-5.43 % of the protein is solubilized and highest amount of protein remains precipitated 50.7-51.57%, respectively. Going towards alkaline pHs the protein solubility increases, until it reaches complete solubility at pHs 11-12 revealing 57 % soluble protein (SP), while no corresponding protein was detected at the same pHs for the precipitate. This pattern for PM protein solubility has been reported for many other oilseed proteins [11, 22-24]. Similar observations have been reported by Lawal and Adebowale [25] for Bambara groundnut. Table 2 also clarifies that highest solubility for the free phenolic compounds (SPC) is at high alkaline pH reaching 3.02 g/100g at pH 12, but the corresponding pH for the precipitate shows that 2.18 mg/ ml were still present, The 2.18 correspond to the (EPP) in the precipitate which were extracted sequentially with both methanol: water (50:50 v:v) and acetone: water (70:30 v:v). Meanwhile, the NEPP or the bound phenolics which were extracted after hydrolysis with conc. HCl in 1- butanol (50 ml/L) in a water bath at 100°C showed that highest binding of phenolic compounds took place at/ or near the IEP pH the protein while at alkaline pHs the binding of the phenolics was at its minimum 0.72, 0.36, 0.08 g/100g meal at pH 10, 11 and 12, respectively. These results prove that preparing a concentrated phenolic extract is not recommended by the method of pH extraction. Odzal *et al.* [15] in a review on protein-phenolic interactions and associated changes, found the lowest solubility of polyphenol-protein complexes occurred at 0.3-3.1 pH units below the isoelectric point of proteins. This is in accordance with our results. Naczk *et al.* [26] studied the effect of pH on the formation of crude canola extract /BSA, fetuin andlysozyme complexes. It was suggested that the optimal pH for the precipitation varies for different proteins and generally

Table 1: Chemical composition of peanut meal (± Standard deviation)

Composition	Peanut meal (%)
Moisture %	8.2 ±0.2
Protein %	57.0 ±0.1
Oil %	0.1 ±0.02
Ash %	5.3 ±0.1
Crude Fiber %	5.63 ±0.2
Nitrogen free extract %	23.77 ±0.1
Phenolic compounds %	6.25 ±0.3

Table 2: The solubility pattern of protein and phenolic compounds present in peanut meal (g/100g meal) at different pH values.

	Supernatant ± SD		Precipitate ± SD		
pH	Soluble protein	Soluble free phenolics	Insoluble protein	Insoluble free phenolics	Insoluble bound phenolics
1	13.65 ±0.1	1.35±0.1	43.35±0.1	2.62±0.2	1.44±0.2
2	9.1±0.2	1.48±0.2	47.9±0.2	2.86±0.2	1.2±0.1
3	9.55±0.1	1.31±0.3	47.45±0.1	2.88±0.3	2.0±0.2
4	6.3±0.1	1.091±0.1	50.7±0.2	2.91±0.2	2.2±0.1
5	5.43±0.2	1.25±0.1	51.57±0.2	2.55±0.1	2.4±0.2
6	14.0±0.1	1.48±0.2	43.0±0.2	2.29±0.3	2.44±0.1
7	17.5±0.1	1.88±0.3	39.5±0.1	2.04±0.2	1.84±0.1
8	52.5±0.1	2.48±0.1	4.5±0.1	2.18±0.1	1.52±0.3
9	53.2±0.3	2.79±0.2	3.8±0.1	1.97±0.2	1.24±0.1
10	54.6±0.2	2.79±0.1	2.4±0.2	2.18±0.2	0.72±0.3
11	57.0±0.1	2.75±0.1	0	2.12±0.3	0.36±0.2
12	57.0±0.3	3.02±0.3	0	2.18±0.1	0.08±0.01

is close to the IEP of the protein. Our results also revealed that lowest solubility of phenolic compounds was between pH 4.0-5.0 (IEP).

These results revealed that preparing a protein isolate from PM by dissolving protein at alkaline pH, then precipitating it at the IEP of the protein will result in a protein isolate containing substantial amounts of phenolic compounds 2.75-3.02 mg/100g. On the other hand, preparing a protein concentrate by washing the PM at its IEP will result in a peanut protein concentrate with high phenolic compounds 4.95-5.11 mg/100g. This finding is confirmed by that of Wagdy *et al*. [27], who found that, while working with JoJoba meal, that the phenolic compounds are solubilized more at the alkaline pH values with the proteins. The work of Rawel *et al.* [17] is in agreement with our results. They were studying the binding of selected phenolic compounds to proteins. They found that the binding parameters were influenced by different factors where e.g. increasing the temperature and ionic strength as well as decreasing pH cause a diminished binding.

Concerning the phenolic compounds in the peanut meal, Table 3 gives a clearer picture. It can be said that extracting all the phenolic compounds can be achieved at

Table 3: Effect of pH on phenolic compounds present in peanut meal (± SD).

pH	Total phenolic compounds (extracted 1)	Soluble phenolics and extractable phenolic compounds (2)	Phenolic compounds extracted by hydrolysis (3)	Other forms of phenolic compounds(4)
1	5.42±0.13	3.98±0.26	1.44±0.09	0.83±0.05
2	5.55±0.14	4.35±0.23	1.2±0.12	0.70±0.08
3	6.2 ±0.24	4.2±0.30	2.0±0.30	0.05±0.12
4	6.21±0.18	4.01±0.19	2.2±0.11	0.04±0.09
5	6.2±0.34	3.8±0.13	2.4±0.09	0.05±0.18
6	6.21±0.33	3.77±0.12	2.44±0.18	0.04±0.21
7	5.76±0.22	3.92±0.26	1.84±0.38	0.49±0.13
8	6.18±0.18	4.66±0.20	1.52±0.26	0.07±0.10
9	6±0.10	4.75±0.22	1.24±0.19	0.26±0.05
10	5.79±0.11	4.97±0.26	0.72±0.27	0.56±0.03
11	5.23±0.32	4.87±0.12	0.36±0.09	1.02±0.16
12	5.28±0.19	5.2±0.26	0.08±0.13	0.97±0.016

1.The sum of the determined phenolics in Table 2

2.The sum of the soluble free phenolics + the insoluble free phenolics from the extracted by solvents from Table 2

3.Bound phenolic compounds extracted by hydrolysis with butanol-HCL.

4.The total phenolics in the Table 1 minus total extractable phenolic compounds column 1.

Table 4: Amino acid composition of peanut meal and its supernatants at different pH values.

		pH values												
Amino Acids	Meal	1	2	3	4	5	6	7	8	9	10	11	12	*FAO /WHO
Argenine	5.487	2.05	2.14	0.32	0.37	0.29	1.22	2.04	3.82	4.14	3.85	3.42	3.43	--
Aspartic	5.523	1.83	1.92	0.45	0.48	0.41	1.32	2.15	3.88	4.13	3.83	3.5	3.42	--
Alanine	1.845	0.46	0.53	0.2	0.21	0.18	0.46	0.74	1.4	1.49	1.35	1.21	1.2	--
Isoleucine	1.487	0.35	0.41	0.12	0.11	0.1	0.41	0.66	1.27	1.31	1.19	1.03	1.05	4.0
Proline	2.489	0.58	0.67	0.16	0.16	0.13	0.49	0.82	1.62	1.79	1.66	1.47	1.51	--
Therionine	1.201	0.31	0.39	0.16	0.16	0.15	0.38	0.58	1.06	1.14	0.94	0.9	0.92	4.0
Glutamic	8.653	2.82	2.83	0.76	0.79	0.71	1.99	2.85	3.81	3.94	3.8	3.67	3.65	--
Glycine	2.874	1.31	1.37	0.77	0.86	0.53	0.6	0.83	1.39	1.54	1.54	1.51	1.52	--
Serine	2.147	0.78	0.83	0.25	0.27	0.19	0.52	0.86	1.65	1.79	1.37	1.48	1.53	--
Cysetine	1.16	0.54	0.5	0.11	0.12	0.12	0.22	0.32	0.65	0.82	1.13	0.73	0.52	--
Valine	1.979	0.42	0.51	0.18	0.16	0.15	0.49	0.77	1.37	1.39	1.3	1.15	1.17	5.0
Phenylalanine	2.85	0.66	0.76	0.24	0.32	0.29	0.61	0.98	1.87	2.14	1.9	1.72	1.65	6.0
Lysine	1.74	0.7	0.81	0.35	0.37	0.29	0.67	0.88	1.26	1.37	1.26	1.09	0.97	5.5
Leucine	2.992	0.84	0.87	0.2	0.2	0.17	0.67	1.14	2.2	2.36	2.18	1.96	1.91	7.0
methionine	0.55	0.12	0.22	0.01	0.03	0.03	0.17	0.26	0.45	0.5	0.45	0.34	0.39	3.5
Histidine	1.29	0.44	0.45	0.14	0.16	0.12	0.32	0.5	0.94	1.01	0.96	0.84	0.87	--
Tyrosine	2.047	0.04	0.35	0.01	0.21	0.14	0.32	0.61	1.41	1.74	1.43	1.05	1.31	--

*Provisional amino acids pattern recommended by FAO/WHO [30]. ND: Not determined.

pHs 3, 4, 5 and 6, where about 6g phenolic compounds /100g meal are extracted (column 1). It is worthy to mention that at these pH values very little protein is extracted or lost at these pH values. Column 3 is the phenolic compounds extracted after the hydrolysis of the precipitate or residue, perhaps these were the bound phenolics that were released upon hydrolysis. Column 4 represents the bound phenolic to other compounds than proteins. Shahidi and Naczk [28] stated that phenolic compounds also bind to carbohydrates and minerals and other food constituents. Haslam *et al.* [7] reported that the interaction of polyphenols and protein may be either reversible or irreversible. Irreversible complexes are usually formed auto catalytically in the presence of oxygen or in the presence of polyphenoloxidases [29] and can lead to the formation of clearly defined new products, as apparent in the case of black tea. Column 4 in Table 3 represents probably these new products. The phenolic compounds extracted by hydrolysis or NEPP in column 3 Table 3 are probably the reversible protein: polyphenol complexes formed according to Haslam et al. [7]. Going back to Table 2 and looking at Table 4 which is the amino acid composition of the PM protein, as well as that of the supernatants resulting at different pH values.

Lysine and the sulphur amino acids are always the concern of scientists working with plant proteins because they are the more important of the essential amino acids since in most of the cases one of them is probably limiting. It is clear from Table 4 that both lysine and sulphur amino acids are limiting. Yet by looking at the table it can be seen that the supernatant resulting from extraction at pH values 8, 9 and10 contain more lysine 1.26, 1.37,and 1.26 g/100g meal, respectively, compared to other pH values, PM contain 1.74 g/100g meal. While, the FAO daily requirements for lysine is 5.5 g/100g. Methionine content was highest at pH values 8, 9 and 10 leveling to 0.45, 0.5 and0.45 g/100g meal, respectively, compared to other pH values, PM contain 0.55g/100g meal. FAO daily requirements for methionine and cysteine are 3.5g/100g meal. Kholeif [31] reported peanut protein to be mainly deficient in methionine, tryptophan and cysteine. The results in Table 4 also indicate that glutamic acid, aspartic acid and arginine are the predominant amino acids. Kholeif [31] also reported arginine and histidine to be abundant compared to egg protein. Abduualrahman [32] found that the amino acid composition revealed that peanut protein was superior with respect to phenylalanine, leucine, isoleucine and valine when compared to FAO requirements. The controversy in the literature regarding the amino acid composition of peanut protein is probably because the difference in types, the land types the type of irrigation and the climate.

Going back to results in Table 4 the difference between the amino acid content of the meal and that at different pH values might be due to the binding of phenolic acid to the protein via the amino acids. Charlton *et al.* [8] reported that early studies of polyphenol/protein binding suggested that polyphenols bound preferentially to proline residues andthat results show that proline is an important binding site, but interactions also occur with arginine and phenylalanine side chains. Rutkowski *et al.* [33] and Shahidi and Naczk [28] stated that products of enzymatic and non enzymatic oxidation of phenolics in seeds, meal or flour may readily react with the å-NH_2 group of lysine and CH2S group of methionine of enzymes or other proteins to form complexes, thus rendering them unavailable to monogastric animals.

In conclusion this study revealed that highest solubility of free phenloic compounds in peanut meal occurred at alkaline pHs, while least solubility is at and the IEP of the protein. Highest binding of proteins with phenolics takes place at or near the IEP of the protein.

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