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Isolation of Protein from Defatted Peanut Meal and Characterize their Nutritional Profile

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Abstract Peanut contains high amount of protein (26%) and protein content increased in the groundnut meal after extraction of oil from it. The proximate analysis defatted peanut meal indicated that it contains 47–55% high quality protein. Peanut protein was isolated from defatted peanut meal by alkaline isoelectric precipitation and physical separation procedures. Peanut protein isolates had a solubility profile similar to that of peanut flour, with minimum solubility observed at pH 3.5–4.5 and maximum solubility at pH 10 and higher. Roasting of peanut reduced all functional properties of defatted peanut flour. Results suggested that peanut protein isolates could be used in food formulations, but would not be suitable for applications requiring high water retention and foaming capacity. It could be a good source of protein fortification for a variety of food products for protein deficient consumers in developing countries as well as a functional ingredient for the peanut industry. The production of peanut protein isolates could also add value to defatted peanut flour, a low value by-product of peanut oil production.

Keywords peanut, protein, isolation, solubility, nutrition

1. Introduction

Groundnut is a rich source of protein with high biological value. However, groundnut is highly digestible both in raw and roasted form [1]. Groundnuts beat most of the snack foods in terms of protein content; as a result, they are widely used as an alternative meat source for many vegetarian recipes. Groundnuts contain 13 different vitamins (vitamin A, vitamin B-complex and vitamin E) along with most of the trace minerals (calcium, zinc and iron). It also contains low amount of salt (1.4 mg per 25 g serve).Most of the peanuts grown in the world usually used for oil production, peanut butter, confections, roasted peanuts and snack products, extenders in meat product formulations, soups and desserts [2].

A lot of by-products are generated in the process of peanut harvest and peanut oil extraction, which are treated as potential pollutants. Nonetheless, only a few of these by-products are used as animal feed and fertilizer. A large portion of peanut meals after oil extraction yielded defatted peanut flour (DPF). DPF is a protein-rich, inexpensive and underutilized by-product from the peanut industry, offering the health and dietary benefits of peanut with less fat [3]. DPF contains 47–55% high quality protein with essential amino acid [4] and lends itself being used in many food applications [5]. Peanut protein isolate (PPI) could develop from DPF for the new product formulation and protein fortification.

Functional properties of proteins are important in food processing and product formulation. Some of these properties are water/oil binding, emulsification, foam formation, viscosity and gelation. Functional properties of protein are influenced by many factors such as pH, temperature and ionic strength. Isoelectric precipitation, alcohol precipitation and hot water extraction are frequently used to develop plant protein isolate/ concentrate. Functional properties of soy protein most extensively studied such as low degree hydrolysis (2–4%) by endo-protease treatment of soy protein resulted in enhanced functional properties of soy flour [6]. Thermal treatments including partial domination with mild hydrolysis of protein was reported to increase the solubility of soy protein, thus enhanced the functional properties of protein such as emulsifying and foaming capacity [7]. A similar process was also found to enhance the solubility and functional properties of wheat protein isolate [8]. Functional properties of other plant protein concentrates/isolates produced from the peas and beans were also studied by a number of investigators [9-11]. Functional properties of peanut protein have been subjected to limited studies that focused mainly on peanut flour [5]. However, limited information is available in the literature on the development and functionality of peanut protein isolate (PPI) as affected by processing. Thus, the objectives of this study were to isolation of protein from defatted peanut meal, to evaluate the effects of solvents, flour to solvent ratio, pH, temperature and time on the production of peanut protein isolates and to determine the functional properties of the protein isolates (raw and roasted peanut) as indicators of its potential uses.

2. Materials and Methods

2.1. Materials

Raw peanuts were collected from the local market of Mymen singh, Bangladesh. Some portion of peanut was roasted and the rest of the peanuts kept raw. Instruments such as trays, grinder, balance, spatula, crucible, desiccators and centrifuge, chemicals (n-Hexane, sulfuric acid, hydrochloric acid, sodium hydroxide, potassium hydroxide, sodium chloride and sodium bi-carbonate) were supplied by the department of Food Technology and Rural Industries.

2.2. Preparation of defatted peanut flour

Peanuts were soaked in 0.5% sodium bicarbonate solution at the 30^{o}C for 24 h in a water bath (JSSB-30T, Korea). The ratio between peanuts and water for soaking was 1:2 [12-13]. The peanuts were drained well afterwards. After discarding the water, the soaked peanuts were dehulled completely by floatation technique through hand rubbing and dried at 60^{o}C for 12 h in cabinet dryer (Modern Laboratory Equipment Co. Inc., Model 1816, USA). The dried peanuts were then cooled at room temperature (25^{o}C) and grind to fine powder using a grinding mill (BUHLER, Model – TII204, Swizerland). The peanut powder then defatted by using a cold extraction for 72 h with petroleum ether followed by n-Hexane for another 24 h. The ratio between of peanuts and solvent was 1:4(w/v). The solvent was changed every 12 h interval. The defatted peanuts were washed at the end with n-Hexane thrice and evaporate the solvent. The peanut powder and defatted peanut meal were analyzed for moisture by Ranganna [14] and protein, fat, crude fiber and ash content as per the methods of AOAC [15].The carbohydrate content of the samples was determined as the total carbohydrate by difference that is by subtracting the measured protein, fat, ash and moisture from 100 [16].

2.3. Extraction of protein from defatted peanut meal

The protein from the defatted peanut oil cake obtained by alkaline extraction at room temperature by using different pH from 4 to 10 [17]. Fifty gram (50 g) of different fractions of defatted groundnut cake and 1 L of water was used along with NaOH (0.2 M)/KOH (0.2M)/NaCl/$NaHCO_3$/deionised water were used for the various extraction. The mixture was stirred at 1200 rpm for 1 h at 30°C and subsequently centrifuged at 3000 rpm for 20 min to remove the insoluble residues. The supernatant was collected and the pH was adjusted to 4.5 with 1N H_2SO_4 to precipitate the proteins. The precipitate was creamy white in color. Further, it was centrifuged at 5000 rpm for 15 min to recover the proteins and was washed repeatedly with water to free it from acid tinge later. It was neutralized to pH 7 using sodium salt. Finally, the proteins were air dried at 40^{o}C for overnight in cabinet dryer (Modern Laboratory Equipment Co. Inc., Model 1816, USA).

2.4 Effect of temperature and time on the yield of peanut protein isolate

The effect of temperature and time on the protein yield was determined by modified method Kain et al. [18]. The effect of different temperature on the yield of protein isolate was evaluated by using a water bath (JSSB-30T, Korea) and set a thermostat in it. The temperature should control during the extraction process. Temperature levels considered in the evaluation of the effect of temperature on protein yield were: 20, 30, 40, 60, 70 and 80°C for 20 to 120 min.

2.5 Effect of flour-to-water ratio on the yield of peanut protein isolate

The flour-to-solvent ratios used were 1:5, 1:10, 1:15, 1:20, 1:25 and 1:30. Extraction was carried out at the optimum pH of 10.0 and a temperature of 40°C for 60 min. One molar NaOH and 1M HCl were used for all pH adjustments [18].

2.6. Determination of functional properties of peanut protein isolates

2.6.1. Protein solubility

Defatted peanut flour was mixed with water at the ratio of 1:20 (w/v) and adjusted the pH 2.0–10.0 with 1 N sodium hydroxide (NaOH) and HCl. The suspension was stirred at room temperature for 1 h followed by centrifugation at 3000 rpm for 15 min. Protein concentration in each supernatant (soluble protein) was determined by adopting AOAC [15] method. The soluble protein content was calculated as gram soluble protein per 100 g flour based on the weight of flour used and supernatant obtained after centrifugation.

2.6.2. Water holding capacities

Water holding capacity was determined according to the modified method described by Beuchat [19]. One gram (1 g) of peanut flour or protein concentrate was weighed into the centrifuge tubes. Ten milliliter (10 ml) distilled water was added to the tube and mixed it properly using vortex for 2 min. The mixture was allowed to stand for 30 min at room temperature followed by centrifuged at 3000 rpm for 20 min. The supernatant was decanted and the centrifuge tube containing sediment was weighed. Triplicate samples were analyzed for each protein concentrate.

$$WHC = \frac{(W_1 - W_2)}{W_0} \qquad \text{(Equation 1)}$$

Where, WHC= Water holding capacity (grams of water per 100 g of protein)

W_1= Weight of the tube plus the dry sample (g), and

W_2= Weight of the tube plus the sediment (g)

W_0 = Weight of the dry sample (g)

2.6.3. Oil absorption capacities

Oil absorption capacity was determined according to the modified method described by Chakraborty [20]. One gram (1 g, W) of protein concentrate was weighed into the centrifuge tubes. Ten milliliter (10 ml, V_1) of vegetable oil was added to the tube and mixed it properly using vortex for 2 min. The protein-oil mixture was allowed to stand for 30 min at room temperature followed by centrifuged at 3000 rpm for 20 min. The supernatant was transferred into a 10 ml graduated cylinder (V_2) and the volume was recorded. Triplicate samples were analyzed for each flour/protein isolates.

$$OAC = \frac{(V_1 - V_2)}{W} \qquad \text{(Equation 2)}$$

Where, OAC= Oil absorption capacity (milliliter of oil per 100 g of protein)

2.6.4. Gelation capacity

Gelation capacity was determined according to the modified method described by Coffman and Garcia [21]. One gram (1) sample was taken in a test tube and 5 ml distilled water was added to the tube. The sample was heated at 90^oC for 1 h in a water bath (JSSB-30T, Korea). The suspensions were cooled rapidly under running tap water to 10 ± 2^oC. The gel strength was determined by inverting the tube. For determining the least gelation concentration, appropriate sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) were prepared. The least gelation concentration was found from the inverted test tube which did not fall down or drop.

2.6.5 Foaming capacity and stability

Foaming capacity (FC) was determined by using the method described by Makri et al. [22]. Concentrations of 1% flour were prepared in de-ionized water and adjusted to pH 7.4 with 1.0 N NaCl and 1.0 N HCl. One hundred (100

ml, V_I) peanut protein concentrate suspension was blended for 3 min using a high-speed blender. The suspension was immediately transferred into a 250 ml graduated cylinder and the volume of foam (V_F) was recorded.

$$FC = \frac{V_F}{V_1} \qquad \text{(Equation 3)}$$

3. Results and discussion

3.1. Chemical Composition of peanut and defatted peanut meals

Peanut contained very high amount of fat, protein and crude fiber. Table 1 shows the chemical compositions of peanut seed. Table 2 shows the chemical composition of defatted peanut meals. Defatting process leads to the removal of the maximum amount of fat content from the peanut meal. The fat content of the defatted meal was found 1.35±0.35%. The fat content of the defatted sesame meal by solvent extraction method was found 1.10% [23].

Table 1: Chemical composition of peanut and defatted peanut meals

Components	Amounts (%)	
	Raw peanut	Defatted peanut meals
Total solid	92.40±1.0	93.95±1.5
Fat	45.80±1.5	1.35±0.3
Protein	24.50±1.2	53.02±1.3
Carbohydrate	16.50±1.5	33.54±1.0
Ash	2.20±1.0	2.50±0.5
Crude fiber	3.19±0.8	3.78±0.7

3.2. Proximate composition of peanut protein isolates

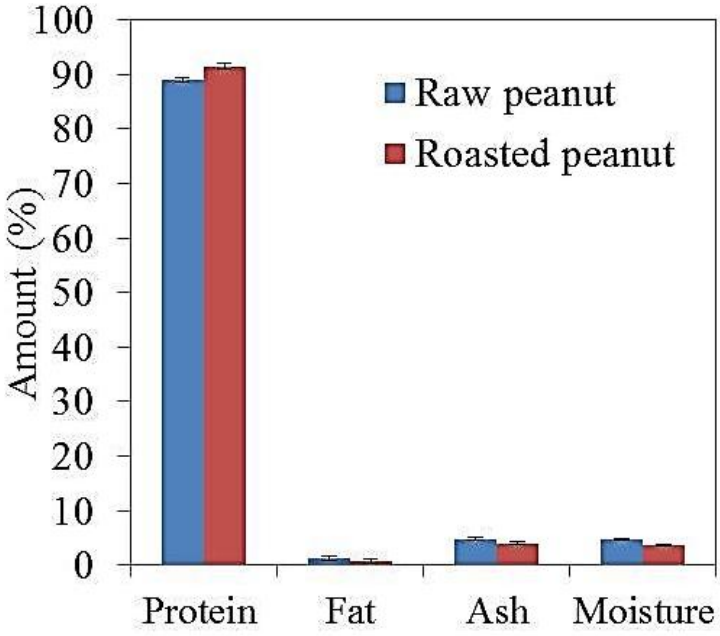

Figure 1: Chemical composition of peanut protein isolates. Bar represents standard deviation

Figure 1illustrates shows the chemical composition peanut protein isolates. Peanut protein isolates from roasted peanut flour had significantly ($p>0.5$) higher protein (91.5%) content and lower amount of fat, ash and moisture content. This study is accordance with the study where the isolated peanut protein has protein content higher than 90% from peanut flour after defatting using hexane [24].

3.3. Isolation of protein from defatted peanut meal

3.3.1. Effect of solvent on protein extraction

Table 2 shows the effect of solvent on the protein recovery from defatted meals. Protein recovery mainly depends on the pH and types of solvent. Maximum amount of protein was recovered by NaOH at pH 9.0 (Table 2).

Table 2: Effect of different solvent on protein yield

Extractant	pH of Dispersion	Recovery of proteins, %
Deionizedwater	7.0	70.4^{d}±0.7
NaCl, 0.5%	7.2	30.0^{e}±0.5
$NaHCO_3$, 0.2M	8.0	75.0^{c}±0.3
NaOH, 0.2M	9.0	85.2^{a}±0.5
KOH, 0.2M	9.3	80.1^{b}±0.8

3.3.2. Peanut protein solubility

Protein solubility is the most important functional property due to its ability to influence the other functional properties of peanuts. Roasting has the desirable and undesirable effects of peanuts and peanut protein. Heating destroys anti-metabolites such as trypsin inhibitor in beans and nuts [25] and amylase inhibitors in legumes, thus improving the bioavailability or digestibility of the protein [26].Roasting also added pleasant flavor/aroma of peanuts and makes peanut palatable. However, roasting may significantly affect the functionality of peanut flour because of partial denaturation of protein. Roasting of peanuts significantly decreased protein solubility in peanut flour in the pH range 3.5–10.0 compared to that in raw peanut flour (Figure 2). Heating of peanut in water at 100–120^{o}C for 15 min decreased the protein solubility [27]. This might be due to the increase of surface hydrophobicity of protein via unfolding of molecules upon heat. The pH had a significant effect on the solubility of peanut protein. The minimum protein solubility was observed at pH 3.5–4.5 and maximum solubility at pH 10 or higher (Figure 2). The extraction of peanut protein was, therefore, conducted at pH 10 as to ensure maximum yield. The use of pH higher than 10 was not desirable because of undesirable changes such as protein discoloration which could affect the functionality and sensory quality of peanut protein concentrate. At pH 4.0 separation of protein from the supernatant through isoelectric precipitation results least solubility of protein. The solubility pattern of peanut protein was found similar to that of soy protein [28], suggesting possible similarities in functional properties and protein composition of these two plant proteins. In fact, the amino acid profiles of peanut protein and soy protein are comparable with the exception of lower lysine level in peanut [4]. Results suggest that protein solubility, to a greater extent, was pH dependent with the lowest solubility was observed for both raw and roasted peanuts at the isoelectric point of pH around 4.0. Protein solubility reduced as pH increased until reached an isoelectric point. At pH above the isoelectric point an increase in protein solubility was observed.

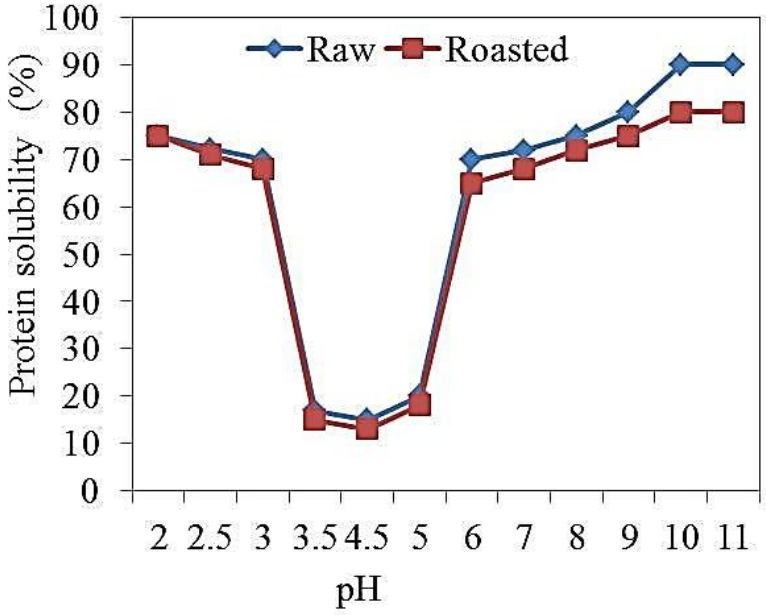

Figure 2: Effect of pH on the protein solubility. Bar represent standard deviation.

At low pH, the carboxyl and amino groups from amino acid chains are protonated in the -COOH and $-NH_3^+$ forms, respectively and the overall charge of most protein molecules are positive. As the pH increases some of the carboxyl groups are dissociated into $-COO^-$ and H^+ and the positive charges associated with the proteins diminish up to the isoelectric point and neutralized [27]. As a result, the protein cannot be hydrated by water due to the changes in its tertiary and quaternary structures, thus, solubility reaches a minimum value. Increasing the pH, the amino groups dissociate into $-NH_2$ and H^+ and the overall protein charge becomes negative due to the presence of $-COO^-$ groups and can consequently be hydrated and dissolved in water. A further increase in the pH, electrostatic repulsion improves and this enhances solubility of protein.

3.3.3. Effect of flour to water ratios on peanut protein yield

Flour to water ratio used in protein extraction also significantly affected the efficiency of protein recovery with maximum recovery achieved at flour to water ratio of 1:20 (Figure 3). There was no significant difference between 1:50 and 1:60 flour to water ratio. Therefore, the 1:20 ratio was used for peanut protein extraction during development of peanut protein isolate.

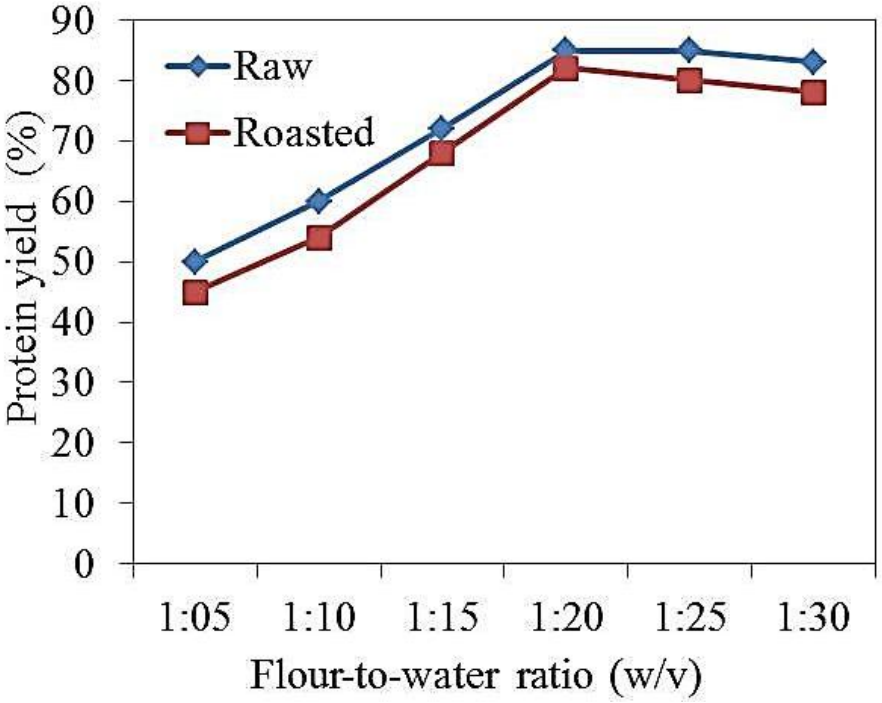

Figure 3: Effect of flour to protein ration (w/v) on the protein yield. Bar represent standard deviation.

Results observe that increase in sample to water ratio above 1:10 had minimal effect on the yield recorded for raw and roasted peanuts. Lower flour to water ratio below 1:10 demonstrated minimum protein extraction yields. The dissolution and/or diffusion kinetics should follow for the extraction of proteins. This kinetics of governing the extraction by a driving force related to the gradient of the component concentration between the solid and liquid phases. The concentration of the material in the liquid at equilibrium could be related to the solid phase by a partition coefficient [29]. However, there was no significant difference between sample ratios of 1:20, 1:25 and 1:30; therefore 1:20 was chosen for optimum extraction.

3.3.4. Effect of pH on peanut protein yield

Figure4 illustrates that lowest yields of proteins were recorded at the isoelectric point of pH 4.5. A gradual increase in yield of protein was observed between pH 5 and 10.Nonetheless, the solubility profiles of protein are pH dependent with minimum solubility in both samples observed between pH 4.5 and 5.5 which is expected as it falls within the precipitation pH range[30]. Both raw and roasted peanuts were recorded high solubility at pH levels of 9 and 10.The solubility of protein isolates, protein concentrate and protein hydrolysate is of great importance in liquid

protein supplements. Hence, an important characteristic of protein isolates used for some food applications is solubility at an appropriate pH to the food or beverage.

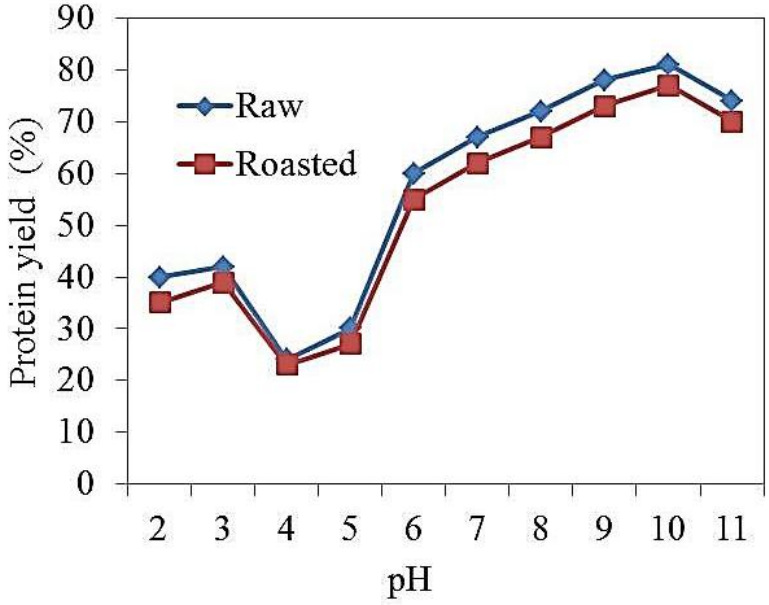

Figure 4: Effect of pH on the protein yield. Bar represent standard deviation

3.3.5. Effect of Temperature and Time

The protein extraction for both raw and roasted peanuts was temperature dependent. Maximum yields of protein were obtained at 40°C. Higher the temperatures (above 40°C) lower the extraction of protein yields (Figure 5). This might be due to the thermal degradation of the proteins. Insoluble aggregations might be formed with sulphur-rich proteins in soybean flour when heated to 70°C and above [31]. However, optimum extraction time for maximum protein yield was around 60 min. nonetheless, increasing the extraction times beyond 60 min suggested lower extraction yields for raw and roasted peanuts (Figure 5).

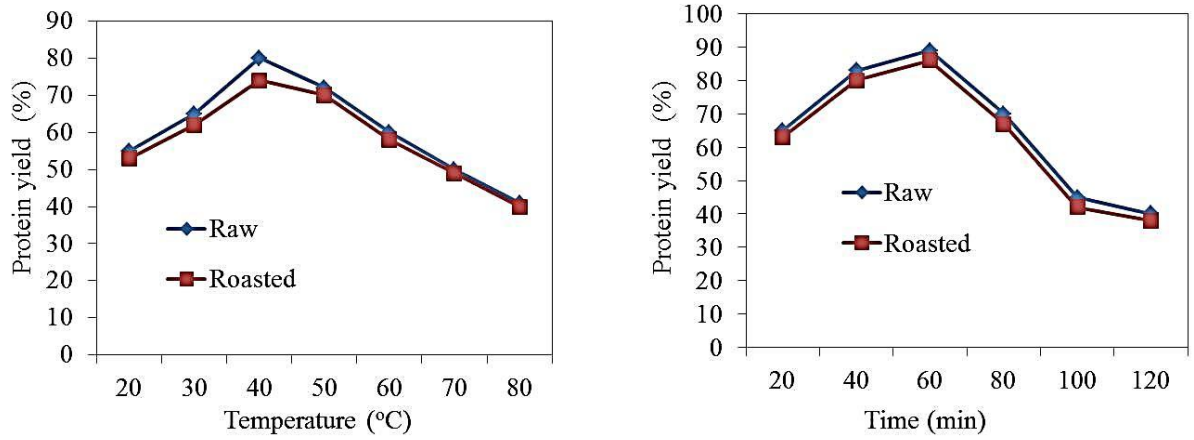

Figure 5: Effect of temperature and time of the extraction process on protein yield. Bar represent standard deviation

3.5. Functional properties of protein isolates

3.5.1 Water/oil holding capacity

Interactions between water-protein and oil-protein are very important in food systems due to their effects on the flavor and texture of foods. Intrinsic factor affecting the water binding capacity of food proteins includes amino acid composition, protein conformation, and surface polarity/hydrophobicity [32]. However, food processing techniques

show important impacts on the protein conformation and hydrophobicity. Water holding capacity and oil absorption capacity both were significantly higher in raw peanut protein isolates (Table 3). This result suggested that during roasting peanut proteins were denatured by high temperature, thus, exposing more hydrophobic sites resulting the reduced water retention capacity of peanut protein. The reduced oil absorption capacity might be due to irreversible denaturation of protein caused by roasting at 175^{o}C. This high temperature treatment might have destroyed both hydrophilic and hydrophobic groups of peanut proteins, thus, reducing both water and oil holding capacity.

3.5.2. Foaming capacity (FC)

The formation of foam is analogous to the formation of emulsions. The mechanism behind the foam formation is water molecules surround air droplets and air is in the non-polar phase. Theoretically, the amphipathic character of protein makes them the good foaming agents that work at the air–water interface to prevent bubble coalescence. However, defatted peanut flour is not a good foaming agent, with a foaming capacity of only 6 ml/100 ml liquid (Table 3), whereas, roasted peanuts showed half of the raw peanuts foaming capacity. Therefore, defatted peanut protein isolates may not be suitable in the food system that requires foaming such as cake and ice cream. Overall, roasting decreased functionality of peanut protein isolates while fermentation significantly increased all functional properties of both raw and roasted peanut flours.

Table 3: Effect of roasting on functional properties of peanut flour

Functional properties (ml/100g)	Raw peanut	Roasted peanut
Water binding capacity	190	170
Oil binding capacity	375	250
Foaming capacity	6	3
Gelation at 12% suspension	Very weak gel	Very weak gel

Conclusion

Higher amount of solubility of the protein isolates both in alkaline and acidic pH suggests that protein isolates from peanuts can be very useful for the formulation of protein rich beverage and bakery food products. Although foaming and gelation capacity is very weak in the peanut protein isolates, high protein solubility indicates that they have promising food applications. Optimum conditions for protein extraction include pH 10.0, temperature 40°C, extraction time of 60 min and flour-to-water ratio of 1:20. Further research should be carried to determine different drying effect such as spray drying, drum drying and vacuum drying on the functional property of isolated protein. It also observes how fermentation of peanut meal effects on protein production and functional property of peanut protein isolates.

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