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Full Length Research Paper

Determination and isolation of protein from different fractions of defatted groundnut oil cake

Kalpana Devi B.[1], Vidhya R.[2]* and Jaganmohan R.[2]

[1]Dr. Mahalingam Centre for Research and Development, Pollachi, India.
[2]Department of Food Product Development, Indian Institute of Crop Processing Technology, Thanjavur, Tamilnadu, India.

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Groundnut is the major oilseed in India. Groundnut has a high amount of protein (26%), and it is a good source of calcium, phosphorus, iron, zinc and boron. After extracting oil, the cake was only used as cattle feed or livestock, hence in the present study protein was isolated by different fractions of defatted groundnut oil cake because of its higher protein content. Sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) used to isolate the protein, among the three British Standard Sieves (BSS), 30 BSS, 52 BSS and 72 BSS had protein content of 14.3, 15.3 and 16.9%, respectively. Low fat content of 8.3% was observed in 52 BSS, which showed higher fiber content of 14.3%. BSS of 72 showed higher fat content of 10% and lower fiber content of 6%. The moisture content of 52 BSS was found to be 5.4%, and 72 BSS and 30 BSS found to be 5.8 and 4.4%, respectively.

Key words: Protein isolation, sodium dodecyl sulphate polyacrylamide gel (SDS PAGE), British standard sieves.

INTRODUCTION

Groundnut or peanut is commonly called the poor man's nut. Groundnut is native to South America and has never been found uncultivated. The botanical name for groundnut, *Arachis hypogaea* Linn, is derived from two Greek words, Arachis means 'a legume' and hypogaea means 'below ground', referring to the formation of pods in the soil. Groundnut is an upright or prostrate annual plant. It is generally distributed in the tropical, sub-tropical and warm temperate zones. Ethnological studies of the major Indian tribes of South America document the widespread culture of groundnut and provide indirect evidence for its domestication long before the Spanish conquest. When the Spaniards returned to Europe they took groundnuts with them. Later traders were responsible for spreading the groundnut to Asia and Africa where it is now is grown between the latitudes 40°N and 40°S.

Groundnut is a source of energy due to its high oil and protein contents. It supplies about 5.6 calories grain, when consumed raw and 5.8 calories grain when consumed roasted. Groundnut is a rich source of protein. Nutritive value is not high because of deficiency in content of certain essential amino acids- methionine, tryptophan, lysine and threonine. It is also a rich source of amino acids and vitamins. Biological value is 55%, protein efficiency ratio: 1.65%, net protein utilization: 43%. Groundnut has good digestibility in both raw and roasted forms (Nagaraj, 1988).

Demand for protein in world is increasing hence more food protein is required from both conventional and new sources of protein. It was very important for protein biochemist to understand what functional properties are and how they can be improved in both existing proteins and new proteins (Abdel Rahman, 1982).

Protein is required for the growth, maintenance and repair of human body tissue, and is one of the essential building blocks of life. Groundnuts beat most snack foods when it comes to protein content. In fact, they have such a high protein content that they are widely used as an al-

*Corresponding author. E-mail: rv_teddy@yahoo.co.in.

ternative to meat in many vegetarian recipes.

According to the latest research, a diet rich in groundnut products can reduce the cholesterol, lower the risk of heart disease and provide protection against cancer. Groundnuts contain 13 different vitamins (including A, the B group and E) along with 26 essential trace minerals, including calcium and iron. Groundnuts also contain zinc, good for protecting brain function, and boron, which can help to maintain strong bones. Groundnuts have a very low salt content-usually only 1.4 mg per 25 g serve.

In groundnut, the proteins are very poorly defined and are contained mainly in two major globulin fractions, arachin and con arachin, which together constitute 97% of the protein of the meal. Each of these globulins consists of several polypeptide species, which are thought to exist together as subunits of large multimeric complexes. In addition, many of the polypeptides appear to have similar molecular weights (Basha and Cherry, 1976).

Groundnuts could help us to loss weight by suppressing hunger naturally. Some nutritional researchers now believe that fat in the small intestine stimulates the release of a chemical transmitter that signals feelings of fullness to the brain, which in turn suppresses hunger. Modern science has now demonstrated that groundnuts are one of the most complete nutritional resources available.

Utilization of groundnut in various food formulations

Groundnut is a good source of oil and protein. The pleasant aroma, nutty flavor and smooth texture of roasted groundnut have found great acceptance (Wood, 1983). In India, edible groundnut flour is used in developing a variety of inexpensive food formulations such as multipurpose food, fortified flour, paustic atta, malted food, chewy candies and high protein biscuits. Groundnut protein can be incorporated into a variety of food products without serious problem in terms of color and flavor. It has unique functional properties. It can be blended with other protein source or fortified with necessary nutrients. Protein isolate has been used in manufacturing of cheese analogues and frozen desserts (Lawhon et al., 1980).

Defatted groundnut oil cake

Defatted groundnut oil cake is a byproduct obtained after extraction of oil. The expeller cake generally contains about 6% oil. The residual oil is recovered through solvent extraction to obtain defatted cake. Defatted cake contains 45 to 60% protein, 20 to 30% carbohydrate, 3.8 to 7.5% crude fiber and 4 to 6% minerals.

Groundnut oil cake showed as a good source of lipid and protein, and the defatted cakes could be used as a protein supplement in human nutrition. Groundnut flour is widely used for culturing molds in the manufacture of antibiotics. The groundnut oil cake contains about 1 to 4% phosphoric acid and 1 to 2% potash, which make it as good organic manure.

The protein content of groundnut ranges from 22 to 32%, depending upon the variety, location and year (Young and Hammons, 1978). Groundnut consists mainly of two globulins namely arachin (93% of defatted seed protein) and co-arachin. The vast food preparation incurporating groundnut to improve the protein level has helped in no small way in reducing malnutrition in developing countries. Groundnut protein is increasingly becoming important as food and feed source, especially in developing countries where protein from animal source are not within the means of the majority of populace.

The major groundnut producing countries of the world are China, India, Nigeria, U.S.A, Indonesia, Argentina, Sudan, Senegal, and Myanmar. These countries account for nearly 70% of production of groundnut. India occupies a prominent position both in area and production of groundnut in the entire world. Of the 7.3 million tons of India's annual groundnut production, about 5 million tonnes are crushed in expeller press to obtain crude edible oil and partially defatted cake contains 35 to 45% proteins, as a byproduct. Over 2 to 2.5 million tonnes of such cake having a potential of about 1 million tonnes of dietary proteins is produced annually as a byproduct in India.

Being unfit for human consumption, it was used mostly as cattle feed while over 20% is exported. The defatted ground nut cake has a rich source of protein and used as a cattle or manure. Attempts have been made to remove pigments and fiber from the market grade cake to obtain high protein edible grade meal (Chavan et al., 1991).

MATERIALS AND METHODS

Collection of materials

The basic material used for the study namely groundnut oil cake *(Arachis hypogaea* L.) was procured from the local market near Thanjavur. Groundnut oil cake is a byproduct obtained after the extraction of oil.

Preparation of defatted groundnut flour

The groundnut oil cake was finally powdered using mixer juicer. It was dried in hot air oven at 60°C for overnight sieved by three different mesh screens, like 30, 52 and 72 BSS. These three different fractions are taken for further process. The works were done in triplicates to concordance in results.

Protein by Kjeldahl method (AOAC, 2000)

Digestion

Place weighed test portion (0.7 to 2.2 g) in a digestion flask (Gerhardr-Turbotherm). Mix with 0.7 g mercuric oxide, 10 g of powdered potassium sulphate or anhydrous sodium sulphate, 1 g of copper sulphate and 25 ml of concentrated sulphuric acid. Place the digestion tubes in digestion chamber and digest the sample until solution clears (2 h for test portions containing organic material). Turn the digester off and remove the tubes. Cool it by adding ice water.

Distillation

Place 40% sodium hydroxide solution in an alkali tank of distillation unit (Gerhardt-Vapodest 20), connect the digestion flask to distilling bulb on condenser and with the tip of the condenser immersed in a standard 4% boric acid solution and add 5 to 7 drops of mixed indicator. Collect the steam of distilled after the distillation completed. Remove the receiving flask. Titrate against standard acid solution. Correct for blank determination on reagents.

Hexane extractable compounds (Oil)

5 g of sample was taken in a thimble. This was extracted in soxtherm fat extractor with hexane for 6 h. After extraction was completed, the oil flask was dried in an air oven for 3 h at 100 to 105°C. Cooled in a dessicator and weighed.

Oil (%) = weight of oil in flask/weight of the sample taken × 100

Crude fiber content (AOAC, 2000)

4 g of defatted sample was weighed and added to 200 ml of 1.25% sulphuric acid held in a 500 ml beaker and glass rot should be tipped in the beaker and boiled for 30 min on a hot plate. Any loss in volume during boiling was made up with distilled water. The solution was filtered and the residues were collected and rinsed with distilled water. To the residue, 200 ml of 1.25% sodium hydroxide was added and boiled for 30 min. The liquor was filtered through a cotton cloth and the residue washed with distilled water until the washing was no alkaline. The residues was dried at 105°C for 3 h and weighed again.

Crude fiber (%): = (B - A/S) × 100

Where, A: weight of empty dish; B: weight of fiber sample + dish; S: weight of defatted sample.

Moisture content

Heat low flat bottomed dishes at 100°C in hot air oven. Place on an asbestos sheet for 2 min and then transfer into a dessicator and leave for half-an hour. Record the weight in analytical balance. Repeat this procedure till constant weight is recorded (with maximum difference of 0.02 g). Weigh 5 g of the whey protein powder and transfer it to a dish and place in an oven thermostatically controlled at 100 to 150°C heat for a stipulated time of 10 to 12 h. Cool in a dessicator for half-an hour and weigh successfully till it shows no further loss.

Weight of the flat bottomed dish + lid = g
Weight of the flat bottomed dish + powder + lid before drying = g
Weight of the flat bottomed dish + powder + lid after drying = g
Weight of the moisture = g
5 g of powder contains 2 g of moisture
100 g of powder = Weight of the moisture/ Weight of the sample*100 = g of moisture.

Ash content (AOAC, 2000)

4 g of well mixed test sample was weighted into a shallow, relatively broad ashing dish (silica crucible), and it was ignited in a furnace at approximately 800°C for 5 h (dull red) until light gray ash result or to constant weight. Then, it was cooled and weighted at room temperature.

Ash content (%) = (B - A/S) × 100

Where, A = weight of empty crucible silica; B = weight of silica crucible + ash; S = weight of sample.

Extraction of protein from groundnut oil cake

The protein of defatted groundnut oil cake was obtained by alkaline extraction at room temperature by varying the pH from 6.8 to 10 according to the method of Taha et al. (1987). For each extraction 50 g of different fractions of defatted groundnut cake and 1 L of water was used along with NaOH (0.2 M)/KaH (0.2 M) / Ca(OH)2/ NaCl/ NaHCO3/ deionised water as appropriate 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 carbohydrate residues. The supernatant was collected and the pH was adjusted to 4.5 with 1N H2SO4 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. The average yield of three replicate was reported.

Electrophoresis

SDS-PAGE of total seed protein was carried out in polyacrylamide slab gels in a discontinuous buffer system according to the method of Laemmle (1970). Vertical gel slabs were prepared in a glass sandwich which was tightened by a set of plastic clips lined with a band of foamed silicon rubber. Separation gel was put into the space between a set of glass plates (up to 2 cm from the top). Small amount of distilled water (120 µl) was added on separation gel gently to prevent gel surface from air and promote fixation. The setup was left for 30 min so that gel was fixed. The separating gels contained 15% by weight of acryl amide and 0.135% by weight of N.N-ethylene-bis-acryl amide in 1 MTris-HCl buffer (pH 8.8) with 0.27% SDS. The gels were polymerized chemically by the addition of 20 µl by volume of tetra methylethylene-diamine (TEMED) and 10% ammonium per sulfate (APS). During the fixation of separation gel, stacking gel was prepared. Stacking gel consisted of 4.5%. The stacking gel was polymerized chemically in the same way as for the separation gel. When separation gel was fixed, distilled water was removed from its top and stacking gel solution poured on it. Combs were fixed into the stacking gel. Combs were put with special care and it was confirmed that there was no air bubble at the bottom of the combs. The set up was left for 15 min so that the stacking solution became gel. Combs, clips and gaskets were removed from glass plates carefully and it was confirmed that there was no any air bubble at this stage. Gel plates were freshly used for electrophoresis but it was also possible that these would be wrapped in aluminum foil and could be used even for one week.

The electrode buffer contained Tris-glycine (9.0 g Tris HCl and 43.2 g glycine /3 L buffer solution at a pH 8.9) with 3.0 g (0.1%) SDS. 15 µl of protein supernatant were applied into the stacking gel sample wells with a micro syringe, followed by 20 µl of reservoir buffer containing bromophenol blue which served as the tracking dye. Electrophoresis was carried out at 70 mA until the bromophenol blue marker reached the bottom of the gel (approximately two and a half hour). In order to check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions.

Staining and destaining

After electrophoresis, the gels were stained with 0.2% (w/v) coomassive brilliant blue R250 dissolved in a solution containing 6% (v/v) acetic acid, 44% (v/v) methanol and water in the ratio of 6:44:50 (v/v) for 1 h. Gels were then destained by washing with a solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and water in the ratio of 5:20:75 (v/v) until the color of background disappeared and electrophoresis bands were clearly visible. After destaining, the gels were dried using Gel.

RESULTS AND DISCUSSION

Moisture content

Moisture contents of three different fractions (72 BSS, 52 BSS and 30 BSS) were 5.8, 5.6 and 5.4% respectively. All these three fractions of defatted Groundnut oil cake characteristically contain low level of moisture content. The low level of moisture content is an advantage when the shelf life is considered.

Fat content

Fat contents of three different fractions (72 BSS, 52 BSS and 30 BSS) of defatted groundnut oil cake were 10, 8.3 and 8.7%. Generally defatted groundnut oil cake had low level of fat content. Among these three fractions, 52 BSS has the lowest level of fat content because the sieve separate the large granules present in the sample. It could help in losing weight by suppressing hunger naturally. Some nutritional researchers now believe that fat in the small intestine stimulates the release of a chemical transmitter that signals feelings of fullness to the brain, which in turn suppresses hunger.

Fiber content

Fiber contents of three different fractions (72 BSS, 52 BSS and 30 BSS) of groundnut oil cake were 6, 14.3 and 9.6%. Dietary fiber is essential to the smooth functioning of our body's waste elimination process. Groundnut has good dietary fiber content so they are very good for our digestion and our bowels.

Protein content

Groundnut characteristically contains high level of protein. Defatted groundnut oil cake contains low level of fat and high rich protein. Protein content of three different fractions was 16.9, 15.3 and 12.3%. High protein of the defatted groundnut makes it good as cake for human consumption, and useful as animal feed.

Ash content

Ash contents of the three different fractions (72 BSS, 52 BSS and 30 BSS) of groundnut oil cake are 9, 11.2 and 10.6%. The ash content are similar to the reports of Nelson and Carlos (1995). The low ash content is indicative of low level of inorganic impurities and qualifies the oil as good source of mineral element.

Sodium dodicyl sulfate polyacrylamide gel electrophoresis (SDS PAGE)

The common molecular weight of groundnut protein is 30 to 34 KD. In order to obtain good resolution of protein of low molecular weight by gel electrophoresis, we used polyacrylamide gel of high density; such a gel system gave good resolution of proteins of low molecular weight but was not suitable for the identification of proteins.

Gel electrophoreses pattern of protein was extracted from defatted groundnut oil cake. Defatted groundnut oil cake contain high amount of protein. Three different British standard sieve fractions such as 72 BSS, 52 BSS, 32 BSS, are loaded in the gel. 72 BSS fraction of defatted groundnut oil cake contain low molecular weight protein. 72 BSS fraction of defatted groundnut oil cake contains 17% protein. Protein content is high in 72 BSS of groundnut oil cake; groundnut oil cake is mainly composed of low molecular weight.

In this study, different fractions of defatted groundnut oil cake had been investigated. All the three fractions are similar in their action. Defatted groundnut oil cake protein analysis by SDS PAGE has proved to be an effective way of revealing the differences and relationship between the three different fractions of British standard sieve proteins (Figures 1, 2, 3 and 4). The protein profile of different fractions of defatted groundnut oil cake observed in the present was compared with that of earlier findings. But in the earlier work, proteins were isolated from the embryo stages of groundnut (Roja-Rani and Venkateswalu, 2000). No other study has been reported in the different fractions of groundnut oil cake.

The present work was initiated to see the protein profile for three different fractions of groundnut. All these three were characterized by same banding pattern. In case of 72 BSS, it contained more band formation on comparing to the other picture BSS. The functionality of groundnut oil cake primarily depends on their fractions of the British Standards Sieve. The high protein 16.9% of 72 BSS of groundnut oil cake makes it good as cake for human consumption, and it also have the potential to add value to the groundnut industry and provide food processors. The nutritive value of peanut protein is much better than the wheat protein (Bodwell, 1979).

A different fraction of defatted groundnut oil cake characteristically contains low level of moisture content (Figure 5). The low level of moisture content is an advantage when the shelf life is considered. Also, the low ash content is inactive of low level of inorganic impurities and qualifies the oil as good source of mineral element.

The crude fiber in this result indicates the ability of groundnut to maintain internal clistention for a normal peristaltic movement of the intentional tract a physiological role which crude fiber plays, diets low in crude fiber is undesirable as it could cause constipation, and such diets have been associated with diseases of colon like piles appendicitis and cancer.

Modern science has now demonstrated that groundnuts are one of the most complete nutritional resources available; the fat content is important in diets as it promotes fat soluble vitamin absorption. It is a high energy nutrient and does not add to the bulk of the diet. Ground-

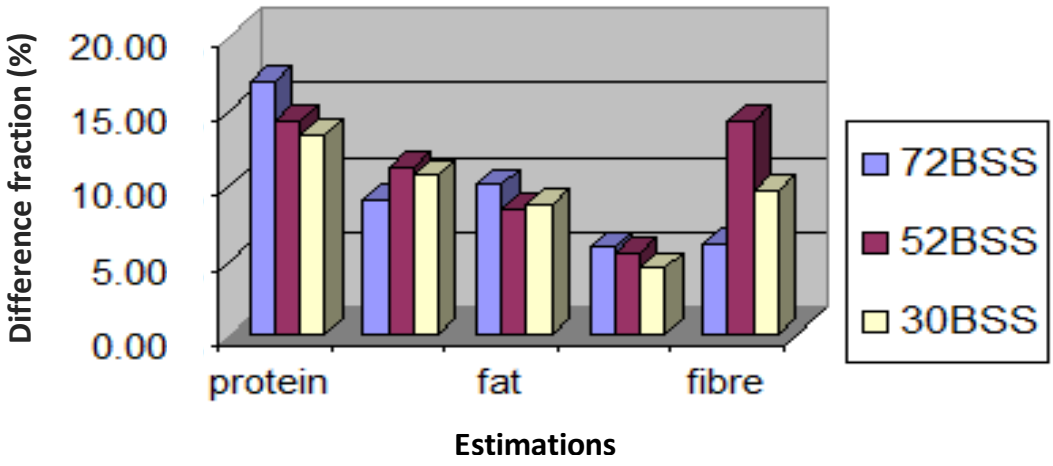

Figure 1. Proximate analysis of different fractions of defatted groundnut oil cake.

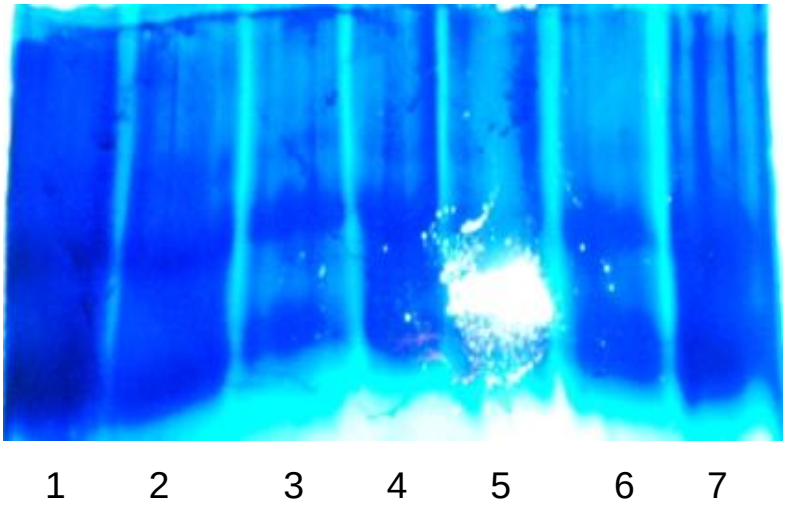

Figure 2. SDS PAGE pattern of protein extracted from 72 BSS fraction of defatted groundnut oil cake.

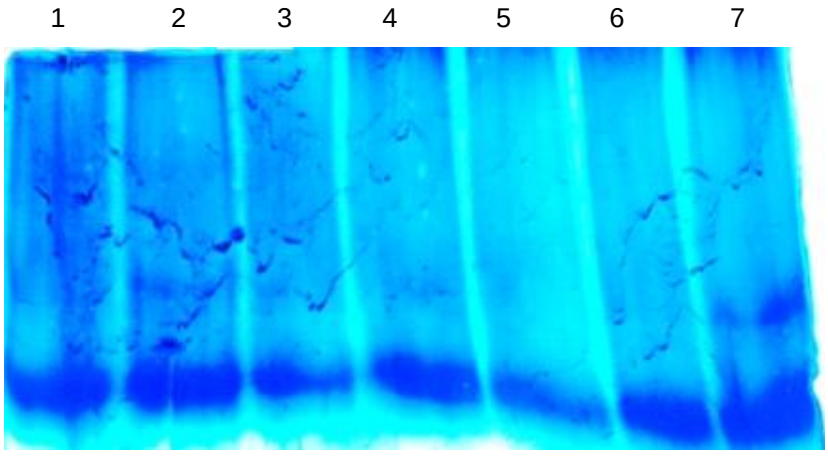

Figure 3. SDS PAGE pattern of protein extracted from 52 BSS of defatted groundnut oil cake.

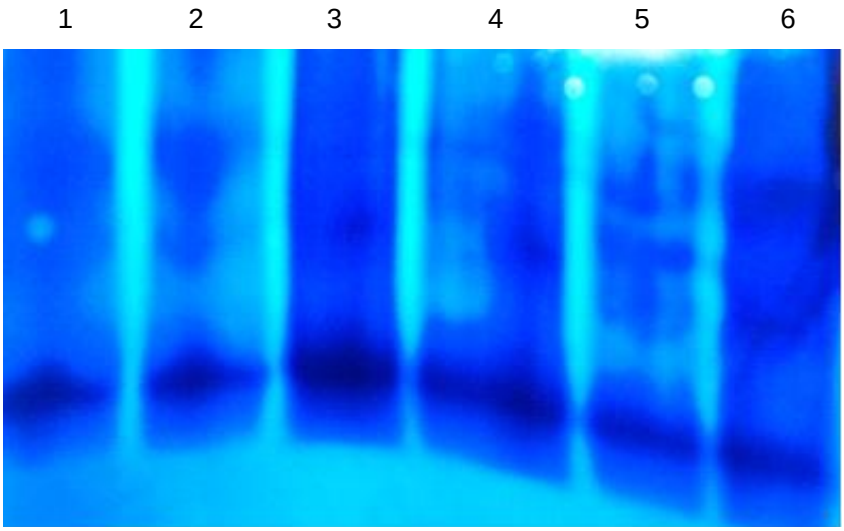

Figure 4. SDS PAGE pattern of protein extracted from 30 BSS fraction of defatted groundnut oil cake.

Figure 5. Picture of defatted groundnut oil cake.

nuts can help in weight loss by suppressing hunger naturally. Some nutritional researchers now believe that fat in the small intestine stimulates the release of a chemical transmitter that signals feelings of fullness to the brain, which in turn suppresses hunger.

This work has enabled us to conclude that the nutritive value of groundnut seeds can be considered as good source of protein. The defatted seeds can be used to prepare food, such as snacks parts, and used in diet to prevent against some mineral deficiency. This will aid to fight against malnutrition, especially protein calorie malnutrition leading to better nutrition and health (Atasie et al., 2009).

The development of groundnut flour from defatted groundnut meal 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 malnutrition remains

Table 1. Proximate composition of three different British standard sieve fraction of defatted groundnut oil cake.

Estimation	72 BSS	52 BSS	30 BSS
Protein (%)	16.9	15.3	14.3
Ash (%)	9	11.2	10.6
Fat (%)	10	8.3	8.7
Moisture (%)	5.8	5.4	4.4
Fiber (%)	6	14.3	9.6

a major health hazard, especially among children.

Conclusion

Defatted groundnut flour was prepared from groundnut seeds using standard procedures. Proximate composition (protein, fat, fiber, ash, moisture) of the defatted flour was studied. Defatted groundnut flour is sieved by three different mesh screens: 30 BSS, 52 BSS and 72 BSS. These three different fractions are taken for further process. Groundnut protein isolate was prepared from defatted groundnut flour by alkaline extraction. Three different fractions of protein isolate were run in SDS PAGE Electrophoresis (Table 1).

The present work was initiated to see the protein profile for three different fractions of groundnut. All these three were characterized by same banding pattern. In case of 72 BSS contain more band formation on comparing to the other picture. The functionality of groundnut oil cake primarily depends on their fractions of the British Standards Sieve. The high protein content 16.9% of 72 British Standard Sieve of groundnut oil cake makes it good as cake for human consumption, and it also has the potential to add value to the groundnut industry and provide food processors.

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