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PROTEIN AND MINERAL COMPOSITION OF SEED MORPHOFORMS OF *DOLICHOS BIFLORUS* L.

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The proximate composition and mineral constituents from seeds of the three morphoforms of *Dolichos biflorus* L were studied in order to evaluate their nutritional performance. Cream variety contained the maximum amount of protein (22.57 %), followed by brown variety (21.64%). The protein variance study showed extreme significance between seeds. The moisture content of the seeds are generally low .This is an indication that they can be stored for a long time without the development of moulds. The content of moisture was high in brown variety (11.69%). The ash content was high in black variety (3.31%). The fat content of black had the highest value (1.14%) than the other two morphoforms; cream had the least (0.42%). Crude fibre varies between 14.5 to 21.9%. Molecular mass of the protein bands revealed by SDS-PAGE for cream morphoform was confined between 7.5 to 43 KDa, while those of brown were extended in the range of 8.5 to 45 KDa. In black samples bands of molecular weight were between 7.5 to 43 KDa. In the present study the amount of minerals was estimated .The minerals analysed were Cr, Cu, Fe, Mn, Na, Ni, Zn, Ca, K, Mg and P. The contents showed extreme significant variation between the seed morphoforms. Thus the present study revealed that the horse gram seeds may provide sufficient amounts of Proteins, fats, fibre and minerals to meet the human requirement. The proximal compositions and mineral variance studied between different morphoforms of *Dolichos biflorus* Linn stress the necessity of categorizing them in to three different varieties .The investigation on comparative analysis of proteins, fats and minerals revealed marked variations between strains studied.

Keywords : Minerals; Morphoforms; Proteins; SDS-PAGE.

Introduction

Studies pertaining to the search of alternative source of nutrition and protein quality are of great importance in tropical developing countries to alleviate hunger and malnutrition particularly in children and pregnant women, as they are most vulnerable[1]. Recently, the analysis of nutritional value of wild plant materials attracted attention due to the fact that they contain significant amount of essential nutrients that can be used for both human consumption and in the formulation of animal feeds. The proximate composition of seed protein of some wild plants showed that, they could be adequately used in the formulation of animal feeds provided the level of their toxic substances is known[2]. For instance, *Amaranthus viridis* had been reported to be an excellent source of protein. Its amino acid composition compared favourably to that of the World Health Organisation (WHO) protein standard[3]. The protein content of the seeds of Mexican wild lupins, lentils *(Splendens, rotundiflorus)* and mango were found to be very high and could be used for animal feeds if alkaloids inherent in them were eliminated[4-6].

Protein is essential for growth and development of living organisms and it constitute 80 - 90% of all organic substances in animal body. Protein quality is measured by the type of amino acids present. There are twenty different types of amino acids, eight of which are essential because they are not manufactured by the animal's body. This includes Lysine, Leucine, Isoleucine, Methionine, Phenylalanine, Threonine, Tryptohan and Valine[6]. The amino acid histidine is essential for the growth and development of children but it is only synthesized by adults. Other non-essential amino acids that are required to maintain health can be synthesized by the body if supplied with necessary nitrogen. These non-essential amino acids include alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, proline, serine and tyrosine[7]. Dietary protein with all the essential amino acids in the proportion required by the body is said

to be a high quality protein. If the protein is low in one or more of the essential amino acids, the protein is of low quality. The amino acid that is in short supply is called limiting amino acid[7]. Generally, many plant proteins are low in one of the essential amino acids. A combination of plant proteins, such as grains, with pulse or seeds leads to a high quality protein which is just as good as protein from animal foods.

Dolichos biflorus L is cultivated in several parts of South India, mainly as dry crop in moderate rainfall areas. *D. biflorus* L, is a slender, sub erect, low growing, common twining creeper, common to most parts of India, and is found up to altitudes of 1000 m. The increase in price of the cereals and other grains necessitated to search intensively for a relatively cheaper ingredient. Among leguminous seeds, horse gram appeared to be one of the potential unconventional foods which are readily available for compounding low cost nutrients. It is also an excellent source of proteins, thus supplementing the staple cereal based diet. Since the phytochemical composition of crops varies with cultivars, soil and climatic conditions of the area, it is imperative to study the composition of the three different morphoforms (Cream, Brown and Black) of horse gram, an important food legume. The proximal compositions analyzed are protein, fat, fibre, moisture, ash and mineral contents in order to highlight their nutritional significance.

Material and Methods

Freshly harvested sun-dried seeds of the cream, Black and brown varieties of Horse gram *(Dolichos biflorus)* were obtained from the Agricultural University,Thrissur. On the basis of seed colour, they will then grouped them into three morphoforms, *viz.* Black, Brown, and Cream, but the farmers use them in mixed form. The seeds were dehulled and then ground to pass a 50-mesh screen. Powdered samples were preserved in air-tight bottles at room temperature. Sub-samples were dried in an oven at 100 ± 10^0C to constant weight, for moisture determination until a constant weight was attained. The difference in initial and final weight of flour was expressed in percentage moisture.

Proximate Composition-Kjeldhal-N was determined and protein content was calculated by multiplying N by the factor 6.25[8]. The other constituents, crude fat and ash, moisture, fibre were estimated by the methods of AOAC[9]. For calculating moisture a clean crucible was dried to a constant weight in air- circulated oven at 105^{0}C. A known weight of the seed sample was placed in the crucible and dried in the oven at 105^{0}C to constant weight for 2 hours. The crucible and its content was cooled in desiccators and weighed. The moisture content was calculated and expressed in percentage. For ash analysis a crucible was pre-heated in a muffle furnace at 500^{0}C cooled in desiccators and weighed. 2g of the sample was transferred into the crucible and weighed. The crucible and its content were kept back in the muffle furnace at 525^{0}C until white ash was obtained after twelve hours. For avoiding statistical error analysis was done in triplicate and mean was calculated.

Mineral Composition- The given sample is dissolved in 3ml Nitric acid and made up into 500ml using HPLC grade water. The filtered solution is analysed with ICP-AES system[9].

Statistical analysis- The data based on three replicates were subjected to analysis of variance (ANOVA) by complete block design[10]. Standard deviation of each individual nutrient of each seed mean was computed and variations among seeds were evaluated by least significance difference (LSD) at 5 % level of probability (P=0.05).

Results and Discussion

The moisture content of the seeds was generally low. This is an indication that they can be stored for a long time without the development of moulds. Brown morphoform seed moisture content was 11.7%. The moisture content of cream observed was 10.5%, followed by black. In the case of *Bauhinia monandra* moisture content (7.55%) was high, but this value fall below the 15% moisture content required as safe storage limit for plant food materials[2].

Ash content is the measure of the total mineral content of a material. From the result (Table 1), the ash content of black morphoform was 3.1%. This value is higher than that of *Detarium microcarpum* (2.77%) but slightly lower to *Moringa oleifera* (3.60%)[2]. Brown morphoform ash content 2.8%) was lower than the value of cream. Total fat content between the morphoforms were 0.42 to 1.14 %. Black morphoform possess 1.14%. Crude fibre also varies between 14.5 to 21.9%. All the seed morphoform showed comparatively higher profile of crude fibre than the common legumes.

Cream morphoform had the highest protein content of 22.6 % (Table 1). The result was compared with other proteins of plant seeds like soya beans *(Glycine max)* 51.4%, groundnut *(Arachis hypogaea)* 51.3% and cottonseed *(Gossypium* spp) 51.5%[11]. Brown morphoform protein observed is 20.64 % followed by black (20.4 %). The results proved that these plants are potential sources of proteins.

Results of electrophoretic patterns of three seed morphoforms such as black, cream and brown are shown

Table 1. Mean data on the proximate composition of the three seed morphoforms.

Nutrients(g/100gm)	Varieties			Calculated F-Value	P- value
	Black	Cream	Brown		
Protein	20.377b±0.07	22.57a±0.5	21.64ab±0.12	41.29	0.0003
Moisture	9.74b±0.04	10.54ab±0.05	11.69a±0.03	1619.9	<0.0001
Ash	3.13a±0.06	2.5b±0.10	2.75ab±0.05	55.907	0.0001
Fat	1.14ab±0.06	0.42b±0.04	0.86b±0.05	176.97	<0.0001
Crude Fiber	21.86b±0.06	14.51a±0.03	20.91b±0.04	24903	<0.0001

abc =Means in the same column with different superscripts differ significantly (P<0.05) ±--standard deviation

Table 2. Mean data on various minerals in the three Horse gram seeds.

Minerals (ppm)	Black	Cream	Brown	CalculatedF-Value	P-Value
Cu	12.72a±0.7	9.50b±0.0	8.75b±0.0	5101.8	<0.0001
Fe	91.41b±0.03	59.73a±0.06	85.40b±0.04	4472	<0.0001
Mn	41.32b±0.03	40.60b±0.08	49.84a±0.06	2126	<0.0001
Na	192.38a±0.03	165.79b±0.01	165.70b±0.02	2385	<0.0001
Ni	6.84c±0.03	8.44b±0.02	10.16a±0.04	9794.4	<0.0001
Zn	44.75b±0.01	43.88a±0.01	44.66b±0.02	4738.2	<0.0001
Ca	2936.33b±0.01	2931.33b±0.01	3098.36a±0.02	1.875	<0.0001
K	6861.88a±0.005	8931.88b±0.005	8182.22c±0.01	3.715	<0.0001
Mg	1523.78c±0.01	1503.98b±0.06	1497.88a±0.02	30.33	<0.0001
P	3356.06a±0.01	2598.71b±0.06	2598.71b±0.06	36.33	<0.0001

abc =Means in the same column with different superscripts differ significantly(P<0.05); ±: standard deviation (Cu-Copper,Fe-Iron,Mn-Manganese,Na-Sodium,Ni-Nickel,Zn-Zinc,Ca-Calcium,K-Pottassium,Mg-magnesium,P-Phosphorous)

in Fig.1. The SDS-PAGE revealed that for cream morphoform seed (lane 2) the MW of the protein bands confined between 7.5 to 43 KDa, while those of brown were extended in the range of 8.5 to 45 KDa. In black samples, bands were in between 7.5 to 43 KDa .

The organic matter content of a plant material is the measure of the total lipids, proteins and carbohydrates. Black morphoform has the highest value (80.11%) of organic material than the other two seeds. The organic matter of cream morphoform was (75.2%). Brown had 55.7% organic matter; this value is lower than that of the other two morphoforms. Generally, the seeds of the three plants can be described as having moderate nutritional value. The antinutritional factors are comparatively very poor (data not shown).

Table -2 shows the results of mineral composition of three morphoforms of *D. biflorus*. Sodium and potassium contents of the meal samples varied from 165.7 to 192.4 ppm and 6861.9 to 8931.8 ppm, respectively. The black seed morphoform had significantly ($P < 0.001$) higher sodium and potassium contents. All morphoforms were significantly different in sodium and potassium contents. Phosphorus was found to be varying from 2598.7 to 3356.1 ppm. The values of phosphorus in all the meal samples were significantly different ($P < 0.001$) from each other. Iron, calcium and copper contents of seed samples

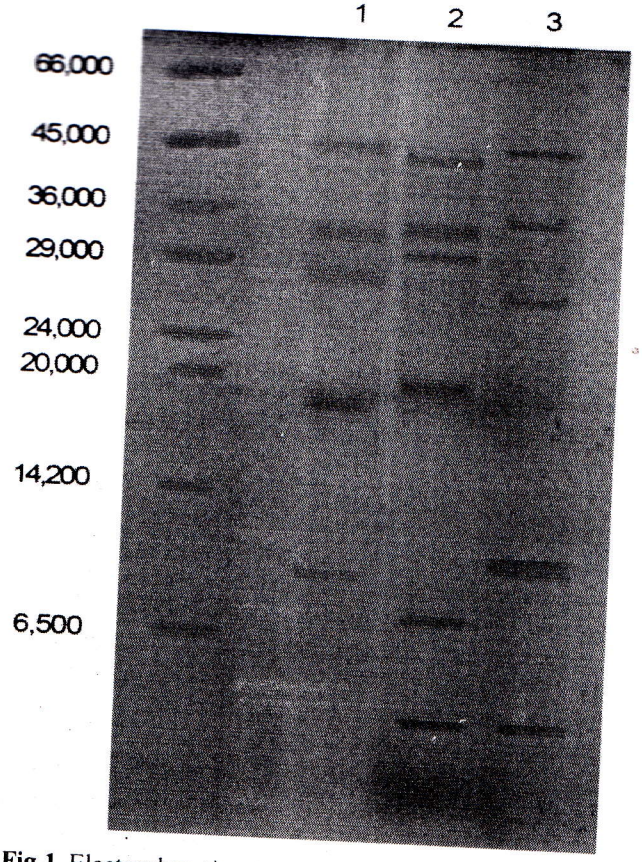

Fig.1. Electrophoretic patterns of three seed morphoforms (1-Brown, 2-Cream, 3-Black)

varied from 59.7 to 91.4 ppm, 2931.3 to 3098.4 ppm and 8.75 to 12.72, respectively. The copper and iron contents in black seed morphoform were significantly higher than others while the calcium content was significantly higher in brown morphoform compared to others. Magnesium content was significantly higher in black morphoform ($P < 0.001$). The samples differ significantly among themselves with regard to all cations tested. The results obtained are comparable to earlier study[12] the mineral content of weaning foods made of sweet potato supplemented with soybean flours.

This study has revealed that the horse gram can contribute useful amount of nutrients including minerals to human diet. Interestingly, the anti-nutritional contents of all the morphoforms were low, than in most legumes seeds. This implies that, the overall nutritional value of the seeds will not be affected. Indeed, these seeds consumed largely by the rural communities in Kerala are not inferior to the conventional popular vegetables and seeds. There is need, however, to determine the vitamins and other nutrients present in the seeds. Understandably, nutrient loss is of great concern during blanching and cooking of seeds, so further study was warranted regarding the effects of cooking and processing procedures on nutrient availability of the seeds. This will help to adequately establish their importance in human nutrition and provide basis for maximum utilization of the plants.

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