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Extraction and Study of Seed Protein Concentrates of Some Legume Plants from the North Eastern UP

Botany

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ABSTRACT

Growing world population pushed the world towards hunger and malnutrition. Cheaper sources of proteins are becoming unavailable to the poor people. New sources of low cost proteins are required to meet the growing demand. The present study revealed the possibility of some new neglected legumes to cope with the emerging problem of protein deficiency. All the five plant species viz. Adenanthera pavonina, Caesalpinia pulcherrima, Delonix regia, Peltophorum ferrugineum and Pongamia pinnata showed the promising results in this direction.

Introduction-

Increasing world population has created a situation of malnutrition especially of protein malnutrition. Recent FAO analysis indicates shifting of people towards cheaper sources of food due to price rise to avoid hunger. This trend severely plunged the population in an undernourished state (FAO report 2013).

Legumes are a good source of plant protein for human and animal nutrition (Vietmeyer, 1986).They belongs to family Leguminosae consisting of three sub family viz- Papilionaceae, Caesalpiniaceae and Mimosaceae. This family of proteinaceous plants include 670 to 750 genera and 18000 to 19000 species of legumes (Polhill et al., 1981). They are just second in rank after Graminae in providing foods to the world population. Several staple foods belong to Leguminosae eg. soybeans, beans, peas, lentils, chickpeas(Duranti, 2006).

Globulins are the major seed storage proteins in legumes. These can be divided into two major groups 7s and 11s globulins according to their sedimentation coefficient (S).These globulin proteins are deficient in sulphur containing amino acids methionine and cysteine (Shewry et.al.,1995). Legume proteins are cost effective substitute to animal protein. However due to their low cost value in comparison to animal protein conventional legumes have been over exploited and in present situation they cannot cope with the growing world demand of proteins.

This led to the exploration of new sources of cheap vegetarian proteins. Several scientific communities worldwide have engaged themselves in the exploration of new sources of proteins and in their studies (Rajaram and Janardhanan, 1992 b ;Pugalenthi et.al,2004,2007;Vadivel et al.,2005). In this context of dearth of vegetarian proteins and the overexploitation of commonly consumed legumes, the present study have been carried out to find some new sources of legume proteins from the North Eastern U.P. Seeds of some legume plants were collected from the locality for their estimation of protein concentrates, total N, protein N, protein content, extractability of protein concentrates and analysis of protein concentrates.

Material and Methods-

Estimation of seed protein concentrates- Seed protein concentrates (SPC) were isolated by the modified method of Molina and Bressani (1973). Six grams of seeds were homogenised with 200 ml. of double distilled water in a waring blender for 30 mins. Homogenate was filtered through double layered muslin cloth. The filtrate was taken in a beaker (1000 ml.) and the initial pH of the extract was noted. 0.1N HCl was added to the filtrate slowly with constant stirring to adjust the final pH 4.5. At this pH protein was coagulated and separated with Whatman filter paper No.1. This crude protein concentrate was repeatedly extracted with Acetone –Ethanol mixture (3;1) and then dried at 60^0C under vacuum.

Estimation of total N- Total N was determined by the micro methods of Doneen (1932). 100 mg of sample was taken in a test tube (2.5x20 cm.) with 1 ml. nitrogen free concentrated sulphuric acid containing 1.0 g of salicylic acid per 20.0 ml. After thorough mixing, it was allowed to stand for 10 mins. Subsequently 0.3 g sodium thiosulphate was added to the mixture and it was heated gently until fumes appeared. The tube was then cooled and 1.0 ml. of perchloric acid containing 0.5g of $CuSO_4$ per 500 ml. was added. The mixture was digested over an electrical Micro-Kjeldahl. Then, the mixture was briskly heated till a clear digest was obtained. The digest from the test tube was transferred to a 100 ml. volumetric flask by washing the tube with double distilled water for several times and the volume was made to 100ml. 1 ml. of this solution was taken in a colorimetric tube with 9 ml. of distilled water and 1.0 ml. of Nessler's reagent as modified by Jackson (1949).The colour intensity was measured in a Spectrophotometer.

A Standard solution of Ammonium Sulphate was treated similarly and its colour intensity was measured as described above.

Total N contents of the digest was calculated by the formula

Total N in the digest= Colorimetric reading for the digest x factor

Total N in the standard

$$\text{(Factor= Total N in the standard= } \frac{\text{Total N in the standard}}{\text{Colorimetric reading of the standard}} \text{)}$$

Colorimetric reading of the standard

Estimation of protein N- 100 mg. of dried seed sample /SPC was homogenised with 10 ml. of 10 % trichloro acetic acid in a mortar and the homogenate was centrifuged at 1500 rpm for 15 min. The supernatant was discarded and the residue was dried in an electric oven at 60^0C. This dried residue was used for determining the protein N by the aforesaid method of Doneen (1932).

Estimation of protein- The total protein was calculated by multiplying the protein N with the factor 6.25.

Per cent extractability of SPC-Per cent extractability of SPC was calculated by the formula-

dry weight of SPC

$$\text{\% extractability of SPC} = \frac{\text{dry weight of SPC}}{\text{Dry matter in the seed sample used for extracting SPC}} \times 100$$

Observations and Discussion-

Seed sample of five legume plants viz. Adenanthera pavonina, Caesalpinia pulcherrima, Delonix regia, Peltophorum ferrugineum and Pongamia pinnata were analysed and the results are shown in Table 1 and Table2. From the table 1 it is clear that the

range of initial pH of all the seed protein extracts is in between 5.9 to 6.7 and is slightly acidic in nature. All the five plant species contain a fairly good amount of proteins in their seeds. The seeds of Pongamia show the minimum value with 16.60 %. The species of Adenanthera and Pongamia show fast precipitation whereas all the remaining species show slow precipitation of proteins.

Table-1 Analysis of biofunctional plant part (Seeds) for contents of dry matter, total N, Protein N and Seed Protein Concentrate (TPC) extractability of the species under study

S.No.	Name of the Plant Species	Dry Matter %	Total N (DM %)	Protein N(DM%)	Protein PN x 6.25	Initial pH of the extract	SPC extractability (%DM basis)
1.	Adenanthera pavonina	88.52	5.87	4.79	29.95	6.6	21.11
2.	Caesalpinia pulcherrima	86.87	7.09	4.69	29.31	6.6	20.55
3.	Delonix regia	86.09	7.03	4.86	30.37	5.9	19.05
4.	Peltophorum ferrugineum	88.82	4.68	3.92	24.50	5.9	34.27
5.	Pongamia pinnata	85.46	4.32	2.65	16.60	6.7	26.61

The analysis of protein concentrates shows a good amount of protein in them. This refers to the solubility of proteins in water and their isoelectric point near pH 4.5(table-2).

Table-2 Analysis of SPC yield from seeds and percent extractability of total N and protein N

S.No.	Source of SPC	Analysis of SPC (% DM basis)			% Extractability	
		Total N	Protein N	Protein	Total N	Protein N
1.	Adenanthera pavonina	12.82	12.20	76.25	46.10	43.87
2.	Caesalpinia pulcherrima	15.35	13.52	84.55	44.49	39.18
3.	Delonix regia	13.75	12.88	80.55	37.25	34.90
4.	Peltophorum ferrugineum	10.94	10.20	63.75	80.10	74.69
5.	Pongamia pinnata	10.32	9.24	57.80	63.56	56.91

Conclusion-

The species under study are wild in nature with enormous amount of seed production. From the study it is evident that they are a good source of water soluble proteins. To remove the hunger from the face of growing population they can be a substitute to the overexploited legumes. However further study is required regarding the nature and toxicity of these proteins before including them in the mainstream of consumption.

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