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Chemical Examination and Biological Evaluation of Proteins Isolated from Some Wild Legumes

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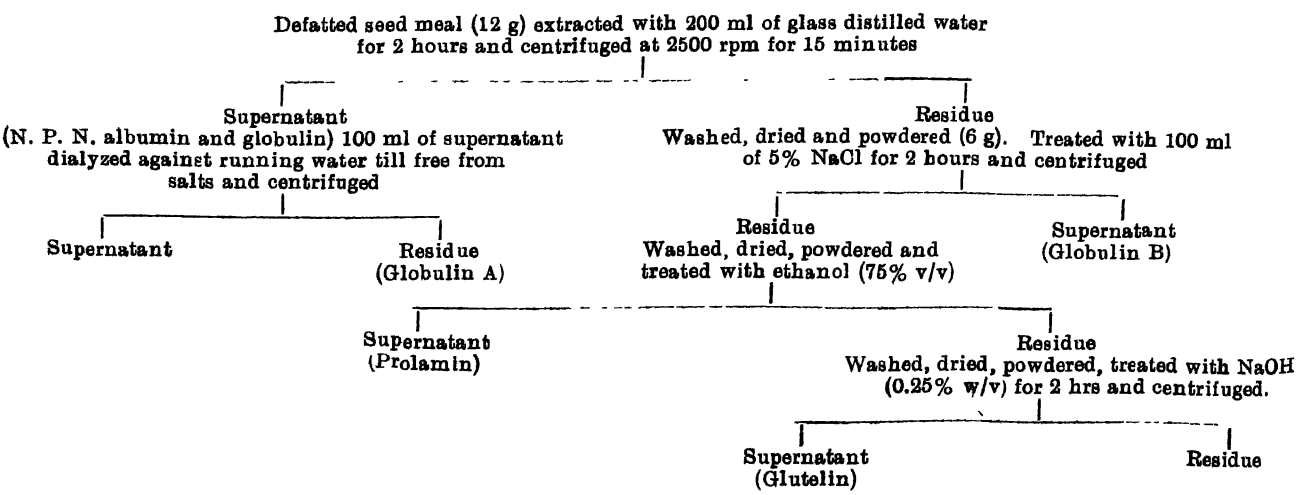
Proteins were isolated from the four varieties of wild leguminous seeds when supplemented with the limiting amino acids methionine and tryptophan and fed to animals, no untoward symptoms were noticed and the diets proved capable of promoting growth.

SINCE legumes constitute the chief source of proteins in the Indian diet[1,2], many leguminous plants are cultivated on a large scale throughout the country. Many wild and uncultivated plants also grow luxuriantly because of favourable soil and climate conditions[3,4]. Faced with protein malnutrition caused by acute food shortage, investigators[5] subjected some of these wild seeds to chemical analysis. Nutritional experiments on albino rats fed whole seed meals at a 10% protein level proved them unpalatable and in some cases toxic. Therefore repetition of feeding experiments were proposed, replacing the whole seed meals with isolated seed proteins, starch and adequate quantities of fats, carbohydrates, vitamins and minerals. However, it was thought desirable to confirm the nutritional efficiency of the globulin type proteins of wild leguminous seeds origin as growth promoters, in animals. Accordingly simple and effective methods for the extraction and isolation of seed protein were standardised and globulin type proteins of considerable purity were isolated from four varieties namely *Mucuna prurita, Mucuna capitata, Cassia tora* and *Cassia occidentalis* seeds.

Materials and Methods :

The wild leguminous seeds were collected from different parts of Bundelkhand Region in Central India and botanically identified. These were powdered to 100 mesh and defatted with petroleum ether (b.p. 60-80°). Fractionation of proteins from seeds were effected by the solubility method of Mitchell[6]. The defatted powder (12 gms) was successively extracted with water, sodium chloride solution (5% w/v), ethanol (75% v/v) and sodium hydroxide (0.25% w/v) until the extracts were negative to the biuret test. Non-protein nitrogen was estimated after precipitating proteins by trichloroacetic acid (2.5% w/v).

The detailed scheme of extraction and fractionation procedure used is represented schematically below :



Nutritive value of protein is expressed in terms of protein efficiency ratio and net protein ratio. The protein efficiency ratio (PER) shows the gain in body weight in g per g protein intake and net protein ratio (NPR) expressed as weight gain of the test animals in relation to the weight loss by the animals provided with nitrogen free diet.

Protein efficiency ratio (PER) and net protein ratio (NPR) were calculated by the expressions as given below :

$$PER = \frac{\text{Gain in body weight in g in 4 weeks}}{\text{Protein intake in g in 4 weeks}}$$

$$NPR = \frac{\text{Gain in weight of experimental animals in g in 4 weeks} + \text{loss in weight of control animals in g in 4 weeks}}{\text{Protein intake by experimental animals in g in 4 weeks}}$$

The protein efficiency ratio and net protein ratio of the isolated purified globulin fractions of the seeds were determined by the rat growth method of Osborne *et al*[7] as modified by Swaminathan[8] on 10 albino rats per group, 4 to 5 weeks old and weighing between 45 to 50 g per rat were used.

A nitrogen-free diet containing practically no nitrogen was prepared from starch which was made adequate with respect to other dietary essentials viz., fats, minerals and vitamins. Experimental diets were prepared at the 10% protein level, the starch in the nitrogen-free diet being replaced by appropriate weighed quantities of the isolated seed proteins.

To study the quality of proteins from defatted seed powders by animal nutrition experiments, lots of 100 g of seed powders were mechanically shaken with sodium chloride solution (5% w/v) for 2 hours at room temperature. The extract, after centrifugation at 2500 rpm for 15 min, was dialyzed against running water for 24 hr. The precipitated protein was centrifuged, redissolved in the minimum quantity of sodium chloride solution, reprecipitated by dialysis to effect purification. The solid obtained after centrifugation was twice washed with ethanol, once with acetone and finally with diethyl ether and dried at room temperature (25-27°).

The leftover diets were individually collected daily in tared beakers bearing the numbers of the rat cages, dried in an oven at 100° to constant weight, and taken into consideration while calculating the daily food intake of each rate.

Results and Discussion

Table 1 indicates that successive extraction of seed meals with water and sodium chloride, solubilizes 76 to 81% of the total nitrogen which consists of globulin. non-protein nitrogen and albumin. Globulin constitutes the major fraction of total proteins of seeds and measured between 56 to 62% of the total nitrogen. Prolamine forms a small fraction 1 to 7% whereas non-protein nitrogen accounts for 10 to 14% and in residue that remains unextracted, nitrogen amounts to 3 to 7%.

TABLE 1—DISTRIBUTION OF TOTAL NITROGEN IN THE DIFFERENT FRACTIONS ISOLATED FROM THE SEEDS

Protein fraction	*Mucuna prurita*	*Mucuna capitata*	*Cassia tora*	*Cassia occidentalis*
Water soluble (alb. + globulin + NPN)	63.4 ± 2.62	62.5 ± 3.16	56 5 ± 2.65	54.4 ± 3.15
Globulin A	42.6 ± 1.05	43 6 ± 2.15	35.35 ± 3.1	40 2 ± 2.25
5 NaCl soluble (Globulin B)	16.4 ± 1.25	18.6 ± 1.18	20 7 ± 2 02	22.2 ± 1.8
Total globulin	59.0	62.2	56.05	59.4
NPN (Non-protein nitrogen)	14.8 ± 1.05	14.6 ± 1.76	10.2 ± 2.15	13.6 ± 1.3
75% ethanol soluble (Prolamine)	1.9 ± 0.4	2.1 ± 0.5	6.1 ± 0.2	7.2 ± 0.24
0.25% NaOH soluble (Glutelin)	10 5 ± 0.8	11.0 ± 0 5	8.4 ± 0.84	7.6 ± 1.16
Residue	3.7 ± 0.42	4.1 ± 0.46	6.88 ± 1.2	5.2 ± 0.8

Table 2 presents the protein efficiency ratio and the net protein ratio of the different isolated seed globulins. In the feeding experiments albumin, prolamine and glutelin fraction were not used as these proteins do not fulfil the requirement of those essential amino acids (tryptophan and methionine) which were lacking in globulin. Studies of the distribution of amino acids in albumin, prolamine and glutelin showed that the methionine and tryptophan were missing in the albumin fraction of all the seeds used[9], prolamine fraction was rich in proline and methionine but tryptophan was totally absent[10]. In all the seeds glutelin was also lacking in these essential amino acids except in *Mucuna prurita* where traces of methionine and tryptophan were found[10], (unpublished data). Growth experiments with young rats revealed that maximum growth was due to casein diets. *Cassia tora*, *C. occidentalis*, *Mucuna prurita* and *M. capitata* seed globulins not only failed to promote growth but could not even maintain the normal weight of animals and caused a decrease in body weight ranging from 7.6 to 8.8 g/rat during the feeding period of four weeks. However, when these were supplemented with the limiting amounts of methionine (0.1%) and tryptophan (0.2%) the animals registered increase weight ranging from 13.5 to 16.4 g/rat during the same period. This suggests that the wild seed globulins are nutritionally much inferior to casein, but on supplementation with the limiting amounts of amino acids, are capable of promoting growth and also maintain nitrogen balance in experimental animals. Moreover, though the nutritional efficiency of a protein depends almost entirely upon its amino acid composition,

TABLE 2—PROTEIN EFFICIENCY RATIO AND NET PROTEIN RATIO OF SOME GLOBULINS ISOLATED FROM UNCULTIVATED LEGUMINOUS SEEDS

Origin of Dietary protein	%N in Globulin	%protein in diet	Protein intake/ rat in 4 weeks (g)	Change in weight/ rat in 4 weeks (g)	Protein efficiency ratio	Net protein ratio
Mucuna prurita	16.8	10.1	9.5±0.55	− 7.8±0.6	—	—
**Mucuna prurita*	17.00		12.2±1.7	+16.4±1.8	1.35	2.44
Mucuna capitata	17.1	9.9	9.8±0.4	− 8.1±0.5	—	—
**Mucuna capitata*	17.3		11.3±1.8	+15.6±2.0	1.38	2.56
Cassia tora	16.9	10.1	8.7±0.5	− 8.8±0.5	—	—
**Cassia tora*	17.1		11.2±2.6	+13.5±0.6	1.20	2.40
Cassia occidentalis	17.0	9.85	9.2±0.4	− 7.6±0.4	—	—
**Cassia occidentalis*	17.2		13.1±1.8	+14.4±0.5	1.10	2.12
Casein	17.8	10.0	16.0±0.5	+40.6±1.8	2.54	3.30
Non-protein diet	—	—	—	−13.4±1.5	—	—

*Supplemented with L-methionine (0.15%) and L-tryptophan (0.2%). Dietary protein was at 10% level.

Non-protein diet consisted of soluble starch (analytical reagent grade) 80 parts ; sources 10 parts ; ground nut oil 6 parts, salt mixture 2 parts ; shark liver oil 5 drops per rat (twice a week). In experimental diet, starch was replaced by dietary protein at 10% level.

Salt mixture prepared by mixing various components in the ratio (g) : $CaCO_3$, 543.0 ; $MgCO_3$, 25.0 ; $MgSO_4$, 16.0 ; K_2SO_4, 67.0 ; KH_2PO_4, 112.6 ; $FePO_4.4H_2O$, 211.5 ; KI, 20.5 ; $MnSO_4$, H_2O, 0.08 ; NaF, 0.03 ; $Al_2(SO_4)_3$, $K_2SO_4.24H_2O$ 1.0 ;

Vitamin mixture supplied the following vitamins per 100 g of diet : Vitamin A, 400 I.U. ; Inositol, 2.2 mg ; Choline chloride, 75 mg ; Niacin, 2.0 mg ; Riboflavin, 0.4 mg ; Pyridoxine-HCl, 0.2 mg ; Thiamine-HCl, 0.4 mg ; Calcium pentothenate, 1.2 mg ; Folic acid, 40 mg ; Vitamin B_{12} 600 μg.

certain other factors cannot be ignored. For example, the physiological state of the experimental animals, and the consequent uncontrollable changes in the rate of availability of amino acids to them are factors, which vary by appreciable margins, and significantly affect the nutritional efficiency of proteins.

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