dimethyl succinate using methanolic sodium methoxide.[4]

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A COMPARATIVE STUDY OF THE NITROGENOUS CONSTITUENTS OF SOME LEGUMINOUS SEEDS

ALTHOUGH all leguminous seeds hitherto examined—edible as well as wild and uncultivated—have appreciable high protein content, their inclusion in animal nutrition has scrupulously been avoided. This is partly due to their unpleasant odour and taste but mainly due to the deleterious physiological effects which they can exert in the presence of anti-growth and other toxic factors if and when present. This investigation was undertaken with a view to explore the possibility of isolating the different protein fractions from such undesirable wild seed constituents in the pure form and to incorporate them in animal nutrition.

The following describes the distribution of nitrogen in the seeds of *Dolichos biflorus*, *Glycine hispida* (edible but not very popular), *Mucuna pruriens* and *Pithecellobium dulce* Benth. (wild and inedible) as well as the extraction, precipitation, fractionation and partial purification of their various protein components by simple methods.

Healthy and dry mature seeds of *Dolichos biflorus* and *Glycine hispida* were bought in Ranikhet, and *Mucuna pruriens* and *Pithecellobium dulce* seeds were bought locally. The seeds were powdered to 100 mesh, defatted with petroleum ether (B.P. 40–60°) and employed for all investigations.

The preliminary analyses, summarized in Table I, were carried out by methods referred in our previous communications.[1-4]

The effect of pH variation (0·2–10) on the extraction of seed-proteins was studied by employing solutions of HCl and NaOH of known concentration and pH. Weighed samples (*ca* 1 g.) in duplicate were mechanically shaken with the extractants (25 ml.) in Erlenmeyer flasks for 2 hours at room temperature (30° ± 1°). The extracts were centrifuged and nitrogen was determined in 5 ml. of the clear supernatant.

TABLE I
Chemical composition of some leguminous seeds

Constituents		*Dolichos biflorus*	*Glycine hispida*	*Pithecellobium dulce*	*Mucuna pruriens*
Moisture	% ..	3·00	2·73	2·36	4·50
Ash	% ..	3·06	5·22	3·00	4·19
Lipids	% ..	2·02	9·64	11·69	8·64
Crude protein (N × 6·25)		27·62	52·80	24·40	27·56
Total soluble carbohydrate	%	6·93	16·48	19·01	..

Figure 1 representing the variation of extraction of nitrogenous components of seed meals

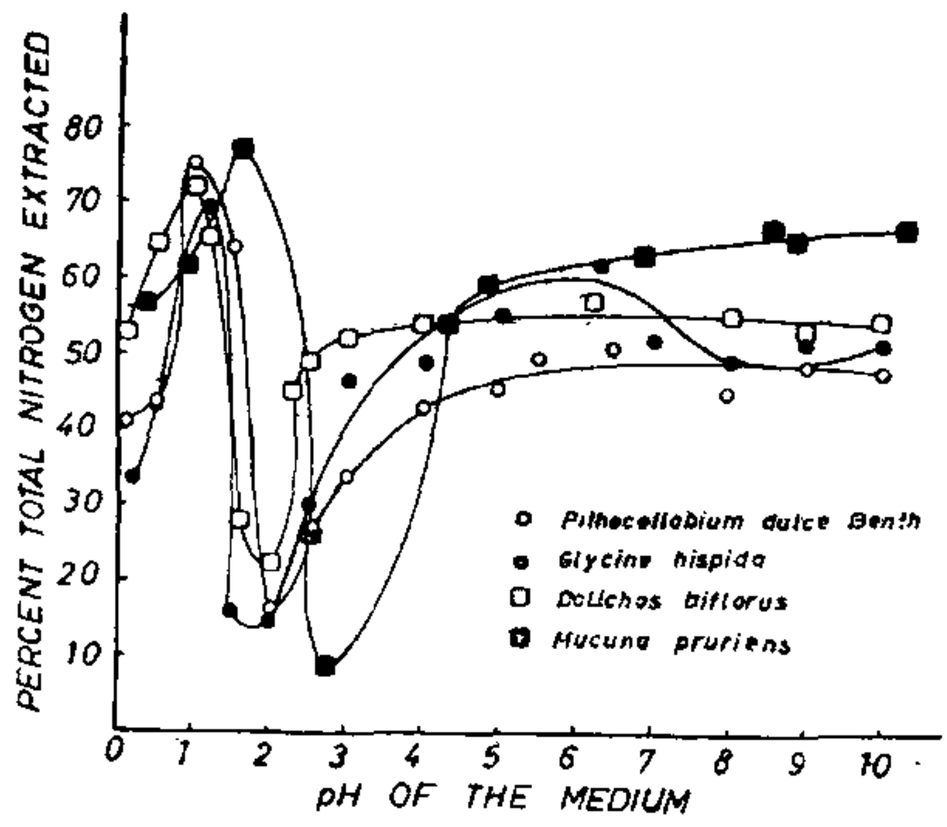

FIG. 1

with the change of pH suggests a method for their isolation. The nitrogenous compounds which mainly consist of proteins and some non-protein nitrogenous compounds (5–6%, Table II) can be maximally extracted with either HCl or NaOH solutions at appropriate pH and then precipitated by adjusting the pH of the medium to that of minimum extraction. However it has been observed by us that these maximally extracted proteins do not get completely precipitated after pH adjustment due to the formation of NaCl which brings about the dissolution of globulins. Therefore, the simplest method for the isolation of seed proteins would be their extraction with NaCl solution at neutral pH and subsequent dialysis of the extract when globulin type of proteins would get precipitated leaving the other soluble ones in solution which could be recovered by suitable precipitants. The proteins thus isolated

seem to be of higher purity since they contain less non-protein materials than the samples of proteins obtained by the other method referred above.

TABLE II

Distribution of total nitrogen in some leguminous seeds

	Dolichos biflorus	*Glycin hispida*	*Pithecellobium dulce*	*Mucuna pruriens*
Protein fractions—water soluble [Alb+Glob (A) +NPN]	69·90	71·03	70·58	79·50
Albumin ..	15·39	3·55	7·45	5·64
Globulin (A) ..	49·77	61·50	58·28	66·91
Globulin (B) (5% NaCl sol-)	13·19	6·21	9·25	16·31
NPN ..	4·73	5·97	4·85	6·95
Total Globulins ..	62·96	67·72	67·53	83·22
Prolamine (75% Ethanol sol.)	2·01	1·02	1·29	0·02
Glutelin (0·25% NaOH)	6·83	6·04	5·17	2·81
Residue ..	8·06	15·69	5·88	1·36

The fractionation of proteins was carried out by successive exhaustive extraction of accurately weighed seed powders (*ca* 3 g.) with glass-distilled water, NaCl (5%, w/v), ethanol (75%, v/v) and NaOH (0·25%, w/v) till the extracts were negative to biuret test. All the extracts were analysed for nitrogen and results were calculated as percentages of the total nitrogen content of the seed powders.

Extractions show that water solubilizes 70–80% of the total nitrogen which is of albumin, globulin and non-protein nitrogen combined origin. Globulins contribute the major fraction of proteins accounting for 63–83% of the total nitrogen, prolamine forms a small fraction and non-protein nitrogen amounts to 5–6%.

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ON THE DEHYDRATION OF ETHYL ALCOHOL OVER ACTIVATED BAUXITE

THE vapour-phase dehydration of ethyl alcohol is a well-studied reaction.[1,2] However, data on a continuous run (particularly over bauxite) are rarely reported in literature.

The bauxite was first washed to remove clay material present and dried to remove free water. It was then taken in a platinum crucible batch by batch and activated at 900° C. to remove combined water and to increase its adsorptive power. The aluminium trihydrate in the bauxite, thus treated, is changed to a new crystalline form of alumina, known as γ-alumina[3] (cubic structure).

A continuous run for 16½ hours was conducted in a fluid-bed flow reactor at atmospheric pressure (using a stainless steel reactor tube 2" i.d. and 18" long). The catalyst used was activated bauxite made as above, its particle size being −65 +80 TSS. The reaction was conducted at 450° C., ethyl alcohol flow rate being maintained at 110 gm./hr. After collecting about 220 l. gaseous product in the drum which had a capacity of 250 l., the run was temporarily discontinued, the drum emptied for making it available again for continuing the run. During the period that the run was temporarily discontinued the catalyst bed was kept heated and maintained at 300° C, thereby giving the bauxite no chance to absorb any moisture from outside atmosphere.

The conversion trend of ethyl alcohol to ethylene is given in Fig. 1. It can be seen from Fig. 1 that at the initial stage (up to 9 hours of run) the conversion (mole %) increased with time, thereafter remaining constant. Though the carbon deposited during the reaction is expected to decrease the catalyst activity, the increase in conversion, as is actually noticed, can be explained on the basis of moisture content in the catalyst employed. Brey and Krieger[4] report that for an alumina base catalyst to be most active for dehydration reaction it should have some optimum moisture content. However, when activating the catalyst at 900° C, it is explained that the same would be left with a moisture content much less than the optimum required as per Brey and Krieger. It is thought that during the reaction (at a much lower temperature) a slow activated adsorption of water would take place increasing its moisture content, which