CLXXX. VEGETABLE PROTEINS.

I. THE PROTEINS OF *DOLICHOS LAB LAB.*

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The field bean (*Dolichos lab lab*; Tamil name, Mochai; Kanarese, Avarai) is a legume which is widely cultivated in South India often as a mixed crop with cereals. The kernel of the seed enters into the diet of many South Indian households, and in the Mysore State the seeds are used as a delicacy when they are green for over four months in the year. The haulm, husk and pods are commonly used as fodder. As the kernel, which is widely used as an article of food and considered to be very nutritious, contains about 24 % of protein hitherto uninvestigated and as the quality of protein plays an important rôle in nutrition, the present work was undertaken.

Experimental.

The seeds used in this investigation belong to the cream-white variety raised in the Mysore State. They were sun-dried, after which the seed coats were removed in a huller and the clean kernel was ground to a fine powder (No. 40), which had the following composition: moisture 10·6 %, ash 2·95 %, ether-soluble extractives 1·57 %, protein 24·44 %, crude fibre 1·18 % and carbohydrate (by difference) 59·26 %.

Distribution of nitrogen in the meal. The nitrogen distribution in the meal was ascertained by extracting it successively with different solvents and the percentages of nitrogen extracted were as follows: water 72·8, 4 % sodium chloride 10·21, 70 % cold ethyl alcohol 1·27, 70 % hot ethyl alcohol 2·54, and 0·4 % sodium hydroxide 12·77; total nitrogen extracted 99·59 %.

All the nitrogen in the meal has been accounted for, and as is evident from the results recorded 72·8 % of the nitrogen is obtained in the aqueous extract, suggesting that probably the protein is the one usually known as "globulin." As this forms the major protein of the seed it formed the first object of study.

Influence of concentration of salt. 5 g. of the vacuum-dried meal were shaken for 3 hours with 50 cc. of salt solution of varying concentration; the extract was centrifuged at the end of that period and the nitrogen in the clear extract was estimated according to Kjeldahl. The results were: water 72·8 %,

5 % sodium chloride 71·57 % and 10 % sodium chloride 71·51 % of the total nitrogen, and indicate that water extracts more than salt solution. Probably the small quantity of electrolyte present in the meal is enough to peptise the protein.

Influence of the period of extraction. 5 g. lots of the vacuum-dried meal were extracted with 50 cc. of water for different periods. Determination of nitrogen in the centrifuged extracts gave the following percentages of the total: 0·5 hour 67·04, 1 hour 71·51, 2 hours 72·8, 3 hours 72·8, 4 hours *75·35*, 5 hours 71·51, and 6 hours 69·96.

Influence of the quantity of meal taken on the extraction. The great rôle played by the quantity of the solid phase in colloid solubility was clearly recognised by Hardy [1905] and Mellanby [1905] who found that the solubility of globulins in dilute solutions of electrolytes depended on the quantity of the solid phase. The quantity of globulin going into solution for the same volume of peptiser increases with increasing quantity of the solid phase although some of the globulin is left. Their experiments clearly bring out the difference between the solubility of molecularly disperse substances and that of colloids. While a molecularly disperse substance has one solubility for a given temperature and given solvent independent of the solid phase, the peptisation of colloids is not similar. The theoretical aspects of the question have been discussed by Ostwald [1927]. Similar behaviour was noticed by the present authors [1929] with the enzyme tyrosinase. The results of experiments to investigate this are given in Table I.

Table I. *The meal was extracted with 50 cc. of water.*

Weight of meal taken in g.	Nitrogen extracted (% of total)
5·0	72·8
7·5	73·22
10·0	75·02
12·5	**81·93**
15·0	75·8

It will be seen that the quantity of nitrogen extracted increases with the quantity of the solid phase, the maximum being attained when four parts of solvent are used for one part of meal.

Preparation of dolichosin.

It was pointed out by Narayanamurti and Ramaswami [1929] that aqueous extracts of the meal turn dark especially in the presence of air, owing to the action of the enzyme tyrosinase. As the action of this enzyme will essentially change the character of the protein with regard to its physical and chemical characteristics the usual methods of preparation of globulins are of no use. To prevent tyrosinase action smaller quantities of meal were extracted with larger quantities of water. 100 g. of the meal were extracted with 1000 cc. of distilled water in a glass-stoppered bottle of about 1200 cc. capacity, the

extract was quickly centrifuged and transferred to a specially constructed electro-dialyser shown in Fig. 1. The electro-dialyser consists essentially of two

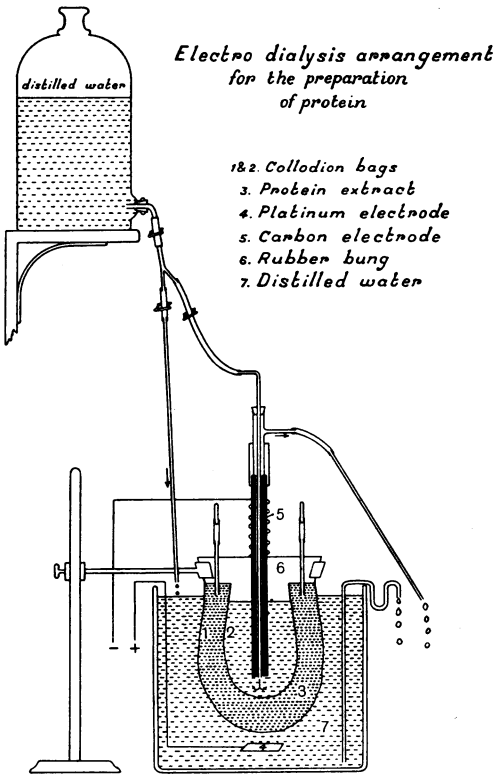

Fig. 1.

collodion thimbles. The outer one holds the protein extract, and distilled water is circulated through the inner one, the whole apparatus being immersed in a beaker of distilled water. A hole bored through the side of the carbon perpendicular to its axis allows water to pass out through the interspace between the wall of the glass tube and the wall of the hole bored through the length of the carbon. Thus continuous circulation of water is effected. By means of a constant level syphon arrangement continuous flow of water in the beaker is effected. The electro-dialysis was started at about 5 v. Enough resistance was introduced into the circuit to ensure a low current passing through. After a few hours the E.M.F. was increased to 10 v. and gradually raised to 30 v. In the course of about 40 hours all the protein had precipitated and the clear liquid above the precipitate was heated to see whether any protein not precipitated could be coagulated. No coagulum was formed. The precipitate was quickly filtered off on a Zsigmondy membrane filter and dried in a vacuum desiccator. As will be seen from the diagram the electro-dialysis was done out of contact with air, the collodion thimble holding the protein extract being

completely filled. Thus tyrosinase action was avoided. The protein was finely powdered and subjected to analysis.

Elementary composition of dolichosin. The results are given in Table II.

Table II. *Percentages on moisture and ash-free basis.*

	I	II	Average
Nitrogen	15·5	15·5	15·5
Carbon	52·0	51·66	51·83
Hydrogen	8·29	8·73	8·51
Sulphur	0·27	0·27	0·27
Phosphorus	0·55	0·55	0·55
Oxygen (by diff.)	23·39	23·29	23·34

Moisture in the protein preparation was 2·71 % and ash 0·66 %. All the sulphur is accounted for as cystine. The nature of the combination of phosphorus in dolichosin is being studied.

Nitrogen distribution in dolichosin. 3 g. of the protein were boiled with 15 cc. of 25 % hydrochloric acid till the reaction mixture no longer gave the biuret reaction. The hydrolysate was analysed according to van Slyke, advantage being taken of all the improvements suggested by Plimmer and Rosedale [1925], Daft [1929] and Linderstrøm-Lang [1927–9]. The results are given in the first column of Table III, the remaining columns give similar figures for proteins of allied species.

Table III.

	Globulin from			
Form of nitrogen	*Dolichos lab lab*	*Dolichos* biflorus*	*Cajanus† Indicus*	*Cicer* aretinum*
Amide	10·49	10·43	10·31	10·42
Humin	1·33	1·36	1·20	0·69
Basic:				
Arginine	16·84	14·45	11·37	19·31
Histidine	1·50	1·44	5·00	1·51
Lysine	8·20	9·25	8·20	8·48
Cystine	0·79	0·43	0·31	0·25
Non-basic:				
Amino	56·35	61·54	62·17	58·23
Non-amino	3·77	1·75	1·90	0·47
Total	99·27	100·65	100·46	99·36

* Narayana. † Sundaram, Norris and Subrahmanyan [1929].

In Table IV are given figures for the amounts of those amino-acids considered to be essential dietary constituents, which are contained in the globulins of *Dolichos* and allied species.

In common with other globulins dolichosin is characterised by a high percentage of basic nitrogen. As regards cystine content it compares favourably with the other globulins, and as regards tryptophan content it is essentially superior to the other globulins. Therefore it is evident that dolichosin is essentially superior to the other globulins and should be of high nutritive value.

Table IV. *Percentages of the essential amino-acids in dolichosin and other globulins as determined by different methods.*

Amino-acid	Globulin from *Dolichos lab lab*	*Dolichos biflorus*	*Cajanus Indicus*	*Cicer aretinum*	Method of determination
Arginine	8·11	7·11	5·72	12·09	Van Slyke
Histidine	0·86	0·84	2·99	0·99	,,
Lysine	6·63	7·64	6·93	7·57	,,
Cystine	1·02	1·81	1·41	0·88	Folin and Looney [1922]
,,	0·96	—	1·39	—	Sullivan [1927]
Tryptophan	2·46	—	—	—	Folin and Looney [1922]
,,	2·59	—	0·12	—	Tillmans, Hirsch and Stoppel [1928]
,,	—	Present	—	0·41	Komm [1926]
Tyrosine	5·68	6·08	—	4·90	Zuwerkalow [1926]
,,	4·86	—	3·16	—	Tillmans, Hirsch and Stoppel [1928]
,,	4·93	—	—	—	Folin and Looney [1922]

Unlike cajanin and the other globulins it can be said to constitute a complete protein food.

The nitrogen distribution of dolichosin indicates that it differs from the protein isolated from *Dolichos biflorus*, principally in the tryptophan and cystine contents.

Summary.

The chief protein of *Dolichos lab lab* constitutes nearly 80 % of the nitrogen in the meal and is obtained in the aqueous extract.

In order to avoid tyrosinase action the protein has been prepared by subjecting the extract to electro-dialysis in a suitably constructed apparatus.

The nitrogen distribution in the protein has been determined and the nutritionally essential amino-acids have been estimated.

The nutritional value of dolichosin, as deduced from its composition, has been shown to be superior to that of the other globulins studied.

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REFERENCES.

Daft (1929). *Biochem. J.* **23**, 149.
Folin and Looney (1922). *J. Biol. Chem.* **51**, 421.
Hardy (1905). *J. Physiol.* **33**, 251.
Komm (1926). *Z. physiol. Chem.* **156**, 161.
Linderstrøm-Lang (1927–9). *Compt. Rend. Lab. Carlsberg*, **17** (9), 25.
Mellanby (1905). *J. Physiol.* **33**, 335.
Narayana. Private communication.
Narayanamurti and Ramaswami (1929). *J. Indian Inst. Sci.* **12A**, 109.
Ostwald (1927). *Kolloid Z.* **41**, 163; **43**, 249.
Plimmer and Rosedale (1925). *Biochem. J.* **19**, 1004.
Sullivan (1927). *J. Biol. Chem.* **74**, xiv.
Sundaram, Norris and Subrahmanyan (1929). *J. Indian Inst. Sci.* **12A**, 193.
Tillmans, Hirsch and Stoppel (1928). *Biochem. Z.* **198**, 379.
Zuwerkalow (1926). *Z. physiol. Chem.* **163**, 185.