STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.

PART III. The Globulins of Bengal Gram (*Cicer Arietinum*, Linn.) and Horse Gram (*Dolichos Biflorus*).

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Bengal gram is an important Indian foodstuff belonging to the order *Leguminosæ*. The vernacular names are :—Sanskrit, *Chenaka*; Hindi, *Chana*; Tamil, *Kadalay*; and Telugu, *Senagalu*. The crop is raised in all parts of India, the upper basins of the Ganges and the Indus being the chief gram-producing areas. Agricultural statistics show that this pulse accounts for about 13 per cent. of the total area (including States) under food crops. It is consumed by all classes of people and is believed to be highly nutritious. In many parts of the country any surplus material is fed to cattle.

Horse gram is one of the cheapest among Indian pulses and is chiefly employed as food for horses and cattle. The vernacular names are :—Sanskrit, *Kulattha*; Hindi, *Kulthi*; Kanarese, *Hurali*; and Tamil, *Kollu*. It is grown in all parts of the country as a catch-crop and thrives with a minimum rainfall. As it enriches the soil with nitrogen, the plant, after harvesting the seed, is generally ploughed in as green manure.

The present investigation of the composition of the globulins which form the chief proteins of the seeds, was undertaken in view of the importance of the pulses in relation to nutrition.

EXPERIMENTAL.

The seeds were sun-dried until crisp and easy to handle. The husk was removed from the Bengal gram and the kernel ground to obtain the flour. The horse gram was ground whole because (*a*) it was not possible to remove the husk and (*b*) the whole seed is invariably fed to animals. Flours passing the 60-mesh sieve were used in the

present investigation, specimens dried at 100° giving the following on analysis, (Table I) :—

TABLE I.

Pulse	Ash	Ether extractives	Crude fibre	Crude protein (N x 6·25)	Carbohydrates (by difference)	True protein (determined separately)
Bengal gram ...	2·45	4·72	1·13	28·14	63·56	25·04
Horse gram ...	3·51	2·32	5·48	26·40	62·29	21·93

Extraction of the Globulins.—Preliminary trials with Bengal gram showed that the optimum concentration of salt solution was 6 per cent. which extracted, in 4 hours at 25–30°, about 88 per cent. of the total nitrogen. In the case of the horse gram, the most suitable concentration was 8 per cent. which extracted nearly 80 per cent. of the total nitrogen in 2 hours.

Preparation of the Globulins.

Extraction.—In each case about one kilogram of the flour was treated with 6–8 litres of the required concentration of sodium chloride solution and stirred vigorously for 2–3 hours. The mixture was then strained through cheese cloth, the liquid poured into large fluted gravity filters and allowed to stand overnight, toluene being added to prevent bacterial action. About 2–3 litres of slightly coloured, opalescent extract was thus obtained.

Precipitation.—The globulins were precipitated from the extract by (1) dialysis, (2) acidification and (3) salting out. The extract was dialysed against cold running distilled water for 4–6 days until the dialysates were free from traces of chlorides. The globulin of the Bengal gram then precipitated readily but that from horse gram did so only on adding a small quantity of acetic acid. The proteins were separated by centrifuging, redissolved in salt solution and dialysed as before. (2) The extract was diluted until cloudy, saturated with carbon dioxide and treated with a few drops of acetic acid to complete the precipitation of protein. (3) This method was adopted only in the case of Bengal gram, the extract being saturated with ammonium sulphate. The precipitated protein was filtered, and washed repeatedly with saturated ammonium sulphate solution. It was then peptised by shaking with sufficient water and dialysed against distilled water until the dialysate was free from sulphate.

Purification.—Suspensions obtained by the different methods were centrifuged and washed repeatedly by triturating with distilled water; they were then dehydrated by washing with graded strength of alcohol, and finally with ether, the dry preparations being powdered and passed through the 100-mesh sieve preparatory to further examination.

General Properties.

The preparations from horse gram were light brown, and those from Bengal gram cream-coloured. All gave reactions characteristic of proteins, and contained sulphur, tyrosine and tryptophan, but no phosphorus. They were completely soluble in dilute alkali and glacial acetic acid. Elementary analysis of the preparations gave the following percentages (Table II), sulphur being determined by the method of Hoffman and Gortner (*J. Amer. Chem. Soc.*, 1923, **45**, 1033).

TABLE II.

	From Bengal gram by			From Horse gram by	
	(1)	(2)	(3)	(1)	(2)
Combined moisture ...	8·73	8·05	7·65	11·50	12·36
Ash	0·33	0·33	0·34	0·48	0·64
Nitrogen *	17·05	17·26	17·17	15·75	15·90
Sulphur *	0·36	0·35	0·36	0·53	0·59

* On ash and moisture free basis.

Nitrogen distribution was determined by the method of Van Slyke as modified by Plimmer and Rosedale (*Biochem. J.*, 1925, **19**, 1004). Arginine in the diamino-fraction was estimated by the method of Plimmer (*Biochem. J.*, 1916, **10**, 115), and sulphur according to Plimmer and Lowndes (*Biochem. J.*, 1927, **21**, 247). Arginine was also estimated directly in the hydrolysate, Plimmer and Rosedale having observed (*Biochem. J.*, 1925, **19**, 1020) that higher values than those by the Van Slyke method are thus obtained. *Free amino-nitrogen* was estimated in 1 per cent. solutions of the globulins in dilute alkali. The Van Slyke micro-apparatus was used and 30 minutes allowed for the reaction. The results of the foregoing determinations are given in Table III.

TABLE III.

	From Bengal gram by			From Horse gram by	
	(1)	(2)	(3)	(1)	(2)
Nitrogen as :—					
Acid Insoluble melanin ...	0·32	0·32	0·48	1·32	1·30
Soluble melanin (absorbed by lime)	0·38	0·30	0·29	0·05	0·05
Amide	10·40	10·40	10·47	10·09	10·78
Basic :—					
Arginine	19·23	19·24	19·47	12·61	11·88
Histidine	1·75	1·12	1·82	1·13	1·75
Cystine	0·24	0·27	0·24	0·41	0·45
Lysine	8·45	8·80	8·22	9·56	8·93
Non-basic :—					
Amino	57·80	58·43	58·45	63·64	63·84
Non-amino	0·49	0·47	0·46	1·88	1·65
Total ...	99·06	99·35	99·90	100·69	100·59
Arginine direct	22·51	22·50	23·17	14·09	14·01
Free amino-nitrogen, direct ...	4·99	5·23	...	4·81	4·27
Half Lysine, N.	4·23	4·40	...	4·78	4·47
Sulphur in basic fraction ...	0·55	0·59	0·54	...	...
Sulphur in non-basic fraction ...	1·60	1·44	1·44	...	...

Tyrosine, Tryptophan and Cystine.

Tyrosine was estimated by the method of Zuwerkalao (*Z. physiol. Chem.*, 1926, **163**, 185) and tryptophan by that of Komm (*Z. physiol. Chem.*, 1926, **163**, 202). The figures for cystine obtained by the Van Slyke method cannot be taken as correct, since, owing to racemisation during hydrolysis, the amino-acid is incompletely precipitated by phosphotungstic acid. Independent determinations of cystine were therefore carried out by the method of Folin and Looney (*J. Biol. Chem.*, 1922, **51**, 421). Tryptophan in the globulin of horse gram could not be estimated by the method of Komm, as the colour obtained with the globulin was different in shade from that with pure tryptophan. The results were as follows :—

TABLE IV.

Percentages of ash and moisture-free protein.

	Globulin from	
	Bengal gram	Horse gram
Tyrosine	4·90	6·68
Cystine	0·88	1·81
Tryptophan	0·41	present
Arginine (Van Slyke) ...	10·27	6·02
Arginine (direct)	12·09	7·11
Histidine	0·99	0·84
Lysine	7·57	7·64

DISCUSSION OF RESULTS.

The figures for the distribution of nitrogen show that the different preparations of each globulin are identical in composition. Arginine, as estimated in the protein hydrolysate, is 2–3 per cent. higher than when estimated in the diamino-fraction, showing that the amino-acid is not completely precipitated by phosphotungstic acid (Plimmer and Rosedale, *loc. cit.*).

The ratio of sulphur in the basic and non-basic fractions of the globulin from Bengal gram is nearly 1 to 3; if all sulphur in the protein had been present as cystine, the ratio should have been 1 to 1·5 (Plimmer and Lowndes, *loc. cit.*). Moreover, since cystine estimated by the method of Folin and Looney (*loc. cit.*) accounts for only two-thirds of the total sulphur, it may be inferred that in addition to cystine, one or more sulphur-containing compounds are present in the non-basic fraction.

The close agreement obtained between the figures for free amino-nitrogen and half the lysine nitrogen in both globulins indicates that the free amino-group of lysine accounts for all the nitrogen in that form so that practically no other free amino-groups are present in the proteins. This result is a contrast with an unpublished observation of the author that in the case of the prolamin of Ragi (*Eleusine coracana*), only one-seventh of the free amino-nitrogen is due to lysine.

Both the globulins contain sufficient amounts of tyrosine, arginine and lysine, while cystine and tryptophan contents are comparatively

ow. A comparison with the composition of similar proteins present n other important Indian pulses (*J. Indian Inst. Sci.*, 1929, **12A**, 193; *. Biol. Chem.*, 1920, **42**, 59) shows that the globulin of the Bengal gram is characterised by a high percentage of arginine and a low one of non-amino nitrogen (proline ?).

SUMMARY.

The globulins of Bengal and horse gram were isolated and analysed by the method of Van Slyke. Tyrosine, tryptophan and cystine were estimated. The arginine content of the Bengal gram, was much higher than those usually met with in the globulins of other Indian pulses. Both proteins were found to contain sufficient amounts of arginine, tyrosine and lysine, but were deficient in cystine and tryptophan.

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