

# CCI. THE NON-PROTEIN-NITROGEN OF PULSES.

## II. PARTITIONING OF THE NITROGEN AND A DETERMINATION OF THE ESSENTIAL AMINO-ACIDS.

BY KAMALA BHAGVAT AND MOTNAHALLI SREENIVASAYA.

*From the Department of Biochemistry, Indian Institute of Science, Bangalore.*

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IN a previous communication [1935] attention was drawn to the presence of an easily assimilable non-protein nitrogenous fraction occurring in the pulses to the extent of 10–55% of the total nitrogen and consisting mostly of the simpler polypeptides. The present study relates to an investigation of the amino-acid make-up of the fraction from the three well-known pulses, *Phaseolus aconitifolius*, *Cicer arietinum* and *Phaseolus mungo*.

### EXPERIMENTAL.

Preliminary trials with *P. aconitifolius* showed that 0.5% acetic acid was the best solvent, yielding the maximum amount of the non-protein-nitrogen. It was found that a 5% solution of sodium chloride, whilst extracting the globulins and albumins of the seed, does not effectively dissolve out the non-protein-nitrogen; further, the sodium chloride which persists in the non-protein fraction after the removal of proteins by heat coagulation or acid precipitation cannot be easily eliminated. 0.5% acetic acid, on the other hand, is not only an efficient solvent for the extraction of the non-protein-nitrogen but can be easily distilled off. It has the further advantage of effecting a coagulation of the globulins during the process of extraction itself.

With a view to determining the percentage of the non-protein-nitrogen (N.P.N.) present in the seed meal, the material (10 g.) was repeatedly (3–5 times) extracted with 100 ml. portions of dilute acetic acid in the cold at 0° to eliminate the possibility of proteoclastic action. Successive extracts obtained by centrifuging the mash were kept separately, warmed up to 60° to coagulate any albumins that might be present, treated with finely disintegrated Seitz asbestos, filtered and the resulting clear filtrate made up to a known volume, an aliquot of which was employed for a determination of the total nitrogen (T.N.). The clear filtrates were found to yield no precipitate or any opalescence on the addition of trichloroacetic acid, showing thereby that all the proteins had been eliminated. In the case of two pulses, *C. arietinum* and *P. mungo*, filtrations of the later extractions became increasingly difficult and yielded turbid filtrates, owing to the simultaneous extraction of increasing amounts of starch whose presence could be detected by the iodine test.

It will be observed from Table I that *P. aconitifolius* has a non-protein-nitrogen content which is about thrice that of *C. arietinum* and about four times that of *P. mungo*. The first extraction removes 54–68% of the N.P.N. present in the seed whilst the yield and purity of the subsequent extracts are low, owing to the extraction of increasing concentrations of carbohydrates whose elimination is

Table I.

Pulse	T.N. as % on the wt. of moisture- free seed	% of N.P.N. on the T.N.	% of N.P.N. obtained at each of the extractions				
			1	2	3	4	5
<i>P. aconitifolius</i>	3.85	34.85	62.62	21.88	8.42	4.32	2.75
<i>C. arietinum</i>	3.50	11.63	68.01	16.55	8.82	6.62	—
<i>P. mungo</i>	3.74	8.86	54.63	22.69	22.69(?)	—	—

extremely difficult and whose presence seriously interferes with the analysis of the N.P.N. In our present study, therefore, we have confined our attention to the investigation of the first extract, for obtaining a large quantity of which the following procedure was adopted.

The seed meal (1 kg.) was extracted for 24 hours at 0° with 10 litres of 0.5% acetic acid. The extract after filtration was warmed to 60°, treated with asbestos and again filtered, a clear protein-free filtrate being thus obtained. The filtrate was concentrated at 45–50° under reduced pressure to about 500 ml., and was then preserved in the ice-chest for subsequent experiments.

A partitioning of the nitrogen by the method of Van Slyke as modified by Damodaran [1931] so as to include the dicarboxylic acid-nitrogen, and an independent estimation of some of the essential amino-acids have been carried out. Arginine was determined by the arginase-urease method of Hunter & Dauphinee [1930] whilst the colorimetric methods of Folin & Marenzi [1929] were adopted with minor modifications for the estimation of tyrosine, tryptophan and cystine. During the estimation of tryptophan, the precipitate of mercury-tryptophan complex was decomposed by H<sub>2</sub>S yielding a light-coloured filtrate which was satisfactory for the colorimetric estimation. In the cystine estimation the precipitation of the mercury compound was carried out at pH 4.6, at which the cystine was quantitatively precipitated as revealed by preliminary experiments with known additions of cystine to the experimental hydrolysate. The mercury compound was suspended in dilute H<sub>2</sub>SO<sub>4</sub> solution and was decomposed by H<sub>2</sub>S. HgS was removed by filtration and the excess of H<sub>2</sub>S from the filtrate eliminated by passing in a current of CO<sub>2</sub>. For the estimation of tyrosine, no special modification in procedure was found to be necessary. Tyrosine and tryptophan were determined on 5 ml. of the non-protein-nitrogen concentrate, which were hydrolysed with 5 ml. of a 20% alkali for 18 hours on a boiling water-bath, whilst for cystine determination, 10 ml. of the material were treated with a sufficient amount of sulphuric acid so that the resulting concentration of the acid in the mixture was 4%. The hydrolysis was allowed to proceed for 3 hours in a metal-bath maintained at 110°. For Van Slyke analysis, however, the hydrolysis was carried out under the same conditions of temperature, time and acid concentration except for the fact that hydrochloric in place of the sulphuric acid was employed. Preliminary trials showed that under these conditions a complete fission of the peptide linkages had occurred with the formation of the minimum amount of humin.

Table II. *Van Slyke partitioning of the nitrogen as percentages of the total.*

Form of nitrogen	Melanin	Amide	Dicarbo- xylic	Basic amino	Basic non- amino	Non- basic amino	Non- basic non- amino	Total
<i>P. aconitifolius</i>	1.24	5.54	23.72	20.56	24.98	25.76	0.51	102.31
<i>C. arietinum</i>	2.34	6.11	13.06	15.22	44.29	19.31	0.04	100.39
<i>P. mungo</i>	2.37	3.41	16.16	11.21	32.46	19.95	16.52	102.10

A study of the above results (Table II) reveals that the N.P.N. fraction of aconite bean has a higher percentage of dicarboxylic acid nitrogen than either of the other two fractions, whilst *C. arietinum* contains the maximum amount of the basic nitrogen. The proline-hydroxyproline content of *P. mungo* appears to be exceptionally high as revealed by the non-amino-nitrogen in the non-basic fraction.

Table III. *Essential amino-acids as percentages of total nitrogen.*

	Arginine (Van Slyke)	Arginine (enzyme)	Tyrosine	Tryptophan	Cystine
<i>P. aconitifolius</i>	12.71	11.85	0.84	0.42	0.22
<i>C. arietinum</i>	45.48	22.92	0.60	0.50	1.13
<i>P. mungo</i>	34.59	16.30	0.78	1.15	3.39

Table III shows that whilst in the case of *P. aconitifolius* there is a fair agreement between the arginine values obtained by the Van Slyke and enzyme methods, there is a great discrepancy between similar pairs of values for the other two pulses, the values by the enzyme method being about half those by the Van Slyke method. This discrepancy points to the existence of bases other than arginine which are capable of yielding ammonia during alkali hydrolysis and which appear to possess no free amino-group capable of reacting with nitrous acid, as revealed by the high values for non-amino-nitrogen (Table II) in the basic fraction. Further work to characterise these bases is now in progress.

The percentage of tyrosine in the non-protein-nitrogen fraction is very much lower than the tyrosine content of globulins associated with the respective seeds, as given by Niyogi *et al.* [1932]. The tryptophan content in general follows the same order as obtains in the globulins, whilst the cystine content of the fraction from *P. mungo* is very much higher than the cystine content of the globulins of the seed as reported by Niyogi *et al.* [1932].

#### SUMMARY.

1. The non-protein-nitrogen fractions of the three pulses, *P. aconitifolius*, *C. arietinum* and *P. mungo*, have been extracted by dilute acetic acid and concentrates prepared for analysis. The nitrogen of the fraction has been partitioned by the method of Van Slyke as modified by Damodaran to include dicarboxylic acid-nitrogen.

2. An independent determination of the essential amino-acids, tyrosine, tryptophan, cystine and arginine by the arginase-urease method has been carried out. The results strongly point to the existence of a new base other than arginine.

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