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FREE AMINO ACID ANALYSIS OF SOME LEGUMES

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Amino acids of leguminous seeds, irrespective of their occurrence in free or protein-bound state are essential factors in nutrition, particularly those assigned as the 'essential amino acid.' The present communication deals the analysis of free amino acid content of fine seeds ; Cassia tora, C. occidentalis, Cassia fistula, Mucuna prurita and M. capitata of Indian origin. The seeds have been collected locally in the Bundelkhand region, particularly the forest of Jhansi district. The total free amino acid content of the seeds in terms of glycine (basic unit) was also estimated by employing the method of Rosen [1].

The seeds of Mucuna capitata was only obtained from the department of chemistry, University of Allahabad, Allahabad. All the powdered seeds were defatted in a Soxhlet apparatus with petroleum ether (60-80° C) and preserved them in air tight bottles. One gram of each of the defatted seed powders was well stirried with ethanol (8-10 ml, 70% v/v) for half an hour. After centrifugation the residue was reextracted with 70% ethanol, spun and the two supernatants combined. This process was repeated 8-10 times till the supernatant was negative to Ninhydrin test. The pooled supernatant was evaporated to dryness in Vacuo, dissolved in distilled water (0·5-1·0 ml), centrifuged and the clear supernatant (2-10 ml) was employed for qualitative and quantitative analysis of free amino acids.

The two-dimentional chromatographic analysis of Datta, Dent and Harris [2] was employed using phenol (80% w/v)– NH_3 and n-Butanol : acetic acid : water (4 : 1 : 5) as the developing solvents. The different amino acids present were confirmed by special spray reagent by Sakaguchi [3], Smith [4], Pant [5], Block etal [6] and Dent [7].

For estimating the total free amino acid content an accurately measured volume of seed extract (2–10 μl) was diluted with glass distilled water (1 ml) followed by the addition of acetate-cyanide buffer (0·5 ml) and ninhydrin solution (0·5 ml) in methyl cellosolve. The tubes were heated in a water bath at 100°C for 20 minutes and quickly diluted by the addition of isopropanol (5 ml) with constant shaking. The solution was cooled to room temperature and colour density read in a Calorimeter at 570 nm with a reagent, black and standard glycine solution.

Table 1. **Qualitative Pattern and Total Content of Free Amino Acids in Leguminous Seeds.**

Amino Acid	Cassia tora/	C. occidentalis/	C. fistula/	M. prurita/	M. capitata
£–alanine	+	+	+	+	+
Arginine	+	+	+	+	+
Aspartic acid	–	+	+	+	–
Cysteic acid	+	–	–	+	–
Glutamic acid	+	+	+	+	+
Glycine	+	+	+	+	+
Histidine	–	–	–	+	+
Leucine-Isoleucine	+	+	+	+	+
Lysine	+	+	+	+	+
Methionine	–	+	–	–	–
Proline	+	+	+	+	+
Phenylalanine	+	+	+	–	–
Serine	+	+	+	+	+
Threonine	+	+	+	+	+
Tryptophan	–	–	+	–	–
Tyrosine	+	+	+	+	+
Valine	+	+	+	–	–
Total free amino acids per 100 gm. seeds	·38	·36	·28	·16	·19

Table 1 represents the qualitative pattern and total content of free amino acid composition of the leguminous seeds analysed. Each seed has its own pattern of amino acids and no single seed was found to be complete with respect to essential amino acids. All the seeds contain 14–17 amino acid in the free state. Table 1 revealed that the various leguminous seeds contain free amino acid in the order of ·13–0·32 g (in terms of glycine) per 100 g of dry seed powder.

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