

NOTE

Estimation of the Amino Acids of Protein from the Seeds of *Kigelia pinnata* DC

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The protein extracted from the defatted seeds of *Kigelia pinnata* DC on hydrolysis gave the amino acids which were identified by chromatographic techniques. Each amino acid of the protein was quantitatively estimated by measuring the optical density with the aid of a spectrophotometer.

Key Words: *Kigelia pinnata* DC, Amino acids, Qualitative, Quantitative.

Kigelia pinnata DC known as common sausage tree in English has been reviewed by many authors¹⁻⁷. The roasted seeds of the plant have been reported to be eaten in times of scarcity. In view of the edible importance, it was thought worth while to analyze the seed protein.

Qualitative analysis: Protein extracted from the defatted seeds with 10% NaCl solution was precipitated from the saline extract at pH between 3.5 to 4.5 (yield 5.1%). The protein isolated was hydrolyzed with 6 N HCl, refluxing for about 24 h at 110°C. The hydrolyzate obtained and decolorized with animal charcoal was dissolved in 10% isopropanol for paper chromatography (ascending and descending), circular paper chromatography⁸ and two-dimensional thin layer chromatography^{9,10}. The presence of alanine, aspartic acid, cystine, glutamic acid, glycine, leucine, lysine, proline and valine was established by comparison with R_f values of the test sample with authentic specimen under identical conditions.

Quantitative analysis: Several methods have been proposed from time to time for quantitative estimation¹¹⁻¹⁵ of the amino acid composition by paper chromatography. In the present investigation, the method of Stein and Moore^{16,17} as modified by Troll and Cannon¹⁸, Moore and Stein¹⁹ and Yemn and Cocking²⁰ has been used to determine the percentage composition of amino acids.

The colour intensity produced by amino acid-ninhydrin reaction is the same for all amino acids at the same dilution except in few cases as shown by Rosen²¹. A standard graph prepared from the optical densities of leucine-ninhydrin reaction at different concentrations was used as a reference to estimate the amino acids present in the test sample (Fig. 1).

Several dilutions were prepared from the stock solution of leucine prepared by dissolving 100 mg in 100 mL of 90% alcohol. Equal amounts of different solutions of varying concentrations were spotted with the help of a micropipette on Whatman No. 1 paper strips and were developed by ascending technique with a solvent

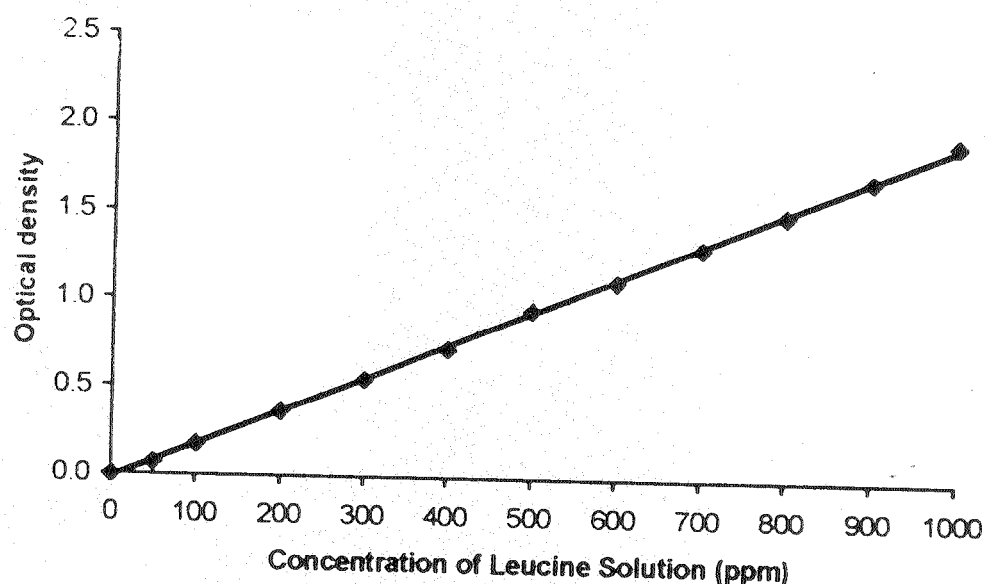


Fig. 1. Colorimetric estimation of amino acids

obtained as the upper layer on shaking a mixture containing *n*-butanol : acetic acid : water (3 : 1 : 1 v/v). The chromatograms were air-dried and sprayed with 0.5% ninhydrin solution. The coloured spots that appeared were cut and eluted with 10 mL of 90% alcohol. After adding 1 mL of ninhydrin to these eluants, they were warmed and cooled under identical conditions. The optical densities of leucine-ninhydrin solutions were measured by means of a spectrophotometer at 570 nm. The optical density of proline was measured at 440 nm as it forms yellow colour with ninhydrin solution.

The optical densities of the amino acids of the test sample were measured under identical conditions and concentration of each amino acid was calculated from the standard reference graph by interpolation method (Table-1). Leucine and glutamic acid were found to be major ones while alanine was present in least concentration in the protein.

TABLE-1
QUANTITATIVE ESTIMATION OF AMINO ACIDS

Concentration of the leucine-ninhydrin solution (ppm)	Optical density observed	Amino acid of the test sample	Optical density observed	Concentration of amino acid (ppm)
1000	1.91	Cystine	0.225	13
900	1.70	Lysine	0.15	9
800	1.50	Glycine	0.175	10
700	1.31	Aspartic acid	0.13	8
600	1.115	Glutamic acid	0.28	16
500	0.95	Alanine	0.09	6
400	0.725	Proline	0.185	11
300	0.55	Valine	0.13	8
200	0.365	Leucine	0.345	19
100	0.175			
50	0.70			

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NOTE

Synthesis of Novel 2',3'-Didehydro-2',3'-dideoxynucleoside

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The structure **4** in Scheme-1 has been wrongly printed. The correct structure is shown below:

