

QUANTITATIVE DETERMINATION OF ASSIMILABLE
LYSINE IN THE PROTEIN OF THE COTTON PLANT
BY THIN-LAYER CHROMATOGRAPHY

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The present paper describes a method for determining the amount of assimilable lysine in the form of the DNP derivative [1] in the protein of the cotton plant which consists in separating ϵ -DNP-lysine from the other DNP-amino acids by chromatography in a thin layer of silica gel.

The dinitrophenylation of the proteins (5-10 mg) and their hydrolysis were performed by the usual method [2]. The acid-soluble fraction of the DNP-amino acids was evaporated and dissolved in acidified acetone, and the solution was filtered and concentrated to 1/3 of its volume and was then deposited in a continuous line (4 cm) on a thin layer of silica gel (15 g of Woelm DC silica gel with 0.75 g of gypsum in 30 ml of distilled water on a plate 20 × 20 cm). The ϵ -DNP-lysine and the DNP derivative of bovine serum albumin [BSA] were taken as standards.

Chromatography was performed in the butan-1-ol-25% NH_4OH - CH_3COOH (8:1:0.1) system twice with intermediate drying at 110°C for 10 min. The band of the silica gel with the adsorbed ϵ -DNP-lysine (Fig. 1) was transferred quantitatively to a centrifuge tube with 4-5 ml of 3 N HCl and the mixture was centrifuged at 5000 rpm. Then the solid matter was washed again with HCl and the absorption of light was measured in the combined eluate at 435 nm in a 1-cm cell on an SF-4A instrument. The amount of ϵ -DNP-lysine present was determined from a calibration curve made by using recrystallized ϵ -DNP-lysine. For the various proteins isolated from cottonseed meal the following figures were obtained: 1.6 ± 0.2% for the water-soluble proteins, 3.0 ± 0.4% for the salt-soluble proteins, 2.0 ± 0.3% for the alkali-soluble proteins, and 2.9 ± 0.3% for the total fodder protein.

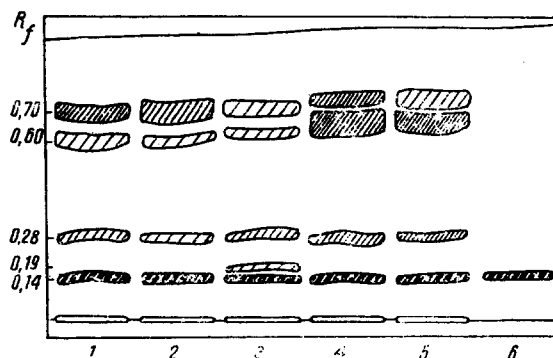


Fig. 1. Thin-layer chromatogram of the acid-soluble DNP-amino acids of protein hydrolyzates: 1) BSA; 2) water-soluble; 3) salt-soluble; 4) alkali-soluble proteins of the cotton plant; 5) fodder protein of the cotton plant; 6) ϵ -DNP-lysine.

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The calculation factor for assimilable lysine found from the value of the ϵ -DNP-lysine of the standard protein - bovine serum albumin [3] - was 0.7. The mean value of the assimilable lysine of the fodder protein isolated by the salt method was $2.1 \pm 0.3\%$ (from 15 experiments), which amounts to 70% of the total amount of lysine determined in a hydrolyzate (6 N HCl, 145°C, 4 h) [4] on an AAA-881 amino-acid analyzer.

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