Estimation of Lysine and Sulfur Amino Acids by Short Ion Exchange Column Method

A relatively inexpensive, short ion exchange column method is described for the determination of lysine, methionine, and cystine, which are probably the commonest limiting amino acids of human diets. Cystine and methionine are determined in their oxidized form to avoid loss during hydrolysis and chromatography.

Lysine and the sulfur amino acids are probably the commonest limiting amino acids of human diets (Miller & Naismith, 1958; F.A.O., 1965). A relatively cheap, quick, and accurate method for their analysis is therefore an advantage in determining the protein value of a diet or dietary component. Such a method, utilizing performic acid oxidation and ion exchange chromatography on a short (40 cm) resin column, is described here.

To avoid the loss of cystine and methionine during acid hydrolysis in the presence of carbohydrates (Schram *et al.*, 1953; Jennings and Lewis, 1969) performic acid oxidation of cystine to cysteic acid and methionine to methionine sulfone is first carried out as described by Lewis (1966). This procedure does not effect the recovery of lysine. The hydrolysis of the material, the preparation of the Zeocarb 225, W.R. 1.5, −200 mesh ion exchange resin column and the preparation of 0.5*M*, pH 2.80 buffer solution are also fully described by Lewis (1966). For successful amino acid separation on the column it is essential that resin of the correct particle size and W.R. (water regain) value is used.

One milliliter of the hydrolyzate (containing between 0.5 and 1.0 mg of N_2) is carefully applied to the top of the resin column and washed in with two 1-ml applications of the pH 2.80 buffer solution. The buffer solution is then washed through the column from a separating-funnel reservoir at a rate of 30 ml per hr under a pressure of 3 pounds per in.^{2} (2 newtons per cm^2) supplied from a cylinder of high-purity nitrogen. The run is carried out at room temperature (18° to 23° C) and the eluate collected in 2-ml fractions by a fraction collector.

After 100 ml of eluant has been collected (fraction no. 50), the run is stopped, the pH 2.80 buffer completely removed from the system and carefully replaced with a 0.5*M*, pH 8.0 sodium citrate buffer, care being taken not to disturb the top of the resin bed. (This buffer is prepared from 40 g of A.R. sodium hydroxide and 69 g of A.R. citric acid made up to 2 l. with deionized water, a final pH adjustment being made using a pH meter. To avoid high blanks, the quantity of sodium hydroxide in the buffer solution should be kept to a minimum. Four milliliters of 0.5% Brij 35 detergent are added to the buffer which is deaerated under vacuum before use.) The pH 8.0 buffer is then washed through the column at room temperature and at a rate of 30 ml per hr.

Under the conditions described above, cysteic acid is washed from the column at an elution volume of 36 to 40 ml, methionine sulfone at an elution volume of 110 to 120 ml, and lysine at an elution volume of 165 to 180 ml. The amino acid elution sequence can be seen in Figure 1; the run takes approximately 6 hr to complete. Ornithine is coincident with ammonia-tyrosine.

The column is regenerated after each run by washing with 50 ml of 0.2*N* NaOH, and then equilibrating with pH 2.80 buffer.

The fractions are developed by the ninhydrin method of Jacobs (1956), development time in the water bath being kept to 20 min to avoid the production of high blank readings. The quantities of cystine and methionine in the hydrolyzate are calculated from the quantities of cysteic acid and methionine sulfone, respectively.

This method of cystine, methionine, and lysine estimation has shown an accuracy of 100 ± 3% when used on intact protein of known composition, and is presently being used in the protein analysis of local plant food materials (Shanley and Lewis, 1969).

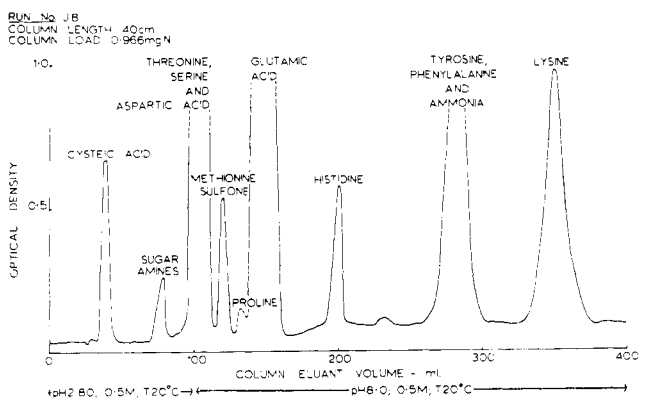

Figure 1. Amino acid resolution pattern of hydrolyzed plant material

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