RAPID ESTIMATION OF LYSINE BY HIGH VOLTAGE ELECTROPHORESIS

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ABSTRACT

High voltage electrophoresis technique has been adopted for rapid estimation of lysine. Pyridine : acetic acid water (100 : 10 : 800 v/v), pH 6·2, gives excellent separation of lysine, argioine, histidine, glutamle acid and aspartic acid. - Each separation takes 20–30 min. at 3,000–4,000 volts. . The values obtained agreed very closely (r-0·0967) with those obtained by the manometric method.

CONSIDERABLE progress has been made in rapid estimation of protein by different techniques. Since lysine happens to be the first limiting amino acid in almost all the cereal grains[1-3], its estimation is qu te important in the improvement of the prote'n quality and in the development of varieties with higher nutritive value. At present lysine is mostly estimated either by employing autoanalyser[4] or by Warburg technique[5]. In both the cases lysine is decarboxylated and the resultant carbondioxide produced is estimated either manometrically or colorimetrically.

When a large number of samples has to be analysed, these two methods cannot be used as screen'ng techniques. The first one requires expensive equipment and the second method is time consuming and enzyme dependent. Colorimetric method for lysine estimation has also been reported[6]. In the present study high voltage electrophoresis has been adopted for the rapid estimation of lysine. In this procedure it is not necessary to completely remove the hydrochloric acid after hydrolysis and the acid can be neutralised by the addition of alkali after the first evaporation. This saves considerable amount of time. The pyridine : acetic acid : water buffer system. (pH : 6·2) gives good resolution for lysine, arginine, histidine, glutamic acid and aspartic acid and all these amino acids can be estimated. Values for lysine as estimated by high voltage electrophoresis and Warburg technique agreed closely.

EXPERIMENTAL

A weighed sample (250 mg) was mixed with 8 ml 6 M HCl in hydrolysing tubes and sealed after evacuation. Hydrolysis of protein was done at 110° C for 18 hr. Hydrolysate was filtered and the filtrate collected was evaporated and the residue was d'ssolved in 1 ml buffer and neutralised NaOH (6 N).

The chromatographic paper (20 × 40 cm) was impregnated with pyridine : acetic acid : water (100 : 10 : 800 v/v) pH 6·2 and the excess buffer was removed, 5 µl of the sample was applied at the centre. Electrophoresis was carried out using Camag High Voltage Electrophoresis equipment at 3000–4000 volts for 20–30 minutes depending on the voltage applied. After this the paper was removed, allowed to dry in air and sprayed with ninhydrin (0·2% in ethanol). The colour was developed at 110° C for 2 minutes. The spots were cut and eluted with 70% acetone and read at 590 nm. The quantity of lysine was calculated from a standard curve prepared in the same way. Lysine was also estimated by conventional Warburg technique[5].

RESULTS AND DISCUSSION

Of the different buffer systems tried pyridine : acetic . acid : water (100 : 10 : 800 v/v) pH 6·2 gave good separation and resolution for the five amino acids, lysine, arginine, histidine glutamic acid and aspartic acid (Fig. 1). Sample was applied in the centre of the paper. Lysine, arginine and histidine migrated towards the cathode while glutamic acid and aspartic acid migrated towards anode. The other amino acids remained very near the origin where the sample was applied. In this estimation the pH of the buffer is quite critical as the lower pH was found to separate other amino acids as well but the separation of lysine from arginine and histidine was not good. At 4000 volts each separation took 20 min. only and three samples in duplicate were applied each time.

The amount of lysine obtained in 19 different samples representing maize endosperm, embryo and pericarp by this technique as well as conventional Warburg technique is shown in Table I. It is seen that the amount of lysine obtained by high voltage electrophoresis is in very close agreement

with the amount obtained by Warburg technique. The correlation coefficient value was 0·987. High voltage electrophoresis technique is much faster than the Warburg technique and complete removal of the acid after hydrolysis is not necessary and acid can be neutralised with alkali after first evaporation thereby saving considerable time.

FIG. 1. Separation of amino acids by High Voltage electrophoresis. Top to bottom : Lysine, arginine, histidine, neutral amino acids, glutamic acid and aspartic acid. Line at the centre represents origin where the samples were applied.

Besides the estimation of lysine, other amino acids, namely, arginine, histidine, glutamic acid and aspartic acid can also be estimated. This is not possible with either Warburg technique[5] or colorimetric estimation for lysine[4,6]. Since this method is rapid, it has a potential to be used as a screening technique for estimation of lysine.

TABLE I

Lysine content in g/100 g as determined by High Voltage Electrophoresis and Warburg technique

Sample No.	High Voltage Electrophoresis Method	Warburg Method
Opaque-2 kernel	0·34	0·31
„	0·32	0·32
„	0·36	0·37
„	0·40	0·39
„	0 39	0·40
„	0·38	0·40
„	0·34	0·33
„	0·32	0·38
Pericarp	0·12	0·12
Opaque–2 Percarp	0·19	0·22
Percarp	0·16	0·16
Endosperm	0·32	0·34
Embryo	0·48	0·50
Endosperm	0·29	0·33
Embryo	0·58	0·59
Opaque–2 Endosperm	0·34	0·36
Opaque–2 Embryo	0·50	0·48
Endosperm	0·30	0·29
Embryo	0·59	0·61

Correlation Coefficient — 0·987**

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