Determination of Available Lysine in Pulses by Thin Layer Chromatography

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ABSTRACT AND SUMMARY

Estimation of available lysine in five pulse samples was carried out by a thin layer chromatographic technique. Results revealed that the available lysine values in all the cases were to some extent lower than the total lysine values.

INTRODUCTION

Among the various vegetable ingredients of our diets, pulses contain a remarkable amount of edible protein. Although essential amino acid composition of these pulses has already been determined by Banerjee (1), data on the contents of available amino acids of these materials have probably not been reported. Datta et al. (2) have shown that a good correlation exists between biologically measured protein quality and chemically measured lysine availability in growing chicks. The present investigation has, therefore, been carried out to estimate available lysine contents of pulses so that these data can be used as a chemical index for the determination of nutritive values of these materials.

EXPERIMENTAL PROCEDURE

Finely ground pulses (0.5 to 1 g) was treated with 2:4-dinitrofluorobenzene (DNFB) according to Rao et al. (3). The mixture was hydrolyzed carefully with 6N HCl for 18 hr at 110 C. After beeing cooled to room temperature, it was filtered through a sintered glass funnel. The residue was washed three to four times with water and the filtrate with the washings was made up to 10 ml. 2 ml of this hydrolysate was extracted four to five times with ether and the aqueous phase was freeze dried. The residue was dissolved in 2 ml of 3% aqueous acetic acid and then passed through an alumina column (30 cm x 2 cm) previously equilibrated with 3% aqueous acetic acid. At a flow rate of 200 ml/hr 3% aqueous acetic acid was passed through the column for 1 hr. The trapped yellow band was collected by eluting with 1% sodium bicarbonate solution and then freeze dried. The residue was redissolved in 2 ml of n-butanol-glacial acetic acid (50:1 v/v). 0.1 ml of this solution was spotted on thin layer plates (25 cm x 8 cm) of silica gel (0.4 mm thickness) and dried at room temperature. The plates were then placed in chambers saturated with n-butanol-glacial acetic acid-water (15:3:8 v/v) solvent system and allowed to develop by ascending technique for 2 hr. After being removed from the chamber, the plates were air dried. Yellow colored ϵ-DNP (2,4-dinitrophenyl) lysine was characterized as a single spot with the help of standard solutions which were cospotted on the plates. For quantitative determination the ϵ-DNP lysine spot was scraped out carefully in a centrifuge tube and taken in 5 ml of 1% sodium bicarbonate solution. The tube was then heated at 60 C on a water bath for 20 min, cooled at room temperature, and centrifuged. The optical density of the supernatant was read at 435 mμ using a DBG Beckman spectrophotometer. A calibration curve was prepared by using standard ϵ-DNP lysine hydrochloride in the range of 0.02-0.10 mg/ml. From this standard curve the available lysine contents in pulses were quantitatively estimated.

RESULTS AND DISCUSSION

The available lysine contents for five pulse samples, procured from the local market, are shown in Table I. It was observed that in all cases available lysine values were lower than the total lysine values. Pulses after being collected from the field are generally dried by heat. As the lysine molecules with free ϵ-amino groups are only nutritionally available, it is likely that during drying some of these free ϵ-groups combined with active substances that are present in these pulses and form complexes to lower the amount of lysine available. Although a substantial part of the total lysine in these pulses was not lost it was found that lysine availabilities in *Lens esculenta, Phaseolous mungo,* and *Lathyrus sativus* were higher than those in *Cicer arientinum* and *Pisum sativus*. We also determined

TABLE I
Available Lysine Contents in Pulses[a]

Pulse	Protein (%)[b]		Total lysine (g/100 g sample)[d]		Available lysine (g/100 g sample)	
	Our result	Reported[c]	Our result	Reported[c]	Present method	Rao et al.'s (3) method
Lens esculenta	22.4 ± 0.4	23.0	1.76 ± 0.6	1.70	1.66 ± 0.2	1.49 ± 0.4
Phaseolus mungo	22.8 ± 0.8	22.0	1.54 ± 0.1	1.60	1.43 ± 0.2	1.24 ± 0.3
Lathyrus sativus	24.2 ± 0.2	25.0	1.98 ± 0.3	1.86	1.82 ± 0.7	1.58 ± 0.8
Cicer arietinum	19.6 ± 0.6	19.0	1.14 ± 0.4	1.02	0.91 ± 0.4	0.82 ± 0.6
Pisum sativus	21.7 ± 0.3	21.0	1.97 ± 0.3	1.86	1.65 ± 0.2	1.43 ± 0.2

[a]Values represent means ± standard deviations from the means.
[b]Determined by micro-Kjeldal method.
[c]Reported by Banerjee (1).
[d]Determined by the method of Moore et al. (6).

TABLE II
Recovery of Pure ϵ-DNP Lysine Hydrochloride after Hydrolysing with 6N Hydrochloric Acid for 18-24 hr[a]

ϵ-DNP lysine hydrochloride added to 6N hydrochloric acid (μg)	ϵ-DNP lysine hydrochloride in the hydrolysate (μg)		Percentage of recovery	
	Present method	Method of Rao et al. (3)	Present method	Method of Rao et al. (3)
40	39.7 ± 0.4	39.4 ± 0.2	99.2 ± 0.9	98.5 ± 0.4
60	59.4 ± 0.2	58.8 ± 0.4	99.0 ± 0.4	98.0 ± 0.7
80	78.1 ± 0.3	77.4 ± 0.5	97.9 ± 0.4	96.8 ± 0.6
100	98.5 ± 0.4	97.3 ± 0.6	98.5 ± 0.4	97.3 ± 0.6

[a]Values represent means ± standard deviations from the means.

TABLE III

Recovery of ϵ-DNP Lysine Hydrochloride Added to Pulse (*Lens esculenta*) Hydrolysate Prepared by the Method of Rao et al. (3)[a,b]

ϵ-DNP lysine added to the hydrolysate (μg)	ϵ-DNP lysine in total hydrolysate (μg)		ϵ-DNP lysine recovered (μg)		Percentage of recovery	
	Present method	Method of Rao et al. (3)	Present method	Method of Rao et al. (3)	Present method	Method of Rao et al. (3)
25	68.6 ± 0.6	62.4 ± 0.3	24.3 ± 0.2	22.3 ± 0.1	97.2 ± 0.9	89.2 ± 0.4
50	92.4 ± 0.8	85.5 ± 0.5	48.1 ± 0.4	45.4 ± 0.4	96.2 ± 0.8	90.8 ± 0.7
75	118.6 ± 0.6	106.5 ± 0.9	74.3 ± 0.2	66.4 ± 0.8	98.8 ± 0.2	88.4 ± 1.0
125	166.8 ± 0.9	153.1 ± 0.4	122.5 ± 0.5	113.0 ± 0.3	98.1 ± 0.4	90.3 ± 0.2

[a]Values represent means ± standard deviations from the means.
[b]The original hydrolysate, before addition of ϵ-DNP lysine HCl, contained 44.3 ± 0.4 or 40.1 ± 0.2 μg of ϵ-DNP lysine according to the present method or the method of Rao et al. (3), respectively.

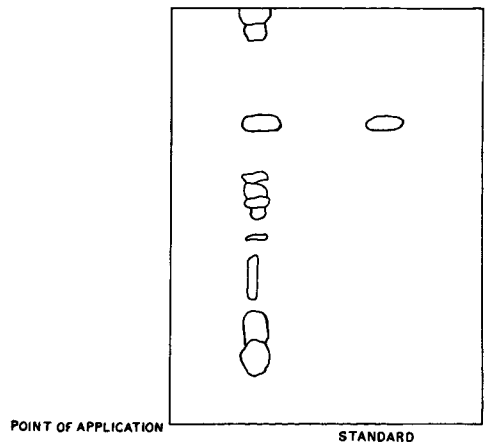

FIG. 1. Thin layer chromatographic separation of ϵ-DNP lysine after passing the pulse hydrolysate through an alumina column.

available lysine contents of pulses by following the column chromatographic method of Rao et al. (3) but obtained lower values in comparison to those determined by present method (Table I).

Two sets of recovery experiments were also carried out. In one set, known amounts of pure ϵ-DNP lysine hydrochloride were added to 6N hydrochloric acid. The solution was then hydrolyzed for 18 to 24 hr. The prepared hydrolysates were then estimated for ϵ-DNP lysine. In the other set, known amounts of pure ϵ-DNP lysine hydrochloride were added to the pulse hydrolysate which was prepared after adding DNFB. The recoveries of the added ϵ-DNP lysine in both cases were estimated according to the method described above and also by following the method of Rao et al. (3). The results of these recovery experiments are tabulated in Tables II and III. The data show that in the first case the recoveries of ϵ-DNP lysine when determined by the present method confirmed those obtained by the method of Rao et al. (3). In the second case, the percentage of ϵ-DNP lysine recovery determined by the method of Rao et al. (3) was lower in comparison to those obtained by the present method. This is why available lysine values of pulses when determined by the method of Rao et al. (3) were poor when compared to those determined by the present method.

In Figure 1 the separation of ϵ-DNP lysine after being passed through an alumina column is shown. An alumina column was used to remove dinitrophenol (4) because it would then not mask the color of ϵ-DNP lysine. Recently Datta (5) has estimated available lysine contents of chick starter rations using DNFB where the interfering components were separated with a Sephadex G-50 column. As Sephadex is costly we used alumina to make the process more economical. Thin layer chromatographic separation of ϵ-DNP lysine was conducted to visually check the purity of this amino acid derivative.

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