

DETERMINATION OF AMINO ACIDS IN RICE (PALDAL)  
BY IONEXCHANGE CHROMATOGRAPHY

Chun Yung Lee, Tae Yung Lee, and Tai Wan Kwon

College of Agriculture, Seoul National University, Suwon, Korea

(Received, Sept. 15, 1961)

The values for amino acids in rice have been reported by several workers and accepted as the bases for the evaluation of rice proteins. The results have been obtained mainly by applying the microbiological assay to the acid hydrolyzates of the milled rice. The presence of carbohydrates, however, invites the considerable loss of several species of amino acids during the acid hydrolysis. Besides the destruction of tryptophan, the mineral acids bring about the diminished yields of arginine, histidine, lysine, cystine, methionine, and serine in the presence of carbohydrates.

Dustin and Moore<sup>1)</sup> et al, however, observed that the hydrolysis of proteins with large excess of 6N HCl in the presence of considerable amount of carbohydrates (for example, one hundred times) gave the good recovery of fifteen amino acids except tryptophan, cystine and methionine in the ion exchange chromatographic determination.

The authors found in the present study that the presence of a large amount of starch resulted in a considerable loss of overall amino acids in the similar treatment of rice as described by Dustin and Moore.

### EXPERIMENTAL

Samples of rice "PALDAL" harvested in 1959 at the Agricultural Experiment Station (Suwon, Korea) were used for the determination of amino acids. The hulled rice contained 1.23 per cent nitrogen. The powdered rice and extracted crude protein were treated. The latter samples were prepared after Block and Bolling<sup>2)</sup>. Ten grams of ground rice were suspended in

90 ml of hot water, the pH being adjusted to 4.5 with 5N acetic acid, and heated for one hour on a boiling water. Then, 180 ml. of cold water were added to the suspension and the pH adjusted to 7.0 with 5N NaOH. After cooling to 37°C., 10-25 ml. of fresh human saliva, clarified by centrifugation, are added. The starch was digested at 37-40°C. over night in the presence of toluene as the preservative.

The nitrogen content was 9.78 per cent.

The hydrolysis of both ground rice and crude rice protein was performed by heating for 24 hours with 6N HCl (200 ml. for 8 mg. nitrogen) at the temperature of 110°C. Each hydrolyzates were concentrated under a reduced pressure to remove excessive HCl. The residue was diluted with distilled water and made up to the final volum 25ml. 1.0ml. out of which was taken for the chromatography.

The amino acids were determined chromatographically, essentially as described by Moore Spackman and Stein<sup>3)</sup> with a modification of authors<sup>4)</sup>. Recovery of  $100 \pm 3 \sim 5$  were obtained for the majority of amino acids.

### RESULTS and DISCUSSION

1. Effect of carbohydrates on chromatography:  
There was considerable difference in the mode of separation of amino acids between the hydrolyzates of ground rice and crude protein preparation as shown in Fig. 1 and Fig. 2. In the first sample the red peak appearing prior to the elution of aspartic acid is marked. The peak is considered to be due to the formation of complex from ninhydrin and unknown substances resulting from hydroly-

sis of carbohydrates and proteins as described by Dustin and Moore et al.<sup>13</sup> In the second sample, on the other hand, no red peak was recognized. This fact and the change in peak sizes of most of amino acids suggest that presence of large amount of starch apparently affected the amino acid determination, nevertheless Dustin's claim that large excess of hydrolyzing acid reduces the losses

of amino acids under the presence of extremely large amount of carbohydrates.

Furthermore, the removal of carbohydrates clearly diminished the interference with the amino acid separation, hence the appearance of peaks of cystine and methionine as well as disappearance of the red peak with good yields of other amino acids were indicated as shown in Fig. 2.

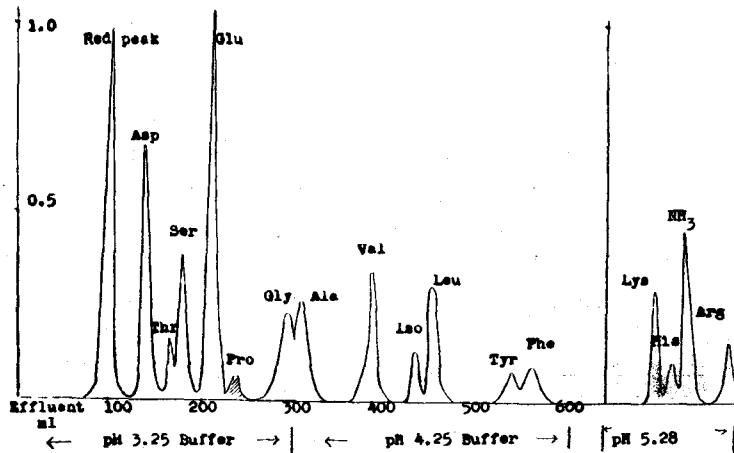


Fig. 1. Chromatographic Fractionation of Amino acids in Hydrolyzate of Rice per ce.

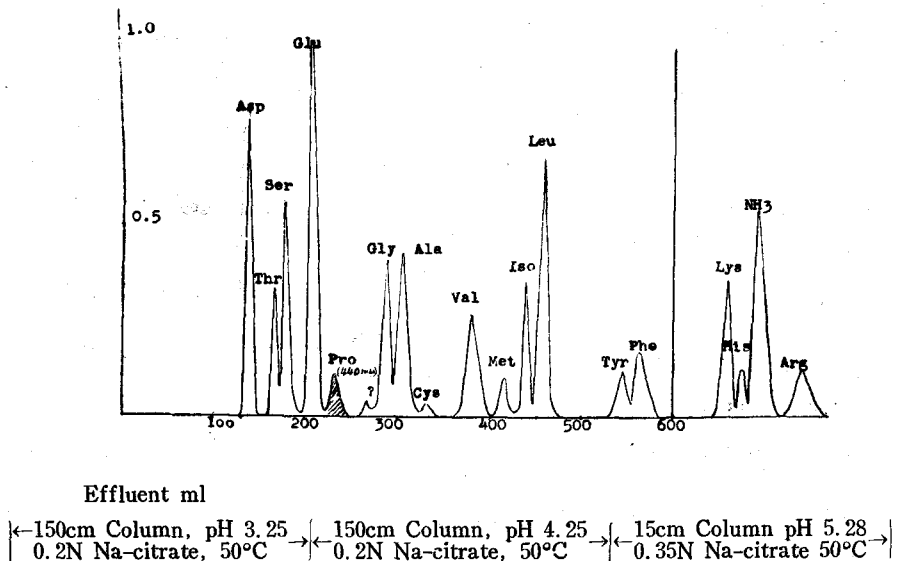


Fig. 2 Chromatographic Fractionation of Amino Acids in the Hydrolyzate of Crude Protein Preparation of Rice

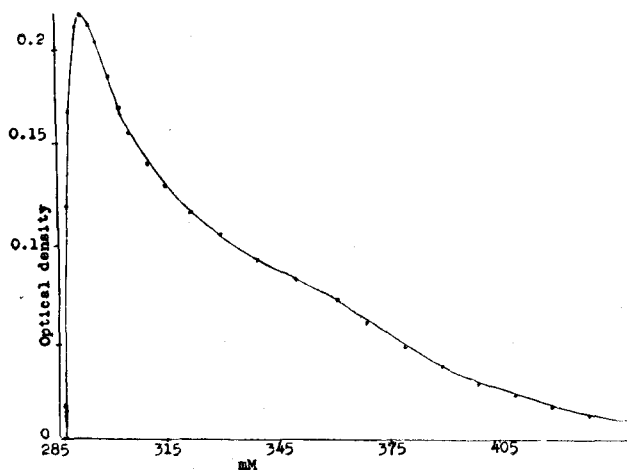


Fig. 3, Ultraviolet Absorption Spectrum of the First Eluate from IR-120 Column. (before Ninhydrin; pH 3.25 Na-citrate Buffer)

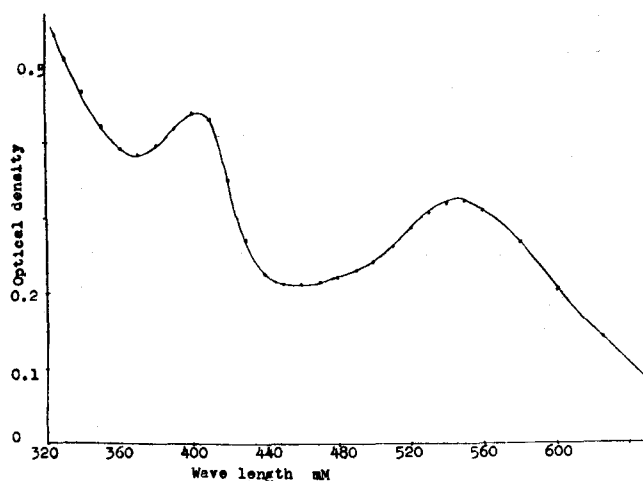


Fig. 4, Absorption Spectrum of the Red Peak

2. Values of aminoacids: Calculating from the results of Fig. 1 the amino acid composition of rice protein may be presented as in Table

1. For the purpose of comparison, the values by several other workers 5,6,7,8) were also included.

**Table 1, The Amino Acid Composition of Rice**

		(g. amino acid per 16.0 g. N)			Kik <sup>8)</sup>
		Block <sup>5)</sup>	Horn <sup>6)</sup>	Tamura <sup>7)</sup>	
aspartic acid	7.59	—	—	9.4	4.82
threonine	3.64	4.1	3.2-4.3	3.4	5.15
serine	6.73	—	—	7.0	4.99
glutamic acid	21.78	24.1	—	23.4	11.31

proline	4.43	—	—	4.3	4.65
glycine	4.47	10.3	—	5.2	7.32
alanine	4.95	—	—	—	—
cystine	1.34	1.4	—	0.8	—
valine	6.56	6.3	6.1—6.7	9.1	7.65
methionine	1.63	3.4	1.3—2.3	1.5	—
isoleusine	5.05	5.3	4.2—5.9	5.5	5.18
leusine	10.63	9.0	7.8—9.0	10.4	9.48
tyrosine	4.71	5.6	5.7—5.8	3.0	5.32
phenylalanine	6.53	6.7	4.2—5.3	8.2	4.82
lysine	3.85	3.2	2.2—4.5	3.3	3.66
histidine	2.32	1.5	2.2—3.9	3.2	3.15
ammonia	(1.91)	—	—	—	—
arginine	6.01	7.2	6.0—8.8	9.0	10.32
total	104.20				

No marked differences were noted between the present results and those of Block <sup>5)</sup>, Horn <sup>6)</sup>, and Tawamura <sup>7)</sup>. The data found by Kik <sup>8)</sup> are rather peculiar especially in the values of glutamic and aspartic acids.

### SUMMARY

The amino acid analyses of ground rice and the crude protein isolated from rice were performed by the most recent method of Moore, Spackman and Stein, using Amberlite IR-120.

The effect of carbohydrates on the hydrolysis of rice was found to be definite in losing several amino acids nevertheless Dustin's claim that large excess of hydrolyzing acid reduces the losses of amino acid.

Consequently the removal of carbohydrates as much as possible is considered to facilitate the amino acid determination.

The amino acid composition of the crude protein of rice was very similar to that previously found by other investigators.

### LITERATURE CITED

- Dustin, J. P., Moore S., Czajkowska, C. and Bigwood, E. J.: *Anal. Chim. Acta* **9**, 256 (1953)
- Block, R. J.: *Amino Acid Handbook*, (Chaeles C. Thomas, Springfield, Ill.) p. 25 (1956)
- Moore, S., Spackman, D. H. and Stein W. H.: *Anal. Chem* **30**, 1185, (1958)
- Lee, K. Y., Kwon, T. W. and Lee, T. Y.: *Bull. Scient. Resear. Inst., Korea* **5**, 119, (1960)
- Block, R. J. and Bolling, D.: *Advances in protein chemistry* **II**, 119, (1945)
- Horn, M. T. et al.: *Cereal Chem.*, **64**, (1955) *ibid*, U.S. Dept. Agr. Misc. Publ. No. 696. (1950)
- Tamura, G. et al.: *J. Agr. Chem. Soc. Japan* **26**, 480, (1952)
- Kik, M.C.: *Arkansas Agr. Expt. Sta. Bull.*, 589; (1957) *ibid*: *J. Agr. Food Chem* **8**, 381, (1960)