AMINO ACID COMPOSITION OF PROTEINS OF "CHAR MAGHAZ" (CUCURBITACEAE SEEDS)

SAJJAD HUSSAIN, MUHAMMAD ARSHAD AND KARIMULLAH

West Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Lahore

(Received July 20, 1964)

The seed proteins of four species of Cucurbitaceae (melon, water melon, cucumber, pumpkin) were analysed. Thirteen amino acids *viz.*, arginine, histidine, lysine, threonine, leucine, isoleucine, valine, tyrosine, tryptophane, phenylalanine, methionine, proline and cystine were identified. As is evident, all the essential amino acids were present in the proteins isolated from the seeds.

Introduction

Summer brings to the people of this sub-continent a refreshing drink 'Sardai', which is commonly taken after some bodily exercise or to check the usual fatigue of burning hot days. 'Sardai' actually is an emulsion formed by blending the "Char Maghaz" i.e. kernels of the seeds of melon (*Cucumis melo*), water melon (*Citrullus vulgaris*), cucumber (*Cucumis sativus*), pumpkin (*Lagenaria vulgaris*) with milk, sweetened and flavoured according to the taste.

Scientifically, in the muscular work, the contractile material consisting of various biologically important compounds including proteins serve in the final step of the conversion of the chemical energy of the food to mechanical energy. Therefore, in the time of stress the body requires some surplus protein which it draws either from blood or is supplied from external resources.

Now the "Char Maghaz", a composition of equal amounts of seeds of melon, watermelon, cucumber and pumpkin are effectively used as tonic,[1] diuretic, as refrigerent for quenching thirst, for stimulating nerves, and a cure for liver and kidney troubles.

All these herbs of *Cucurbitaceae* family grow abundantly in the plains of Indo-Pakistan subcontinent. The fruits are eaten up but the seeds generally go waste.

Noe and Fowden[2] have reported the presence of β-Pyrazol-1-ylalanine in water melon seeds. In consideration of the important use of these seeds and with a view to studying the presence of some unidentified amino acids, it was desirable to undertake a study of these seeds, with special reference to their proteins.

Experimental

The seeds, after removal of the seed coat, were washed with water to remove insoluble matter like sand, dirt etc., and dried at room temperature. The total nitrogen contents of each species were determined by the Kjeldahl method.

Isolation of Crude Protein.—The clean and dry seeds were then subjected to coarse grinding in a disc mill to facilitate the removal of oil. The oil was removed by extracting with petroleum ether (b.p. 80-100°C.) in a soxhlet extractor for about six hours.[3] The oil-free powder was soaked in sufficient volume of 1 percent sodium hydroxide and allowed to stand overnight. The alkaline paste was ground, squeezed through doublefold muslin cloth and centrifuged to remove the starchy material present in it. The supernatent liquid containing most of the proteins, was acidified to pH 3.8 by the addition of 3 N. HCl. The precipitate was removed by centrifugation at 2500×g and the supernatent layer checked for complete precipitation of the protein.

The protein paste was finally washed with 95 per cent alcohol and allowed to dry in an air oven at 60°C.

Preparation of Protein Hydrolysate.[4]—Hydrolysis of crude protein (1.0 g.) was done by boiling with 80 ml. of 6N H_2SO_4 under reflux for 20 hours. The hydrolysate was then brought to pH 11.0 by adding finely powdered Ba(OH)2 with constant stirring. After centrifugation the unreacted Ba(OH)2 was removed and the pH of the contents was adjusted to 4.0 by adding dilute H_2SO_4. Any $BaSO_4$ formed during acidification was centrifuged. The filtrate so obtained was concentrated to dryness on a water bath and the protein hydrolysate was taken in 20 ml. of 10 per cent isopropyl alcohol solution in water.

Amino Acids.—The amino acid composition was established using the two dimensional ascending chromatographic technique. The equipment used was a multi-sheet chromatographic tank.

The solvents employed for the development of chromatograms are:-

1. n-Butanol	:	Glacial Acetic Acid	:	Water
100		24		100
2. Phenol	:	Water		
80		20		

Developing Reagent.—It was prepared by dissolving 0.25 mg. of ninhydrin in 100 ml. redistilled acetone and 7.0 ml. of glacial acetic acid.

Fixing Solution.—It was made by mixing 1.0 ml. of saturated copper nitrate solution, 0.02 ml. of 65 percent nitric acid and 99 ml. of acetone.

Development of Chromatograms.—For the identification and quantitative determination of amino acids, the sheets of 20×20 cm. were cut from Whatman I filter paper. Standard solutions of known aminoacids (Merck) containing 5 mg. per ml. were prepared. A known volume of these solutions was applied with ultramicropipette so as to obtain the spots of significant optical density on development of a chromatogram. It was observed that 20μg. of an acid applied to the paper resulted in a spot of desired colour intensity.

The amino acids present in the hydrolysates were separated by paper chromatography. Guide chromatograms were run along with unknown sample.

After a comparative study of the R_f values for identification purposes, the spots on these chromatograms were cut and eluted for quantitative analysis.

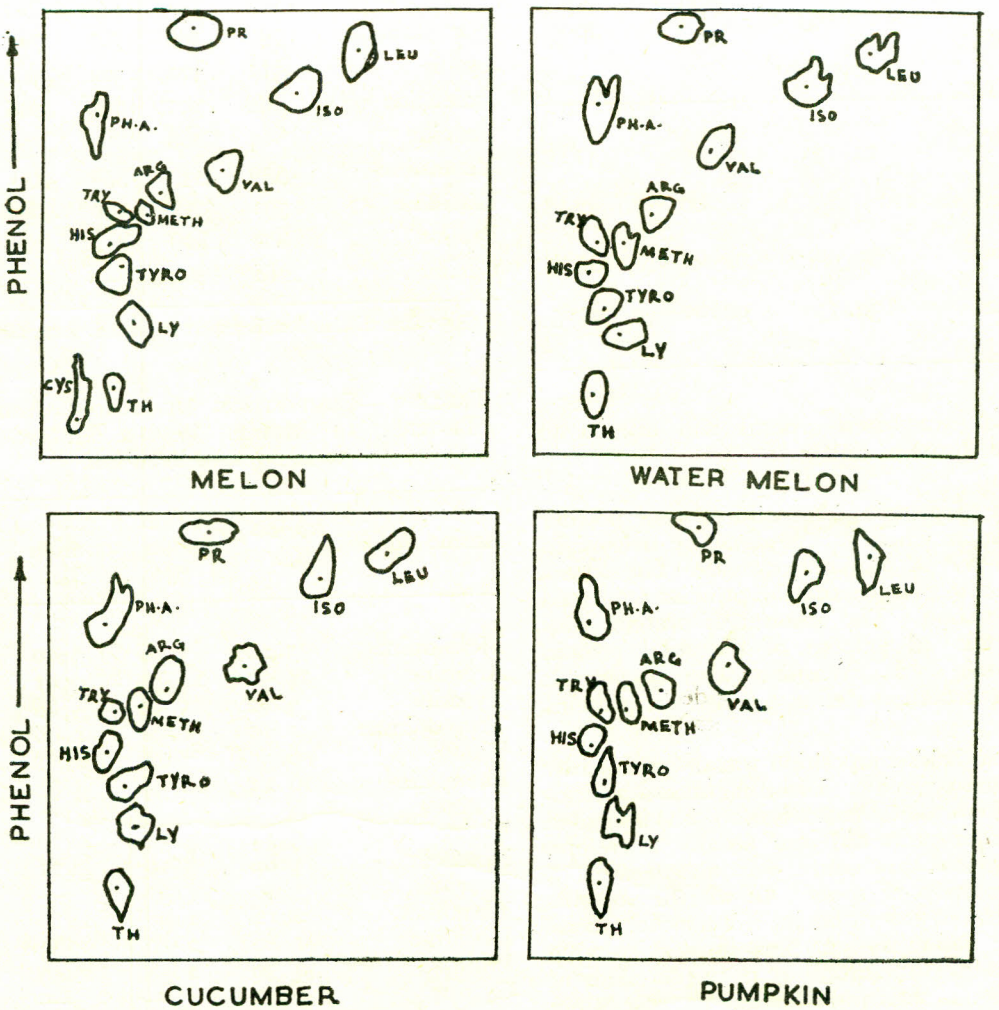

Fig. 1.—Chromatograms showing amino acids in various protein hydrolysates.

Results and Discussion

The seeds of melon, watermelon, pumpkin and cucumber were fairly soft and could easily be ground to thick oily paste. To avoid an interference in the subsequent process of paper chromatography, oil-free samples were used. The oil percentages present in these seeds are recorded in Table 1.

TABLE 1.—OBSERVED OIL AND PROTEIN PARTS OF SEEDS.

Seeds	Total protein of seed (N×5.85) %	Protein Purity (N×5.85) %	Recovered oil %
Melon	35.75	96.00	41.1
Water melon	39.75	94.00	42.3
Cucumber	38.00	94.00	35.6
Pumpkin	39.75	92.00	47.5

The paste was then ground and filtered to collect the protein isolate.

Isolation of protein from oil-free seed powder was maximum when it was done by over-night soaking in 1 percent sodium hydroxide. The extract was adjusted to pH 3.8 and the proteins separated by centrifugation. The amount of proteins thus recovered was 48 percent of the total protein present in the seed. Washing with 95 percent alcohol dehydrated as well as purified the protein.

The amino acid composition was determined by paper chromatography. Uni-dimensional ascending method revealed about ten spots, but indicated some overlapings in different areas. Therefore, two dimensional procedure was adopted for a distinct resolution of spots. Of all the solvents tried (a) phenol: water and (b) n-butanol: glacial acetic acid: water system proved most suitable to achieve better separation of 13 amino acids in the case of melon seed and twelve in the case of the rest.

TABLE 2.—COMPARISON OF THE R_f VALUES OF THE STANDARD AMINO ACIDS WITH THE ACID HYDROLYSATES OF CHAR MAGHAZ SEEDS.

Amino acids	Standard amino acid	Melon protein hydrolysate	Water melon hydrolysate	Cucumber protein hydrolysate	Pumpkin protein hydrolysate
Methionine	.680	.678	.677	.672	.671
Arginine	.721	.720	.722	.725	.725
Tyrosine	.552	.549	.549	.553	.551
Tryptophane	.630	.625	.626	.630	.630
Valine	.723	.721	.722	.713	.711
Phenylalanine	.790	.788	.785	.783	.789
Lysine	.511	.521	.521	.512	.514
Isoleucine	.792	.791	.790	.786	.779
Leucine	.814	.800	.809	.799	.814
Threonine	.350	.349	.351	.351	.352
Histidine	.591	.579	.590	.589	.589
Proline	.822	.818	.822	.820	.824
Cystine	.261	.263	—	—	—

(R_f values tabulated with respect to phenol: water solvent).

TABLE 3.—COMPOSITION OF AMINO ACIDS IN PROTEINS OF MELON, WATER MELON, CUCUMBER, AND PUMPKIN.

Amino Acid	Melon %	Water melon %	Cucumber %	Pumpkin %
Methionine	3.20	3.58	3.10	2.90
Arginine	14.51	14.50	13.91	13.81
Tyrosine	5.62	5.58	5.66	5.80
Tryptophane	1.10	1.30	1.00	1.12
Valine	6.50	7.10	6.51	6.62
Phenylalanine	10.23	10.54	9.86	10.11
Lysine	3.44	3.54	4.10	3.40
Isoleucine	4.80	5.10	4.21	5.23
Leucine	5.40	4.91	—	5.40
Threonine	2.12	1.90	2.10	2.31
Histidine	1.80	2.10	1.83	1.78
Proline	2.30	2.51	2.49	2.45
Cystine	0.80	—	—	—

The compositions of amino acids were then determined using a recent technique as indicated earlier and the following results were obtained:

Tables 2, 3 indicate the presence of at least twelve amino acids in the four different species of seeds under study. However, the presence of any unknown amino acid could not be detected. The presence of all the essential amino acids in these proteins seems to be responsible for their nutritional and medicinal use. The incorporation of these seeds in the bakery products or in a mixed vegetable cooking at home can improve the quality of the vegetable protein.

References

1. *Indian Medicinal Plants*, Volume II, pp. 1116, 1140, 1145.
2. F.F. Noe and L. Fowden, Biochem. J., **77**, 543 (1960).
3. A.M. Altschul, *Processed Plant Proteins Foodstuffs* (Academic Press Inc., New York, 1958), p. 45.
4. Mohammad Saeed, *et al.*, Pakistan J. Sci Res., **13**, 4, 145 (1961).