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AMINO ACID COMPOSITION OF PROTEIN CONCENTRATE PREPARED FROM LEAVES OF SUSBANIA AEGYPTICA (JANTAR)

MUHAMMAD NAZIR and MUHAMMAD SAEED

P.C.S.I.R. Laboratories, Ferozepur Road, Lahore 16

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Preparation of protein concentrate from the leaves of *Susbania aegyptica* (Jantar) is reported. Amino acid analysis of the product showed that it contains threonine, isoleucine, phenyl analine, lysine, methionine and tryptophane to the extent of 2.8, 4.65, 15.7, 7.75, 4.5 and 6.75% respectively, in addition to other amino acids. Feasibility of incorporation of these amino acids into cereal products is discussed. Of special importance are lysine and methionine, commonly lacking in cereals.

The presence of protein in plants was first demonstrated by Roulle as early as 1773.[1] The extraction of proteins from leaves was suggested by Ereky[2] and Pirie.[3] After that, considerable literature has appeared on the subject.[4–10] Akeson and Stahmann reported that biological value of leaf protein concentrate was higher than beef, casein, soyabean and yeast.[11] Duckworth and Woodham[12,13] studied the nutritive value of leaf protein concentrate as supplementary source of proteins for chicks and rats. Nazir and Shah[14] tested the extractability of protein nitrogen from some leafy plants and observed that leguminous and cruciferous plants were best sources of easily extractable proteins.

Susbania aegyptica (Jantar) plant, grown for animal feeding in tropical countries, appeared to present a suitable material for preparing edible protein concentrate. However, the knowledge of amino acid composition of protein of the plant material under investigation was essential before suggesting it for human consumption. This paper reports amino acid composition of *Susbania aegyptica* (Jantar) leaf proteins extractable by weak alkali treatment method.

Experimental

Leaves.—*Suabania aegyptica* (Jantar) plants were collected from the experimental plots of the Laboratories. Both wildly grown and cultivated plants are available in this country. Age of the plant at the time of harvesting was 150 days.

Preparation of Leaf Protein Concentrate.—The leaves were stripped off from tough stems and minced in the mincing machine after soaking in 0.2% sodium carbonate solution. The homogenous mass obtained was squeezed through double folds of muslin cloth for extracting the juice. The juice extracted was concentrated at 70 to 80°C, acidified to pH 4.0 with dilute sulphuric acid and allowed to stand for 4 hr for complete precipitation of proteins. The proteineous material precipitated was separated from the supernatant by centrifugation at $2000 \times g$ for 20 min, washed twice with distilled water (adjusted to pH 4.0) followed by one washing with 95% ethanol. It was dried in oven at 60°C for 12 hr.

Methods of Analysis

All pH adjustments were carried out with pH meter. Nitrogen was estimated by microkjeldahl method using a mixture containing 9 parts K_2SO_4, 1 part $CuSO_4$ and 0.2 part SeO_2. Moisture was determined by drying the sample at 100°C for 48 hr. Total sugars were determined by Folin and Wu method.[16] Fibre was estimated by Henneberg acid–alkali method; ash, by direct incineration method, and sulfur by Osborn peroxide method. All these methods have been described by Winton.[17]

The standard solutions of amino acids were prepared by dissolving 10 mg of each amino acid in 1 ml of 10% isopropyl alcohol and 1 drop of concd hydrochloric acid.

The acid hydrolysate of leaf protein concentrate was prepared as described by Block *et al.*[18]

Amino acid composition of protein hydrolysate was determined by two dimensional paper chromatography, following the procedure described by Cramer.[19] Mixture of n-butanol, glacial acetic and distilled water in ratio 50:10:40 was used as solvent I. Mixture of phenol and distilled water in ratio 80:20 used as solvent II. Chromatogram was developed with 0.2% ninhydrin solution in acetone containing 5 ml glacial acetic acid. Ninhydrin colour was rendered permanent for reasonable period by spraying the chromatogram with mixture of 0.2 ml 10% HNO_3 and 1 ml saturated copper nitrate solution in 99 ml acetone.

The spots were cut out, eluted in 3–5 ml quantity of methanol and the optical density was measured at 500 mμ with Bausch and Lomb colorimeter.

Results and Discussion

Susbania aegyptica leaf-protein concentrate. contains 40.8% protein, 9.1% moisture, 3.4% total sugars, 0.98% sulphur, 13.2% ash and 4.2% fiber content. Analysis of other constituents such as polysaccharides, fat, nucleic acid was not carried out, as it was beyond the scope of this study.

The chromatographic analysis is shown in the Fig. 1 and Table 1. A total of 15 amino acids were detected and estimated. Of the special significance are the growth-promoting lysine and methionine which were estimated to be 7.75 and 4.5% respectively. These amounts are comparable with cow's milk which contains lysine 8.3% and methionine 3.0%.[20]

The methionine contents of Jantar protein are higher than those of other proteins reported in the literature. This seems to be a special feature of Jantar proteins which make them a relatively more suitable source from methionine-deficient cereal

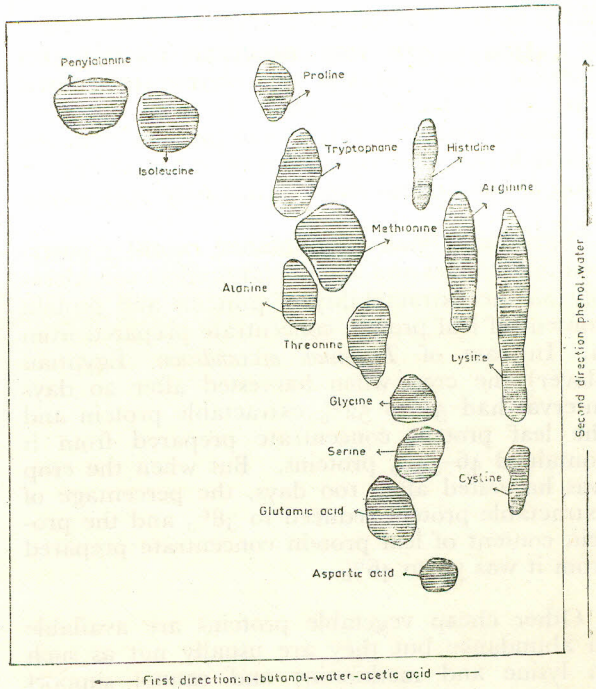

Fig. 1.—Two dimensional chromatogram of hydrolysed protein prepared from *Susbania aegyptica* (Jantar) leaves.

TABLE 1.—AMINO ACID COMPOSITION OF ACID HYDROLYSATE OF PROTEIN CONCENTRATE PREPARED FROM *Suabania aegyptica* (*jantar*) Leaves

Amino acids	*Rf* values of std. amino acids with respect to water phenol solvent	*Rf* values of acid hydrolysate with respect of water phenol sonvent	Percentage of amino acids in the crude protein*	Percentage of amino acids based on pure proteins†
Aspartic acid	0.125	0.12	3.4	8.5
Glutamic acid	0.24	0.23	3.72	9.30
Serine	0.25	0.26	0.96	2.40
Glycine	0.36	0.37	1.39	3.45
Threonine	0.38	0.36	1.12	2.80
Isoleucine	0.78	0.77	1.87	4.65
Phenyl alanine	0.80	0.81	6.3	15.7
Lysine	0.53	0.53	3.10	7.75
Histidine	0.64	0.64	0.37	0.925
Arginine	0.638	0.632	0.83	2.07
Methionine	0.624	0.62	1.80	4.50
Alanine	0.58	0.58	2.35	5.85
Tryptophane	0.64	0.645	2.7	6 75
Cystine	0.22	0.21	0.80	2.0
Proline	0.87	0.87	—	—

*Crude extract had 40% protein

†These values derived from those of column 4 by multiplying with 2.5 show per cent amino acid based upon 100% proteins.

based diets. The presence of lysine to the extent of 7.75% is also very significant because this essential amino acid is not present in desired amounts in cereal proteins. It may, however, be added that total extractable proteins from the Jantar leaves were lower because of harvesting the crop at the highest stage of maturity (150 days).

Nazir and Shah (unpublished work[15]) observed that species and age of plants effect considerably on the extractability of proteins and protein content of leaf protein concentrate prepared from it. In case of *Trifolium alexandrium* (Egyptian clover) the crop when harvested after 20 days interval had 51 to 52% extractable protein and the leaf protein concentrate prepared from it contained 46–54% proteins. But when the crop was harvested after 100 days, the percentage of extractable protein reduced to 38% and the protein content of leaf protein concentrate prepared from it was 39 to 46%.

Other cheap vegetable proteins are available in abundance but they are usually not as such in lysine and methionine and cannot support proper growth and development.[21] Extraction of protein on large scale from the leaves of *Suabania aegyptica*, therefore, can to some extent meet the protein deficiency in general.

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