AMINO ACID COMPOSITION OF SOME WILD LEGUMES

RADHA PANT, C. RAJAGOPALAN NAIR, K. S. SINGH AND G. S. KOSHTI
Department of Biochemistry, Allahabad University

ABSTRACT

Twenty-eight non-edible wild leguminous seeds were analysed for their proximate composition and free amino acid pattern. Distribution of total nitrogen in the various fractions of protein isolates were studied. Essential amino acids in the seed meal hydrolysates were assayed.

Tryptophan, cystine and methionine invariably appear to be the most common limiting amino acids in most of the seeds. No single seed has been found to be nutritionally complete with respect to essential amino acid content.

SEVERAL novel sources of proteins that have hitherto not been employed in human nutrition are now being investigated[1] in view of increasing demands of proteins by a growing world population. For example, oil seeds such as pea nut, cotton seed, sesame, soy bean, sunflower seed, and coconuts, etc., have been employed as sources of proteins. All-vegetable mixtures rich in protein content are prepared commercially and sold at low cost[2]. Other unconventional protein sources that are presently being developed and nutritionally evaluated include various algae like chlorella and blue alga, seafoods like weeds and planktons, yeasts and micro-organisms. Nevertheless, apart from their nutritional efficacy, cost of production and availability, the question of their acceptance by the consumers is an important factor in recommending them for human use. Some of these novel proteins for instance, possess unpleasant odour, acrid taste and unattractive colour.

Dry legumes have occupied an important position in human dietary since agriculture began and man adopted a settled life[3]. Leguminous plants belong to the second largest family of seed plants consisting of about 600 genera with 13,000 species. Of these however, only some twenty species have been considered suitable for human consumption and have hitherto been investigated by nutritionists while the rest have grossly been discarded and branded as "wild and non-edible" till recently[4,5]. The presence of cyanogenetic factors[6], hemaglutinins and toxic substances that produce lathyrism are some of the reasons for such downright disregard for these protein-rich sources in an age of severe protein scarcity and malnutrition.

Many of these wild leguminous plants grow most abundantly in tropical countries that are also the chief centres of protein undernutrition. They survive even under inhospitable and acute adverse ecological conditions and yield annually large quantities of seed-bearing pods of all sizes upto a meter in length with thick, fat and protein-rich cotyledons.

Therefore, with a view to explore the possibility of their inclusion in animal dietary a number of wild leguminous seeds were collected, botanically identified and investigated chemically to explore their nutritional efficiency.

MATERIALS AND METHODS

Wild leguminous seeds of *Acacia arabica, Acacia catechu, Acacia melanoxylon, Acacia suma, Albizzia lebbek, Albizzia moluccana, Albizzia odoratissima, Albizzia richardiana, Bauhinia alba, Bauhinia accuminata, Bauhinia macrostachya, Bauhinia malabarica, Bauhinia monandra, Bauhinia variegata, Cassia absus, Cassia grandis, Cassia marginata, Cassia obtusifolia, Cassia occidentalis, Cassia renigera, Cassia siamea, Crotalaria juncea, Crotalaria medicaginea, Dolichos biflorus, Erythrina indica, Glycine hispida, Mucuna pruriens* and *Pithecellobium dulce* were collected and botanically identified. They were powdered to 100-mesh in a hand grinder and stored in air-tight bottles. Moisture, ash, minerals, ether extractives and crude proteins were determined as described previously[7,8]. Niacin was assayed by the method of Swaminathan[9] and ascorbic acid, according to Roe and Kuether as described in Practical Physiological Chemistry by Hawk, Oser and Summerson[10].

Nitrogen-free extract percentage was calculated by subtracting the total of the percentages of crude protein, ether extractives, crude fibre and ash on moisture-free basis from 100. This presumably constitutes the total carbohydrate percentage.

Seed powders were defatted with petroleum ether (B.P. 60–80° C) in a Soxhlet extractor.

Extraction and isolation of free amino acids from seed powders were made by stirring the defatted seed powder (1·0 gm) with warm ethanol (10 ml, 70%, v/v) for 30 minutes. After centrifugation the residue was re-extracted with ethanol, centrifuged and the two supernatants combined. This process was repeated (8–9 times) till the supernatant was negative to ninhydrin test. The pooled supernatant was then evaporated to dryness *in vacuo*, dissolved in distilled water (0·5–1·0 ml), centrifuged and the clear supernatant (2–10 μl) was employed for qualitative free amino acid analysis by paper partition chromatography on Whatman No. 1. filter-paper sheets.

Free amino acids were detected by the two-dimensional technique of Datta, Dent and Harris[11] employing phenol (80%, w/v)-NH_3 and butan-1-ol-acetic acid-water (4 : 1 : 5) as developing solvents. The chromatograms after development were sprayed with ninhydrin (0·1%, w/v) in butan-1-ol. The identity of the various amino acids was confirmed by using specific spray reagents[12].

Preparation of protein hydrolysates from seed meals for amino acid analysis: 0·10 gm of the dry residue obtained after the isolation of free amino acids was hydrolyzed with hydrochloric acid (7 ml, 6 N) in an evacuated sealed tube by heating it in an air oven maintained at a temperature of 100–110° C for about 20–22 hours till the hydrolysate was negative to burette test. Under the conditions employed, complete hydrolysis of the proteins to amino acids was achieved. The acidic hydrolysate was made acid-free to pH 4–5 by repeated distillation *in vacuo*. The residue dissolved in a known volume of water, was employed both for qualitative and quantitative amino acid estimations.

Presence of amino acids in the seed protein hydrolysates was detected as described above. Tryptophan was tested and identified in the alkaline hydrolysate prepared by refluxing another sample of free amino acid-free seed powder with sodium hydroxide (10 ml, 5 N) till negative to the burette test.

Quantitative estimations of amino acids in seed protein hydrolysates were made by the elution method of Price[13]. The chromatograms spotted with the seed meal protein hydrolysates and developed by the two-dimensional technique, were dipped in ninhydrin solution (0·5%, w/v) in acetone-acetic acid mixture (1%, v/v) and heated at 90° C for 30 minutes. The amino acid spots were identified by comparison with chromatograms of known reference amino acids run simultaneously under identical conditions.

The coloured amino acid spots of identified amino acids were cut individually into small pieces taking care not to contaminate them and eluted with aqueous ethanol (7 ml, 60%, v/v) in centrifuge tubes. The tubes were well-agitated with a glass rod and centrifuged at 1500 r.p.m. for about 10 min. and the optical density of the supernatants were measured at 750 mμ against a reagent blank prepared from a paper treated in an identical manner. All estimations were made in triplicates. The percentage error by this method was $\pm$ 8.

Proline was measured at 440 mμ. Amino acids were calculated from a calibration curve prepared earlier from standard glycine solutions of known concentration.

Tryptophan was assayed in the alkaline hydrolysate by employing the method of Inglis and Leaver[14].

For the fractionation and isolation of proteins from defatted seed meals Mitchell's[15] solubility method as detailed in an earlier communication[16] was adopted. The isolated globulins and albumins were purified by the method of Esh and De[17]. Electrophoresis of the purified proteins was performed on an LKB 3276 unit employing Carl Schleicher and Schull No. 2043 13 (120 g/m^2) filter-paper strips using several buffer solutions (acetate buffer pH 5·0, citrate buffer pH 6·0 and phosphate buffer ph 8·0).

Results and Discussion

Table I reveals that barring a few varieties of Cassia quite a number of wild inedible leguminous seeds could be good sources of proteins.

Qualitative amino acid analyses in the seed powders showed the presence of 9–18 amino acids in the free state. They were α-alanine, α-amino-butyric acid, arginine, aspartic acid, citrulline, cysteic acid, cystine, glutamic acid, glutamine, glycine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, proline, hydroxy-proline, serine, taurine, threonine tryptophan, tyrosine and valine. Each seed appears to have its own pattern although most of them in general, showed the absence of the essential amino acids phenylalanine, tryptophan and the sulphur containing methionine as well as cystine and cysteic acid. Some of them also revealed the presence of some unidentified ninhydrin positive substances.

Qualitative amino acid analyses of the seed protein hydrolysates revealed them to possess more or less similar amino acid patterns consisting of 15–19 usually occurring amino acids. Among the essential amino acids methionine and tryptophan singly or jointly appeared to be the common limiting factors in general. In addition, the other sulphur containing amino acid cystine also was observed to be present only in few seeds.

Unlike the green leafy vegetables which are well balanced with respect to all essential amino acids except methionine, practically all the legume proteins investigated lack mainly in sulphur containing

TABLE I

Proximate chemical composition of some nonedible leguminous seeds
(Results expressed as percentage on dry weight basis)

SEED	Crude Protein	Ether Extractives	Nitrogen free Extract	Crude fibre	Total ash	Calcium mg/100 g	Phosphorus mg/100 g	Iron mg/100 g	Niacin mg/100 g	Ascorbic acid mg/100 g
Acacia arabica	26·4	3·3	62·9	2·7	4·7	673	420	4·95	3·17	4·51
Acacia catechu	44·2	6·6	41·6	3·5	4·1	578	445	5·15	1·63	6·11
Acacia melanoxylon	39·7	5·5	47·0	2·9	4·9	707	412	7·19	2·93	6·98
Acacia suma	31·9	5·5	54·0	3·7	4·9	718	396	7·10	2·45	6·93
Albizzia lebbek	39·5	6·8	45·3	4·2	4·2	172	620	11·50	5·21	1·75
Albizzia moluccana	33·5	5·6	52·5	3·8	4·6	732	365	4·90	1·46	6·58
Albizzia odoratissima	38·6	6·7	47·2	2·9	4·6	682	338	3·81	1·06	2·83
Albizzia richardiana	40·0	7·4	45·3	3·5	3·8	189	570	12·80	4·62	2·68
Bauhinia alba	30·7	15·4	46·6	3·2	4·1	273	192	3·91	2·84	1·62
Bauhinia accuminata	31·8	18·2	43·7	2·9	3·4	285	165	3·20	4·13	1·08
Bauhinia macrostachya	22·8	20·7	50·9	2·1	3·5	105	267	8·10	12·82	4·26
Bauhinia malabarica	27·0	17·8	48·6	3·2	3·4	142	299	13·30	9·20	6·84
Bauhinia monandra	31·0	22·7	39·5	3·7	3·1	121	201	14·40	15·81	4·28
Bauhinia variegata	34·1	16·5	43·1	3·2	3·1	312	120	4·29	3·91	1·00
Cassia absus	36·8	6·8	49·4	2·6	4·4	135	680	22·40	3·21	2·56
Cassia grandis	12·8	5·0	75·6	3·7	2·9	124	203	8·10	6·59	2·88
Cassia marginata	16·5	6·8	68·4	4·6	3·7	149	191	11·36	9·15	6·28
Cassia obtusifolia	23·9	5·2	60·9	4·[illegible]	5·2	153	215	10·57	8·38	3·56
Cassia occidentalis	35·2	5·0	51·3	3·8	4·7	160	226	10·98	7·59	4·29
Cassia renigera	12·9	3·5	75·8	4·5	3·3	133	178	12·67	9·03	5·42
Cassia siamea	21·8	6·9	62·6	5·3	3·4	155	430	8·20	3·82	3·10
Crotalaria juncea	32·4	2·8	59·6	2·1	3·1	201	326	7·91	2·95	1·39
Crotalaria medicaginea	47·5	3·4	43·2	2·6	3·3	167	352	8·57	3·15	2·05
Dolichos biflorus (Edible)	21·3	2·3	69·6	2·9	3·9	269	360	9·20	2·07	0·65
Erythrina indica	23·1	15·7	53·1	3·5	4·6	214	201	12·27	1·28	1·97
Glycine hispida (Edible)	46·8	21·1	23·6	3·2	5·3	317	272	10·79	3·14	1·17
Mucuna pruriens	29·3	9·0	53·7	4·1	3·9	238	159	13·52	3·64	4·78
Pithecellobium dulce	24·4	11·7	57·2	3·7	3·0	254	570	3·55	4·87	1·62

amino acids—methionine and cystine as well as in tryptophan. However, many of them appear to be fairly good sources of lysine, threonine and leucine-isoleucine and other essential amino acids. Nevertheless, in the total absence of one or two essential amino acids these proteins cannot possibly be expected to possess nutritive value.

Table III shows that successive extraction of seed meals with water and sodium chloride, solubilizes 41–86% of the total nitrogen consisting of albumin, globulin and non-protein nitrogen. Globulin forms the major fraction of total nitrogen. Prolamin constitutes a small fraction (1–5%), non-protein nitrogen accounts for 4–18% while 1–6% remains unextracted in the residue.

Electrophoresis of purified globulin fractions invariably afforded mainly one band. The present findings are in conformity with the previous reports[17,18] on the nature of seed proteins isolated in the manner described in the present investigation.

TABLE II

Essential amino acid content of some nonedible leguminous seeds
(Results expressed as g amino acid/16 g N)

SEED	His.	Lys.	Met.	Cys.	Phe.	Tyr.	Phe. + Tyr.	Leu + Ile	Val.	Thr.	Tp.*	Total
Acacia arabica	3·7	4·3	0·4	0·49	3·6	1·58	5·18	8·8	4·2	3·3	+	30·37
Acacia catechu	2·3	4·2	0·5	..	3·8	1·2[illegible]	5·08	9·4	3·0	2·8	+	27·28
Acacia melanoxylon	2·1	3·8	+	0·70	1·6	1·98	3·59	7·3	3·6	2·1	—	23·38
Acacia suma	2·9	2·9	2·0	—	3·8	1·39	5·19	6·8	2·5	1·9	0·4	24·59
Albizzia lebbek	2·8	5·8	1·3	—	3·7	0·90	4·60	8·6	2·0	1·8	+	27·70
Albizzia moluccana	2·3	6·2	0·9	—	5·6	0·78	6·38	7·2	1·8	2·4	—	27·18
Albizzia odoratissima	2·2	5·2	1·2	—	5·0	0·70	5·70	7·2	1·6	1·5	1·4	26·00
Albizzia richardiana	3·8	7·8	0·8	—	4·0	0·49	4·49	8·9	3·3	3·3	+	32·39
Bauhinia accuminata	1·7	5·8	0·3	0·27	4·3	1·64	5·94	6·9	6·2	4·3	1·1	32·51
Bauhinia alba	1·2	4·9	0·5	0·30	3·3	1·65	4·95	7·9	4·2	2·3	+	26·25
Bauhinia macrostachya	1·6	4·7	0·4	—	1·5	0·77	2·27	5·6	3·4	1·6	+	19·57
Bauhinia malabarica	1·9	3·6	+	—	2·0	0·33	2·33	4·2	3·5	2·9	—	18·43
Bauhinia monandra	2·2	4·7	+	—	1·4	0·94	2·34	7·7	4·8	2·2	—	23·94
Bauhinia variegata	2·3	4·8	0·4	0·29	3·4	1·75	5·15	6·2	6·0	4·2	—	29·34
Cassia absus	1·5	3·1	1·2	—	3·2	0·81	4·01	4·6	5·6	4·5	—	24·51
Cassia grandis	1·5	3·9	1·0	—	1·4	0·48	1·88	6·9	2·5	2·8	1·1	21·58
Cassia marginata	1·1	4·3	1·5	—	2·8	0·38	3·18	8·9	2·4	2·0	—	23·38
Cassia obtusifolia	2·8	6·5	0·6	—	2·8	1·63	4·43	7·1	2·3	2·9	1·6	28·23
Cassia occidentalis	1·2	7·5	0·5	—	2·2	1·25	3·45	6·8	3·7	3·8	1·2	28·15
Cassia renigera	2·5	4·4	1·1	—	3·7	0·68	4·38	6·1	3·6	1·3	0·7	24·08
Cassia siamea	3·1	4·4	1·3	—	1·1	0·23	1·33	6·4	3·5	3·7	1·2	24·93
Dolichos biflorus	3·1	3·8	0·3	0·79	3·4	1·13	4·53	2·4	4·6	2·8	1·1	23·24
Glycine hispida	2·7	3·9	0·4	0·83	3·9	1·44	5·34	7·2	5·6	3·3	1·4	30·67
Mucuna pruriens	2·8	1·7	0·3	—	1·3	0·63	1·93	7·5	2·6	1·3	1·3	19·43
Pithecellobium dulce	2·6	4·0	0·2	1·13	4·1	2·07	6·17	5·6	4·7	2·4	1·8	28·60

\+ Present

— Absent

* Estimated in alkaline hydrolysates of seed meals.

TABLE III

Distribution of nitrogen in some nonedible leguminous seed proteins
(Expressed as per cent of total N)

SEED	Water-soluble (Alb+ Glob+ NPN)	Albumin	Globulin (A)	Non-protein nitrogen (NPN)	5% NaCl soluble (Globulin B)	Total Globulin (A+B)	75% Ethanol soluble (Prolamin)	0·25% NaOH soluble (Glutelin)	Residue
Acacia arabica ..	41·3	7·1	21·1	13·1	25·3	46·4	1·2	21·1	3·1
Acacia catechu ..	46·9	6·2	26·3	14·4	26·0	52·3	3·2	16·4	7·5
Acacia melanoxylon ..	44·8	5·9	20·5	18·4	22·6	43·1	3·6	24·7	4·3
Acacia suma ..	40·8	4·1	24·7	12·0	25·8	50·5	4·6	20·3	8·5
Albizzia lebbek ..	78·4	13·9	47·9	14·8	8·8	58·5	0·9	4·3	7·6
Albizzia moluccana ..	50·4	6·8	30·0	13·6	28·6	58·6	2·3	12·6	6·1
Albizzia odoratissima ..	49·1	5·7	29·3	14·1	27·8	57·1	2·7	15·2	5·2
Albizzia richardiana ..	85·9	17·5	53·2	15·3	2·9	56·1	0·4	2·4	8·4
Bauhinia accuminata ..	60·5	8·6	46·5	5·4	18·6	65·1	1·4	12·2	7·3
Bauhinia alba ..	67·4	13·8	47·6	6·1	22·4	70·0	3·2	2·7	4·2
Bauhinia macrostachya ..	73·2	7·2	61·0	5·0	9·3	70·3	2·4	9·1	6·0
Bauhinia malabarica ..	59·9	6·7	39·8	13·4	20·9	60·7	3·1	7·3	8·8
Bauhinia monandra ..	49·2	5·1	39·7	4·3	17·0	56·7	3·5	20·1	10·2
Bauhinia variegata ..	72·7	4·6	60·0	8·1	12·5	72·5	2·1	8.6	4·1
Cassia absus ..	46·0	8·7	33·0	4·3	18·8	51·8	0·7	28·4	6·1
Cassia grandis ..	46·1	10·3	28·0	8·0	16·4	44·4	2·5	32·3	8·5
Cassia marginata ..	47·0	15·3	26·4	5·3	18·8	45·2	1·2	28·4	4·5
Cassia obtusifolia ..	47·8	9·5	32·5	5·8	13·2	45·7	0·8	29·4	8·8
Cassia occidentalis ..	52·5	12·5	33·8	6·2	12·7	46·5	0·9	25·4	8·5
Cassia renigera ..	51·8	14·8	32·1	4·9	15·4	47·5	1·6	25·0	6·2
Cassia siamea ..	49·1	12·6	27·6	9·7	17·6	45·2	4·7	23·0	5·6
Dolichos biflorus ..	69·9	15·4	49·8	4·1	13·2	63·0	2·0	6·8	8·1
Glycine hispida ..	71·0	3·5	61·5	6·0	6·2	67·7	1·0	6·0	15·7
Leucina glauca ..	58·6	8·6	35·8	14·2	29·1	64·9	1·8	30·1	3·0
Mucuna pruriens ..	79·5	5·6	66·9	6·9	16·3	83·2	Traces	2·8	1·4
Pitheocellobium dulce ..	70·5	7·4	58·3	4·8	9·2	67·5	1·3	5·2	5·9
Phaseolus aconitifolius ..	52·3	5·1	33·2	13·6	23·6	56·8	3·8	12·3	7·2

ACKNOWLEDGEMENT

This research was financed in part by grant No. FG-In-188 by the U.S. Department of Agriculture, Agricultural Research Service under PL 480.

1. Alan Berg, D., "Malnutrition and National Development," *Foreign Affairs,* October 1967.
2. Bressani, R. L. B., Elias Aguirre, A. and Scrimshaw, N. S., "All-vegetable protein mixture for human feeding. II. The development of INCAP vegetable mixture nine," *J. Nutr.*, 1961, 74, 281.
3. Autert, Marcel, In : *Legumes in Human Nutrition,* (Eds.) W. R. Aykroyd and Joyce Doughty, FAO Nutritional Studies, No. 19, 1964 (Preface).
4. Aykroyd, W. R. and Doughty, Joyce, (Eds.), *Legumes in Human Nutrition,* FAO Nutritional Studies, No. 19, 1964, p. 1.
5. — and —, *Ibid.*, No. 19, 1964, p. 13.
6. Food and Agriculture organization of the United Nations, *Report of the FAO/CCTA Technical Meeting on Legumes in Agriculture and Human Nutrition in Africa, Dukavu, Belgian-Congo,* 1958, Rome ; *Ibid.*, W. R. Aykroyd and Joyce Doughty (Eds.), *Legumes in Human Nutrition,* FAO Nutritional Studies, No. 19, 1964, p. 61.
7. Pant, R. and Bishnoi, P. L., *Curr. Sci.*, 1967, 36, 376.
8. — and Kapur, A. S., *Ann. Biochem. Exptl. Med.*, 1963, 23, 95.
9. Swaminathan, M., *Ind. J. Med. Res.*, 1939, 26, 427.
10. Hawk, P. B., Oser, B. L. and Summerson, W. H., *Practical Physiological Chemistry,* 12th Ed., J. and A. Chruchill, London, 1954, p. 1138.
11. Datta, S. P., Dent, C. E. and Harris, H., *Science,* 1950, 112, 62.
12. Pant, R. and Agrawal, H. C., *Ind. J. Exptl. Biol.*, 1965, 3, 133.
13. Price, G. M., *Biochem. J.*, 1963, 86, 372.
14. Inglis, A. S. and Leaver, I. H., *Anal. Biochem.*, 1964, 7, 10.
15. Mitchell, C. A., *Allen's Commercial Organic Analysis,* Vol. 8, Churchill, London, 1948.
16. Pant, R. and Tulsiani, D. R. P., *Agri. Food. Chem.*, 1969, 17, 361.
17. Esh, G. C. and De, T. S., *Symposium on Proteins, Central Food Technological Research Institute, Mysore, India,* 1960, p. 35.
18. Tawde, S. and Cama, H. R., *Ibid.*, 1960, p. 8.