the problem of measuring small peak areas. The low yields of TCA precipitable nitrogen is not unique to *Prosopis* pods as the pods of *Acacia moniliformis* and the pulp of *Pithecellobium dulce* yielded no discernible TCA precipitate.

Difficulties were encountered with the g.l.c. analyses for methionine. Sometimes the methionine peak area on g.l.c. from the protein hydrolysate declined with successive injections over a 6 h period. During this same period the peak area for methionine in standards did not decline relative to the other amino acids. In such cases the highest and first peak area is used to calculate the methionine values and these values are listed in parentheses in Table 3. Perhaps the methionine antioxidant of March[14] could have prevented this destruction.

In terms of repeatability, the standard deviation, as percent of the mean, for all the amino acids minus Cys, His and Trp averaged 6% for four runs done on the same day.

4. Discussion

Data are presented on the protein and amino acid composition of the major seed proteins of tree legume seeds. The seeds chosen for analyses have been, or are presently used, as food for humans or livestock in some area of the world.[1] Since the use of these trees is mainly in poor, developing countries there is a paucity of biochemical and nutritional data on these seeds. The results reported here are in good agreement with the protein[15] and amino acid content of *Prosopis*[12] and the amino acid[11] and nutritional data[10] of *Parkia* species. Amino acid composition data of the seeds of *Acacia*, *Gleditsia*, *Pithecellobium* and *Leucaena* have not, to our knowledge, been reported.

That these seeds came from plants of unknown nutritional status may be important. Sulphate is a limiting nutrient on some soils where legumes are grown,[16,17] and since sulphate deficient legumes have been reported to have lowered levels of methionine, cysteine and tryptophan rich proteins,[18] it is possible that the low levels of cysteine, methionine and tryptophan we have observed are the results of the nutritional rather than genetic status of the plant.

The data presented here show that there is a great deal of diversity among the tree legumes. For instance, the protein content ranges from 16 to 69% of dry weight, and the methionine, cysteine and tryptophan content respectively range from 44 to 121 mg/gN, 14 to 145 mg/gN, and 40 to 109 mg/gN. The diversity we have found is in a very small sample of potential breeding stock. For example, only 2 of a possible 20 species of *Prosopis* and one out of 90 varieties of *Leucaena leucocephala* have been analysed.

The use of tree legumes as food or fodder is not without problems. For instance *Leucaena* contains an alkaloid, mimosine, which is a depilatory if eaten in excess[19] although ferrous sulphate has been shown to counteract the depilatory action in pigs.[20] The Djenkol beans (*Pithecellobium lobatum*), although a prized Indonesian food[9] will cause cystitis if eaten in excess. The causative agent is thought to be an oil.[9] If cattle are given a diet consisting solely of *Prosopis* pods they may die with a ball of compacted pods and unbroken seeds in their rumen.[21,22] Perhaps if the *Prosopis* seeds which contain 69% of the protein in the pod[23] and are indigestible because of the seed coat, were broken prior to feeding, sufficient nitrogenous substances would be released into the rumen to allow digestion of the fibrous pods. Pig feeding trials, with ground mesquite pods as 50% of the ration, show no toxicity.[23] Studies indicate that *Prosopis* pods have a higher protein digestibility coefficient than alfalfa hay.[24,25]

Although the number of species analysed in this work is small some new data emerge. First, a seed with 29% protein in the seed embryo is promising since this protein content is as high as any reported in 759 species in a systematic sampling of the plant kingdom.[26] Second, the protein from *Pithecellobium lobatum* has an amino acid composition that should make it much more nutritious than the average legume or cereal. For instance the chemical scores, derived by comparison with hen's egg protein,[27] are 0.79, 0.58, and 0.47 for *P. lobatum* protein, casein and soy protein respectively. It seems obvious that intensive studies, including for example, feeding studies of *P. lobatum* protein, should be made with this extraordinary group of plants.

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Protein and Amino Acid Composition of Tree Legume Seeds

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Pods and seeds of tree legumes are widely used as food in developing countries since tree legumes can be grown with minimal inputs of capital and energy.[1] The data presented here show that the protein content of the seed of different legumes ranges from 16 to 69% of the dry weight, and methionine, cystine and tryptophan content ranges from 44 to 121 mg/gN, 14 to 156 mg/gN and 40 to 109 mg/gN respectively. The chemical score of the protein from *Pithecellobium lobatum* was 0.79 which compared to 0.58 and 0.47 for casein and soy protein respectively. This variability in protein content and in amino acid composition indicates that there is sufficient genetic variability in the family to permit improvement by selection and breeding.

1. Introduction

Leguminous trees have been suggested as food sources since their production would require minimal inputs of capital, fuel and energy.[1] In this study we present data on the protein, and amino acid composition of the seeds of a number of leguminous trees that are currently being used as human food or for livestock. We have found variations in protein content and in amino acid composition indicating there is sufficient genetic diversity in the legume family so that a breeding and selection programme could enhance the food value of tree legumes.

2. Experimental

The seeds for protein and amino acid analysis were obtained from several sources and we have used the botanical name provided by the collector. *Parkia clappertoniana*, *P. roxburghii*, *Pithecellobium sonorae*, *P. saman*, *P. dulce*, *Prosopis juliflora*, *P. stephaniana*, *Leucaena leucocephala* and *Gleditsia triacanthos* were obtained from the USDA, Northern Regional Research Laboratory, Peoria, Illinois. *Parkia javanica*, *Pithecellobium lobatum*, *Acacia auriculaeformis* and *Leucaena glauca* were collected in Indonesia by Dr Peter Murphy, Michigan State University. *Acacia moniliformis* and *Prosopis chilensis* were obtained through Mr Howard Hyland, USDA, Northeastern Laboratory, Beltsville, MD, from Argentinean collectors.

In most cases it was necessary to remove the seed from its coat (endocarp). The seeds were crushed in an hydraulic press, wrapped in miracloth and extracted with hexane in a soxhlet distillation apparatus for 24 h. Following extraction the embryo could be removed manually from the endocarp. The seed embryo, which in the case of legumes is virtually the entire seed, was ground sufficiently fine to pass a 40 mesh sieve. Protein assays were made of the seed powder by Kjeldahl analysis[2] using a factor of 6.25 to convert nitrogen to protein.

Since carbohydrates increase the destruction of hydroxy-amino acids during acid hydrolysis,[3] we prepared a low carbohydrate crude protein fraction. The preparation of the protein fraction for analysis of amino acids, other than tryptophan, cystine and histidine, was performed as previously described.[4] The protein extraction technique used for Cys, His and Trp was as follows: a sample estimated to contain at least 20 mg of protein was homogenised in a Teflon-pestle tissue grinder in approximately 6 ml of 0.05 M sodium pyrophosphate pH 8.2, 0.4 M-NaCl, and 0.005 M dithioerythritol. After grinding for 5 min, the suspension was centrifuged at 1200 *g* for 4 min. The super-

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natant fluid was transferred to a 30 ml Corex tube, and the pellet extracted twice more. This procedure is an improvement of that previously described.[4]

The combined supernatant volume from the homogenate was measured, and an aliquot equivalent to 20 μg protein taken for Kjeldahl analysis.[5] After addition and mixing of 3 g of solid trichloroacetic acid (TCA), the Corex tube was placed in a 95°C water bath for 3 min. The tube was then centrifuged at 15 000 *g* for 3 min. The volume was measured and an aliquot was taken to measure TCA soluble nitrogen.[5] The supernatant fluid was then discarded and the pellet was resuspended in 5 ml of 10% TCA. The resuspended pellet was sedimented at 15 000 *g* for 3 min. The supernatant fluid was discarded and the 10% TCA wash, which is designed to remove NaCl, repeated once more. The TCA was then removed from the pellet by resuspending in 5 ml of chloroform: methanol 28:72 (density of 1) and centrifuging at 15 000 *g* for 3 min. The chloroform–methanol wash was repeated once more and the pellet dried *in vacuo*. (Peroxide contaminants in the previously described ether-based method[4] may oxidise cystine and tryptophan.)

Protein hydrolysis for amino acids other than Cys, His and Trp was as previously described[4] using approximately 5 mg of protein and 1.0 ml of 6 N-HCl. Prior to hydrolysis 200 μg of norleucine was added as an internal standard. After hydrolysis, duplicate aliquots of 0.4 ml were taken for Kjeldahl analysis and duplicate aliquots of 20 μl were derivatised for g.l.c. These Kjeldahl analyses were performed as previously described.[3]

For g.l.c. analysis, duplicate and alternating injections were made from each of the two derivatisations so there were a total of 4 replicates per hydrolysate. A standard amino acid mixture was also derivatised and analysed immediately before and after the unknowns so that relative weight responses (RWR),[4] could be established. This method of cutting out and weighing peaks on the recorder chart paper must be used if an integrating recorder capable of measuring asymmetric peaks is not available. The amino acid composition was then calculated from the average of the four replicates, the RWR coefficients, and the Kjeldahl analyses.

Determination of cysteine, histidine and tryptophan was as previously described[6] following S-alkylation of protein prepared as described above. Nitrogen determinations could not be made on the hydrolysate, owing to the residual ammonium bicarbonate buffer. Thus, Kjeldahl analyses were performed on the dried protein. The protein (400–600 μg) was weighed on a Cahn electrobalance and transferred to hydrolysis vials. N-propanol (50 μl) was then added to promote wetting by the NH_4HCO_3 buffer. Analyses of Trp, Cys and His were performed in triplicate using isotope dilution of the n-propyl esters as previously described.[6] Column contamination was eliminated before each analysis by a 10 μl injection of acetic anhydride and a sufficient delay to permit emergence of histidine-like contaminants.

Nitrogen determinations on the fluid aliquots were performed by a colorimetric micro-Kjeldahl assay employing the dichloroisocyanurate-salicylate reagent.[5]

3. Results

Since the nitrogen content of the hexane extracted seed powder (Table 3) and of the buffer extract were known, the percentage of nitrogen extracted by the buffer could be calculated and these data are presented in Table 1. It can be seen that, with one exception, at least 70% of the nitrogen was extracted by the procedure described. The nitrogen in the supernatant fluid after TCA precipitation and centrifugation was also measured and an appreciable amount of the extracted nitrogen was found not to be TCA precipitable (Table 1). This was unexpected since the conditions described will precipitate greater than 90% of the extractable nitrogen from soybeans. It was of interest to know whether the nitrogen not precipitable by TCA consisted of amino acids and alkaloids or of glycosylated protein, which is known to be TCA soluble.[7] Consequently, the TCA supernatant fluid from the pods of *Prosopis juliflora* and from the seeds of *Leucaena leucocephala* were chosen for a nitrogen distribution study as these both had a large percentage of TCA soluble nitrogen. From Table 2 it can be seen that approximately 20% of the TCA soluble nitrogen was non-dialysable. In a preliminary experiment sugar and amino acid analysis of a hydrolysate of non-dialysable material

Table 1. Extractability and TCA precipitability of legume seed proteins

	Buffer extractable N	TCA soluble N
	N in seed powder (%)[a]	Buffer Extractable N (%)[b]
Parkia clappertoniana	28	38
roxburghii	91	33
javanica	84	23
Pithecellobium sonorae	106	36
saman	81	50
dulce	97	43
lobatum	65	50
Acacia moniliformis	91	27
auriculaeformis	75	36
Prosopis chilensis	85	16
juliflora (seeds)	79	22
juliflora (pods)	70	73
Leucaena leucocephala	106	42
glauca	69	16
Gleditsia triacanthos	86	16
Glycine max	90	9

[a] The figures in the first column represent the N soluble in the buffer extract divided by the total N in the hexane-extracted seed powder (column 1, Table 3) used for preparation of the extract and expressed as a percentage.

[b] The second column is the percent of N in the buffer extract[a] remaining soluble after making the buffer to 15% trichloroacetic acid (TCA). That two of the values for % N are greater than 100% is experimental error.

Table 2. Distribution of trichloroacetic acid soluble nitrogen after dialysis[a]

	Prosopis juliflora		*Leucaena leucocephala*	
	N (mg)	%	N (mg)	%
Hexane extracted meal	3.2	100	3.8	100
Combined buffer extract	2.2	69	3.9	103
Hot TCA soluble	2.1	64	1.5	40
Not dialysable	0.42	13	0.29	7.7
Dialysable	1.5	47	1.2	31.0

[a] Hexane-extracted 40 mesh powder from *Prosopis juliflora* pods (without seeds) and *Leucaena leucocephala* seeds were used for this study. Dialysis was three times for 24 h each time against 20 volumes of glass distilled water. The N in the hexane-extracted meal is taken as 100%

showed the presence of arabinose, galactose and galacturonic acids, as well as hydroxyproline and a number of other amino acids, suggesting that the non-dialysable material contained glycoproteins. The presence of much more ammonia in the hydrolysate than could be accounted for as amides of glutamic and aspartic acid is reminiscent of other legume glycoproteins.[8] The TCA precipitable substance was 62% protein by dry weight as an average for all the samples. The remainder presumably was polysaccharide, but this was not studied.

G.l.c. chromatographic profiles of Cys, His and Trp in a hydrolysate of legume protein are shown in Figure 1. This profile of *Prosopis chilensis* protein is similar to that of lysozyme[6] except the legume

amino acids were chromatographed as the n-propyl esters instead of the methyl esters. The propyl esters are preferable since the two peaks preceding S-carboxyethyl cysteine (CySR) are separated from CySR, while they are not with the methyl esters. It should be noted that, with the exception of the other amino acids on the chromatogram, Tyr, Lys and Arg, the chromatogram is free of contaminating peaks.

As can be seen from Figure 1 exact coincidence of retention time between standards and the corresponding amino acids from a protein hydrolysate was not observed. The protein hydrolysate amino acids emerged later than standards with the difference roughly proportional to retention time. This difference in retention time is probably due to the 10 fold more material injected for the protein hydrolysate assay than for the standard amino acid mixture since similar differences are observed using similar amounts of standards. The identity of the standards was checked by g.c.–m.s.[6]

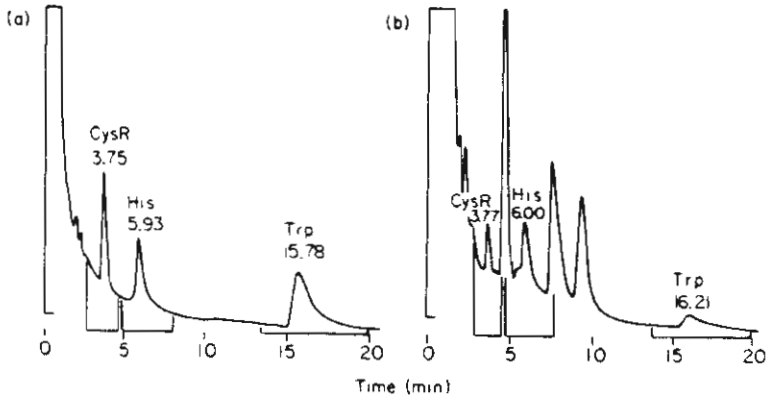

Figure 1. (a) Recorder tracing of the flame detector response of a gas–liquid chromatogram of approximately 1 μg of S-[^{14}C] carboxyethylcysteine, ^{14}C-histidine and ^{14}C-tryptophan chromatographed as the N-acetyl-O-n-propyl esters. Chromatography condition are identical to those reported in ref. 6 except that the oven temperature was 215°C. The bars under the peaks indicate the times of radio-isotope collection. The retention times are above the peaks in min. (b) Gas–liquid chromatogram of an aliquot of derivatised *Prosopis chilensis* seed amino acids. Approximately 50 μg of amino acids were injected.

The results of the protein and amino acid composition determinations are compared with the FAO provisional values in Table 3. As can be seen, the protein content varies from 16 to 69%. In general the larger seeds seemed to have a lower protein content than the smaller ones. It is apparent that the majority of the legumes are low in the sulphur amino acids-methionine and cysteine, as well as in tryptophan. The single exception is the protein from *Pithecellobium lobatum* the Djenkol bean.[9] While the Djenkol bean is about 15% low in methionine, it has a much higher level of cysteine than do the remainder of the seed proteins. Since the cysteine can substitute for a portion of the methionine requirement, the large amount of cysteine in the Djenkol bean could perhaps overcome the methionine deficiency.

The amino acid composition data presented here for several *Parkia* species (African locust bean) are in fair agreement with that of Fetuga *et al.*[10] and Busson.[11] The amino acid composition previously reported for *Prosopis*, known as mesquite or algaroba[12] are in good agreement with our data except for the hydrophobic amino acids. In the earlier study the acid hydrolysis of the *Prosopis* protein was for only 4 h,[12] and this would explain their lower values for the hydrophobic amino acids. Hydrophobic residues are sometimes not hydrolysed until 72 h,[13] and increasing the 20 h hydrolysis time used here might increase the yield of the essential amino acids valine, and isoleucine in *Prosopis* seed. The amino acid composition of the protein from *Prosopis* pods is better than that of the seed. However, in terms of nutrition, it must be remembered that: the total N in the pod is, on a weight basis, an order of magnitude lower than that in the seed; the extractability of nitrogen from the pod is lower than that of the seed; the percent of dialysable buffer-extractable nitrogen is several fold higher in the pod than the seed; and an accurate analysis of pod protein is more difficult owing to

Table 3. Protein and amino acid composition of tree legume seeds (mg/gN)

	Prot. (%)[a]	ala	gly	val	thr	ser	leu	ile	pro	met	phe	asp	lys	tyr	glu	arg	trp	cys	his
Acacia auriculaeformis	38	307	296	300	224	391	648	308	378	74 (90)	335	764	551	357	1144	496	46	35	164
Acacia monliformis	55	320	236	258	205	333	584	270	410	50	242	687	311	354	894	556	56	49	115
Gleditsia triacanthos	58	297	309	321	207	325	450	271	285	64 (100)	242	608	371	243	1453	631	51	88	123
Leucaena glauca	39	317	297	305	209	332	540	289	310	77	321	707	385	273	1167	563	86	28	184
Leucaena leucocephala	46	295	286	263	226	367	484	266	319	59	314	652	377	298	1095	631	62	34	161
Parkia clappertoniana	16	291	283	294	236	417	585	340	339	95	351	943	500	404	1121	284	53	92	93
Parkia javanica	44	318	296	324	201	327	522	263	315	44 (55)	325	613	460	298	1161	366	40	19	150
Parkia roxburghii	41	303	276	288	170	297	494	239	299	50 (71)	307	568	385	278	1088	356	43	22	127
Pithecellobium saman	38	333	286	270	253	392	607	266	363	81 (97)	300	673	493	357	808	400	73	53	136
Pithecellobium sonorae	44	305	292	197	231	370	704	245	391	82	312	780	618	374	1150	500	66	49	154
Pithecellobium dulce	28	351	291	296	289	352	607	308	369	121 (146)	321	709	519	342	888	441	81	27	157
Pithecellobium lobatum	16	321	286	384	279	394	538	321	398	121	285	669	335	300	618	167	109	156	176
Prosopis chilensis	69	278	233	242	152	255	432	221	350	47	247	475	240	208	1041	645	77	62	163
Prosopis juliflora (seeds)	60	274	249	203	130	254	441	220	363	57	246	503	250	204	1261	730	61	14	83
Prosopis juliflora (pods)	6.5	339	318	317	232	329	533	299	347	76 (95)	272	611	330	263	848	330	112	38	136
Prosopis stephianta	56	352	282	321	168	375	594	306	283	78	271	696	351	283	1179	569			
FAO Provisional Score				270	180		306	270		144	180		270	180			90		

[a] Insufficient material was available for the tryptophan, cystine and histidine analysis of *Prosopis stephaniana*. Protein was calculated for the defatted seed minus seed coat as N × 6.25.

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