Relationship between total sulphur and sulphur amino acids in chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* [L.] Millsp.)*

R. JAMBUNATHAN and U. SINGH

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), ICRISAT Patancheru PO 502 324, Andhra Pradesh, India

(Received 4 March 1981; in revised form 8 September 1981)

Key words: amino acids, chickpea, pigeonpea, sulphur

Abstract. Total sulphur was determined in seed meal of 30 chickpea and 24 pigeonpea cultivars by the wet digestion procedure and by using the Leco sulphur analyser. Methionine and cystine were determined after performic acid oxidation in an amino acid analyser. The two methods used for total sulphur determinations were highly correlated ($r = 0.943$). Percent meal protein was significantly correlated ($r = 0.476$) with total sulphur in chickpea but not in the case of pigeonpea. Total sulphur content exhibited a significant positive correlation ($r = 0.651$) with sulphur amino acids of pigeonpea when expressed as percent of protein but not in the case of chickpea. Correlation coefficients between total sulphur and sulphur amino acids when the results were expressed as percent of sample were positive for both chickpea ($r = 0.494$) and pigeonpea ($r = 0.534$). The amount of sulphur in methionine and cystine accounted for 54.8% of the total sulphur in chickpea and for 75.5% in pigeonpea. In both chickpea and pigeonpea, methionine was positively and significantly correlated with cystine when they were expressed either as percent of sample or as percent of protein.

The Protein Advisory Group of the United Nations recommended that in addition to the improvement of productivity, adaptability, and yield stability of food legumes, increased attention should be paid to improve their food value and acceptability. Food legumes are a rich source of protein and the nutritional deficiencies of protein arise, in general, from low sulphur amino acid content [3]. However, progress in genetically improving the nutritional quality of legumes has been slow. This, to a great extent, is due to nonavailability of a reliable rapid method for the estimation of sulphur amino acids. Also, the ion exchange chromatographic technique for the accurate analysis of methionine and cystine is slow and tedious as it involves a performic acid oxidation procedure.

Several methods have been reported for the determination of methionine and cystine [6–8]. However, the suitability of these methods for screening large numbers of samples has not been properly evaluated. The microbiological method has been used effectively as a means for screening large number of

*Submitted as J.A. no. 159 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Qual Plant Plant Foods Hum Nutr 31:109–117 (1981) 0377–3205/81/0312–0109 $01.35.

samples of common beans (*Phaseolus vulgaris* L.) for methionine content [11, 12]. Total sulphur content has been suggested as an indicator of sulphur amino acids in some grain legumes. Porter et al. [14], using five lines of dry bean (*Phaseolus vulgaris*), reported a significant relationship between total sulphur and sulphur amino acids. Sandhu et al. [15] reported a coefficient of determination of 0.89 between total sulphur and sulphur amino acids in six lines of chickpea. However, Evans and Boulter [5] obtained a poor correlation coefficient between total sulphur and sulphur content of sulphur amino acids in 36 cultivars of cowpea. Bhatty et al. [2] reported that total sulphur was not a satisfactory indicator of the levels of sulphur amino acids in four species of legumes (*Phaseolus vulgaris, Vicia faba, Pisum sativum* and *Lens culinaris*). However, such studies have not been carried out systematically on pigeonpea and chickpea. At ICRISAT, a large number of germplasm accessions of chickpea and pigeonpea are available. As the improvement of nutritional quality is one of the objectives of ICRISAT, the relationship between total sulphur and sulphur amino acids was studied using a limited number of chickpea and pigeonpea cultivars to find out if total sulphur could be considered a reliable screening criterion.

Materials and Methods

Seed samples of 30 cultivars of chickpea (*Cicer arietinum* L.) and 24 cultivars of pigeonpea (*Cajanus cajan* [L.] Millsp.) were analysed (Table 1). Whole seeds were soaked in water overnight at 5°C, and then seed coats were removed manually. Decorticated split seed (dhal) samples were dried at 70°C in an oven and were ground in a Udy cyclone mill to pass through a 60-mesh sieve. The samples were defatted in a Soxhlet apparatus using hexane and then dried. Total nitrogen was determined by the micro-Kjeldahl procedure [1] and crude protein calculated using a factor of 6.25.

Determination of sulphur

Total sulphur was determined by the wet digestion method according to Tabatabai and Bremner [16] with the following modifications. A 1 g sample was digested with 10 ml of nitric acid in a Tecator digestion tube (250 ml) for 30 min at 100°C in a block digester. After cooling, 6 ml of 70% perchloric acid was added and digestion continued for 60 min at 235°C. The contents were allowed to cool, 10 ml of 6 N HCl was added and the volume made up to 250 ml. To 15 ml of this aliquot, 250 mg of finely ground barium chloride was added and after mild shaking for 10 min, the percent transmittance (T) of the turbid suspension was measured at 420 nm in a Spectronic-20 spectrophotometer. The quantity of sulphate in the aliquot was read from a standard graph (prepared by using potasium sulphate as the standard).

Total sulphur content of the dhal samples was also determined by the Leco sulphur analyser (Leco Corporation, St. Joseph, Michigan, USA).

Table 1. List of chickpea and pigeonpea cultivars used in the experiment

		Chickpea		
No. 59	12-071-05093	P-1213-2	Chafa	G-24
NEC 1196	P-134-1	P-1231	C-104	L-550
NEC 1572	P-416	P-1363-1	C-214	K-4
NEC 1614	P-1081	P-1630	NEC-34	P-3090
NEC 1713	P-1081-1	Annegiri	BEG-482	NP-34
T-3	P-1181-A	Chana	BR-70	Kaka
		Pigeonpea		
No. 148	4685/1	BDN-2	Prabhat	As-71-37
Bhandra coll	NP(WR)-15	Sharda	T-21	KWR-1
Pant A-2	T-17	BDN-1	DH-74-1	T-7
UPAS-120	Badalkhadi coll	Gwalior-3	Mukta	Hy-3C
Pakhanjore coll	Bhedaghat coll	C-11	Hy-2	

Samples were subjected to combustion in a stream of oxygen and the released sulphur dioxide was measured colorimetrically according to the procedure described in the manual. Ten determinations were carried out on randomly selected samples on each of chickpea, cv. G-130, and pigeonpea, cv. Sharda, to estimate the standard deviation and coefficient of variation of these procedures.

Recovery experiments using methionine and cystine (obtained from BDH, England) were also carried out by using both methods. Five milligrams of methionine and 5 mg of cystine were weighed and added to samples before digestion in the case of the wet digestion method and before combustion in the case of the Leco analyser. Ten milligrams of each amino acid were added when assayed separately. Recovery assays were repeated eight times.

Amino acid analysis

Methionine and cystine were determined as methionine sulphone and cysteic acid after performic acid oxidation according to Moore [13]. Fifty milligrams of each sample were used for hydrolysis and the amino acids were analysed in a Beckman Model 120C amino acid analyser.

Results and Discussion

Comparison of methods of sulphur determination

The standard deviations and coefficients of variation of the wet digestion and the Leco analyser procedures are shown in Table 2. The means, standard deviations and coefficients of variation were higher for the Leco analyser than for the wet digestion method but the results obtained by both these procedures were highly correlated ($r = 0.943^{**}$) with each other. The results of recovery experiments are shown in Table 3. Methionine gave a slightly lower recovery by the wet digestion procedure for both chickpea and pigeonpea while cystine gave excellent recoveries with both methods. The average re-

Table 2. Sulphur estimation by wet digestion method and the Leco sulphur analyser (g/100 g meal)[a]

	Wet digestion		Leco analyser	
	Chickpea	Pigeonpea	Chickpea	Pigeonpea
Minimum	0.205	0.125	0.217	0.133
Maximum	0.225	0.150	0.251	0.162
Mean	0.215	0.136	0.238	0.146
± SE	0.0022	0.0019	0.0043	0.0041
CV (%)	3.16	4.48	5.60	8.92

[a]Minimum, maximum, and mean values of ten determinations; SE, standard deviation of estimation; CV, coefficient of variation.

Table 3. Recovery of sulphur from methionine and cystine[a]

Compound	Wet digestion		Leco analyser	
	Chickpea	Pigeonpea	Chickpea	Pigeonpea
	Recovered			
Methionine	91.3 ± 5.7	91.6 ± 3.5	95.4 ± 3.2	97.3 ± 4.7
Cystine	99.9 ± 3.6	96.5 ± 4.0	97.1 ± 4.6	97.2 ± 5.3
Methionine and cystine	94.3 ± 4.4	101.0 ± 6.2	98.8 ± 6.6	97.8 ± 4.0

[a]Mean values (± standard deviations) of eight determinations.

covery values (%) for methionine together with cystine were 94.3 and 101.0 for chickpea and pigeonpea respectively by the wet digestion procedure and 98.8 and 97.8 for chickpea and pigeonpea respectively when assayed by the Leco analyser method (Table 3). Based on these findings, the wet digestion method results were used for correlation studies between total sulphur and sulphur amino acids.

Variation in total sulphur and sulphur amino acids

A comparison of the individual values of total sulphur, methionine, cystine and the sulphur content of sulphur amino acids in relation to the total sulphur content in 30 chickpea cultivars showed that total sulphur as percent of sample varied between 0.17 and 0.27 with a mean value of 0.22, showing a difference of about 57% between the lowest and highest value (Table 4). Total sulphur as percent of protein varied between 0.82 and 1.41 with a mean value of 1.13 and the relative difference between the lowest and highest value was about 72%. Total sulphur amino acids as percent of protein varied between 2.02 and 2.63 with a mean of 2.31 while the values expressed as percent of sample were found to vary between 0.36 and 0.57, the variation being about 57%. The sulphur content of methionine and cystine accounted for 54.8% of total sulphur while the individual values ranged between 41.0 and 67.6% (Table 4). This indicates that a considerable amount of the total sulphur was present in forms other than sulphur amino acids.

A comparison of total sulphur, methionine, cystine and the sulphur content of sulphur amino acids in relation to the total sulphur content in 24 pigeonpea cultivars showed that total sulphur as percent of sample varied between 0.14 and 0.19, the variation being about 36% between the lowest and highest values (Table 4). Total sulphur amino acids as percent of protein varied between 1.76 and 2.55 with a mean of 2.11. When expressed as percent of sample they varied between 0.38 and 0.57 with a mean of 0.47, the variation being about 50%. The amount of sulphur in methionine and cystine together accounted for 75.5% of total sulphur, ranging from 59.2 to 84.6% (Table 4).

Chickpea showed a larger variation in protein content than pigeonpea, though the mean protein content of chickpea was lower. The mean values for total sulphur expressed as percent of sample (0.22) or as percent of protein (1.13) were higher in chickpea than in pigeonpea, which had the values 0.16 and 0.74 respectively. However, the species did not differ in mean values for sulphur amino acids expressed either as percent of sample or as percent of protein. Also, the sulphur content of methionine and cystine accounted for a higher proportion of the total sulphur in pigeonpea (75.5%) than in chickpea (54.8%). It was obvious that both crops had considerable amounts of other sulphur compounds in addition to methionine and cystine and apparently chickpea had higher extraneous sulphur compounds. Bhatty et al. [2] reported that sulphur in sulphur amino acids formed between 44.8 and 66.5% of the total meal sulphur in other legumes.

Correlations between total sulphur and sulphur amino acids

The correlation coefficients among the constituents are shown in Table 5. In chickpea, on a whole-sample basis, the percentage protein and total sulphur were significantly positively correlated with percentage cystine, methionine and cystine plus methionine and with each other. The correlation between percentage protein and cystine plus methionine was 0.809,** indicating that about 65% variation in these amino acids can be attributed to the levels of protein in the sample. When expressed as percentage of protein in sample, the correlation of protein with methionine was significant and negative while with cystine and cystine plus methionine, it was negative but insignificant. The correlation of total sulphur as percent of sample with cystine, methionine, cystine plus methionine as percent of protein was insignificant, indicating that any rapid method of estimating total sulphur may not yield reliable information on the sulphur amino acid contents of the sample.

In pigeonpea, total sulphur was correlated with cystine, methionine and cystine plus methionine on a whole-sample basis, while protein was correlated with methionine and to a lesser magnitude ($r = 0.392^*$) with cystine plus methionine.

When expressed as a percentage of protein in the sample, the correlation of protein with sulphur amino acids was insignificant. Total sulphur as percent

Table 4. Total sulphur (TS), methionine (Met) and cystine (Cys) in dhal samples of 30 chickpea cultivars and 24 pigeonpea cultivars

Crop	Protein (%) (N X 6.25)	Total sulphur (% of sample)	Methionine (% of protein)	Cystine (% of protein)	S in Met (% of TS)	S in Cys (% of TS)	S in Cys + Met (% of TS)
Chickpea:							
Range	16.18–25.20	0.17–0.27	0.89–1.14	1.13–1.51	17.78–25.91	23.26–45.25	41.04–67.61
Mean	19.99	0.22	1.04	1.27	21.49	33.35	54.84
± SD[a]	0.18	0.007	0.04	0.04	0.41	0.66	1.07
Pigeonpea:							
Range	19.40–25.92	0.14–0.19	0.78–1.15	0.94–1.42	24.41–38.25	35.65–52.16	59.23–84.62
Mean	22.37	0.16	0.93	1.18	29.45	46.07	75.52
± SD[a]	0.23	0.006	0.04	0.03	0.54	0.70	1.24

[a] Standard deviation of estimation.

Table 5. Correlation coefficients among protein, total sulphur and sulphur amino acids in 30 chickpea and 24 pigeonpea cultivars

	Protein (%)	Cystine (g/100 g sample)	Methionine (g/100 g sample)	Cystine + methionine (g/100 g sample)	Cystine (g/100 g protein)	Methionine (g/100 g protein)	Cystine + methionine (g/100 g protein)
				Chickpea			
Protein (%)	–	0.719**	0.845**	0.809**	−0.309	−0.645**	−0.487
Total sulphur (g/100 g sample)	0.476**	0.402*	0.578**	0.494**	0.043	0.094	0.016
Total sulphur (g/100 g protein)	−0.611**	−0.460**	−0.361	0.466**	0.390**	0.612**	0.522**
				Pigeonpea			
Protein (%)	–	0.269	0.489*	0.392*	−0.308	−0.262	−0.214
Total sulphur (g/100 g sample)	−0.150	0.554**	0.453*	0.534**	0.616**	0.612**	0.651**
Total sulphur (g/100 g protein)	−0.745**	0.175	−0.064	0.096	0.593**	0.458**	0.566**

*Significant at 5% level.
**significant at 1% level.

Table 6. Correlation coefficients between cystine and methionine in 30 chickpea and 24 pigeonpea cultivars

	Correlation coefficients				
	Methionine	Cystine + methionine	Cystine	Methionine	Cystine + methionine
	(g/100 g sample)		(g/100 g protein)		
			Chickpea		
Cystine[a]	0.756**	0.969**	0.201	−0.277	0.009
Methionine[a]		0.893**	0.079	−0.148	−0.130
Cystine[b]	–	–	–	0.686**	0.941**
Methionine[b]	–	–	–	–	0.890**
			Pigeonpea		
Cystine[a]	0.801**	0.956**	0.829**	0.743**	0.956**
Methionine[a]	–	0.940**	0.516**	0.838**	0.693**
Cystine[b]	–	–	–	0.780**	0.958**
Methionine[b]	–	–	–	–	0.926**

[a] g/100 g sample.
[b] g/100 g protein.
** Significant at 1% level.

of sample showed a significant positive correlation with cystine, methionine and cystine plus methionine when expressed as percent of protein. This differs from the results obtained with chickpea and indicates the possibility of using total sulphur content as an index of higher sulphur amino acids in pigeonpea.

Since cystine can partially replace methionine [4] the levels of cystine and methionine were considered together. In both chickpea and pigeonpea, the increase in protein content was associated with a decrease in protein sulphur and pigeonpea showed a higher correlation. A similar relationship had been reported by Evans and Boulter [5]. Jermy et al. [10] reported that in peas it was desirable to determine the sulphur amino acids as percent of protein in order to minimise any environmental effect on genetic variation on these amino acids. Gupta et al. [8] reported low and insignificant correlation between total sulphur and methionine when expressed as percent of protein in chickpea cultivars. Our results are in agreement with these observations but are in contrast with Sandhu et al. [15].

Relationship between cystine and methionine

Methionine, cystine, and methionine plus cystine together when expressed as percent of protein or as percent of sample showed highly significant correlations with and among each other (Table 6). Methionine and cystine when expressed as percent of sample were correlated with each other for both chickpea (0.756) and pigeonpea (0.801). When expressed as percent of protein, methionine was significantly correlated with cystine in chickpea ($r = 0.686$) as well as in pigeonpea ($r = 0.780$). Moreover, cystine and

methionine were correlated with each other when expressed either as percent of protein or as percent of sample. This indicates that perhaps screening for one of the sulphur amino acids might be considered depending on the objectives, keeping in mind the values of coefficients of determination between methionine and cystine when expressed as percent of sample for chickpea (0.57) and pigeonpea (0.64).

Acknowledgments. The authors gratefully acknowledge the technical assistance of R. Seetha, S. Gurtu and S. Surya Prakash.

References

1. Association of Official Analytical Chemists (1975) Official methods of analysis, 12th edn. Washington DC: AOAC
2. Bhatty R, Finlayson AJ, MacKenzie SL (1977) Total sulphur as a screening criterion for sulphur amino acids in legumes. Can J Plant Sci 57: 177–183
3. Bressani R (1972) Legumes in human diets and how they might be improved. In: Nutritional improvement of food legumes by breeding. Pro Symp sponsored by Protein Advisory Group (PAG) of the United Nations held at FAO, Rome, Italy 30 July 1972, pp 15–42
4. Eppendorfer WF (1971) Effect of S, N and P on amino acid composition of field beans (*Vicia faba*) and responses of the biological value of the seed protein to S-amino acid content. J Sci Food Agric 22: 501–505
5. Evans IM, Boulter D (1974) Chemical methods suitable for screening for protein content and quality in cowpea (*Vigna unguiculaty*) meals. J Sci Food Agric 25: 311–322
6. Finlayson AJ, MacKenzie SL (1976) A rapid method for methionine determination in plant materials. Anal Biochem 70: 397–402
7. Gerhrke GW, Neuner T (1974) The determination of methionine. J Assoc Official Anal Chem 57: 682–688
8. Goa J (1961) Microdetermination of cystein plus cystine in protein. Acta Chem Scand 15: 853–855
9. Gupta VP, Ramanujam S, Kaul AK (1974) Stability analysis in respect of protein, sulphur and protein value index of seed and its implication in adaptation of chickpea (*Cicer arietinum* L.). Acta Biol Yugosl Genetika 6: 247–261
10. Jermyn WA, MacKenzie SL, Slinkard AE (1979) Methods of reporting methionine content in peas. Can J Plant Sci 59: 231–232
11. Kelly JF (1971) Genetic variation in the methionine levels of mature seeds of common beans (*Phaseolus vulgaris* L.). J Am Soc Hortic Sci 96: 561–563
12. Leleji OI, Dickson MH, Hackler LR (1972) Effect of genotype on microbiologically available methionine content of bean seeds. Hortic Sci 7: 277–278
13. Moore S (1963) On the determination of cystine as cysteic acid. J Biol Chem 238: 235–137
14. Porter WM, Axtell J, Keim WF (1972) Quantitative relationships between total sulphur and methionine and cystine in legumes. In: Nutritional improvement of food legumes by breeding. Proc Symp sponsored by PAG, held at the FAO, Rome, Italy, p 319
15. Sandhu SS, Keim WF, Hodges HF, Nyquist WE (1974) Inheritance of protein and sulphur content in seeds of chickpeas. Crop Sci 14: 649–652
16. Tabatabai MA, Bremner JM (1970) A simple turbidimetric method of determining total sulphur in plant materials. Agron J 62: 805–806