

TECHNICAL NOTE

Protein quality of Asian beans and their wild progenitor, *Vigna sublobata* (Roxb)

Iqtidar A. Khalil^a & Saleem Khan^b

Department of Agricultural Chemistry and Human Nutrition, North-West Frontier Province Agricultural University, Peshawar, Pakistan

(Received 1 February 1994; revised version received and accepted 3 May 1994)

The small seeded tropical Asian beans of the so-called mung group (*Vigna* species), mung bean (green or golden gram, *V. radiata*), and urd bean (black gram, *V. mungo*), and their wild progenitor *V. sublobata* (wild bean), were assessed for their nutritional composition and protein quality. The protein content of these legumes varied from 20.7 to 23.8%. It was rich in lysine and the aromatic amino acids. However, S-containing amino acids were most deficient for children's diets. Tryptophan was the second limiting amino acid. The protein score ranged from 40 to 64% of the reference protein recommended for child nutrition. In the adult human diet beans can fulfil the requirements of all essential amino acids and thus can serve as supplemental sources of protein in cereal based diets.

INTRODUCTION

Food legumes are economic sources of protein and calories in mixed cereal diet. The small seeded Asian beans are less exploited as a protein source in human nutrition. These beans of the so-called mung group (Allen & Allen, 1980) include: *Vigna radiata* (L.) Wilczek (green or golden gram = mung bean), and *V. mungo* (L.) Hepper (black gram or urd bean). The putative progenitor of both these species is *V. sublobata* (Roxb), which grows wild and hence is referred to as the wild bean.

Some work has been done on the amino acid profile of the obscure germplasm of certain *Vigna* species (Rajaram & Janardhanan, 1992; Mohan & Janardhanan, 1993). Such studies are needed for the nutritional improvement of the existing germplasm through selection and breeding. The present work was also undertaken to examine the nutritional quality of protein of the two cultivated Asian beans and their wild progenitor, collected from the North-West Frontier Province (NWFP) of Pakistan.

^aPresently: Research Fellow, Department of Crop Production and Agriculture Technology, University of Udine, Via delle Scienze 208, 33100-Udine, Italy.

^bPresently: Research Scholar, Department of Food Science and Nutrition, University of Padova, Via Giustiniani-2, 35100 Padova, Italy.

MATERIALS AND METHODS

Mature seeds of cultivated beans (*V. radiata* (L.) Wilczek, and *V. mungo* (L.) Hepper) & wild bean (*V. sublobata* Roxb) seeds were collected from three locations of NWFP. Each sample was ground in a Willy mill to 60 - mesh size. Moisture, crude protein (N × 5.7) crude fat, crude fibre and ash contents were determined by the standard methods of AOAC (1990). Carbohydrate content as nitrogen-free-extract was calculated by difference. Mineral analysis was done after wet acid oxidation of each sample in nitric:perchloric acid (5:2) mixture. Calcium was determined by EDTA titration, and phosphorus by the molybdenum blue method (Chapman *et al.*, 1961). Sodium and potassium were estimated by flame photometry, and iron by a spectrophotometric method (Khalil & Manan, 1990).

For amino acid analysis, 300 mg of air-dried, finely ground seed samples were hydrolysed in 6 M HCl according to the procedure detailed perviously (Khalil & Rahman, 1984). The hydrolysate was dissolved in citrate buffer (pH 2.2) and analysed by ion exchange chromatography (LKB automatic amino acid analyser). Cystine was determined as cysteic acid after oxidising the samples with performic acid and subsequent hydrolysis according to the method of Moore (1963). Since tryptophan is destroyed during acid hydrolysis, it was estimated by the colorimetric method (Friedman &

Finely, 1971). The limiting amino acids in each seed protein were computed with reference to standard amino acid pattern of the FAO/WHO (1985). Protein quality scores were then calculated on the basis of amino acid in greatest deficit. Biological value of proteins was estimated by the equation described by Block and Mitchell (1946). Protein digestibility (%) *in vitro* was determined by using proteolytic enzymes, pepsin followed by pancreatin digestion (Akeson & Stahmann, 1964).

RESULTS AND DISCUSSION

The proximate and mineral composition of beans (Table 1) indicate that the cultivated beans (*Vigna radiata* and *V. mungo*) had significantly ($P < 0.05$) higher crude protein content (23.1–23.8%) compared to their wild progenitor (20.7%). The latter contained higher fibre content, and was better than *V. mungo* as a source of mineral matter. It contained a higher amount of iron and lesser sodium content as compared to cultivated species. Mung bean was the richest in calcium and phosphorus, followed by wild bean and urd bean. Potash was found to be the main ash contributing factor. Sodium was present in a much smaller quantity than both calcium and phosphorus in all three cultivars tested. A higher Na:P ratio was observed in cultivated beans compared to their wild progenitor. However, the Ca:P ratio was the same in the three legumes tested.

These observations reflect that, like other food legumes, such as field pea (*Pisum sativum*) (Jabeen *et al.*, 1988), chickpea (*Cicer asietinum*) (Singh *et al.*, 1991) and cowpea (*Vigna sinensis* = *V. unguiculata*) (Mohan & Janardhanan, 1993), small seeded Asian beans can also contribute significant amounts of pro-

tein, fibre and mineral matter in vegetarian diets. The much lower Ca:P ratio may affect the bioavailability and utilisation of these nutrients (Rickett *et al.*, 1970). Hence, supplementation with rich dietary calcium sources may be required to enhance food efficiency of the small seeded Asian beans.

The amino acid composition of the three *Vigna* species is shown in Table 2. It is evident that these legumes contained all the essential amino acids required in human nutrition. They are particularly rich in lysine and the aromatic amino acids (phenylalanine and tyrosine). Mung bean and wild bean were significantly ($P < 0.05$) better than urd bean with respect to their lysine content. However, the latter contained more tryptophan and the S-containing amino acids, methionine and cystine.

The essential amino acid profile of *Vigna* beans is comparable with other food legumes. The lysine, phenylalanine and tyrosine contents were comparatively higher than those of soybean (Zarkadas *et al.*, 1993), chickpea (Singh *et al.*, 1988) and cowpea (Mohan & Janardhanan, 1993). However, the proteins of these legumes were better than *Vigna* cultivars with respect to their methionine, cystine and tryptophan contents. When the contents of these amino acids were compared with the FAO/WHO (1985) amino acid reference profile of protein for human consumption, it was found that the three *Vigna* species tested can fulfil the requirements of all the essential amino acids in adult human diet. However, in the diet of children (aged 2–5 years) proteins of all legumes were deficient in S-containing amino acids. Protein of *Vigna* cultivars were also deficient in tryptophan. Methionine and cystine were the most limiting amino acids in all the legumes except chickpea, which was most deficient in threonine. The per cent deficiency of S-containing amino acids in

Table 1. Proximate and mineral composition of Asian bean (*Vigna*) cultivars

Nutrients ^a	Mung bean (<i>V. radiata</i>)	Urd bean (<i>V. mungo</i>)	Wild bean (<i>V. sublobata</i>)	±S.E. ^b
Major (%)				
Moisture	8.2	7.8	8.5	0.21
Crude protein	23.1	23.8	20.7	0.75
Crude fat	2.2	1.8	2.0	0.07
Crude fibre	4.8	5.2	5.7	0.16
Ash	3.4	2.8	3.2	0.08
NFC ^c	58.3	58.6	59.9	—
Energy (10 ¹ kcal kg ⁻¹)				
Protein calories	92	95	83	—
Total calories	345	346	340	—
Mineral constituents (10 ² mg kg ⁻¹)				
Na	6.8	5.1	4.5	0.21
K	275	208	230	6.5
Ca	20.7	16.2	18.5	0.70
P	31.5	24.5	28.2	0.86
Fe	2.5	2.2	3.2	0.08
Na:P ratio	0.22	0.21	0.16	—
Ca:P ratio	0.66	0.66	0.66	—

^aOn air-dried basis.

^bStandard error of each mean (6 d.f.).

^cNitrogen-free-extract (carbohydrate) calculated by difference.

wild bean was the highest, followed by urd bean and mung bean. The protein of these three legume cultivars scored much lower than that of soybean, chickpea and cowpea. Hence, the biological value of protein was also lower in *Vigna* species than that of the other legumes. Protein *in vitro* digestibility of *Vigna* beans varied from 70 to 75%, the highest value recorded in mung bean and the lowest in urd bean protein.

It is noteworthy that except S-containing amino acids and tryptophan, proteins from *Vigna* species were also comparable with hen's egg (Table 2). Deficiency of methionine and cystine was also observed in other food legumes, such as field pea (*Pisum sativum*) (Jabeen *et al.*, 1988), lentil (*Lens culinaris*) (Peace *et al.*, 1988), and large-seeded beans (*Phaseolus vulgaris* L.) (Tezoto & Sgarbieri, 1990). More recent work (Mohan & Janardhanan, 1993) also revealed deficiency of S-containing amino acids in certain *Vigna* species other than those tested in this study. However, tryptophan deficiency is rarely reported in the literature.

The protein-amino acid relationship in the three *Vigna* cultivars, collected from three locations was studied by computing the co-efficient of correlation (γ) and co-efficient of determination (r^2). The S-containing amino acids and histidine were positively related to the protein contents. Although these relationships were non-significant, they were important since earlier work (Jabeen *et al.*, 1988) revealed a highly significant negative correlation between protein and the S-containing amino acids in field pea. The relationships between different amino acids were also studied. The S-containing amino acids (methionine and cystine) were positively related to histidine and isoleucine. Lysine and tryptophan had a negative correlation ($r = -0.48$),

while methionine and cystine had a positive association ($r = 0.61$). These correlations could further be exploited in the breeding and selection of beans.

CONCLUSION

From the preceding discussion it appears that the proteins of beans are rich in lysine, but deficient in S-containing amino acids and tryptophan (only in children's diet). Since cereals are most deficient in lysine but contain sufficient methionine and cystine (Khalil & Rahman, 1984), beans can serve as a good protein and lysine supplement in cereal diet for children. Amino acid deficiencies in both the foods could be balanced by formulation. Among cereals, maize is also deficient in tryptophan (Saxena *et al.*, 1984) like beans. Hence this fact should not be ignored while formulating a cereals-legume diet for children.

ACKNOWLEDGEMENT

The authors would like to thank Mr Vincenzo Montalbano, Istituto di Semeiotica Medica, Università di Padova, Italy for his generous advice and for providing a computer facility for writing this manuscript.

REFERENCES

- Akeson, W. R. & Stahmann, M. A. (1964). A pepsin-pancreatin digest index of proteins. *J. Nutr.*, **82**, 257-60.
 Allen, O. N. & Allen, E. K. (1981). *Leguminosae*. Macmillan, London.

Table 2. Essential amino acid profile and protein quality score of Asian beans in comparison with other legume proteins, hen's egg protein and the FAO/WHO (1985) amino acid reference profile for human consumption

Amino acid (% of protein)	Mung bean	Urd bean	Wild bean	Soy bean ^a	Chick pea ^b	Cow pea ^c	Hen's egg ^d	Ref. Profile	
								Child	Adult
Histidine	3.2	3.5	3.4	3.2	2.7	2.3	2.2	1.9	1.6
Isoleucine	4.6	5.0	4.0	4.9	4.3	2.4	5.4	2.8	1.3
Leucine	7.4	7.0	6.1	8.1	7.1	8.0	8.6	6.6	1.9
Lysine	7.7	6.8	7.6	6.5	6.1	6.6	7.0	5.8	1.6
Methionine + cystine	1.6	2.0	1.4	2.3	2.1	2.0	5.7	2.5	1.7
Phenylalanine + tyrosine	9.3	9.0	10.0	8.6	7.8	7.8	9.3	6.3	1.9
Threonine	3.4	3.6	3.8	3.4	2.6	4.0	4.7	3.4	0.9
Tryptophan	0.8	1.0	0.9	1.1	1.4	—	1.7	1.1	0.5
Valine	5.2	4.8	5.7	4.4	4.4	5.5	5.1	3.5	1.3
Limiting-amino acid ^e	S	S	S	S	Thr	S	—	—	—
Deficiency%	36	48	60	8	24	20	—	—	—
Protein score (%)	64	52	40	92	76	80	100	100	—
Biological value	79	72	64	97	87	89	100	100	—
Protein <i>in vitro</i> digestibility (%)	75	70	72	—	—	—	100	100	—

^aZarkadas *et al.* (1993). ^bSingh *et al.* (1988).

^cCow pea (*Vigna sinensis* = *V. unguiculata* (L.) Walp, Mohan & Janardhanan (1993). ^dFAO/WHO (1985).

^eMost limiting amino acid with reference to FAO/WHO (1985) reference profile for children (age 2-5 years); S = sulphur containing amino acids (methionine - cysteine); Thr = threonine.

- A.O.A.C. (1990). *Official Methods of Analysis*, (5th edn), ed. K. Helrich. Arlington, Virginia.
- Block, R. H. & Mitchell, H. H. (1946). The correlation of the amino acid composition of proteins with their nutritive value. *Nutr. Abst Reviews*, **16**, 249–79.
- Chapman, D., Homer, P. & Parker, F. (1961). *Methods of Analysis of Soils, Plants and Water*. Division Agricultural Science, University of California.
- FAO/WHO (1985). Energy and protein requirements. Tech. Rep. 72, WHO, Geneva.
- Friedman, M. & Finely, J. W. (1971). Methods of tryptophan analysis. *J. Agric. Food Chem.*, **19**, 626–31.
- Jabeen, T., Iqbal, P. & Khalil, I. A. (1988). Amino acid and mineral composition of pea cultivars grown in Peshawar. *Pakistan J. Agric. Res.*, **9**, 171–5.
- Khalil, I. A. & Manan, F. (1990). *Chemistry-one (BioAnalytical Chemistry)*. Taj Kutub Khana, Peshawar, Pakistan.
- Khalil, I. A. & Rahman, S. (1984). Effect of boron on the amino acid composition of wheat. *J. Agric. Sci. (Cambridge)*, **103**, 75–80.
- Mohan, V. R. & Janardhanan, K. (1993). Chemical composition and nutritional evaluation of two little-known species of vigna. *Food Chem.*, **48**, 367–71.
- Moore, S. (1963). On the determination of cystine and cysteic acid. *J. Biol. Chem.*, **238**, 235–7.
- Peace, R. W., Keith, M. O., Sarwar, G. & Botting, H. G. (1988). Effect storage on protein nutritional quality of grain legumes. *J. Food Sci.*, **53**, 439–41.
- Rajaram, N. & Janardhanan, K. (1992). Agrobotanical and biochemical studies of some less known vigna spp. *Acta Botanica Indica*, **20**, 143–9.
- Rickett, R. E., Campbell, J. R., Weinman, D. E. & Tumbleson, M. E. (1970). Effect of three calcium: Phosphorus ratios on performance of growing Holstein steers. *J. Dairy Sci.*, **53**, 898–903.
- Saxena, A. R., Sharma, A. K., Sehgal, K. L. & Bakhshi, A. K. (1984). Proximate composition and nutritive value of some improved varieties of maize of Punjab. *Indian J. Nutri. Diet.*, **21**, 233–40.
- Singh, D. K., Rao, A. S. & Singh, R. (1988). Amino-acid composition of storage proteins of a promising chickpea (*Cicer arietinum* L.) cultivar. *J. Sci. Food Agric.*, **43**, 373–9.
- Singh, U., Subramaniam & Kumar, J. (1991) Cooking quality and nutritional attributes of some newly developed cultivars of chickpea (*Cicer arietinum*). *J. Sci. Food Agric.*, **55**, 37–46.
- Tezoto, S. S. & Sgarbieri, V. C. (1990). Protein nutritive value of a new cultivar of bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.*, **38**, 1152–6.
- Zarkadas, C. G., Yu, Z., Voldeng, H. D. & Minero-Amador, A. (1993). Assessment of the protein quality of a new high protein soybean cultivar by amino acid analysis. *J. Agric. Food Chem.*, **41**, 616–23.