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Amino Acid Composition of Storage Proteins of a Promising Chickpea (*Cicer arietinum* L) Cultivar

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ABSTRACT

The amino acid compositions of flours made from the cotyledons and from the whole seeds of a disease-resistant, stable and high-yielding cultivar of chickpea (Cicer arietinum *L*) *cv H75-35, known locally as Gaurav, have been analysed. These, together with similar analyses of the albumin, globulin, legumin and vicilin protein fractions, have been compared with those of other legumes. Seed protein content was 19·8 % with globulin constituting 62·4 % of the total seed protein. The ratios of albumin to globulin and legumin to vicilin were 1:4 and 6:1, respectively. The proportion of basic amino acids in the albumin was low whereas the reverse was true in the globulin. The legumin fraction seems to be superior in terms of total essential amino acids to those from other sources. Sulphur amino acids were the most limiting, followed by tryptophan or threonine depending on the fraction. However, the ratio of methionine to cystine was high (2·76:1).*

The extent of the sulphur amino acid deficiency was assessed, and possible approaches for its improvement are outlined.

Key words: Chickpea, protein fractions, amino acid composition.

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1 INTRODUCTION

Chickpea (*Cicer arietinum* L) is an important tropical legume. The Indian crop constitutes nearly 85% and 79% of the total chickpea production in Asia and the world, respectively. Hence, evolving a stable, high-yielding and nutritionally well balanced cultivar is an important goal for chickpea breeders in India. A stable and high-yielding cultivar, H75-35, known locally as Gaurav, has been released in recent years (Dahiya *et al* 1983). The objective of the present investigation was to analyse the amino acid composition of the major storage protein fractions of this promising cultivar in order to provide some basic information which could be utilised for the improvement of amino acid balance. A comparison has also been made with other legumes to reflect the relative nutritional status of the present cultivar with respect to amino acid balance.

2 EXPERIMENTAL

2.1 Seed material

Seeds of chickpea cv H75-35 were procured from the Department of Plant Breeding, Haryana Agricultural University, Hisar, India.

2.2 Preparation of flour

Seeds, with or without seed coat, were ground in a Udy-Cyclone mill using 80-mesh screen. The flour was defatted with hexane, air dried and used for further analysis.

2.3 Protein fractionation

Methods were similar to those of Krishna *et al* 1979, Matta *et al* (1981) and Krishna and Bhatia (1985).

2.3.1 Separation of albumin and globulin fractions

Defatted cotyledon flour (500 mg) was extracted with 10 ml of 0·1 M borate buffer (pH 8·3) by shaking in a water bath (1 h), centrifuging at 12 000 × g for 20 min and extracting the residue twice with 5 ml of the same buffer. The three supernates were pooled and dialysed against 25 mM sodium citrate buffer (pH 4·6) for 15 h and centrifuged as before. The supernate and pellet were the albumin and globulin fractions, respectively.

2.3.2 Separation of globulin into legumin and vicilin

Globulin precipitates were dissolved in the 0·1 M borate buffer (pH 8·3) containing 0·2 M sodium chloride, dialysed against 25 mM sodium citrate buffer (pH 4·6) for 15 h and centrifuged. The precipitate and supernate were legumin and vicilin, respectively.

2.4 Nitrogen estimation

The nitrogen contents of the whole seed protein and the various fractions were determined by a micro Kjeldahl method (AOAC 1975).

2.5 Amino acid composition

Amino acid composition of the protein samples was determined by the procedure outlined in an ICRISAT progress report (ICRISAT 1982) using a Beckman 119 CL analyser connected to an HP 3390A integrator.

2.6 Tryptophan estimation

Tryptophan was estimated colorimetrically according to Friedman and Finley (1971).

2.7 Chemical evaluation of protein

Chemical scores were calculated as recommended by FAO/WHO (1973). Essential amino acid index (EAAI) and biological value (BV) were calculated according to Oser (1959).

3 RESULTS AND DISCUSSION

3.1 Protein content and relative distribution of storage protein fractions

Protein content of Gaurav seed flour was found to be 198·3 g kg^{-1} and that of cotyledon flour 209·2 g kg^{-1}. Chickpea cultivars are known to show a wide variation (116–311 g kg^{-1}) in seed protein content (Jambunathan and Singh 1979). Thus, the protein content of the present cultivar is similar to those reported for chickpea generally.

Globulin was found to be the major fraction, comprising 624 g kg^{-1} of the total seed protein which is lower than that reported by Srivastava *et al* (1980). The ratio of albumin to globulin was 1:4, quite high compared with several other legumes, viz pigeon pea (Singh and Jambunathan 1980), black gram (Padhye and Salunkhe 1979) and pea (Grant *et al* 1976). Legumin constitutes most of the globulin fraction (823 g kg^{-1}) giving a legumin to vicilin ratio of 6:1. This ratio is apparently superior to that observed in faba bean (Boulter 1983), pea (Muller and Werner 1979) and cowpea (Bliss 1975), which show legumin to vicilin ratios of 2·3–4:1, 1·3:1 and 1·9:1, respectively, but similar to that of french bean reported by Hall *et al* (1979).

3.2 Amino acid composition

In the seeds of various legumes (Mosse and Pernollet 1982) total essential amino acid content varies from 230 to 430 g kg^{-1} protein. Variation also exists among different cultivars of chickpea and is of the range 336–412 g kg^{-1} protein (ICRISAT 1982). It is evident from Table 1 that the essential amino acid content of the present variety is quite high (357 g kg^{-1} protein). However, as in many other legumes, there is a deficiency of methionine and cysteine. Together the sulphur-containing amino acids constitute only 2·07 % of the total amino acids. Nevertheless, a positive aspect of the sulphur amino acid content of Gaurav seeds is the high methionine:cysteine ratio (2·76:1). This ratio has been shown to vary between 0·73:1 and 1·25:1 in other cultivars of chickpea and between 0·41:1 and 1·76:1 in other legumes (Mosse and Pernollet 1982). Similar results were obtained for cotyledon flour.

TABLE 1
Amino Acid Compositions of Chickpea Flour and the Major Storage Protein Fractions

Amino acid	*Amino acid content*[a] *(g kg*$^{-1}$ *protein)*					
	Whole seed flour	*Cotyledon flour*	*Albumin*	*Globulin*	*Legumin*	*Vicilin*
Essential						
Threonine	25·7	34·0	46·3	34·5	30·4	24·6
Cysteine	5·5	8·8	17·9	10·8	6·0	11·3
Valine	43·7	40·1	49·5	52·5	48·2	74·0
Methionine	15·2	17·7	9·1	10·4	6·6	Trace
Isoleucine	43·2	40·0	38·2	52·4	47·0	18·0
Leucine	70·9	70·7	68·1	81·3	79·0	79·3
Tyrosine	27·6	30·1	33·0	34·1	23·1	35·3
Phenylalanine	50·0	53·7	44·6	66·0	66·2	80·6
Lysine	61·1	63·0	44·9	69·3	57·9	59·3
Tryptophan	13·6	14·6	8·2	10·3	14·1	9·7
Non-essential						
Aspartic acid	94·8	110·2	114·7	133·1	126·3	96·0
Serine	29·6	44·7	149·8	53·2	41·3	40·0
Glutamic acid	159·7	157·6	139·2	171·7	173·0	148·0
Proline	40·7	39·7	50·2	52·1	43·6	Trace
Glycine	35·9	36·6	45·9	41·6	36·9	39·3
Histidine	26·8	27·0	33·3	31·0	27·3	25·3
Arginine	89·6	89·7	52·3	88·2	85·9	59·3
Alanine	47·1	45·4	48·8	50·9	38·9	48·0
Total	880·7	923·6	994·0	1043·4	951·7	848·0

[a]Each value is a mean of two determinations.

In comparison with earlier work (Jackson *et al* 1969; Mosse and Pernollet 1982) the proportion of basic amino acids in Gaurav seed albumin appears low; the reverse is true for the globulin fraction. Chickpea legumin is, in general, superior to other legumins in terms of essential amino acids.

3.3 Chemical score, essential amino acid index and calculated biological value

It is evident from Table 2 that the sulphur amino acids are first limiting in all the fractions, with valine or threonine the second or third limiting amino acids, depending on the fraction. These observations are at variance with some earlier reports (Khan *et al* 1979; Srivastava *et al* 1980; ICRISAT 1982) in which threonine was the first limiting with tryptophan or methionine the second or third limiting amino acids.

EAAI and the BV for cotyledon flour were higher (75·4 and 70·5) compared with whole seed flour (60·8 and 54·6). Among the protein fractions vicilin and globulin had the lowest and highest EAAI and BV, respectively.

TABLE 2
Chemical Score, Essential Amino Acid Index and Biological Value of Chickpea Flour and Storage Protein Fractions

Amino acid	*Whole seed flour*	*Cotyledon flour*	*Albumin*	*Globulin*	*Legumin*	*Vicilin*
Isoleucine	108	100	95	131	117	120
Leucine	101	101	97	116	112	113
Lysine	111	114	82	126	105	96
Methionine + cysteine	59	75	77	60	36	32
Phenylalanine + tyrosine	129	139	129	166	148	193
Threonine	64	85	115	86	76	61
Tryptophan	136	146	82	103	141	97
Valine	87	80	99	105	96	148
Essential amino acid index	60·8	75·4	68·5	76·4	69·5	66·1
Biological value	54·6	70·5	62·9	71·6	64·0	60·3

3.4 Magnitude of deficiency of sulphur amino acids *vis-à-vis* the FAO suggested level

It may be inferred from the amino acid composition that the quantity of sulphur amino acids has to be increased 2·32-fold in legumin if cotyledon flour is to be raised to the status of FAO reference protein and if the entire improvement is to be made through legumin alone at a constant proportion in the flour. Vicilin, the nutritionally poorest fraction, would require still greater improvement. It may also be calculated that even if all the globulin, or the balance of the total protein (total protein less albumin and globulin), is replaced by albumin, then cotyledon flour would still be deficient in terms of sulphur amino acids.

Thus, in view of the above calculations and several biochemical considerations involved in the genetic engineering of storage proteins (Rao and Singh 1986), it appears that large alterations in the quantity and/or quality of different protein fractions are required to alleviate the sulphur amino acid deficiency in the seeds of the present variety and presumably also in other legumes. Hence, attempting to increase the desired amino acids in the free pool, as suggested by Miflin *et al* (1982), seems to be a more promising approach than attempting to change the overall amino acid make-up of individual protein fractions. However, from the rapid and significant advances in plant molecular biology in general, and in knowledge of the structure, organisation, expression and regulation of seed storage proteins in particular (Larkins 1982; Van Vloten-Doting *et al* 1985), some newer approaches to protein improvement in legume seeds may emerge.

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