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Factors affecting the protein quality of pigeonpea (*Cajanus cajan* L.)

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Abstract. Pigeonpea occupies an important place in human nutrition as a source of dietary proteins in several countries. Some of the important factors that affect the protein quality of pigeonpea have been reviewed and summarised in this paper. Among important food legumes, pigeonpea contained the lowest amount of limiting sulphur amino acids, methionine and cystine implicating the importance of these amino acids in protein quality improvement program. Large variation existed in the levels of protease inhibitors of pigeonpea varieties. The concentration of these inhibitors were significantly higher in some of the wild relatives of pigeonpea. Protein digestibility of cooked pigeonpea meal remained low and this could be due to the presence of certain compounds other than trypsin inhibitors. Pigeonpea polyphenolic compounds adversely affected the activity of digestive enzymes and this would affect the protein quality of pigeonpea. The protein quality of pigeonpea was greatly influenced by storage and processing practices.

Introduction

Pigeonpea (*Cajanus cajan* L.), also called redgram, is among the important grain legumes which are grown and consumed in the tropics and the semi-arid tropics of the world. Besides the improvement of productivity, adaptability and yield stability of grain legumes, the improvement of nutritional quality of proteins has also been emphasized by the Protein Advisory Group (PAG) of the United Nations [1].

Worldwide attention has been paid to improve the nutritional quality of grain legumes [2–5]. Information on the nutritional aspects of pigeonpea is scanty and limited systematic efforts have been made to identify factors affecting its nutritional quality. This paper attempts to review and discuss the recent work on protein and amino acids, antinutritional factors, storage, and processing practices that affect the protein quality in pigeonpea.

Dietary use

India accounts for about 85% of the world's supply of pigeonpea. Other

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countries where pigeonpea is an important legume are Kenya, Malawi, Uganda, Thailand and Phillipines. In India, it is mostly consumed after dehusking in the form of dhal (decorticated split cotyledons) after cooking in water to a desirable softness whereas in some African countries whole seeds of pigeonpea are consumed after boiling. The developing green seeds shelled out of green pods harvested are also used as a vegetable in India, and some African, Latin American and Caribbean countries. Raw pigeonpea is processed into dhal by suitable milling processes. Pigeonpea husk which is a byproduct of milling process is mostly used as animal feed in India.

Protein and amino acids

The protein quality of a crop is primarily expressed in terms of its protein content and levels of essential amino acids. In most of the food crops, genetic variability for protein content is always considered an important factor towards improvement of protein quality by selections and breeding. Protein content of 43 commonly cultivated varieties of pigeonpea ranged between 17.9 and 24.3 percent for whole grain samples and between 21.1 and 28.1% for dhal, split decorticated cotyledon samples indicating a small variation [6]. High environmental influence on protein content and also a negative correlation between yield and percent protein have been reported in pigeonpea [7]. Under such circumstances, it may be possible to select lines with higher seed yield while maintaining percent protein near average and this strategy may yield in more protein per unit area. However, more recent efforts have suggested the possibility of developing high protein cultivars in pigeonpea through use of wild relatives as a source of high protein [8]. Maturity of the crop plays an important role in protein accumulation during seed development. The protein content of late maturing cultivars was greater than the early maturing cultivars of pigeonpea [9]. Environment and agronomic practices influence the protein quality of pigeonpea to a considerable extent and this should be kept in mind while breeding for protein quality [10].

The biological value of dietary protein which is defined as the fraction of absorbed nitrogen retained in the body for maintenance and growth is one of the most useful measurements of protein quality. Unfortunately, pigeonpea has the lowest biological value (Table 1). Eggum (1973) reported that biological availability of amino acids plays an important role in determining the nutritive value of plant proteins [11]. Experimental evidence has indicated conclusively that the biological value of legume seeds is postively and significantly correlated with sulphur containing amino acids. It was further suggested that protein quality of the processed legumes could be accurately estimated from their sulphur amino acids [4]. By expressing the essential amino acids of various legumes as percentages of the 1973 FAO/WHO provisional pattern [12], it was reported that the sulphur amino acids and tryptophan are the most limiting amino acids of legumes and the lowest values were for pigeonpea [13–14]. No large variation appear to exist in amino acid composition of wild relatives and cultivated species of pigeonpea (Table 2). However, the comparison of dhal and whole seed samples revealed considerable differences. But it may be pointed out that this comparison is based on the analyses carried out in two different laboratories and any inference should be made with a caution. The methionine and cystine contents of whole seed samples were higher and this was attributed to the status of sulphur in the soils [15].

This indicates that the selection of high protein cultivars, although desirable, cannot solve by itself the protein quality inadequacy of pigeonpea and a more balanced amino acid pattern with respect to sulphur amino acids and tryptophan is needed. In general, the increase of limiting essential amino acids does not parallel the increase in protein content. Negative relationships have usually been found in legumes between protein percentage and methionine content per unit of protein [3]. However, it is interesting to note that there was no strong relationship between methionine (g/16 g N) and protein percentages in pigeonpea [16]. This observation could be useful in a breeding program where a higher protein concentration is often emphasized.

Like other grain legumes, the storage proteins, globulins, constitute about 65% of the total seed protein of pigeonpea (Table 3). However, a comparison of low and high protein species of pigeonpea did not reveal large variation in seed protein fractions [17]. The globulin proteins are most deficient in sulphur amino acids in pigeonpea [18]. Albumin fraction, although representing a small proportion, is a very rich source of methionine and cystine. Therefore, the selection of cultivars containing greater concentration of this fraction will be desirable to improve the protein quality of pigeonpea. Higher levels of sulphur-containing amino acids in glutelin than in globulins of pigeonpea also suggest that cultivars with higher ratio of glutelin to globulin should be identified to improve the protein quality [18].

In conclusion, it is pointed out that although the possibility of producing high yielding lines with both high protein quantity and quality cannot be ruled out, the breeder may have to settle for lines with high yielding capacity with a content of protein and sulphur containing amino acids not less than what is normal for pigeonpea at present.

Antinutritional factors

Having discussed the well-documented deficiency of sulphur-containing amino acids in pigeonpea, it becomes imperative to discuss the role of antinutritional factors or antiphysiological substances which could be responsible for low protein digestibility. Of the various antinutritional factors that are found in grain legumes, trypsin and chymotrypsin inhibitors, amylase inhibitors and polyphenols (commonly known as tannins) are very important

Table 1. Nutritional quality of some legume crops

Legume species	Indicators of nutritional quality					
	Limiting amino acids					
	Gross deficiency	Marginal deficiency	Chemical score (A/T)	Biological value (%)	Protein efficiency ratio	Net protein utilization (%)
Broad bean	Met, Cys	Try	28	55	–	48
Chick pea	Met, Cys	Try	40	52–78	1.1–2.2	52–64
Common bean	Met, Cys	Try, Val	34	45–73	0.0–1.9	31–47
Common pea	Met, Cys	Try	37	48–66	0.3–2.2	41–50
Cow pea	Met, Cys	Ileu	41	49–66	–	35–51
Groundnut	Met, Cys	Ileu, Thr	43	51–63	1.5–1.8	52–55
Pigeon pea	Met, Cys	Try	27	46–74	0.7–1.8	52
Soybean	Met, Cys	Val	47	68–77	0.7–2.4	58–71

Source: Bozzini and Silano (1983). Reference No. 5. Met = Methionine. Cys = Cystine and Try = Tryptophane.

Table 2. Amino acid composition (g/100 g protein) of wild relatives and cultivated species of pigeonpea

Amino acids	Wild relatives[a]				Cultivated species (cultivars)			
	Atylosia albicans	*A. scarabaeoides*	*Flemingia grahamiana*	*Rhynchosia rothi*	Dhal[a]	Whole seed[b]		
					T-21	Sharda	Mukta	Pusa Ageti
Lysine	7.10	6.17	6.31	6.82	7.06	6.17	5.84	7.27
Histidine	3.27	3.44	3.61	3.62	4.21	3.66	3.39	3.99
Arginine	5.98	8.07	6.21	7.72	7.89	7.13	7.42	7.27
Aspartic acid	10.64	10.78	10.09	10.96	10.74	8.75	9.14	8.46
Threonine	3.46	4.29	3.66	4.19	4.24	3.06	3.14	3.36
Serine	4.83	5.73	5.06	5.31	6.30	4.63	4.00	4.98
Glutamic acid	25.08	23.84	22.75	18.93	24.71	18.28	17.66	19.54
Proline	4.25	4.76	4.26	5.10	3.90	4.13	4.62	3.95
Glycine	3.53	4.79	5.84	4.48	4.57	3.66	3.67	3.85
Alanine	3.24	5.27	5.72	4.21	5.02	4.60	5.05	4.83
Cystine	0.97	1.31	1.16	1.58	1.03	2.29	1.98	1.94
Valine	4.71	5.18	4.84	5.71	5.70	4.88	5.08	5.05
Methionine	1.16	1.17	1.86	0.75	1.82	1.67	1.58	1.82
Isoleucine	3.66	4.40	4.23	4.40	4.06	3.44	4.45	4.18
Leucine	8.31	9.60	8.76	8.39	8.70	6.48	7.24	7.21
Tyrosine	2.75	3.27	2.75	3.28	3.18	2.74	2.77	3.45
Phenylalanine	10.02	9.26	12.19	8.20	10.01	7.91	8.34	8.58
Tryptophan	–	–	–	–	–	0.54	0.75	0.64
Protein (%)	30.5	28.4	29.3	28.7	24.2	22.8	23.4	22.6

Source: a, Singh et al. (1981); b, Chatterjee and Abrol (1975). Reference No. 15.

Table 3. Distribution of protein fractions in different components of pigeonpea

Component	Amount (%)	Protein (%) (N X 6.25)	Non Protein Nitrogen (%)	Protein fractions (%)			
				Albumin	Globulin	Glutelin	Prolamin
Embryo	0.7	49.6	6.2	17.0	52.7	21.3	2.7
Cotyledon	85.3	22.2	9.5	11.4	64.5	18.2	3.5
				(4.7)	(1.7)	(2.3)	(0.7)[a]
Seed coat	14.3	4.9	27.4	2.6	26.3	32.8	4.2
Whole seed	–	20.5	12.8	10.2	59.9	17.4	3.0

[a]Values within parenthesis indicate methionine + cystine (g/6g N).

Source: Singh & Jambunathan (1982). Reference No. 18.

in case of pigeonpea. Large variation in the trypsin and chymotrypsin inhibitors has been reported among the cultivated and wild species of pigeonpea [19]. The high levels of protease inhibitors in some of the wild species are evident from the results shown in Table 4. It is pointed out that the use of such wild species as a source of high protein in breeding program to develop high protein cultivars should be made very carefully and all intergeneric lines obtained from crosses of cultivated species with wild species should be tested for the levels of protease inhibitors.

It is evident that trypsin inhibitory activity of pigeonpea is much lower than in common bean, lima bean and soy bean when compared under similar assay conditions (Table 5). Although the improved protein efficiency ratio of the heat treated samples of pigeonpea meal could suggest the role of some heat labile factors, the reasons for low protein digestibility of heated pigeonpea meal samples could not be explained [20]. The protease inhibitors in pigeonpea were destroyed to a certain extent by heat treatment but complete destruction of these factors was possible only when heated under acidic conditions [21]. Further, it can be assumed that in cooked foods of grain legumes these factors are not responsible for low protein digestibility [22]. In view of these observations, additional studies are required to find out the reasons for low protein digestibility of heated pigeonpea meal.

Polyphenolic compounds have been reported to influence the nutritive value of food crops by affecting the utilization of proteins. Recent studies indicated that pigeonpea condensed tannins ranged between 0 to 0.2% [23]. But pigeonpea seeds contain considerable amount of polyphenolic compounds which may or may not be tannins (Table 6) and most of these compounds are located in seed coat. Pigeonpea polyphenols inhibited the activities of trypsin, chymotrypsin and amylase enzymes to a large extent [24]. Also, the polyphenolic compounds of cultivars with dark testa colour showed more inhibitory activity than those with light testa colour in pigeonpea. Studies have demonstrated the effects of seed coat colour on the protein quality of beans and suggested the possible role of heat resistant tannins and other polyphenols as trypsin inhibitors [25]. Although the nutritional role of such compounds remain unclear in pigeonpea, experimental evidence suggest that the polyphenolic compounds of pigeonpea adversely affect the activities of digestive enzymes and that this effect will have nutritional implications in terms of nutrient utilization [24].

Storage and processing practices

Factors like storage and processing practices influence to a certain extent the actual content, availability and utilizability of essential nutrients which determine the nutritional potential of the diet. The grain legumes stored in conventional storage structures are generally attacked by the insects and molds which cause considerable destruction of the grain. Studies have shown

Table 4. Protein content, trypsin and chymotrypsin inhibitors and protein digestibilities in cultivars of pigeonpea and the wild relatives

Cultivars/species	Protein N X 6.25 (%)	Trypsin inhibition		Chymotrypsin inhibition		In vitro protein digestibility (%)
		(Units/mg meal)	(Units/mg protein)	(Units/mg meal)	(Units/mg protein)	
Cajanus cajan cultivars						
Pant A-2	24.4	12.5	69.7	5.0	27.8	57.9
UPAS-120	23.1	12.9	71.3	4.2	23.1	59.5
Baigani	26.2	15.1	67.1	3.5	15.3	64.1
Mean	24.6	13.5	69.4	4.2	22.1	60.5
Wild species						
Atylosia scarabaeoides	27.8	14.2	60.4	14.2	60.9	67.8
A. albicans	28.5	19.4	81.9	22.0	92.4	62.6
A. volubilis	27.1	25.8	121.4	11.5	60.9	52.6
A. cajanifolia	29.1	14.9	61.3	5.9	24.2	56.0
Rhynchosia rothii	27.6	82.4	445.7	20.9	113.2	40.9
Mean	28.0	31.3	154.1	14.9	70.3	56.0

Source: Singh and Jambunathan (1981b). Reference No. 19.

Table 5. Effect of heat on the digestibility and protein efficiency ratio of legumes having trypsin inhibitor activity

Legume	Trypsin inhibitor activity X10-4 units/g	Digestibility (%)		Protein efficiency ratio	
		Raw	Heated	Raw	Heated
Common bean	4.25	56.0	79.5	a	0.8
Hyacinth beans	4.38	56.5	81.6	a	1.3
Soybean	4.15	70.1	85.4	1.3	2.4
Lima bean	4.04	34.0	51.3	b	0.7
Pigeonpea	2.77	59.1	59.9	0.7	1.6
Cowpea	1.91	79.0	82.6	1.6	2.2
Lentil	1.78	88.3	92.6	0.4	1.2

[a]Loss in weight occurs on these diets.
Source: Liener (1979). Reference No. 20.

that insect pests significantly decreases the protein efficiency ratio (PER) of pigeonpea [26–27] and this was only attributed to low levels of lysine and threonine in infested materials [27]. Methods of storage also influence the protein quality of stored grains. The effect of halogen compounds on the nutritive value of proteins when such compounds are used in sufficient concentration to kill pests have been reviewed and the use of such compounds has been criticised with particular reference to the losses they cause to the sulphur amino acids [28]. Since low levels of these amino acids are a serious problem in pigeonpea, the use of such compounds as fumigant will have adverse effect on the protein quality of pigeonpea. This situation should be viewed and attempts, therefore, should be made to study the effects of such compounds on protein quality in pigeonpea.

Considerable nutritional losses occur during primary processing which includes removal of seed coat from raw pigeonpea to obtain dhal. During this process of milling the proteins are lost to a certain extent [29]. Such losses occur due to the presence of protein rich outer layers on the cotyledons of pigeonpea [8] and these layers are lost as a result of abrasive action of roller mill during processing of raw pigeonpea into dhal. Amino acid analysis conducted on different products that were obtained during processing indicated that measurable amount of lysine, sulphur amino acids and tryptophan are lost during this process (Singh, 1984 unpublished data). This shows that protein quality of pigeonpea undergoes both qualitative and quantitative changes as a result of dehulling process. In case of secondary processing which involves cooking, the destruction of some of the antinutritional factors has been the important advantage derived from heat treatment as discussed earlier. Protein denaturation by heat has also been reported to improve the digestibility by the proteases. But excessive heating, reduced the nutritive value of protein possibly by promoting such reactions as amide cross linking of amino acid side-chains, particularly of lysine amino groups with carboxyl groups of aspartic acid and glutamic acids [30]. Although it depends on the

Table 6. Cultivaral differences in the enzyme inhibitory property of polyphenols of pigeonpea

Cultivar	Testa colour	Polyphenols (mg/g sample)	Enzyme inhibition (%)[a]			
			Trypsin	Chymotrypsin	Amylase	
					Human saliva	Hog pancreas
HY-3C	White	3.7	37.9	36.0	34.5	21.8
NP(WR)-15	White	6.0	40.5	38.6	32.7	19.7
C-11	Light brown	14.2	91.5	90.3	86.0	80.9
BDN-1	Brown	15.2	90.3	91.6	79.4	69.3
No-148	Brown	14.9	88.0	85.9	75.8	68.5
Mean	–	10.8	69.7	68.5	61.7	52.0
SE ±	–	0.2	2.1	1.7	1.4	1.3

[a]Based on assay carried out using 200 ug polyphenols for trypsin and chymotrypsin and 250 ug polyphenols for amylase inhibitions.
Source: Singh (1984). Reference No. 24.

method of cooking, protein quality of pigeonpea was improved more by moist heat than by dry heat treatment as available lysine was less in roasted than in boiled and pressure cooked pigeonpea [31]. Significant losses of methionine on heating soybean protein during boiling have been reported by earlier workers [32]. No meaningful data are available on the effect of cooking on the protein quality of pigeonpea. It is, therefore, desirable to find out the duration and optimum conditions of heating to derive maximum nutritional advantages of cooking of pigeonpea.

References

1. PAG (Protein Advisory Group) Statement 22 (1973). Upgrading human nutrition through the improvement of food legumes. In: Milner M (ed). Nutritional improvement of food legumes by breeding, United Nations, New York, pp 349–380
2. Bressani R (1972) Legumes in human diets and how they might be improved. In: Milner M (ed). Nutritional improvement of food legumes by breeding, United Nations, New York, pp 15–42.
3. Bliss FA, Hall TC (1977) Food legumes – compositional and nutritional changes induced by breeding. Cereal Foods World 22:106–112
4. Eggum BO, Beames RM (1983) The nutritive value of seed proteins. In: Gottschalk W, Muller HP (eds). Seed proteins – Biochemistry, genetics nutritive value. The Hague, Dr W Junk, pp 499–531
5. Bozzini A, Silano V (1978) Control through breeding methods of factors affecting nutritional quality of cereals and grain legumes. In: Friedman M (ed). Nutritional improvement of food and feed proteins. Plenum Press, New York and London, pp 249–274
6. Singh U, Jambunathan R (1981a) Methods of protein estimation in pigeonpea and relationship between whole seed and dhal protein. J Sci Food Agric 32:705–710
7. Dahiya BS, Brar JS, Bhullar BS (1977) Inheritance of protein content and its correlation with grain yield in pigeonpea (*Cajanus cajan* [L.] Millsp.). Qual Plant Plant Foods Hum Nutr 27:327–334
8. Reddy LJ, Green JM, Singh U, Bisen SS, Jambunathan R (1979) Seed protein studies on *Cajanus cajan Atylosia* spp. and some hybrid derivatives. In: Proc Sym Seed protein improvement in cereals and grain legumes. IAEA/FAO, Munich, West Germany pp 105–117

9. Tripathi RD, Srinivastava GP, Misra MC, Sinha ST (1975) Comparative studies on the quality characteristics of early and late cultivars of redgram (*Cajanus cajan* L.). Ind J Agric Chem 8:57–61
10. Singh L, Sharma D, Deodhar AD (1974) Effect of environments on the protein of seeds and implication in pulse improvement. Ind J Gen and Pl Breeding 34:764–770
11. Eggum BO (1973) Biological availability of amino acid constituents in grain protein. In Nuclear techniques for seed protein improvement, IAEA, Vienna, p 422
12. FAO/WHO (Food and Agriculture Organization/World Health Organization) (1973). Energy and protein requirements. Report of a Joint FAO/WHO Ad Hoc Expert Committee. WHO technical report series 522, Geneva, Switzerland, pp 63
13. Burr HK (1975) Pulse protein. In: Friedman M (ed). Protein nutritional quality of foods and feeds. Dekker Inc, New York, p 119
14. Eggum BO (1977) Nutritive value of food crops and factors affecting the utilization of dietary protein and energy. Second FAO/SIDA Seminar on Field Food Crops in Africa and the Near East, Lahore, Pakistan
15. Chatterjee SR, Abrol YP (1975) Amino acid composition of new varieties of cereals and pulses and nutritional potential of cereal-pulse combinations. J Food Sci & Technol 12:221–227
16. Jambunathan R, Singh U (1981) Relationship between total sulphur and sulphur amino acids in chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). Qual Plant Plant Foods Hum Nutr 31:109–117
17. Singh U, Jambunathan R, Santosh Gurtu (1981) Seed protein fractions and amino acid composition of some wild species of pigeonpea. J Food Sci Technol 18:83–85
18. Singh U, Jambunathan R (1982) Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). Qual Plant Plant Foods Hum Nutr 31:347–354
19. Singh U, Jambunathan R (1981b) Protease inhibitors and *in vitro* protein digestibility of pigeonpea (*Cajanus cajan* L.) and its wild relatives. J Food Sci Technol 18:246–247
20. Liener IE (1979) Protease inhibitors and lectins. In: Neuberger A, Jukes TH (eds). Biochemistry of nutrition. University Park Press, Baltimore, Vol 27, pp 97–122
21. Sumathi S, Pattabiraman TN (1976) Natural plant enzyme inhibitors: Part II – Protease inhibitors of seeds. Ind J Biochem & Biophys 13:52–56
22. Bressani R, Elias LG (1977) The problem of legume protein digestibility. In: Hulse JH, Rachie KO, Billingsley LW (eds). Nutritional standards and methods of evaluation for food legume breeders: IDRC-TS7e, pp 61–72
23. Price ML, Hagerman AE, Butler LG (1980) Tannin content of cowpeas, chickpeas, pigeonpea and mung beans. J Agri Food Chem 28:459–461
24. Singh U (1984) The inhibition of digestive enzymes by polyphenols of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). Nut Rep Int 29:745–753
25. Elias LG, de Fernandez DG, Bressani R (1979) Possible effects of seed coat polyphenols on the nutritional quality of bean protein. J Food Sci 44:524–527
26. Parpia HAB (1972) Utilization problems in food legumes In: Milner M (ed). Nutritional improvement of food legumes by breeding. United Nations, New York, pp 281–285
27. Daniel VA, Rajan P, Sanjeevarayappa KV, Srinivasan KS, Swaminathan M (1977) Effect of insect infestation on the chemical composition and protein efficiency ratio of the proteins of Bengal gram and red gram. Ind J Nutr & Diet 14:70–73
28. Synge RLM (1976) Damage to nutritional value of plant proteins by chemical reactions during storage and processing. Qual Plant Plant Foods Hum Nutr 28:9–26
29. Singh U, Jambunathan R (1980) A survey of the methods of milling and consumer acceptance of pigeonpeas in India. In Proc International Workshop on Pigeonpeas. Vol 2, p 149, 15–19 Dec, 1980. ICRISAT Center, Patancheru, A.P. 502 324, India
30. Carpenter KJ, Booth VH (1973) Damge to lysine in food processing: Its measurement and its significance. Nutr Abs Rev 43:423–430
31. Geervani P, Theophilus F (1980) Effect of home processing on nutrient composition of certain high yielding legume varieties. Ind J Nutr & Diet 17:443–446
32. Shemer M, Perkins EG (1975) Degradation of methionine in heated soybean protein and the formation of B-methyl mercaptopropionaldehyde. J Agr Food Chem 23:201–205