

Food Chemistry 73 (2001) 209-215



www.elsevier.com/locate/foodchem

Nutritional and anti-nutritional attributes of the under-utilized legume, *Cassia floribunda* Cav.

V. Vadivel*, K. Janardhanan

Seed Technology Laboratory, Department of Botany, Bharathiar University, Coimbatore — 641 046, (T.N), India

Received 27 August 1999; received in revised form 8 October 2000; accepted 8 October 2000

Abstract

Three germplasm seed samples of the under-utilized legume, *Cassia floribunda* Cav., collected from three locations of South India were analyzed for proximate composition, mineral profiles, protein fractions, amino acid profiles of total seed protein, in vitro protein digestibility and certain anti-nutritional factors. The accessions contain (%) 19.9–21.7 crude protein, 2.1–3.1 crude lipid, 11.7–13.8 total dietary fiber, 3.4–5.6 ash and 58.0–60.5 carbohydrates (by difference). Globulins and albumins constituted the predominant fractions of the seed protein. The contents of essential amino acids, valine, phenylalanine, tyrosine, isoleucine, histidine and lysine, were high in all three germplasm seed samples compared to FAO/WHO (1991), [FAO/WHO. (1991). *Protein quality evaluation*. Rome, Italy: Food and Agricultural Organization of the United Nations requirement pattern. In vitro protein digestibility ranged from 81.4-85.9%. Anti-nutritional factors such as total free phenolics, tannins, L-DOPA (3,4-dihydroxyphenylalanine), trypsin inhibitor activity, chymotrypsin inhibitor activity and haemagglutinating activity also were analyzed and resulting levels found were not considered a threat to human health if the seeds were properly processed. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Cassia floribunda; Under-utilized legume; Amino acid profiles; In vitro protein digestibility; Protein fractions; Anti-nutritional factors

1. Introduction

The wide prevalence of protein-calorie-malnutrition in developing countries including India is of great concern not only to agricultural scientists but also to the concerned governments (Olsen, 1975). The continuous increase in population growth and inadequate supply of protein lead to malnutrition among people in developing countries (Siddhuraju, Vijayakumari & Janardhanan, 1995). To meet the protein demands in developing countries, where animal protein is grossly inadequate, intensive efforts to find alternative sources of protein from under-utilized leguminous plants adapted to adverse conditions have been generated. Besides, underutilized legume seeds served as low-cost protein sources to alleviate the protein-energy-malnutrition among people living in developing countries (Amubode & Fetuga, 1983; Siddhuraju et al.).

E-mail address: botkj@bharathi.ernet.in (V. Vadivel).

The Cassia floribunda Cav., presently evaluated under-utilized legume, is predominantly available as perennial shrub up to 1300 m above sea level, in Indian sub-continent (Katoch & Bhowmik, 1983). Its tender shoots and pods are cooked as vegetable and seeds are pickled by the northwest Himalayan tribals, Gujjars and Gaddies (Singh, 1996). Its mature seeds are also consumed as a pulse by the tribal people of Kolli hills, Salem District, Tamil Nadu, South India (Janardhanan, 1993). The earlier investigations on this wild legume give an account of proximate composition, mineral profiles, protein fractions and anti-nutritional factors like total free phenolics, tannin and lectins (Katoch & Bhowmik; Janardhanan). Because of its attractive chemical composition (Katoch & Bhowmik; Janardhanan), C. floribunda merits to be studied further as an alternative low-cost source of protein to alleviate the proteincalorie-malnutrition among the economically weaker sections of population in developing countries including India. The present work is a confirmatory and more detailed study of data reported earlier on C. floribunda. Nonetheless, the earlier investigators do not seem to have made any attempt to decipher the amino acid profiles of the

^{*} Corresponding author. Present address: Indian Cardamom Research Institute, Spices Board, Myladumpara, Kailasanadu — 685 553, (Kerala), India. Fax: +91-422-422-387.

total seed protein, to evaluate in vitro protein digestibility and to examine anti-nutritional factors like L-DOPA, trypsin inhibitor activity and chymotrypsin inhibitor activity of this legume.

2. Materials and methods

2.1. Sampling

Three germplasm seed samples of mature pods of *C. floribunda* were collected from natural stands of three locations of South India viz., Erattupattai, Kottayam district and Kumlza, Pathanamthitta district of Kerala state in March, 1994 and Kargal, Uttarakannada district of Karnataka state in March, 1995. After drying in the sun thoroughly, the pods were thrashed to remove seeds and then dried in open sunlight for 3 days. The seeds, after thorough cleaning and removal of broken seeds and foreign materials, were stored in airtight plastic containers at room temperature (25±2°C) for analysis.

2.2. Proximate composition

Moisture content of the seeds was estimated by taking 100 transversely cut seeds at a time and the weight was taken before and after incubation in a hot-air oven [Toshniwal Brothers (SR) Private LTD, Chennai, India] at 80°C for 24 h, followed by cooling in a desiccator. Oven-dried and air-dried seeds were powdered separately in a Wiley mill (Scientific Equipment, Delhi) to 60-mesh size and stored in screw-capped bottles at room temperature; three samples for each germplasm were analyzed and the results expressed on dry weight basis. Nitrogen content was estimated by the micro-kjeldahl method (Humphries, 1956) and crude protein was calculated (N \times 6.25). The contents of crude lipid, and ash were estimated by AOAC (1975) methods. Total dietary fiber (TDF) was estimated by the non-enzymatic-gravimetric method proposed by Li and Cardozo (1994). To determine TDF, duplicate 500 mg of ground samples were taken into separate 250 ml beakers. To each beaker, 25 ml of distilled water was added and gently stirred until samples were thoroughly wetted, i.e. no clumps were noticed. The beakers were covered with Al foil and allowed to stand for 90 min without stirring in an incubator maintained at 37°C. After that 100 ml 95% ethanol was added to each beaker and allowed to stand for 1 h at room temperature (25±2°C). The residue was collected under vacuum in preweighed crucible containing filter aid. The residue was washed successively with 20 ml 78% ethanol, 10 ml 95% ethanol and 10 ml acetone. Crucible containing the residue was dried ≥ 2 h at 105°C then cooled \geq 2 h in a desiccator and weighed. One crucible-containing residue was used for ash determination at 525°C for 5 h in a muffle furnace [Tempo

Instrument & Equipments (I) Private Ltd, Bombay, India]. The ash-containing crucible was cooled for ≥ 2 h in a desiccator and weighed. The residue from the remaining duplicate crucibles was used for crude protein determination by micro-kjeldahl method as mentioned above. The TDF was calculated as follows:

$$TDF\% = 100 \times \frac{W_r - \frac{P+A}{100} \times W_r}{W_s}$$

where W_r , mg residue; P, % protein in residue; A, % ash in residue; and W_s , mg sample.

Nitrogen-free-extract (NFE) was obtained by difference (Muller & Tobin, 1980). Energy value of the seed was estimated (in kJ) by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, respectively (Siddhuraju et al., 1995).

2.3. Mineral analysis

Five hundred mg of the ground legume seeds were digested with a mixture of 10 ml concentrated nitric acid, 4 ml of 60% perchloric acid and 1 ml of concentrated sulphuric acid. After cooling, the digest was diluted with 50 ml deionized distilled water, filtered with Whatman No. 42 filter paper and the filtrates made up to 100 ml in a glass volumetric flask with deionized distilled water. All the minerals, except phosphorus, were analyzed from triple acid digested samples by atomic absorption spectrophotometry (Perkin-Elmer, Model 5000) equipped with different lamps (Issac & Johnson, 1975). For calibration curves, measurements were plotted by aspirating into the flame samples of solutions containing known concentrations of the element had been determined, measuring the absorption (emission) of solution, and then constructed graphs in which the measured absorption (emission) were plotted against the concentrations of the solution. Zero reading was adjusted by spraying deionized distilled water. For the preparations of the standard solutions, stock solutions were prepared from the pure metal or from the pure metal oxide (Sigma standards, Sigma Chemical Co., Poole, UK) by dissolution in a suitable acid solution; the solids used were of the highest purity. Phosphorus content in the triple acid digested extract was determined colorimetrically (Dickman & Bray, 1940).

2.4. Isolation, fractionation and estimation of total seed proteins and soluble proteins

Total protein was extracted by a modified method of Basha, Cherry and Young (1976). The ethanol treatment was omitted to save the prolamin fraction. The extracted proteins were purified by precipitation with cold 20% (w/v) trichloroacetic acid (TCA) and estimated by the method of Lowry, Rosenbrough, Farr

and Randall (1951). The albumin and globulin fractions of the seed protein were extracted and separated according to the method of Murray (1979). The prolamin fraction was extracted from the residual pellet by treating the pellet with 80% ethanol (1:10 w/v) overnight. After centri-fugation (MB 20 model, 1985, Bombay, India) (20,000 g for 20 min at room temperature) the supernatant containing prolamins was air-dried and dissolved in 0.1 M NaOH. The resulting pellet was extracted with 0.4 M NaOH (1:10 w/v) overnight and centrifuged as above. The supernatant was designated as glutelins. All four fractions so obtained were precipitated and washed with cold 10% (w/v) TCA. All samples were redissolved in 0.2 M NaOH and protein content was determined by Lowry et al. method.

2.5. Amino acid analysis

Total seed proteins extracted by the modified method of Basha et al. (1976) were purified by cold 20% (w/v) TCA precipitation. A 30 mg protein sample was hydrolyzed by 6 N HCl (5 ml) in an evacuated sealed glass tube, which was kept in an air oven [Toshnimal Brothers (SR) Private Ltd, Chennai, India] maintained at 110°C for 24 h. The sealed tube was broken, and the acid removed completely by repeated flash evaporation after the addition of de-ionized water. Dilution was effected by means of a citrate buffer pH 2.2, to such an extent that the solution containing 0.5 mg protein/ml. The solution was passed through a millipore filter (0.45 μm; Waters Millipore, Mississauga, ON, Canada). The amino acid analysis was performed using an automated precolum derivation with O-phthaldialdehyde (OPA) using reverse phase high-performance liquid chromatography (Model 23250, ISCO, USA) with spherisorp C_{18} coloum (4.6×250 mm; ISCO, USA) and ISCO-dual pump, by the method of Rajendra (1987). The flow rate was 1.5 ml/min with florescence detection (excitation 305–395 nm and emission 430–470 nm). The contents of the different amino acids recovered are expressed as g 100 g⁻¹ protein. The essential amino acid score was calculated as follows:

Essential amino acid score =

grams of essential amino

acid in 100 g of test protein

grams of essential amino acid in 100 g of

FAO/WHO (1991) reference pattern

2.6. Determination of in vitro protein digestibility (IVPD)

This was determined using the multi-enzyme technique (Hsu, Vavak, Satterlee & Miller, 1977). Calculated control (casein) and sample weight were weighed out,

hydrated in 10 ml of distilled water and refrigerated (5°C) for 1 h. The pH of protein containing samples and enzymes were all adjusted to 8.0 at 37°C. The IVPD was determined by the sequential digestion of the protein containing sample with a multi-enzyme mixture (trypsin, α -chymotrypsin and peptidase) at 37°C followed by protease at 55°C. The pH drop of the samples from pH 8.0 was recorded after 20 min of incubation. The IVPD was calculated according to the regression equation Y = 234.84 - 22.56 (X), where Y = % digestibility, X = pH drop.

2.7. Analysis of anti-nutritional compounds

The anti-nutritional compounds, total free phenolics (Bray & Thorne, 1954), tannins (Burns, 1971) and the non-protein amino acid, L-DOPA (3,4-dihydroxyphenylalanine; Brain, 1976) were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade, Rackis, McGhee and Puski (1974). One trypsin inhibitor unit (TIU) was expressed as an increase of 0.01 absorbence unit per 10 ml of reaction mixture at 410 nm. Trypsin inhibitor activity was defined in terms of trypsin units inhibited per mg protein. Chymotrypsin inhibitor activity was assayed in a 0.1 M phosphate buffer, pH 7.6, extract of acetone-treated meal by the procedure of Kakade, Swenson and Liener (1970). One chymotrypsin unit was expressed as an increase of 0.01 absorbence unit at 275 nm in 10 min per 10 ml of the reaction mixture. Chymotypsin inhibitor unit (CIU) was defined in terms of chymotrypsin units inhibited per mg protein. Haemagglutinating activity of albumin and globulin fractions of seed protein was also assayed (Liener, 1976).

3. Results and discussion

The results of proximate analysis are shown in Table 1. Crude protein range (19.9–21.7%) of *C. flor-*

Table 1 Proximate composition of the seeds of three different germplasm seed samples of *Cassia floribunda* (g 100 g⁻¹ seed flour)^a

Component	Location					
	Erattupattai	Kumlza	Kargal			
Moisture	4.4±0.2	6.0±1.7	5.7±0.1			
Crude protein						
(Kjeldahl N×6.25)	19.9 ± 0.8	19.9 ± 1.0	21.7 ± 0.6			
Crude lipid	2.7 ± 0.3	2.1 ± 0.9	3.1 ± 0.3			
Total dietary fiber	11.7 ± 0.4	12.3 ± 1.2	13.8±1.4			
Ash	5.6 ± 0.1	5.2 ± 0.7	3.4 ± 0.1			
Nitrogen-free-extract	60.1	60.5	58.0			
Calorific value (kJ 100 g ⁻¹ DM)	1438	1422	1444			

^a Mean±standard error of three replicates, expressed on dry weight

ibunda accessions is comparable to that of Cassia laevigata (Siddhuraju et al., 1995); C. obtusifolia (Mohan & Janardhanan, 1995) and C. sieberiana (Amubode & Fetuga, 1983). Janardhanan (1993) and Katoch and Bhowmik (1983) reported 10.4 and 24.7% of protein, respectively, in C. floribunda. The notable difference in the protein content within a species is probably due to different growing conditions from where the seeds are collected. Crude lipid ranged from 2.1 to 3.1%; this range is higher than that of C. nodosa (Amubode & Fetuga). Nonetheless, crude fat range does not qualify these seeds as an oil-rich legume, especially when compared with groundnuts and soybeans (Narasinga Rao, Deosthale & Pant, 1989). Total dietary fiber ranged from 11.7-13.8%. The range in ash content of this legume (3.4–5.3%) would be important to the extent that it contains nutritionally important mineral elements, which are shown in Table 2. It appears that C. floribunda seed samples have a high range of carbohydrates because of its low fat content. Groundnuts and soybeans have lower carbohydrate values of 26.1% and 20.9%, respectively (Narasinga Rao et al.).

The data on mineral profiles are given in Table 2. Potassium, as in most legumes, is the predominant mineral. In general, contents of calcium and phosphorus are found to be higher than that of recommended dietary allowances of calcium (400 mg) and phosphorus (400 mg) for children by the Indian Council of Medical Research (ICMR); whereas the levels of microminerals (Fe, Cu, Zn and Mn) are lower than the Indian RDA figures (ICMR, 1992). The ICMR recommended 19 mg iron, 15 mg zinc, 2.2 mg copper and 5.5 mg manganese for Indian children. The variability in the content of minerals for the same species may be related to genetic origin, geographical source and the levels of soil fertility.

Total proteins and protein fractions of the three accessions are presented in Table 3. The three accessions presently investigated are found to contain more true proteins than that of *C. floribunda* (Janardhanan, 1993)

Table 2 Mineral profiles of three different germplasm seed samples of $\it Cassa floribunda (mg 100^{-1} seed flour)^a$

Name of the mineral	Location					
	Erattupattai	Kumlza	Kargal			
Sodium	42±4.7	51±3.7	136±6.4			
Potassium	913±7.3	877 ± 4.4	573±8.4			
Calcium	549 ± 8.3	426 ± 3.8	538±3.5			
Magnesium	318 ± 8.4	306 ± 2.9	289±95.4			
Phosphorus	503 ± 7.4	638 ± 6.4	603 ± 6.7			
Iron	4.4 ± 0.4	5.7 ± 0.8	3.6 ± 1.0			
Copper	0.1 ± 0.7	0.2 ± 0.4	0.3 ± 0.7			
Zinc	1.7 ± 0.3	1.8 ± 0.7	2.0 ± 0.5			
Manganese	1.0 ± 0.5	0.7 ± 0.6	1.0 ± 0.5			

^a Mean±standard error of three replicates, expressed on dry weight basis.

and *C. laevigata* (Siddhuraju et al., 1995). Seed protein fractionation showed that the globulins and albumins together constitute the major bulk of seed proteins as in many other legumes (Boulter & Derbyshire, 1976; Murray, 1979).

Nutritive value of dietary protein is determined by the pattern and quantity of essential amino acids present. The presence of one or more of the essential amino acids in adequate amounts would increase the nutritive value of the protein. Hence, the seed protein as a source of amino acids can usually be assessed by comparison with the FAO/WHO (1991; Table 4) suggested pattern of essential amino acids. The data on the amino acid pattern of total seed protein show that threonine content of all the three germplasm seed samples and leucine content of Erattupattai and Kargal germplasm seed samples seem to be deficient; whereas, valine, phenylalanine, tyrosine, isoleucine, histidine and lysine contents of all the three germplasm seed samples and leucine content of Kumlza germplasm are found to be higher than FAO/WHO requirement pattern. In general, the amino acids profiles are incomplete; it is because some amino acids are destroyed during the preparation of the samples by acid digestion.

Studies on IVPD for *Cassia* species/germplasm seed samples are meager (Siddhuraju et al., 1995). IVPD of *C. floribunda* ranged from 81.4 to 85.9%. This range seems to be higher compared to cultivated legumes such as *Cajanus cajan* (Rajyalakshmi & Geervani, 1990) and *Cicer arietinum* (Attia, El-Tabey Shehata, Aman & Hamza, 1994). These two legumes are used extensively in typical Indian diets and are expected to play a significant role in improving protein nutrition in India.

Protein quality is affected by factors that interact with the intestinal tract such as protease inhibitors, phytate, lectins, tannins and saponins that reduce protein digestibility and amino acid absorption. These substance unless destroyed by heat or some other suitable treatment can exert adverse physiological effects when ingested by man and animals (Liener, 1994). On the

Table 3 Seed total proteins and protein fractions (g 100 g^{-1}) of three different germplasm seed samples of *Cassia floribunda*^a

Name of the protein fraction	Location					
protein fraction	Erattupattai		Kumlza		Kargal	
	Seed flour	Seed protein	Seed flour	Seed protein	Seed flour	Seed protein
Total protein	16.3±0.5	100	15.5±0.3	100	17.7±0.6	100
Albumins	4.1 ± 0.2	25.2	3.2 ± 0.1	20.5	4.7 ± 0.2	26.6
Globulins	8.1 ± 0.5	49.7	9.6 ± 0.4	61.5	9.9 ± 0.7	55.9
Prolamins	1.1 ± 0.1	6.7	1.0 ± 0.1	6.5	1.1 ± 0.4	6.2
Glutelins	3.0 ± 0.2	18.4	1.8 ± 0.1	11.5	2.0 ± 0.1	11.3

 $^{^{\}rm a}$ Mean $\pm {\rm standard}$ error of three replicates, expressed on dry weight basis.

Table 4 Amino acid profiles of acid hydrolyzed purified total seed proteins of three different germplasm seed samples of *Cassia floribunda* (g 100 g^{-1} protein)

Amino acid	Erattupattai	Essential amino acid score	Kumlza	Essential amino acid score	Kargal	Essential amino acid score	FAO/WHO (1991) requirement pattern
Aspartic acid	12.38		13.39		10.83		
Glutamic acid	16.78		17.98		18.82		
Alanine	3.64		3.01		3.27		
Valine	4.41	126.00	4.25	121.43	4.38	125.14	3.5
Glycine	4.85		5.57		5.74		
Arginine	6.34		6.67		6.85		
Serine	3.95		3.34		4.04		
Cystine	N.D		N.D		N.D		2.5
Methionine	0.84		0.93		0.83		2.5
Threonine	2.38	70.00	2.29	67.35	2.87	84.41	3.4
Phenylalanine	5.34	155.56	4.92	110.00	5.82		6.3
Tyrosine	1.94	155.56	2.01	110.00	1.87	122.06	
Isoleucine	3.24	115.71	2.89	103.21	3.83	136.78	2.8
Leucine	6.43	97.42	6.67	101.06	6.23	94.39	6.6
Histidine	5.12	269.47	4.13	217.37	5.27	277.36	1.9
Lysine	6.67	115.00	6.24	107.59	6.47	111.55	5.8
Tryptophan	$N.D^a$		N.D		N.D		1.1
Proline	N.D		N.D		N.D		

a N.D, not detected.

contrary, it has been suggested that consumption of low levels of certain anti-nutrients may produce health benefits while avoiding some of the adverse effects associated with their large intake (Thompson, 1988). In view of this, in the present investigation an attempt has been made to detect the presence of certain anti-nutritional factors such as total free phenolics, tannins, L-DOPA, trypsin inhibitor activity, chymotrypsin inhibitor activity and phytohaemagglutinating activity (Table 5). Trypsin inhibitors and chymotrypsin inhibitors are the predominant protease inhibitors found throughout the plant kingdom particularly among legumes (Ryan, 1981). Protease inhibitors have been reported to be the cause of growth depression (Liener & Kakade, 1980). Protease inhibitors not only cause growth depression, but also hypertrophy and hyperplasia of pancreas in rats and chicks, when fed directly or as raw meal (Ge & Morgan, 1993; Liener & Kakade, 1980). Trypsin inhibitors are low molecular weight proteins capable of binding to and inactivating the digestive enzyme, trypsin (Bacon, Lambert, Matthews, Arhur & Duchene, 1995). The presence of this inhibitor in animal diets (including human diets) can lead to pancreatic enlargement, reduced digestibility, and reduced absorption of amino acids and minerals (Liener & Kakade; Yavelov, Finlay, Kennedy & Troll, 1983; Gatel & Grosjean, 1990). Substances possessing the property to agglutinate red blood cells are known as phytohaemagglutinins (lectins). They are a class of proteins or glycoproteins characterized by their ability to bind particular sugar residues that belong to polysaccharide moieties of glycoproteins, glycolipids, polysaccharides or simple glycosides (Murray, 1984). The lectins are capable of agglutinating animal and/or

human erythrocytes and stimulating mitogeny in resting lymphocytes (Hankins & Shannon, 1978). There are over 600 species of plants belonging to Leguminosae which exhibit haemagglutinating activity. The haemagglutinins seem to exert their toxic effect on recipient man/animal by interfering with digestion and absorption of food (Jaffe, 1980) and by causing a disruption of the brush border of duodenal and jeynal erythrocytes (Pusztai, Clarke, Grant, King & Stewart, 1981). Higuchi, Suga and Iwai (1983) demonstrated that lectins bind to the intestinal mucosa impairing digestion and absorption of nutrients. The lectins reduce the protein digestibility by inhibiting the digestive enzymes (Thompson, Tenebaum & Hui, 1986). Total free phenolics and tannins ranged from 0.3 to 0.4% and from 0.3 to 0.6%, respectively. These ranges are comparable to that of an earlier report in the same legume (Janardhanan, 1993). For consumption, people would de-hull the seed before producing flour; this of course would remove most of the tannin. L-DOPA ranged from 1.1 to 1.9% (Table 5). In Mucuna pruriens, an under-utilized legume, rich in L-DOPA, significant reduction in its content by dry heat treatment has been demonstrated (Siddhuraju, Vijayakumari & Janardhanan, 1996). Soaking, cooking and autoclaving also resulted in reducing L-DOPA (Vijayakumari, Siddhuraju & Janardhanan, 1996).

Table 5 also presents the protease inhibitor activities derived from enzymatic measurements. Although *C. floribunda* accessions exhibit high values for trypsin inhibitor activity (16.4–17.4 TIU mg⁻¹ protein) and chymotrypsin inhibitor activity (8.7–12.1 CIU mg⁻¹ protein), these values seem to be low compared to *Cajanus cajan* var. Pant A-2 and UPAS-120 (Singh &

Table 5
Data on anti-nutritional factors of the seeds of three different germplasm seed samples of *Cassia floribunda*

Component		Location			
		Erattupattai	Kumlza	Kargal	
Total free phenolics (%) ^a		0.3 0.1	0.3 0.2	0.4 0.5	
Tannins (%) ^a		0.3 0.2	0.6 0.3	0.4 0.1	
L-DOPA (%) ^a		1.9 0.6	1.1 0.9	1.6 0.2	
Trypsin inhibitor activity (TIU mg	g ⁻¹ protein) ^b	16.4	17.4	16.8	
Chymotrypsin inhibitor activity (C	CIU mg ⁻¹ protein) ^b	11.4	8.7	12.1	
Phytohaemagglutinating activity ^b					
Name of the protein fraction	Erythrocytes from the human blood group	Heam	Heamagglutinating activity ^c		
Albumins	A	+	+	+	
Albumins	В	+	+	+	
Albumins	O	+	+	+	
Globulins	A	++	+ +	+ +	
Globulins	В	++	+ +	+ +	
Globulins	O	++	+ +	+ +	

- ^a Values are means of triplicate determinations expressed on dry weight basis. ±Standard Error.
- ^b Values of two independent experiments. TIU, trypsin inhibitor unit; CIU, chymotrypsin inhibitor unit.
- c +, Clumping, pellet partially disperses; + +, clumping, no dispersion of pellet.

Eggum, 1984). Both the inhibitors are thermolabile and their inhibitory activity can be reduced considerably by thermal treatments (Liener, 1994). The seed protein fractions, albumins and globulins in *C. floribunda* exhibited haemagglutinating activity without any specificity against the human "ABO" system. In general, globulins exhibit strong agglutinating activity compared to albumins. The lectin activity can be easily eliminated by dry or wet thermic treatments (Liener).

In conclusion, this study reveals that the chemical composition of all three accessions of C. floribunda seems to be similar to or higher than that of Cassia species/accessions reported earlier. Besides, they register higher levels of certain amino acids and minerals compared to recommended levels. In vitro protein digestibility of this legume is also found higher than that of certain common legumes. The presence of anti-nutritional factors identified in this current report should not pose a problem to human health if the seeds are properly processed. In view of the overall nutrient and chemical composition, seeds of C. floribunda may be an economic and alternative protein source to alleviate protein malnutrition among the socially-economic lower classes of the population in developing countries, including India.

Acknowledgements

We are grateful to the Government of India, Ministry of Environment & Forests, New Delhi, India for financial assistance (Grant No. 14/52/89-MAB/RE dated 26.03.1992).

References

Amubode, F. O., & Fetuga, B. L. (1983). Proximate composition and chemical assay of the methionine, lysine and tryptophan concentrations of some forest tree seeds. *Food Chemistry*, 12, 67–72.

AOAC. (1975). Official methods of analysis (11th edn.). Washington DC, USA: Association of Official Analytical Chemists.

Attia, R. S., El-Tabey Shehata, A. M., Aman, M. E., & Hamza, M. A. (1994). Effect of cooking and decortication on the physical properties, the chemical composition and the nutritive value of chickpea (*Cicer arietinum L.*). Food Chemistry, 50, 125–131.

Bacon, J. P., Lambert, N., Matthews, P., Arthur, A. E., & Duchene, C. (1995). Genotype, environment and genotype x environment effects on trypsin inhibitor in peas. *Journal of the Science of Food and Agriculture*, 67, 101–108.

Basha, S. M. M., Cherry, J. P., & Young, C. T. (1976). Changes in free amino acids, carbohydrates and proteins of maturing seeds from various peas (*Arachis hypogaea*) cultivars. *Cereal Chemistry*, 53, 583–597.

Boulter, D., & Derbyshire, D. (1976). The general properties, classification and distribution of plant proteins. In G. Norton, *Plant proteins* (pp. 3–24). London: Butterworths.

Brain, K. R. (1976). Accumulation of L-DOPA in cultures from *Mucuna pruriens. Plant Science Letters*, 7, 157–161.

Bray, H. C., & Thorne, W. V. (1954). Analysis of phenolic compounds. Methods of Biochemical Analysis, 1, 27–52.

Burns, R. R. (1971). Methods for estimation of tannins in grain, *Sorghum. Agronomy J.*, 63, 511–512.

Dickman, S. R., & Bray, R. H. (1940). Colorimetric determination of phosphate. *Ind. Eng. Chem. And Ed.*, 12, 665–668.

FAO/WHO. (1991). *Protein quality evaluation*. Rome, Italy: Food and Agricultural Organization of the United Nations.

Gatel, F., & Grosjean, F. (1990). Composition and nutritive value of peas for pigs: a review of European results. *Livestock Production Science*, 26, 155–175.

Ge, Y. C., & Morgan, R. G. H. (1993). The effect of trypsin inhibitor on the pancreas and small intestine of mice. *British Journal Nutri*tion, 70, 333–345.

- Hankins, C. N., & Shannon, L. M. (1978). The physiological and enzymatic properties of a phytoheamagglutinin from mung beans. *Journal of Biological Chemistry*, 253, 7791–7797.
- Higuchi, M., Suga, M., & Iwai, K. (1983). Participation of lectin in biological effects of raw winged bean seeds on rats. Agricultural Biological Chemistry, 47, 1879–1886.
- Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A multi-enzyme technique for estimating protein digestibility. *Journal* of Food Science, 42, 1269–1271.
- Humphries, E. C. (1956). Mineral components and ash analysis. In K. Paech, & M. V. Tracey, *Modern methods of plant analysis*, Vol. 1 (pp. 468–502). Berlin: Springer Verlag.
- ICMR. (1992). Daily requirements and recommended dietary allowances for Indians. New Delhi, India: Expert Committee of Indian Council of Medical Research, ICMR Offset Press.
- Issac, R. A., & Johnson, W. C. (1975). Collaborative study of wet and dry ashing techniques for the elemental analysis of plant tissue by Atomic Absorption Spectrophotometer. *Journal of the Association* of Official Analytical Chemists, 58, 436–440.
- Jaffe, W. G. (1980). Phytohaemagglutinins. In I. E. Liener, *Toxic constitutes of Plant Food Stuffs* (pp. 69–101). New York, USA: Academic Press.
- Janardhanan, K. (1993). Chemical composition and nutritional value of the tribal pulse, Cassia floribunda Cav. Adv. Plant Sci., 6, 137– 144.
- Kakade, M. L., Swenson, D. H., & Liener, I. E. (1970). Note on the determination of chymotrypsin and chymotrypsin inhibitor activity using casein. *Analytical Biochemistry*, 33, 255–258.
- Kakade, M. L., Rackis, J. J., McGhee, J. E., & Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. *Cereal Chemistry*, 51, 376–382.
- Katoch, B. S., & Bhowmik, K. B. D. (1983). Biological evaluation of Cassia seeds (Cassia tora and Cassia floribunda) as poultry feed ingredients. Indian Veternary Journal, 60, 390–395.
- Li, B. W., & Cardozo, M. S. (1994). Determination of total dietary fiber in foods and products with little or no starch, non-enzymatic-gravimetric method: Collaborative study. J. AOAC Int., 77, 687–689.
- Liener, I. E. (1976). Phytohaemagglutinins (phytolectins). Annual Review of Plant Physiology, 27, 291–319.
- Liener, I. E. (1994). Implications of antinutritional components in soybean foods. CRC Ctirical Reviews in Food Science and Nutrition, 34, 31–67.
- Liener, I. E., & Kakade, M. H. (1980). Protease inhibitors. In I. E. Liener, *Toxic constituents of plant foods* (pp. 7–71). New York, USA: Academic Press.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951).
 Protein measurement with the folin-phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
- Mohan, V. R., & Janardhanan, K. (1995). Chemical determination of nutritional and antinutritional properties in tribal pulses. *Journal of Food Science and Technology*, 32, 465–469.
- Muller, H. G., & Tobin, G. (1980). Nutrition and food processing. London: Croom Helm Ltd.

- Murray, D. R. (1979). The seed protein of kowhai, *Sophora microphylla* AIT. *Zeitschrift fuer Pflanzenphysiologie*, 93, 423–428.
- Murray, D. R. (1984). Accumulation of seed reserves of nitrogen. In Murray, D. R., *Seed Physiology*, Vol. 1, (pp. 83–137) *Development* Academic Press, New York, USA.
- Narasinga Rao, B. S., Deosthale, Y. G., & Pant, K. C. (1989). Nutritive value of Indian foods. Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research.
- Olson, R. E. (1975). Protein-calorie nutrition. New York, USA: Academic Press.
- Pusztai, A., Clarke, E. M. W., Grant, G., King, T. P., & Stewart, J. C. (1981). Nutritional evaluation of kidney beans (*Phaseolus vulgaris*): Chemical composition, lectin content and nutritional value of selected cultivars. *Journal of the Science of Food and Agriculture*, 30, 843–848.
- Rajendra, W. (1987). High Performance Liquid Chromatographic determination of amino acids in biological samples by precoloum derivatization with O-phthaldialdehyde. Journal Liquid Chromatography, 10, 941–955.
- Rajyalakshmi, P., & Geervani, P. (1990). Studies on tribal foods of South India: Effect of processing methods on the vitamin and in vitro protein digestibility (IVPD) of cereals/millets and legumes. Journal of Food Science and Technology, 27, 260–263.
- Ryan, C. A. (1981). Protease inhibitors. In P. K. Stumpf, & E. E. Conn, The biochemistry of plants. A comprehensive treatise, Vol. 6. Protease and nucleic acids (pp. 351–370). New York, USA: Academic Press.
- Siddhuraju, P., Vijayakumari, K., & Janardhanan, K. (1995). Nutritional and antinutritional properties of the under-exploited legumes Cassia laevigata Wild and Tamarindus indica L. J. Food Comp. Analys., 8, 351–362.
- Siddhuraju, P., Vijayakumari, K., & Janardhanan, K. (1996). Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC.). *Journal of Agricultural and Food Chemistry*, 44, 2636–2641.
- Singh, P. B. (1996). Wild edible plants of Mandi district in Northwest Himalaya. In S. K. Jain, *Ethnobiology in human welfare* (pp. 22–25). New Delhi, India: Deep Publications.
- Singh, U., & Eggum, B. O. (1984). Factors affecting the protein quality of pigeonpea (*Cajanus cajan L.*). *Qualitas Plantarum Plant Foods for Human Nutrition*, 34, 273–283.
- Thompson, L. U. (1988). Antinutrients and blood glucose. *Food Technology*, 42, 123–132.
- Thompson, L. U., Tenebaum, A. V., & Hui, H. (1986). Effect of lectins and mixing of proteins on rate of protein digestibility. *Journal of Food Science*, 51, 150–152.
- Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1996). Effect of different post-harvest treatments on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) DC. *Int. J. Food Sci. Nutr.*, 47, 263–272.
- Yavelov, J., Finlay, T. H., Kennedy, A. R., & Troll, W. (1983). Bow-man-Birk soybean protease inhibitor as an anti-carcinogen. *Cancer Research*, 43, 2454–2459.