

## Nutritional Quality of Vegetable Pigeonpeas [*Cajanus cajan* (L.) Millsp.]: Dry Matter Accumulation, Carbohydrates and Proteins

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### —ABSTRACT—

Since average dry matter accumulation of green pigeonpea seeds intended for use as a vegetable was 65.6% of the total in matured seeds, green seeds were collected prior to physiological maturity. Green seed contained less starch and more dietary fiber than did mature seed. Flatulence causing oligosaccharides were present in a lower amount in green seed. Trypsin inhibitor activity (TIA) was more in mature seed whereas green and mature seed differed little in chymotrypsin inhibitor activity (CIA). The mean value for *in vitro* protein digestibility (IVPD) of green seed was more than that of mature seed. The green seed had a greater amount of tryptophan and threonine and the sulphur containing amino acids, methionine and cystine. It is concluded that the green seeds of pigeonpea genotypes are nutritionally better than their mature seeds.

### INTRODUCTION

LEGUME SEEDS are important for supplying protein to diets in many parts of the world. Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a nutritionally important grain legume of tropical and sub-tropical regions of the world. While pigeonpea is predominantly consumed in the form of cooked dhal (decorticated dry split seeds) along with cereals, it remains a common practice in several countries to consume the developing green seeds shelled out of fresh pods. In India, developing green pigeonpea seeds are used fresh as a vegetable in several states (Singh et al., 1977). The collection of pigeonpea pods is generally made 25–30 days after flowering. Large seeded varieties are preferred for this purpose. The green pigeonpeas, mostly processed by canning and freezing, are consumed in some Caribbean and Latin American countries (Mansfield, 1980). Although reports are available on the methods of processing green pigeonpea seeds (Sanchez et al., 1961), limited information is available on the nutritional quality of green seeds, particularly for those cultivars used as a vegetable in India.

In legumes, there are two main aspects of protein quality (Boulter et al., 1976). First, the amino acid composition relative to the F.A.O. reference pattern (FAO/WHO, 1973) and second, digestibility and the presence of antimetabolic proteins (Liener, 1969). A large variation in protease inhibitors and *in vitro* protein digestibility of pigeonpea and its wild relatives has been reported earlier (Singh and Jambunathan, 1981a). Improvement of the nutritional quality of pigeonpea is one of the objectives at ICRISAT (Jain et al., 1980). Besides breeding for improved grain type, the development of pigeonpea cultivars suitable for vegetable use has been emphasized recently in an International Pigeonpea Workshop held at ICRISAT (Jain et al., 1980). We have examined the nutritional composition of green seed of pigeonpea for use as a vegetable. This paper reports the dry matter and nutritional quality of carbohydrates and proteins in green seeds and compares the results with those of the mature seed.

### MATERIALS & METHODS

#### Materials

Nine pigeonpea genotypes with a range of seed size (ICPL-102, ICPL-114, ICPL-119, ICPL-122, ICPL-128, ICPL-212, ICP-6997, ICP-7035 and cv. C-11) grown in unreplicated plots were used for this study. These were grown on black soil at ICRISAT Center, Patancheru, near Hyderabad during the 1980–81 rainy season. The crop was grown by using normal cultural practices. No irrigation or fertilizer was applied. The homogenous lots of pods containing green seeds were collected at the stage when pigeonpeas are generally harvested for use as a vegetable by growers. Green seeds were shelled out of these pods manually. Mature seed samples of these same genotypes were collected. The fresh weight of the green seeds was recorded. The material was freeze-dried and moisture content determined. Dried green and mature seed samples were ground in a Udy cyclone mill using a 0.4 mm screen. After defatting in a Soxhlet apparatus, using hexane, the material was used for chemical analyses.

#### Methods of analysis

Crude fiber was determined according to the method of AOAC (1975). The procedure described by Van Soest and Wine (1973) was followed for the estimation of neutral detergent fiber. The analysis of dietary fiber as an estimate of unavailable carbohydrates was carried out according to the method of Southgate et al. (1978).

Soluble sugars were extracted in a Soxhlet apparatus using 80% ethanol and estimated colorimetrically by the phenol-sulphuric acid method (Dubois et al., 1956). Thin-layer chromatography (TLC) was used to estimate glucose and fructose, sucrose, raffinose, stachyose and verbascose. TLC was carried out on a silica gel plate in the ascending fashion. The plates of 500  $\mu$  thickness were prepared using a slurry of silica gel-G in water and activated before use by heating it at 105°C for 35 min. The solvent used consisted of chloroform:acetic acid:water (6:7:1 v/v). After separation, sugar spots were detected on the TLC plates by spraying with aniline-diphenylamine reagents. The TLC plates were scanned in a densitometer and sugar concentrations were calculated in comparison with sugar standards which were run simultaneously under identical conditions. The following were the sources for the oligosaccharide standards: raffinose and stachyose (Sigma Chemical Co., USA) and verbascose (70% pure, a gift from Nestlé Products, Technical Assistance Co., Ltd., Switzerland) and other sugars of analar grade were used as standards.

Starch content in the dry residue, left after extraction of soluble sugar with 80% ethanol, was determined by enzymatic hydrolysis (Singh et al., 1980).

**Amylase inhibitor activity.** The inhibitor activity of pancreatic amylase (obtained from Sigma Chemical Co., USA) was carried out according to the method of Jaffe et al. (1973). Amylase inhibitor was extracted by shaking a defatted sample with 0.02M phosphate buffer pH 6.9 (1:10 w/v) for 2 hr at room temperature. The suspension was then centrifuged at 10,000  $\times$ g for 15 min at room temperature. The supernatant was then heated for 10 min at 70°C, centrifuged again at 10,000  $\times$ g for 15 min at room temperature, and the supernatant tested for amylase inhibitor activity.

Determination of *in vitro* digestibility of meal starch was determined using pancreatic amylase according to the procedure described by Singh et al. (1982). A suitable amount of defatted meal (50 mg) was dispersed in 1.0 ml of 0.2M phosphate buffer, pH 6.9. Pancreatic amylase (20 mg) was dissolved in 50 ml of the same buffer and 0.5 ml was added to a sample suspension. Maltose was used as the standard and the values were expressed as mg of maltose released per g of sample.

**Amino acid analysis.** A suitable quantity of sample (50 mg) was refluxed for 24 hr in 50 ml 6N HCl. After evaporating the HCl, the

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Table 1—Fresh and dry weight and moisture of green and mature seed of 9 pigeonpea cultivars

Varieties	Pod color	Seed color (mature)	Green seed			Mature seed			Green seed as % of mature seed (dry wt basis)
			Fresh wt (mg/seed)	Dry wt (mg/seed)	Moisture (%)	Fresh wt (mg/seed)	Dry wt (mg/seed)	Moisture (%)	
ICPL-102	Mixed <sup>a</sup>	Cream	238.0	91.4	61.5	153.5	130.9	14.7	69.8
ICPL-114	Mixed	Cream	198.4	75.3	62.0	130.8	117.0	10.5	64.3
ICPL-119	Mixed	Lt brown	216.2	80.8	62.6	146.4	129.4	11.6	62.4
ICPL-122	Mixed	Lt brown	200.6	82.1	59.1	143.4	129.0	10.0	63.4
ICPL-128	Green	Brown	226.7	86.4	61.8	140.8	123.8	12.1	69.8
ICPL-212	Mixed	Cream	206.9	90.4	56.4	144.6	126.0	12.2	71.7
ICP-6997	Green	Brown	208.4	78.9	62.1	142.5	125.7	11.9	62.6
ICP-7035	Purple	Dk brown	327.5	120.6	63.2	221.5	190.8	13.9	63.2
cv. C-11	Mixed	Brown	154.8	66.4	57.1	118.7	103.8	12.6	64.0
Mean			198.6	85.8	60.7	149.1	130.7	12.2	65.6

Table 2—Mean and range values of seed coat, fibers, starch, amylase inhibitors and *in vitro* starch digestibility of green and mature seed of 9 pigeonpea cultivars

Constituent	Maturity stage	Range	Mean	S.D
	Mature	9.03-16.70	13.57	
Crude fiber (%)	Green	7.93- 8.67	8.19	0.13
	Mature	5.89- 7.02	6.57	
Neutral-detergent fiber	Green	18.18-23.40	20.67	0.74
	Mature	13.50-18.74	16.56	
Dietary fiber (%)	Green	22.08-24.76	23.08	0.60
	Mature	17.92-21.60	20.11	
Starch (%)	Green	46.60-50.99	48.39	0.78
	Mature	50.82-55.20	52.96	
Amylase inhibitors <sup>a</sup>	Green	15.89-19.26	17.28	1.25
	Mature	22.51-34.15	26.87	
<i>In vitro</i> starch digestibility <sup>b</sup>	Green	50.89-56.92	53.06	0.93
	Mature	32.43-40.10	36.22	

<sup>a</sup> Units/g meal

<sup>b</sup> Expressed as mg maltose released/g meal

<sup>c</sup> Standard deviation of the difference

\* Significant at 5% level

\*\* Significant at 1% level

residue was dissolved in citrate buffer (pH 2.2). The amino acids were analyzed in a Beckman 120C amino acid analyzer. The mean coefficient of variability for the different amino acids ranged between 1.96 and 13.02% except for cystine where it was 22.12%.

Determination of tryptophan was carried out colorimetrically according to Spies and Chambers (1949) with minor modifications as follows: a suitable amount of sample (20–25 mg) was placed in a test tube which, after the addition of 10 ml of solution of dimethyl-aminobenzaldehyde (3 mg/ml of 19N H<sub>2</sub>SO<sub>4</sub>), was incubated in the dark at room temperature (25 ± 2°C) for 18 hr. After incubation, 0.1 ml of 0.45% solution of sodium nitrite was added and the tube allowed to stand at room temperature for 30 min before reading the density at 590 nm in Spectronic-21 spectrophotometer.

**Trypsin inhibitor activity.** The trypsin inhibitor activity (TIA) was assayed according to Kakade et al. (1969). Trypsin inhibitor was extracted by shaking 200 mg of defatted material with 10 ml of 0.1M phosphate buffer (pH 7.6) at room temperature for 1 hr. The extract was diluted fourfold. Aliquots containing 0.2, 0.4, 0.6 and 0.8 ml were assayed for trypsin inhibitor activity. Protein content of the extract was determined according to Lowry et al. (1951).

**Chymotrypsin inhibitor activity.** Chymotrypsin inhibitor activity (CIA) was assayed according to Kakade et al. (1970). Chymotrypsin inhibitor was extracted as described above for trypsin except that 0.1M borate buffer at pH 7.6 was used. Protein content of the extract was determined according to Lowry et al. (1951).

***In vitro* protein digestibility.** The determination of *in vitro* protein digestibility was carried out using pepsin and pancreatin enzymes (Sigma Chem. Co., USA) according to Singh and Jambunathan (1981b).

**Polyphenolic compounds (polyphenols).** Polyphenols were extracted from each defatted sample (500 mg) by refluxing with 50 ml of methanol containing 1% HCl for 4 hr. The extract was concentrated in a rotary flash evaporator and brought to 25 ml with methanol HCl. The amount of phenolic compounds were estimated as tannic acid equivalent according to the Folin-Denis procedure (Swain and Hillis, 1959).

## RESULTS & DISCUSSION

### Dry matter, fibers and starch contents

The moisture content of green seed ranged from 56.4–63.2% indicating a small variation between the average of the samples (Table 1). Dry matter content of green seed varied from 75.3–120.6 mg/seed and of the mature seed from 117.0–190.8 mg/seed for these genotypes. The dry weight of the green and mature seeds of cv C-11 was 66.4 and 103.8 mg/seed, respectively. The dry matter accumulation of the green seeds was about 65% of the dry matter contained by the fully mature seeds (Table 1). This shows that the seeds were collected considerably before physiological maturity, which is the stage for maximum dry matter accumulation during seed development. This lack of physiological maturity was also revealed by the lower levels of starch in the green seed compared to that in the mature seed (Table 2). In a previous study, percent starch in the dehulled pigeonpea seed was maximum in samples collected at 28 days after flowering (Singh et al., 1980).

A different trend between the green and mature seed indicated that the levels of crude-, neutral detergent-, and dietary-fiber in the developing seed were greater than in the mature seed. No clear differences between the large seeded pigeonpeas and the small-seeded one (C-11) were observed with respect to the levels of starch and various fibers (Table 2).

### Amylase inhibitors and *in vitro* starch digestibility (IVSD)

Amylase inhibitors of green seeds were significantly lower than those of mature seeds (Table 2). Nutritionally, these inhibitors will be of little importance in countries where green seeds are consumed after cooking as amylase inhibitors become inactive when they are heated at 100°C (Hernandez and Jaffe, 1968). However, these inhibitors will be of considerable importance where fresh green seeds are consumed.

IVSD, expressed as mg maltose released/g meal, ranged between 50.89 and 56.92 with the mean being 53.06 for these genotypes (Table 2). Significantly lower values for IVSD were obtained in the mature seed samples. The lower IVSD values of mature pigeonpea seed could be due to two factors: higher levels of amylase inhibitors in mature seeds, and a more complex starch-protein matrix as a result of seed maturation. The maturity of the seed has been reported

to influence the susceptibility of starch to enzymes in wheat (Kulp and Mattern, 1973). Further studies will be needed to accurately determine these relationships in pigeonpea.

### Soluble sugars

The green seeds of pigeonpea contained more total soluble sugars than did mature seeds (Table 3). The relative concentration of reducing sugars and nonreducing sugars were significantly different in green and mature seeds. The percent soluble sugars was higher in green seeds than in mature seeds which agrees with the earlier results of Singh et al. (1980) who reported that percent soluble sugars increased in the early stages of seed development and then declined.

Using TLC it was found that glucose, fructose and sucrose were the predominant sugars of green seeds and that the concentration of these sugars declined as the seed matured (Table 3). Glucose and fructose were estimated together as these sugars could not be resolved completely. A large variation was observed in the levels of glucose, fructose and sucrose in the green seed among the genotypes tested (Table 3). ICP-7035 contained the highest amount of glucose + fructose (36.44%) and the lowest amount of sucrose (29.04%) in the developing seed. Such differences disappeared in the mature seed of this line. The variations observed in the levels of soluble sugars among vegetable-pigeonpea cultivars and their importance to their consumer taste suggest that attempts should be made to select vegetable cultivars having a suitably high level of these sugars.

Raffinose and stachyose were present in very low amounts in the green seed whereas they were detected in greater concentration in the mature seed (Table 3). Another oligosaccharide, verbascose, was not detected in the developing seed but was the predominant sugar in the mature seed. This clearly indicates that these oligosaccharides accumulate in the seed during the later stages of maturation. Food legumes are regarded as notorious inducers of flatulence when consumed in large quantities. In particular, the hydrogen component of intestinal gas is formed by the fermentation of low molecular weight galactosido-oligosaccharides-raffinose, stachyose and verbascose (Hellendoorn, 1969). The consumption of pigeonpea as a vegetable seems to be better than as a mature seed in view of the remarkably low amount

of flatulence causing oligosaccharides in green seeds. Furthermore, the variation in the levels of oligosaccharides in mature seed among pigeonpea lines suggest that attempts should be made to screen and then select cultivars with low amounts of these oligosaccharides.

### Protease inhibitors and *in vitro* protein digestibility (IVPD)

While no large differences in the chymotrypsin inhibitor activity between green and mature seed was observed, trypsin inhibitor activity of mature seed was remarkably higher than that of the green seed (Table 4). This indicates an accumulation of trypsin inhibitors in mature seed. The *in vitro* protein digestibility of the green seed was more than that of the mature seed while a reverse trend was observed for the polyphenolic compounds. This indicated that polyphenolic compounds may have interfered with the *in vitro* protein digestibility in these samples. Polyphenolic compounds showed a large variation among these genotypes. ICP-7035 had the highest amount of polyphenolic compounds in both green and mature seeds.

Polyphenolic compounds are mostly present in the seed coat of grain legumes (Singh and Jambunathan, 1981b) and these compounds are known to inhibit the activity of digestive enzymes (Griffiths, 1979; Singh, 1984). In view of these observations the polyphenolic compounds have nutritional implications as green pigeonpea seeds are normally consumed without decortication.

The nutritive value and protein digestibility of gram legumes are improved by processing or cooking as these treatments destroy the heat labile antinutritional factors (Bressani, 1972). Among these factors, trypsin and chymotrypsin inhibitors have been studied in detail (Fener, 1979). Variation observed in the levels of protease inhibitors and IVPD suggest that cultivars with a lower amount of such inhibitors which are associated with better protein digestibility should be selected in a breeding program.

### Amino acid composition

The protein content of mature seeds was lower than those of green seed (Tables 5 and 6) which might be due to an accumulation of starch during the later stages of maturation (Singh et al., 1982). Considerable differences between green and mature seed were observed nutritionally as the levels of some essential and nonessential amino acids changed with maturation. On a dry weight basis, the green

Table 3—Mean and range values of soluble sugars in green and mature seed of 9 pigeonpea cultivars

Constituent	Maturity stage	Range	Mean	SD <sup>b</sup>
Total soluble sugars (%)	Green	4.70- 5.54	5.09	0.18**
	Mature	2.32- 4.13	3.14	
Reducing sugars (%)	Green	1.24- 2.06	1.59	0.10**
	Mature	0.21- 0.54	0.31	
Non-reducing sugars (%)	Green	3.15- 4.04	3.50	0.15**
	Mature	2.11- 3.59	2.83	
Glucose + Fructose <sup>a</sup>	Green	12.30-36.44	18.37	2.55**
	Mature	0.84- 3.90	2.11	
Sucrose <sup>a</sup>	Green	29.04-65.72	52.60	3.88**
	Mature	15.59-30.23	21.16	
Raffinose <sup>a</sup>	Green	1.25- 9.50	6.19	1.34**
	Mature	10.34-17.30	12.92	
Stachyose <sup>a</sup>	Green	2.04-11.90	4.08	1.19**
	Mature	12.29-19.40	15.72	
Verbascose <sup>a</sup>	Green	-	-	-
	Mature	20.98-27.50	24.89	

<sup>a</sup> Expressed as g/100g meal total soluble sugars

<sup>b</sup> Standard deviation of the difference

\*\* Significant at 1% level

Table 4—Mean and range values of protease inhibitors, *in vitro* protein digestibility and polyphenols of green and mature seeds of 9 pigeonpea cultivars

Constituent	Maturity stage	Range	Mean	SD <sup>d</sup>
Trypsin inhibitor <sup>a</sup>	Green	2.40- 3.86	2.80	0.55**
	Mature	8.07- 12.08	9.88	
Trypsin inhibitor <sup>b</sup>	Green	26.44- 45.44	32.97	4.88**
	Mature	67.96-103.52	87.11	
Chymotrypsin inhibitor <sup>a</sup>	Green	1.91- 3.05	2.55	0.19
	Mature	2.07- 3.63	2.96	
Chymotrypsin inhibitor <sup>b</sup>	Green	12.75- 22.15	18.16	1.46
	Mature	16.21- 26.71	20.19	
<i>In vitro</i> protein digestibility <sup>c</sup>	Green	63.89- 72.09	66.80	1.53*
	Mature	52.51- 63.14	58.50	
Polyphenols (mg/g)	Green	6.68- 12.49	8.62	1.10
	Mature	6.05- 18.34	10.60	

<sup>a</sup> Units inhibited/mg meal

<sup>b</sup> Units inhibited/mg extracted protein

<sup>c</sup> Percent digestible nitrogen

<sup>d</sup> Standard deviation of the difference

\* Significant at 5% level

\*\* Significant at 1% level

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Table 5—Amino acid composition (g/100g protein) of green seed of 4 pigeonpea cultivars

Amino acid	ICPL-128	ICP-6997	ICP-7035	C-11
Lysine	6.37	6.53	7.25	7.25
Histidine	4.22	5.31	4.41	5.59
Arginine	6.71	7.73	7.89	8.64
Aspartic acid	10.43	10.20	11.71	10.05
Threonine	3.94	4.01	4.03	5.34
Serine	4.97	4.53	5.50	4.91
Glutamic acid	17.29	18.21	18.34	16.15
Proline	4.13	5.06	5.07	4.41
Glycine	3.89	4.04	4.00	4.08
Alanine	5.46	5.20	5.06	4.11
Cystine	0.99	1.12	1.44	1.02
Valine	4.65	5.62	4.92	6.21
Methionine	1.42	1.65	1.51	1.63
Isoleucine	4.24	5.32	4.18	5.38
Leucine	7.48	9.15	8.28	8.31
Tyrosine	2.48	3.62	3.25	3.21
Phenylalanine	5.64	7.11	8.14	5.38
Tryptophan	0.91	0.90	0.73	1.10
Total	95.58	105.01	104.23	102.87
Protein (%)	21.15	20.24	21.26	21.30

Table 6—Amino acid composition (g/100g protein) of mature seed of 4 pigeonpea cultivars

Amino acid	ICPL-128	ICP-6997	ICP 7035	C-11
Lysine	6.90	6.25	6.73	6.95
Histidine	4.48	3.62	4.10	3.54
Arginine	7.24	5.58	5.66	6.88
Aspartic acid	10.34	9.13	8.91	9.16
Threonine	3.59	3.52	3.65	3.21
Serine	4.65	4.14	4.47	4.09
Glutamic acid	18.64	17.23	18.71	18.46
Proline	2.27	2.13	2.33	2.37
Glycine	2.78	2.77	2.88	3.11
Alanine	3.61	3.37	3.85	4.23
Cystine	0.90	0.80	1.19	0.77
Valine	4.03	3.07	3.78	3.92
Methionine	1.30	1.40	1.39	1.18
Isoleucine	3.23	3.13	3.14	3.34
Leucine	7.02	6.81	6.35	6.82
Tyrosine	3.03	2.60	2.94	2.64
Phenylalanine	9.22	9.19	9.54	8.18
Tryptophan	0.86	0.73	0.67	0.86
Total	98.08	95.07	90.29	88.71
Protein (%)	19.24	18.34	17.63	20.03

seed contained a greater amount of the sulphur containing amino acids, methionine and cystine, tryptophan, threonine, leucine and isoleucine than did the mature seed. The values for methionine and cystine reported here are probably low as these amino acids are partially destroyed during hydrolysis. Tyrosine and phenylalanine when considered together were considerably higher in the mature seed. Among the nonessential amino acids, proline, glycine and alanine were higher in the green seed. This study shows that the protein quality, measured as a function of the level of essential amino acids, is better in the green seed than in the mature seed. Studies involving animal feeding experiments are required; however, to have a more precise idea of the protein nutritional quality of green and mature pigeonpea seed.

Although the overall effects of green pigeonpea consumption on its nutritive value should be studied, the nutritional composition of green seeds appeared to be superior to that of mature seeds when the results were expressed on a dry weight basis. Also, since there are differences among genotypes, these variations should be studied and utilized to develop pigeonpea cultivars suitable for use as a vegetable.

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