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# Nutritional and antinutritional evaluation of raw and processed seeds of a wild legume, *Canavalia cathartica* of coastal sand dunes of India

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# Abstract

Seeds of a wild legume, *Canavalia cathartica* collected from coastal sand dunes of the southwest coast of India were processed (roasted and cooked) and analyzed for proximate composition, mineral constituents, protein fractions, amino acid profiles, fatty acids and some antinutritional factors. Raw, roasted and cooked seeds contained 35.5%, 30.5% and 29.2% crude protein; 52.8%, 65.3% and 65.4% crude carbohydrates; 1.3%, 1.4% and 1.4% crude lipids; 1.7%, 1.6% and 1% crude fibre and 3.1%, 3% and 3.1% ash, respectively. Among the minerals, potassium was the highest (895, 821 and 190 mg/100 g), followed by phosphorus (137, 112 and 99 mg/100 g) and calcium (84, 70 and 44 mg/100 g). Among the true protein fractions of raw seeds, globulins (18.3 g/100 g) and albumins (7.3 g/100 g) were the major seed proteins. Essential amino acids, threonine, valine, methionine + cystine, isoleucine, leucine, phenylalanine + tyrosine and lysine, were above the FAO/WHO pattern in raw seeds. In roasted and cooked seeds, essential amino acid score ranged between 54 (threonine) and 224 (methionine). Essential amino acids, leucine, phenylalanine and lysine, in raw seeds were more than those of whole egg protein, soybean and rice. Total phenolics slightly declined in cooked seeds. Seeds did not possess tannins and trypsin inhibitors. Proteins of raw seeds proteins essential amino acids, here activity, which was lowered in processed seeds. The current study demonstrated that seeds of *C. cathartica* were high in protein, essential amino acids and low in saturated fatty acids and anti-nutritional factors.

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Keywords: Canavalia cathartica; Sand dunes; Wild legume; Nutritive value; Proteins; Amino acids; Saturated fatty acids; Roasting; Cooking

# 1. Introduction

Malnutrition in children and lactating women in developing countries is common, due to inadequate supply of protein diets, and this is of great concern to scientists and governments (Coulter et al., 1988; Olsen, 1975; Pelletier, 1994). Such protein requirements, where animal protein is inadequate, can be compensated using wild legumes adapted to adverse conditions (Amubode

\* Corresponding author. *E-mail address:* sirikr@yahoo.com (K.R. Sridhar). & Fetuga, 1983; Rao, 1994; Siddhuraju, Vijayakumari, & Janardhanan, 1992; USNAS, 1975; Vadivel & Janardhanan, 2001). Even though many wild legume plant species have been identified, their utilization is limited due to lack of nutritional information (Viano et al., 1995; Vijayakumari, Siddhuraju, & Janardhanan, 1994). Investigations on economically viable wild legumes as alternative foods broaden the protein sources for human nutrition. Adaptation to adverse environmental conditions, resistance to pests and adequate nutritional qualities are the major advantages of wild legumes (Maikhuri, Nautiyal, & Khali, 1991). Attempts

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have been made to explore the nutritional status of wild legumes in India (Janardhanan & Nalini, 1991; Maikhuri et al., 1991; Mohan & Janardhanan, 1995; Pandey & Srivastava, 1990; Rajaram & Janardhanan, 1991; Siddhuraju, Vijayakumari, & Janardhanan, 1995).

Canavalia cathartica was the most common wild legume on the Coastal Sand Dunes (CSDs) of the southwest coast of India (Arun, Beena, Raviraja, & Sridhar, 1999). Coastal fisher folk occasionally utilize tender pods under conditions of severe shortage of food. There are insufficient data on the nutrient status of C. cathartica although some species of Canavalia have been evaluated (Bressani, Brenes, Gracia, & Elias, 1987; Ekanayake, Jansz, & Nair, 1999, 2000; Rajaram & Janardhanan, 1992a). Besides distribution of C. cathartica on the CSDs, it is also widely distributed in plantations adjacent to CSDs as well as in mangrove habitats. Due to lack of information on the nutritional potential of C. cathartica of coastal sand dunes, the current study emphasizes the proximate composition, mineral constituents, proteins, amino acids, fatty acids and antinutritional qualities of raw and processed seeds. Seed processing followed includes, household roasting and cooking in view of convenience, saving cooking fuel and enhancement of nutritive value for coastal dwellers of developing countries.

#### 2. Materials and methods

#### 2.1. Seed samples and processing

Dried seeds of C. cathartica Thouars were obtained from coastal sand dunes of Thalapady (12°45' N, 74°45' E), west coast of India, during the summer (February-March, 2002). The seeds were sun-dried for three days. Mean weights and dimensions of seeds were determined. Seeds were divided into three parts. The first set of the seeds was cut and dehulled, milled (30 mesh) and stored in air-tight glass containers and designated as raw seed samples. The second set was roasted on a sand bath at 180 °C for 20 min. After attaining room temperature, roasted seeds were cut, dehulled, milled and stored. A third set of seeds was cut and dehulled. The cotyledons were soaked in freshwater for 1 h and subsequently cooked in a pressure cooker for 30 min with 1:3 (v/v)freshwater. Cooked cotyledons were sun-dried, milled and stored.

#### 2.2. Proximate composition

Moisture content of seed powders was determined after attaining constant weight at 100 °C. Total nitrogen and the crude protein content ( $N \times 6.25$ ) were determined by the microKjeldahl method (Humphries, 1956). Crude lipid (Soxhlet extraction), crude fibre and ash contents (gravimetric) were determined by on employing AOAC methods (AOAC, 1990). Total crude carbohydrate was calculated as outlined by Müller & Tobin (1980) [100 – (crude protein + crude lipid + crude fibre + ash)]. Gross energy (kJ) was estimated by multiplying the percentages of crude protein, lipid and carbohydrates by the factors 16.7, 37.7 and 16.7, respectively.

#### 2.3. Mineral constituents

Seed flour was digested with concentrated nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v) and mineral constituents (sodium, potassium, calcium, magnesium, iron, copper, zinc and manganese) were determined by atomic absorption spectrophotometry (GPC 902, Australia) by the method outlined in AOAC (1990). Total phosphorus (as orthophosphate) was determined by the ascorbic acid method after acid digestion and neutralization by phenolphthalein indicator and combined reagent (APHA, 1995). Absorbance was read at 880 nm (Bausch and Lomb Spectronic 21) with KH<sub>2</sub>PO<sub>4</sub> as standard.

# 2.4. Protein isolates

Total protein of raw seed flour was extracted, based on the method outlined by Basha, Cherry, & Young (1976); to save the prolamine, ethanol treatment was omitted. Proteins were purified by precipitation with 20% TCA and estimated according to Lowry, Rosebrough, Farr, & Randall (1951). The albumin and globulin fractions were separated, based on Murray (1979). The rest of the pellet was treated with 80% ethanol (1:10 w/v) overnight and centrifuged (20,000g, 20 min); the prolamine-containing supernatant was air-dried, dissolved in 0.1 N NaOH (1:10 w/v), centrifuged (20,000g, 20 min) and the supernatant thus obtained was designated as glutelin. The protein fractions obtained were precipitated with TCA and redissolved in 0.2 N NaOH and protein content was determined (Lowry et al., 1951).

# 2.5. Proteins separation

Proteins (100 µg) were dissolved (100 µl) in buffer consisting of 60 mM tris–HCl, pH 6.8, 10% (w/v) glycerol, 2% (w/v) SDS and 10% (v/v) mercaptoethanol. Samples were boiled for 2 min at 100 °C, cooled and 2 µl 50% (w/v) bromophenol blue solution were added (Miersch, Kullertz, & Henning, 1998). Soluble protein separation was carried out using one-dimensional SDS–PAGE prepared in a 5% (w/v) stacking gel and 13.5% (w/v) separating gel (Laemmli, 1970) using a BROVIGA Mini Vertical Slab Gel Electrophoresis unit (Balaji Scientific Services, Chennai, India). Identical amounts of proteins were loaded on to each lane of gel and run for 3 h at 70 V and the gels were stained with Coomassie Brilliant Blue R-250.

# 2.6. Amino acid analysis

The method described by Hofmann, Gehre, & Jung (2003) was followed to assay amino acids. Seed flours (15 mg) were hydrolyzed with 15 ml 6 N HCl for 4 h at 145 °C, cooled and HCl eliminated in a rotaryevaporator (RE121, Büchi Laboratoriumstechnik AG, Switzerland) combined with a diaphragm vacuum pump (MC2C, Vacuubrand GmbH, Germany). Internal standard trans-4-(aminomethyl)-cyclohexanecarboxylic acid was added to each sample for quantitative analysis of amino acids. The derivatization step consisted of esterification with trifluoroacetylation. Samples were dried using CH<sub>2</sub>Cl<sub>2</sub>. Later, 12 ml of fresh acidified isopropanol (acetyl chloride, 3 ml + 2-propanol, 12 ml) were added and the mixture was heated at 110 °C for 1 h. Samples were cooled and filtered through glass fibre paper and the reagent was eliminated with a gentle stream of helium at 45 °C, followed by combined evaporation with aliquots of CH<sub>2</sub>Cl<sub>2</sub>. Dried residue was acetylated with 300 µl trifluoroacetic anhydride overnight at room temperature. Amino acids were determined using a gas chromatography-combustion-isotope ratio mass spectrometer (GC-C-IRMS) (GC: Hewlett-Packard 5890 Series II; IRMS: MAT 252; Finnigan Bremen, Germany).

The essential amino acid (EAA) score was determined by employing the formula: glycol succinate on Supelcoport 80/100 isothermically at 165 °C. Conditions for the analysis were: carrier gas, N<sub>2</sub>; injector temperature, 225 °C; detector temperature, 265 °C and oven temperature, 200 °C.

#### 2.8. Antinutritional qualities

Total phenolics of the seed flours were assessed after twice extracting with 50% methanol in a water bath at 95 °C for 10 min (Rosset, Bärlocher, & Oertli, 1982). The pooled extract was made up to 10 ml; 0.5 ml extract was mixed with an equal quantity of distilled water and treated with 5 ml Na<sub>2</sub>CO<sub>3</sub> (in 0.1 N NaOH). After 10 min, 0.5 ml of Folin–Ciocalteu reagent (diluted 1:2 with distilled water) was added and read at 725 nm. Tannic acid was used as standard. Tannins were assayed by the radial diffusion method, using bovine serum albumin for precipitation (Hagerman, 1987).

Trypsin inhibitory activity of the seed flour was determined by enzymic assay (Kakade, Rackis, McGhee, & Puski, 1974). Phytohemagglutinating activity was determined by using a trypsin-treated rabbit erythrocyte suspension (Hankins, Kindinger, & Shannon, 1980). For preparation of rabbit erythrocytes, Alsever's solution (60 mM glucose, 40 mM citric acid and 70 mM NaCl) was used as an anticoagulant. The pH was adjusted to 6.1 with 1 N HCl and the solution was autoclaved prior to use. Three ml of blood were collected from 6 month old rabbits (New Zealand White), by ear vein puncturing (Gorden, 1981) directly into a graduated tube containing 1 ml Alsever's solution. The blood was mixed immediately

EAA score =  $\frac{\text{Grammes of EAA in 100 g test protein}}{\text{Grammes of EAA in 100 g FAO/WHO (1991) reference pattern}} \times 100.$ 

#### 2.7. Fatty acid analysis

The method outlined by Garces & Mancha (1993) was followed to determine fatty acid methyl esters (FAMEs) of the seed flour. Samples weighing 50 mg, together with AOCS 1 and 2 (internal standard), were transferred to tubes with teflon-lined caps and methylated with methylating mixtures containing methanol, benzene, DMP and  $H_2SO_4(37:20:5:2)$  (v/v). About 2.1 ml of mixture and heptane up to a total volume of 5 ml were added to the sample and placed in water bath at 80 °C for 2 h. After heating, tubes were cooled and shaken to separate two phases. One ml of the upper layer, containing the FAMEs, was injected into the gas liquid chromatograph (GLC) (Sigma Instruments, Baroda, India) in a glass column packed with 5% ethylene

with the solution to avoid clotting. The solution containing erythrocytes was centrifuged at 1000g for 5 min at 4 °C. The erythrocytes were washed thrice with phosphate buffered saline (PBS: 10 mM sodium phosphate buffer, pH 7.2, containing 150 mM NaCl), centrifuged at 1000g for 5 min at 4 °C. The cells were then treated with 50  $\mu$ g ml<sup>-1</sup> of trypsin (0.04 BTEE units/mg solid) for 1 h at room temperature and centrifuged. The treated erythrocytes were washed thrice with excess PBS by centrifugation. The trypsin-treated erythrocytes were suspended in PBS to make a 2% (v/v) cell suspension. For hemagglutination assay, trypsintreated erythrocyte suspension (2% in PBS) was used (Hankins et al., 1980). Two-fold serial dilutions of 50 µl crude lectin solution (10-50 µg) with 0.3 M NaCl were incubated with 50 µl erythrocyte

suspension in a microtitre plate for 30 min at 30 °C and were examined for agglutination under a microscope.

# 2.9. Statistical analysis

Differences in proximate composition, minerals and total phenolics of raw vs. processed and roasted vs. cooked seed flours were assessed by *t*-test (MICRO-STAT, Statosoft Inc., 1995).

# 3. Results

Seed, cotyledons and seed coat weights and dimensions of C. cathartica are given in Table 1. Cotyledons constitute about 72% of the intact seed and the rest is seed coat. Proximal constituents of raw and processed seeds are listed in Table 2. Moisture (9.2%) decreased more in cooked (4%) than in roasted (2%) seeds. Crude protein (35.5%) declined more in cooked (6%) than roasted (5%) seeds. Crude lipid and ash contents did not alter much between raw and processed seeds. Crude fibre declined in cooked seeds (1.7 vs. 0.96%). Crude carbohydrates (52.8%) and gross energy values (1520 kJ 100 g<sup>-1</sup>) increased (65.3–65.4% and 1618–1630 kJ 100  $g^{-1}$ , respectively). Moisture, crude protein, crude carbohydrates and energy value were significantly different between raw and processed seeds (p < 0.05), so also was crude fibre within processed seeds. Crude lipid and ash contents did not vary significantly between raw and

Table 1

Physical characteristics of *Canavalia cathartica* seeds  $(n = 20; mean \pm SD)$ 

dimension Percent
.1 100
.1 71.6
.03 28.4
.26 –
.24 –
.09 –
.16 –

Table 3

Mineral composition of seed flours of *Canavalia cathartica* on dry weight basis (mg 100 g<sup>-1</sup>; n = 5; mean  $\pm$  SD)<sup>A</sup>

Minerals	Raw seeds	Roasted seeds	Cooked seeds
Sodium	$49.2 \pm 1.13^{a}$	$43.8 \pm 8.15^{\rm ac}$	$24.1 \pm 4.63^{bd}$
Potassium	$895 \pm 2^{a}$	$821 \pm 104^{\rm ac}$	$190 \pm 4.27^{bd}$
Calcium	$83.7 \pm 1.265^{a}$	$69.9 \pm 7.41^{bc}$	$44.0 \pm 9.83^{bd}$
Phosphorus	$137 \pm 1.22^{a}$	$112 \pm 7.18^{bc}$	$99.4 \pm 11.4^{bd}$
Magnesium	$5.3 \pm 1.6^{\mathrm{a}}$	$4.55 \pm 0.42^{\rm ac}$	$3.58 \pm 0.41^{bd}$
Iron	$2.88\pm0.35^{\rm a}$	$2.45 \pm 0.31^{\rm ac}$	$2.18 \pm 0.25^{\rm bc}$
Copper	$0.35 \pm 0.32^{\mathrm{a}}$	$0.13 \pm 0.05^{\rm bc}$	$0.1 \pm 0.01^{\rm bc}$
Zinc	$11.4 \pm 1.16^{a}$	$7.44 \pm 1.6^{bc}$	$0.91 \pm 0.29^{\rm bc}$
Manganese	$1.36\pm0.35^{\rm a}$	$1.22\pm0.09^{\rm ac}$	$0.79 \pm 0.26^{bd}$

<sup>A</sup> Figures across the columns with different letters are significantly different (p < 0.05, *t*-test).

processed seeds (p > 0.05), but between roasted and cooked seeds, moisture, crude fibre and gross energy differed significantly (p < 0.05). Among the minerals (Table 3), potassium was the highest, followed by phosphorus and calcium, in raw as well as processed seeds. Drastic decline in minerals was seen between raw and cooked seeds. Calcium, phosphorus, copper and zinc were significantly different between raw and processed seeds (p < 0.05). Except for iron, copper and zinc, the minerals differed significantly (p < 0.05) within processed seeds.

True protein content of raw seed flour was 28.7%, of which globulins were highest (18.3%), followed by albumins (7.3%) (Table 4). Separation of raw seed proteins by SDS–PAGE resulted in 14 fractions, ranging from 9-91 kDa. Four fractions of roasted seeds ranged from 33.3 to 52.6 kDa, while a smear was seen in cooked seeds with unclear bands, indicating partial denaturation.

Table 4

True protein fractions of raw seed flour of *Canavalia cathartica* on dry weight basis (g 100 g<sup>-1</sup>; n = 5; mean  $\pm$  SD)

	· · · ·	
Protein fractions	$g \ 100 \ g^{-1}$	Percentage
True protein	$28.6 \pm 0.35$	100
Albumins	$7.28 \pm 0.16$	$25.5 \pm 0.56$
Globulins	$18.3 \pm 0.24$	$64 \pm 0.96$
Prolamins	$0.3 \pm 0.02$	$1.05 \pm 0.05$
Glutelins	$2.7 \pm 0.04$	$9.53 \pm 0.19$

Table 2

Proximate composition of seed flours of *Canavalia cathartica* on dry weight basis  $(n = 5; \text{mean} \pm \text{SD})^A$ 

Component	Raw seeds	Roasted seeds	Cooked seeds
Moisture (%)	$9.18 \pm 0.13^{\rm a}$	$7.18 \pm 0.86^{\rm bc}$	$5.32 \pm 0.48^{bd}$
Crude protein (g 100 $g^{-1}$ )	$35.5 \pm 0.82^{\rm a}$	$30.5 \pm 3.19^{\rm bc}$	$29.2 \pm 0.38^{\rm bc}$
Crude lipid (g 100 $g^{-1}$ )	$1.3 \pm 0.18^{\rm a}$	$1.38 \pm 0.15^{\rm ac}$	$1.36 \pm 0.15^{\rm ac}$
Crude fibre (g 100 $g^{-1}$ )	$1.7 \pm 0.23^{a}$	$1.66 \pm 0.27^{\rm ac}$	$0.96 \pm 0.21^{bd}$
Ash (g 100 g <sup>-1</sup> )	$3.08 \pm 0.14^{\rm a}$	$3 \pm 0.16^{\rm ac}$	$3.1 \pm 0.16^{\rm ac}$
Crude carbohydrates (g 100 $g^{-1}$ )	$52.8 \pm 0.74^{\rm a}$	$65.3 \pm 2.44^{\rm bc}$	$65.4 \pm 0.38^{\rm bc}$
Energy value (kJ 100 $g^{-1}$ )	$1520 \pm 6.1^{a}$	$1618 \pm 8^{bc}$	$1630 \pm 8^{bd}$

<sup>A</sup> Figures across the columns with different letters are significantly different (p < 0.05, t-test).

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Table 5							
Amino acid composition of	acid-hvdrolvzed	seed flour of	Canavalia	cathartica (g	$100 \text{ g}^{-1}$	protein: $n = 3$ .	mean)

Amino acid	Raw seeds		Roasted seeds		Cooked seeds		FAO/WHO pattern <sup>b</sup>	Whole egg protein <sup>c</sup>
	Content	EAA score <sup>a</sup>	Content	EAA score <sup>a</sup>	Content	EAA score <sup>a</sup>		
Glutamic acid	13.9		9.8		8.43			12.7
Aspartic acid	17.4		7.5		6.23			9.6
Serine	4.16		2.78		2.42			7.6
Threonine	3.77	111	2.02	59.4	1.82	53.5	3.4	5.1
Proline	3.80		2.96		2.47			4.2
Alanine	4.35		2.87		2.55			5.9
Glycine	3.68		2.2		2.03			3.3
Valine	4.79	137	2.9	82.9	2.23	63.7	3.5	6.9
Cystine	5.14		4.13		3			5.9
Methionine	1.72	274	1.47	224	1.11	164	2.5 <sup>d</sup>	3.4
Isoleucine	4.13	148	2.57	91.8	1.9	67.9	2.8	6.3
Leucine	9.39	142	5.1	77.3	3.9	59	6.6	8.8
Tyrosine	3.27		0.28		0.24			4.2
Phenylalanine	5.89	146	5.8	96.5	5.65	93.5	6.3 <sup>e</sup>	5.7
Tryptophan	ND		ND		ND		1.1	1.7
Lysine	11.8	204	6.05	104	4.58	79	5.8	7
Histidine	ND		ND		ND		1.9	2.4
Arginine	3.18		2.96		1.92			6.1

ND, not detected.

<sup>a</sup> EAA score, essential amino acid score (FAO/WHO, 1991).

<sup>b</sup> FAO/WHO pattern (FAO/WHO, 1991).

<sup>c</sup> Whole egg protein (FAO, 1968).

<sup>d</sup> Methionine + cystine.

<sup>e</sup> Phenylalanine + tyrosine.

Amino acid compositions of the raw and processed seed flours (in g 100 g<sup>-1</sup> true protein), whole egg protein (FAO, 1968) and FAO/WHO reference pattern EAA (FAO/WHO, 1991) are given in Table 5. Essential amino acids, leucine, phenylalanine and lysine, exceeded

# Table 6

Fatty acid composition of seed flours of *Canavalia cathartica* (mg g<sup>-1</sup> lipid; n = 3, mean)

Fatty	Raw	Roasted	Cooked seeds
acid	seeds	seeds	
Saturated fatty acids			
Lauric acid $(C_{12:0})$	_	_	0.2
Tridecanoic acid $(C_{13:0})$	_	0.53	0.34
Myristic acid (C <sub>14:0</sub> )	_	0.01	_
Pentadecanoic acid (C <sub>15:0</sub> )	_	0.64	0.5
Stearic acid $(C_{18:0})$	281.6	0.22	0.02
Tricosanoic acid $(C_{23:0})$	_	_	0.01
Pentacosanoic acid (C <sub>25:0</sub> )	_	1.56	_
Polyunsaturated fatty acids			
Myristoleic acid $(C_{14:1})$	_	1.1	0.77
Palmitoleic acid (C <sub>16:1</sub> )	_	56.73	40.68
Oleic acid $(C_{18:1})$	714	3.34	0.14
Linoleic acid (C <sub>18:2</sub> )	_	81.35	76.35
Linolenic acid $(C_{18:3})$	_	-	33.64
Eicosadienoic acid (C <sub>20:2</sub> )	_	35.72	19.57
Arachidonic acid (C <sub>20:4</sub> )	_	_	0.08
Eicosapentaenoic acid (C <sub>20:5</sub> )	_	0.4	0.6
Sum of saturated fatty acids	281.6	2.96	1.07
Sum of polyunsaturated fatty acids	714	178.64	171.83
P/S ratio <sup>a</sup>	2.53	60.35	160.59

-, not detected.

<sup>a</sup> Ratio of polyunsaturated/saturated fatty acids.

those of whole egg protein. In raw seeds, most EAA are above the FAO/WHO pattern (FAO/WHO, 1991) and EAA score ranged between 111 (threonine) and 274 (methionine). There was only minimal loss of cystine + methionine, isoleucine, tyrosine + phenylalanine and lysine, even after processing. The EAA score ranged between 59 (threonine) and 224 (methionine) in roasted seeds while, in cooked seeds, it was 54 (threonine) and 164 (methionine). Fatty acid composition revealed that stearic and oleic acid were higher in raw seeds (Table 6). Cooked seeds possessed the essential fatty acids, linoleic, linolenic and arachidonic acid, while roasted seeds had only linoleic acid. The polyunsaturated to saturated fatty acid ratio progressively increased from raw (2.5), roasted (60.4) to cooked (161) seeds.

Table 7

Antinutritional components of seed flour of *Canavalia cathartica* (total phenolics: g 100 g<sup>-1</sup>; n = 5; mean ± SD)

		,	
Component	Raw seeds	Roasted seeds	Cooked seeds
Total phenolics <sup>a</sup> Tannins Trypsin inhibition activity	1.49 ± 0.01 <sup>a</sup> NP NP	1.53 ± 0.02 <sup>bc</sup> NP NP	1.29 ± 0.06 <sup>bd</sup> NP NP
Phytohemagglutinin activity	+++	++	++

NP, not present; +++, red blood cells clumped strongly; ++, clumpy patches.

<sup>a</sup> Figures across the columns with different letters are significantly different (p < 0.05, *t*-test).

A slight decline in total phenolics was seen between raw (1.5%) and cooked (1.3%) seeds (Table 7). Significant difference (p < 0.05) was seen in total phenolics between raw and processed and within-processed seeds. Tannins and trypsin inhibitors were absent. Proteins of raw seeds showed strong agglutination of rabbit RBC, while processing decreased the extent of agglutination.

#### 4. Discussion

Crude protein of raw and processed seeds of C. cathartica is higher than or equivalent to common pulses: Cajanus cajan, Cicer arietinum, Phaseolus aureus, Phaseolus mungo and Vigna anguiculata (Gupta & Wagle, 1978; Jambunathan & Singh, 1980; Nwokolo, 1987; Nwokolo & Oji, 1985) and wild legumes: Bauhinia spp., Canavalia spp., Mucuna spp. and Vigna spp. of India (Rajaram & Janardhanan, 1993). Carbohydrate was higher, while crude lipid, crude fibre and gross energy values were lower than Canavalia ensiformis and Canavalia gladiata, respectively (Rajaram & Janardhanan, 1993). Although crude lipid of raw seeds was low, oleic acid content exceeded that of C. ensiformis and C. glad*iata.* (714 vs. 225–368 mg  $g^{-1}$  lipid) (Mohan & Janardhanan, 1994). Even though cooking drastically drained some of the minerals, sodium and manganese exceeded values in raw seeds of C. ensiformis and C. gladiata (Rajaram & Janardhanan, 1993).

Globulin concentrations of C. cathartica were higher than C. ensiformis and C. gladiata, while albumins were higher than C. gladiata (Rajaram & Janardhanan, 1993). Amino acid profile of raw seeds of C. cathartica is more or less higher or on a par with whole egg protein (FAO, 1968). The EAA of raw seeds surpassed the FAO/WHO pattern (FAO/WHO, 1991). Cystine + methionine, isoleucine, tyrosine + phenylalanine and lysine in roasted seeds and cystine + mehtionine and tyrosine + phenylalanine in cooked seeds were higher or almost equivalent to the FAO/WHO pattern. The EAA of raw seeds of C. cathartica is higher than that of C. ensiformis and C. gladiata of India (Rajaram & Janardhanan, 1992a) and Central America (Bressani et al., 1987). Valine, methionine, leucine, phenylalanine and lysine exceeded values in *Glycine max* (Bau et al., 1994), while threonine, leucine, phenylalanine and lysine were higher than Oryza sativa (Ekanayake et al., 1999). Cystine + methionine and phenylalanine concentrations in processed seeds were more than those in raw seeds of C. ensiformis and C. gladiata of India (Rajaram & Janardhanan, 1992a). Methionine and lysine concentrations in raw and processed seeds in our study were higher than or equivalent to those in many wild legumes of India  $(4.6-11.8 \text{ vs. } 5.2-6.7 \text{ g} 100 \text{ g}^{-1} \text{ protein})$  (Mohan & Janardhanan, 1995; Rajaram & Janardhanan, 1992b; Siddhuraju et al., 1995; Vijayakumari et al., 1993). Methionine and phenylalanine concentrations of processed seeds were above those in Glycine max (Bau et al., 1994); so also were phenylalanine and lysine of O. sativa (Ekanayake et al., 1999). Albumins and globulins of C. cathartica seeds constituted the major seed proteins. High albumin content might have resulted in elevated EAA in C. cathartica. According to Murray & Roxburgh (1984), high levels of albumins are nutritionally significant, due to elevated sulphur-containing amino acids (cystine and methionine). However, C. ensiformis and C. gladiata of India and Central America were deficient in sulphur-containing amino acids (Bressani et al., 1987; Rajaram & Janardhanan, 1992a). Our study revealed adequate cystine and methionine in raw, as well as processed, seeds of C. cathartica (1.11-5.14 g 100 g<sup>-1</sup> protein). Bressani et al. (1987) demonstrated significant improvement of nutritional quality of raw seeds of C. ensiformis and C. gladiata by pressure cooking and roasting (protein digestibility: 47.9 vs. 76.4–78.7%). Methionine supplementation has been advocated by Bressani et al. (1987) to meet the deficiency of sulphur-containing amino acids Canavalia seeds. However, fortification is difficult in legumes if cooked prior to consumption according to Salunke (1982). Such supplementation is not necessary as adequate amounts of cystine and methionine are present in cooked seeds of C. cathartica. Unsaturated fatty acids in human diet lower the risk of cardiovascular diseases (Ezeagu, Petzke, Lange, & Metgea, 1998). Roasting and cooking of C. cathartica seeds elevated unsaturated fatty acids (P/S ratio: raw, 2.5; roasted 60.4; cooked, 161). Cooked seeds consisted of essential fatty acids, linoleic, linolenic and arachidonic acids.

Antinutritional factors, such as amylase inhibitors, antivitamin factors, cyanogens, goitrogens, lectins and tannins are heat-labile (Liener, 1980), while alkaloids, cyanogenic glucosides, pyrimidine glucosides, flavones, isoflavones, saponins and toxic amino acids are heat-stable (Ezeagu et al., 1998). In the current study, out of the antinutritional factors analyzed, tannins and trypsin inhibitor activities were absent and total phenolics were low. Strong hemagglutinating activity of raw seed proteins decreased after processing. Hemagglutinins (lectins) combine with cells lining the intestinal mucosa and interfere with nutrient absorption. It is possible to inactivate lectins of seeds of C. cathartica by dry or wet heat treatments as advocated by Liener (1980). In fact, boiling C. cathartica seeds of Nigeria at 100 °C for 3 h inactivated lectins (Akpapunam & Sefa-Dedeh, 1997).

Evaluation of nutritional features of raw and processed *C. cathartica* seeds of coastal sand dunes in the current study revealed that these seeds are promising sources of high protein, carbohydrate, energy, EAA and low saturated fatty acids. To meet mineral deficiency, processed seeds of *C. cathartica* have to be supplemented. *C. cathartica* possesses agronomical features desirable for cultivation in tropical habitats. Further investigations, directed toward improvement of nutritional qualities through simple traditional processes, might help in developing *C. cathartica* for an alternative protein diet.

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# References

- Akpapunam, M. A., & Sefa-Dedeh, S. (1997). Some physiological properties and antinutritional factors of raw, cooked and germinated jack bean (*Canavalia ensiformis*). Food Chemistry, 59, 121–125.
- 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. (1990). Official methods of analysis (15th ed.). Washington, DC, USA: Association of Official Analytical Chemists.
- APHA. (1995). Standard methods for examination of water and waste water (19th ed.). USA: American Public Health Association.
- Arun, A. B., Beena, K. R., Raviraja, N. S., & Sridhar, K. R. (1999). Coastal sand dunes – A neglected ecosystem. *Current Science*, 77, 19–21.
- Basha, S. M. M., Cherry, J. P., & Young, C. T. (1976). Changes in free amino acid, carbohydrates and proteins of maturing seeds from various pea nut (*Arachis hypogaea* L.) cultivars. *Cereal Chemistry*, 53, 586–597.
- Bau, H. M., Vallaume, C. F., Evard, F., Quemener, B., Nicolas, J. P., & Mejean, L. (1994). Effect of solid state fermentation using *Rhizophus oligosporus* sp. T-3 on elimination of antinutritional substances and modification of biochemical constituents of defatted rape seed meal. *Journal of Science of the Food and Agriculture*, 65, 315–322.
- Bressani, R., Brenes, R. S., Gracia, A., & Elias, L. G. (1987). Chemical composition, amino acid content and protein quality of *Canavalia* spp. seeds. *Journal of Science of the Food and Agriculture*, 40, 17–23.
- Coulter, J. B., Suliman, G. I., Omer, M. I., MacFarlane, S. B., Moody, J. B., & Hendrickse, R. G. (1988). Protein-energy malnutrition in northern Sudan: Clinical studies. *European Journal of Clinical Nutrition*, 42, 787–796.
- Ekanayake, S., Jansz, E. R., & Nair, B. M. (2000). Literature review of an underutilized legume: *Canavalia gladiata* L. *Plant Foods for Human Nutrition*, 55, 305–321.
- Ekanayake, S., Jansz, E. R., & Nair, B. M. (1999). Proximate composition, mineral and amino acid content of mature *Canavalia* gladiata seeds. Food Chemistry, 66, 115–119.
- Ezeagu, I. E., Petzke, K. J., Lange, E., & Metgea, C. C. (1998). Fat content and fatty acid composition of oils extracted from selected

wild gathered tropical plant seeds from Nigeria. Journal of the American Oil Chemists Society, 75, 1031–1035.

- FAO. (1968). Amino acid composition of foods and biological data on proteins. Rome, Italy: Food and Agricultural Organization of United Nations.
- FAO/WHO. (1991). Protein quality evaluation. Reports of a joint FAO/WHO Expert Consultation. Rome, Italy: Food and Agriculture Organization of the United Nations.
- Garces, R., & Mancha, M. (1993). One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Analytical Biochemistry*, 211, 139–143.
- Gorden, L. K. (1981). A reliable method for repetitively bleeding rabbits from the central artery of the ear. *Journal of Immunological Methods*, 44, 241–245.
- Gupta, C. N., & Wagle, D. S. (1978). Proximate composition and nutritive value of *Phaseolus mungoreus*. A cross between *Phaseolus mungo* and *Phaseolus aureus*. Journal of Food Science and Technology, 15, 34–35.
- Hagerman, A. E. (1987). Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology*, 13, 437–449.
- Hankins, C. N., Kindinger, J. I., & Shannon, L. M. (1980). Legume alphagalactosidases which have haemagglutinin properties. *Plant Physiology*, 65, 618–622.
- Hofmann, D., Gehre, M., & Jung, K. (2003). Sample preparation techniques for the determination of natural <sup>15</sup>N/14N variations in amino acids by gas chromatography–cumbustion–isotope ratio mass spectrometry (GC–C–IRMS). *Isotopes in Environmental and Health Studies*, 39, 233–244.
- Humphries, E. C. (1956). Mineral composition and ash analysis. In K. Peach & M. V. Tracey (Eds.). *Modern methods of plant analysis* (Vol. 1, pp. 468–502). Berlin: Springer.
- Jambunathan, R. & Singh, U. (1980). Studies on Desi and Kabuli chickpea (*Cicer arietinum*) cultivars. 1. Chemical composition. In *Proceedings of the international workshop on chickpea improvement*. ICRISAT, Hyderabad, India, February 28–March 2, 1979.
- Janardhanan, K., & Nalini, K. (1991). Studies on the tribal pulse *Entada scandens* Benth: Chemical composition and antinutritional factors. *Journal of Food Science and Technology*, 28, 249–251.
- 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.
- Laemmli, U. K. (1970). Cleavage of structure proteins during assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- Liener, I. (1980). Heat-labile antinutritional factors. In J. Summerfield & A. H. Bunting (Eds.), *Advances in legume science* (pp. 151–170). Kew: Royal Botanic Gardens.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with folin-phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
- Maikhuri, R. K., Nautiyal, M. C., & Khali, M. P. (1991). Lesserknown crops of food value in Garhwal Himalaya and a strategy to conserve them. FAO/IGPBR Plant Genet Resources Newsletter, 86, 33–36.
- Miersch, J., Kullertz, G., & Henning, H. (1998). Protein phosphorylation in polysomes of pumpkin cotyledons after coumarin treatment. *Botanica Acta*, 111, 316–324.
- Mohan, V. R., & Janardhanan, K. (1994). The biochemical composition and nutrient assessment of less known pulses of the genus *Canavalia*. *International Journal of Food Sciences and Nutrition*, 45, 255–262.
- Mohan, V. R., & Janardhanan, K. (1995). Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna. Food Chemistry*, 52, 275–280.
- Müller, H. G., & Tobin, G. (1980). Nutrition and food processing. London: Croom Helm Ltd..
- Murray, D. R. (1979). Seed proteins of Kowhai Sophora microphylla AIT. Zeitschrift Fur Pfalnzenphysiology, 93, 423–428.

- Murray, D. R., & Roxburgh, C. M. (1984). Amino acid composition of the seed albumins from chickpea. *Journal of Science of the Food and Agriculture*, 35, 893–896.
- Nwokolo, E. (1987). Nutritional evaluation of pigeon pea meal. *Plant Foods for Human Nutrition, 37*, 283–290.
- Nwokolo, E., & Oji, D. I. M (1985). Variation in metabolizable energy content of raw or autoclaved white and brown varieties of three tropical grain legumes. *Animal Food Science and Technology*, 13, 141–146.
- Olsen, R. E. (1975). *Protein-calorie nutrition*. New York, USA: Academic Press.
- Pandey, V. N., & Srivastava, A. K. (1990). Seed protein yield from some *Crotalaria* spp. and in vitro nutritional quality of that from *C. juncea. Plant Foods for Human Nutrition*, 40, 195–200.
- Pelletier, D. L. (1994). The potentiating effects of malnutrition on child mortality: epidemiologic evidence and policy implications. *Nutrition Review*, 52, 409–415.
- Rajaram, N., & Janardhanan, K. (1991). Chemical composition and nutritional potential of the tribal pulses *Bauhinia purpurea*, *B.* racemosa and *B. vahlii. Journal of Science of the Food and* Agriculture, 55, 423–431.
- Rajaram, N., & Janardhanan, K. (1992a). Nutritional and chemical evaluation of raw seeds of *Canavalia gladiata* (Jacq) DC. and *C.* ensiformis DC: The under utilized food and fodder crops in India. *Plant Foods for Human Nutrition*, 42, 329–336.
- Rajaram, N., & Janardhanan, K. (1992b). The chemical composition and nutritional potential of the tribal pulse *Abrus precatorius* L. *Plant Foods for Human Nutrition*, 42, 285–290.
- Rajaram, N., & Janardhanan, K. (1993). Ex situ conservation of genetic resources of tribal pulsed and their wild related species. *FAO/IBPGR Plant Genetic Resource News Letter*, 91/92, 29–32.
- Rao, P. U. (1994). Nutrient composition of some less-familiar oil seeds. *Food Chemistry*, 50, 379–382.

- Rosset, J., Bärlocher, F., & Oertli, J. J. (1982). Decomposition of conifer needles and deciduous leaves in two Black Forest and two Swiss Jura streams. *Internal Revue Gesamten Hydrobiologe*, 67, 695–711.
- Salunke, D. K. (1982). Legumes in human nutrition: Current status and future research needs. *Current Science*, 51, 387–394.
- Siddhuraju, P., Vijayakumari, K., & Janardhanan, K. (1992). The biochemical composition and nutritional potential of the tribal pulse *Alysicarpus rugosus* (Willd.) DC. *Food Chemistry*, 45, 251–255.
- Siddhuraju, P., Vijayakumari, K., & Janardhanan, K. (1995). Nutritional and antinutritional properties of the underexplored legumes *Cassia laevigata* Willd. and *Tamarindus indica* L. *Journal of Food Composition and Analysis*, 8, 351–362.
- Statosoft Inc. (1995). STATISTICA for Windows. Tulsa, Okla., USA.
- US National Academy of Sciences. (1975). Under-exploited tropical plants with promising economic value. Report of adhoc panel of the Advisory Committee on Technology Innovation, USA.
- Vadivel, V., & Janardhanan, K. (2001). Nutritional and anti-nutritional attributers of the under-utilized legume, *Cassia floribunda* Cav. Food Chemistry, 73, 209–215.
- Viano, J., Masotti, V., Gaydou, E. M., Bourreil, P. J. L., Ghiglione & Giraud, M. (1995). Compositional characteristics of 10 wild plant legumes from Mediterranean French pastures. *Journal of Agricultural and Food Chemistry*, 43, 680–683.
- Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1993). Chemical composition and nutritional potential of the tribal pulse (*Bauhinia malabarica* Roxb.). *Plant Foods for Human Nutrition, 44*, 291–298.
- Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. (1994). Nutritional assessment and chemical composition of the lesser known tree legume, *Acacia leucophloea. Food Chemistry*, 50, 285–288.