Nutritional qualities of ripened beans of mangrove wild legume *Canavalia cathartica* Thouars

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Raw and pressure-cooked ripened beans of *Canavalia cathartica*, a wild mangrove legume of southwest coast of India were evaluated for nutritional qualities. Crude protein of raw beans (25.9%) was higher than some edible legumes, wild legumes and cereals. Raw and cooked beans contained low crude lipids (1.5-2.7%), high crude fiber (8.3-9.1%), crude carbohydrates (58.5-73.3%), starch (44.7-52%) and calorific value (1516-1517 kJ/100 g). Magnesium, zinc and manganese of raw and cooked beans meet the NRC/NAS requirements for infants. Although cooking drained crude protein (25.9 to 13.8%) of beans, total sugars and non-reducing sugars did not alter. Among essential fatty acids, raw beans consisted of linoleic and linolenic acids, while in cooked beans linoleic and arachidonic acids. Except for sulphur-amino acids, tryptophan and histidine, both raw and cooked beans met the FAO/WHO/UNU essential amino acid (EAA) pattern for adults, adequate EAA score and protein digestibility corrected to amino acid score (PDCASS). Total phenolics and tannins in beans were low, while beans devoid of orthodihydric phenols and trypsin inhibitors. Phytohemagglutinin activity of raw beans decreased to about 50% on cooking. The *in vitro* protein digestibility (53.7 vs. 82.3%) and starch digestibility (1.01 vs. 6.64 mg maltose/hr/100 g) of raw beans drastically improved on cooking, indicating the possibilities to use the ripened beans as food for human or livestock after appropriate processing.

Key words: *Canavalia cathartica*, essential amino acids, fatty acids, mangroves, minerals, protein digestibility, ripened beans, starch digestibility, wild legume

Introduction

Developing countries have high demand for protein-rich food due to their teeming population, cereal-based diet and scarcity of fertile land (Sadik, 1991; Weaver, 1994). Even though 14 types of edible legumes are cultivated in India, protein-energy malnutrition is still one of its serious problems. Wild legumes are important for food security, nutrition, agricultural development and rotation of crops to improve the nation's economy. Many wild legumes are known for

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inexpensive proteins, calorific value, essential amino acids, essential fatty acids, fiber and vitamins. Among the legumes, the genus *Canavalia* consists of 51 species with a wide pantropical distribution (Smartt, 1990). *Canavalia cathartica* is an under explored wild legume in India and spread over in mangroves and sand dunes of the southwest coast (Arun *et al*., 1999, 2003; Bhagya *et al*., 2005). In and around mangroves and plantations adjacent to coastal sand dunes, *C. cathartica* grow naturally or cultivated for green manure and mulch. It occasionally serves as pasture for livestock and the coastal dwellers use the tender pods or ripened beans as vegetables (Arun *et al*., 1999; Seena and Sridhar, 2006). This study aimed to evaluate and compare the nutritional composition, *in vitro* protein and starch digestibility of raw and pressure-cooked ripened beans of mangrove *C. cathartica*.

Materials and methods

Beans and processing

Mature yellow pods of *C. cathartica* Thouars (Fig. 1a) were harvested during post-monsoon season (December, 2003) from the mangroves of the River Nethravathi (12º50'27" N, 74º51'45" E), Southwest India. The ripened beans (Fig. 1b) were separated from the pods and physical properties (fresh and dry weights and dimensions) were recorded. The beans were divided into two sets. The first set was sun dried and powdered (30 mesh, Wiley Mill) and stored in air-tight containers. Beans of the second set were pressure-cooked in household pressure-cooker with potable water (1:3 v/v), sun dried, powdered and stored.

Proximate composition and minerals

The moisture of the bean flours was assessed gravimetrically on drying at 100°C until attaining constant weight. Total nitrogen and the crude protein (N × 6.25) were determined by micro-Kjeldahl method (Humphries, 1956). Crude lipid (Soxhlet extraction), crude fiber and ash were estimated using AOAC (1990) methods.

Total carbohydrate was calculated based on Müller and Tobin (1980):

Total carbohydrates (%) = 100 – [crude protein (%) + crude lipid (%) + crude fiber (%) + ash (%)]

Gross energy was calculated according to Osborne and Voogt (1978):

Gross energy kJ/100g DM = [crude protein (%) × 4] + [crude lipid (%) × 9] + (crude carbohydrates (%) × 4]

To estimate ascorbic acid, a known volume of extracted sample (in 0.4% oxalic acid) was titrated against 2,6-dichlorophenol indophenol dye (Sigma) in the presence of 0.4% oxalic acid and ascorbic acid (Sigma) in 0.4% oxalic acid (0-500 μg) served as standard (Roe, 1954).

Fig. 1. Mature pods (a) and ripened beans (b) of mangrove wild legume *Canavalia cathartica* of Nethravathi mangrove, Southern India.

To extract minerals, the bean flours were digested with HCl (1:4 v/v), ashed and filtered. The sodium, potassium and calcium were determined by flame emission photometry (Systronics, Mediflame 127 Sr. # 2083, India) according to AOAC (1990). Magnesium, iron, copper, zinc, manganese and selenium were estimated using atomic absorption spectrophotometer (GBC 904AA, Germany) (AOAC 1990). The total phosphorus was determined as orthophosphate by ascorbic acid method after acid digestion and neutralization

using phenolphthalein indicator and combined reagent (APHA, 1995). The absorbance was read at 880 nm (Spectronic 21 D, Miltonroy, India) and KH_2PO_4 (Merck) served as standard.

Protein and carbohydrate fractions

Total protein of raw and cooked bean flours was extracted as per Basha *et al*. (1976) with a slight modification. The protein fractions were extracted (1:10 w/v) with different solvents (albumins, distilled water; globulins, 0.25M NaCl; prolamins, 70% ethanol; glutelins, 0.05N NaOH), incubated (28°C, 1hr) and centrifuged (20,000 g, 15 min). Extracted proteins in the supernatants were precipitated with 10% trichloroacetic acid (TCA) (Merck), centrifuged (20,000 g, 10 min) and decanted. The precipitate was digested to determine nitrogen (Humphries, 1956) and protein (N×6.25). The non-protein nitrogen was estimated on precipitating protein in bean flour (100 mg in 10 ml 10% TCA) (Sadasivam and Manickam, 1992), centrifuged, supernatant was collected and the process was repeated. The pooled supernatant was made up to 25 ml with TCA (10%) and nitrogen was estimated by micro-Kjeldahl method (Humphries, 1956).

To estimate the total sugars, defatted flour (100 mg) was extracted with 30 ml ethanol (80%) on boiling (10 min) (Dubois *et al*., 1951). Cooled ethanol extract was decanted, concentrated (70-80ºC) and distilled water (5 ml) was added. To the above mixture Dowex 50 H^+ (cationic gel) (Sigma) (3 ml) was added, shaken (1 hr, 28ºC) and allowed to settle (4ºC, 12 hr). An anionic gel, Dowex 1 (formate-form) (Sigma) (3 ml) was added to the decanted supernatant and the process was repeated as with cationic gel. The supernatant was made up to 5 ml with distilled water and total sugars were estimated according to Dubois *et al*. (1951).

Reducing sugars were estimated based on Nelson (1944). Supernatant with free sugars (1 ml) was mixed with reaction mixture (1 ml) [25:1, copper reagent A (sodium carbonate, 25 g; sodium potassium tartrate salt, 25 g; sodium bicarbonate, 20 g and sodium sulphate, 200 g in 800 ml distilled water diluted to 1000 ml) copper reagent B (15% copper sulphate, in 2 drops of concentrated sulphuric acid/100 ml)] and boiled (20 min). On cooling, arsenomolybdate reagent (1 ml) was added and the volume was made up to 25 ml with distilled water. The absorbance was read after 15 minutes at 500 nm using spectrophotometer (Spectronic 21 D, Miltonroy, India) with maltose (Sigma) (0-100 μg) as standard. Non-reducing sugars was calculated by subtracting reducing sugar from total sugars.

Starch was determined based on the method by Clegg (1956). Defatted bean flour (100 mg) was extracted on boiling (10 min) with ethanol (30 ml, 80%), dried (70°C, 4 hr) residue was digested (28°C, 15 min) with $HClO_4$ (52%, 10 ml), made up to 25 ml with distilled water and filtered (Whatman # 1). Total sugars and starch (total sugars × 0.9) was determined by Dubois method (1951). To a known volume of digested sample (20 µl made up to 1 ml with distilled water), phenol (5%, 1 ml) and H_2SO_4 (36 N, 5 ml) was added and the absorbance was read at 490 nm using spectrophotometer (Spectronic 21 D, Miltonroy, India) with D-Glucose (Sigma) (20-100 µl) as standard.

Amino acids and fatty acids

The method outlined by Hofmann *et al*. (1997, 2003) was followed to determine amino acids of the bean flours. Known quantity of samples was hydrolyzed (4 hr, 145ºC) with 6 N HCl (15 ml). After cooling, HCl was eliminated in a rotoevaporator (Büchi Laboratoriumstechnik AG RE121; Switzerland) combined with a diaphragm vacuum pump (MC2C; Vacuubrand GmbH, Germany). Trans-4-(Aminomethyl)-cyclohexanecarboxylic acid (Aldrich, 85765-3; 97% purity) was added to each sample as internal standard. Derivatization step was done by esterification with trifluoroacetylation (Brand *et al*., 1994). Standards were dried using CH_2Cl_2 under a gentle stream of helium and slow heating in an oil bath (40-60°C) to remove traces of water. Freshly prepared acidified isoporpanol (acetyl chloride, 3 ml + 2-propanol, 12 ml) was added (12 ml) and the mixture was heated (110°C, 1 hr). The cooled samples were filtered through glass fiber paper and the reagent was eliminated with a gentle stream of helium (60°C), followed by combined evaporation with aliquots of CH_2Cl_2. Dried residue was acetylated with trifluoroacetic anhydride (200 µl) overnight at room temperature. Amino acids were determined using a Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometer (GC-C-IRMS/MS). The GC-C-IRMS/MS measurements were carried out with a Hewlett-Packard 58590 II gas chromatograph, connected via a split with a combustion interface to the IRMS system (GC-C-II to MAT 252, Finnigan MAT; Germany) for the isotopic determination of nitrogen and via a transfer line with a mass spectrometer (GCQ, Finnigan MAT; Germany) for qualitative analysis and quantification of the amino acids. The capillary column of GC was a 50 m × 0.32 mm i.d. × 0.5 µm BPX5 (SGE), operating with the carrier gas flow of 1.5 ml/min with following temperature and pressure: initial 50°C (1 min), increased to 100°C at 10°C/min (10 min), increased to 175°C at 3°C/min (10 min), increased to 250°C/min (10 min); head pressure, 13 psi (90 kpa).

The essential amino acid (EAA) score was determined as follows: EAA score = [EAA in 100 g test protein (g)] ÷ [EAA in 100 g FAO/WHO/UNU (1985) reference pattern (g)] × 100

Fatty acid methyl esters (FAMEs) were estimated by the method followed by Garces and Mancha (1993). The bean flours (50 mg) along with standard fatty acids [American Oil Chemists Society (AOCS); Merck, Germany] were taken in tubes with teflon-lined caps and methylated with a mixture containing methanol; benzene; 2, 2-dimethoxypropane (DMP) and H_2SO_4 (37:20:5:2) (v/v). The sample was placed in water bath at 80ºC for 2 hr and the cooled mixture was made up to a total volume of 5 ml with heptane and shaken to separate two phases. The upper layer containing FAMEs was injected (1 ml) to Gas liquid chromatograph (GLC) (Sigma Instruments, Baroda, India) in a glass column (Silar, 10%) packed with ethylene glycol succinate (5%) on Supelcoport (80/100 isothermically, 200ºC). Analysis was performed at the following conditions: carrier gas, N_2; injector temperature, 225ºC; FID detector temperature, 265ºC; oven temperature, 200ºC; flow rate, N_2, 35 ml/min; H_2, 30 ml/min; O_2, 75 ml/min. Polyunsaturated and saturated fatty acid ratio (P/S ratio) was calculated on dividing sum of saturated fatty acids by sum of polyunsaturated fatty acids.

Antinutritional factors

Total phenols of beans were estimated as per Rosset *et al*. (1982). A known volume (0.5 ml) of extracted sample (50% methanol, 95ºC, 10 min) was mixed with equal quantity of distilled water and treated with Na_2CO_3 (in 0.1 N NaOH, 5 ml). After 10 min, Folin-Ciocalteus reagent (diluted 1:2 with distilled water, 0.5 ml) (Merck) was added and read at 725 nm with tannic acid (Merck) as standard.

Orthodidydric phenols were estimated by the method of Mahadevan (1966). Defatted bean flour was fractionated by ion exchange chromatography using both cation and anion exchange resins. The anionic gel was extracted with formic acid (6 N) and made up to a known volume (1 ml). Formic acid extract (250 µl) was mixed with hydrochloric acid (0.05 N, 1 ml), Arnow's reagent (1 ml) (sodium nitrate, 10 g; sodium molybdate, 10 g in distilled water, 100 ml), distilled water (10 ml), and sodium hydroxide (1 N, 2 ml). The pink color developed immediately after the addition of the alkali was read at 515 nm using spectrophotometer (Spectronic 21 Miltonroy) with caeffic acid (Sigma) (20-100 µl) as standard.

Tannins were determined by vanillin-HCl method (Burns, 1971). The bean sample (1 g) was treated with methanol (10 ml, 28ºC, 12 hr), vortexed

and decanted. The process was repeated with the precipitate. The pooled supernatant was made up to 25 ml. The extract (1 ml) was treated with reagent mixture (5 ml) (1:1, 4% vanillin in methanol and 8% concentrated HCl in methanol). After 20 min the color developed was read at 500 nm (Spectronic 21, Miltonroy, India) with catechin (Sigma) (50-250 µg) as standard.

The trypsin inhibitory activity was assayed by enzymatic method of Kakade *et al*. (1974). Known quantity of raw and cooked bean flours (1 g) was extracted with NaOH (0.01 N, 50 ml) and made up to 2 ml with distilled water. Trypsin solution (2 ml) (4 mg in 200 ml 0.001 M HCl) was added to each sample and incubated (37ºC, 10 min). To each tube, BAPNA (5 ml) (40 mg N-a-Benzoyl-DL-Arginine p-nitroanilide hydrochloride (Aldrich, 85711-4; purity, ≥99%) in dimethyl sulfoxide (1 ml) diluted to 100 ml with tris buffer (37ºC) was added and later (10 min) the reaction was terminated by adding acetic acid (30%, 1 ml), mixed thoroughly, filtered and the absorbance was measured at 410 nm against reagent blank (1 ml) [(acetic acid, 30% + trypsin (2 ml) + distilled water (2 ml) + BAPNA (5 ml)].

To test phytohemagglutination activity, trypsin-treated suspension of rabbit erythrocyte was used (Hankins *et al*., 1980). Alsever's solution (glucose, 60 mM; citric acid, 40 mM; NaCl, 70 mM) was used as an anticoagulant (pH 6.1, autoclaved). Three ml blood was collected from six-month-old rabbit (New Zealand White) by ear vein puncturing and taken into a graduated tube containing Alsever's solution (1 ml). The blood suspension was mixed gently and centrifuged (1,000 g, 5 min, 4ºC). It was rinsed thrice with phosphate buffered saline (PBS) (sodium phosphate, 10 mM; NaCl, 150 mM; pH 7.2) and centrifuged (1000 g, 5 min, 4ºC). The erythrocytes were treated with trypsin (50 µg/ml) (0.04 BTEE units/mg solid) (1 hr, 28ºC) and centrifuged to remove trypsin. Trypsinized erythrocytes were washed thrice with excess PBS by centrifugation to prepare 2% cell suspension (Hankins *et al*., 1980). Two-fold serial dilutions of 25 µl (50 mg sample/ml PBS) crude lectin in saline (0.3 M NaCl) were mixed with trypsinized rabbit erythrocyte suspension (50 µl, 2%) on hemagglutination slabs and incubated (28ºC, 30 min). The slabs were observed for agglutination under a low power microscope. The highest dilution, which showed positive hemagglutination was considered as the titre value. The amount of protein present at this dilution represents nearly the minimum quantity of protein necessary for agglutination and is defined as one unit. Specific activity is defined in this method as the number of units per milligram protein.

Protein and starch digestibility

The *in vitro* protein digestibility was estimated according to Akeson and Stahmann (1964). Samples (100 mg each) defatted test flours were incubated (37°C, 3 hr) with pepsin (Sigma, 3165 units/mg protein) (1.5 mg/2.5 ml 0.1N HCl) followed by inactivation (0.25 ml 1N NaOH). Incubation was continued (24 hr, 37°C) with trypsin (Sigma, 16,100 units/mg protein) and α-chymotrypsin (Sigma, 76 units/mg protein) (2 mg each/2.5 ml potassium phosphate buffer, pH 8.0, 0.1M) followed by inactivation (0.7 ml 100% TCA). Zero-time control was maintained by inactivating the enzyme before addition of substrate. The inactivated reaction mixtures were centrifuged and supernatant was collected. The residue was washed (2 ml 10% TCA) and centrifuged. The combined supernatant was extracted with 10 ml diethyl ether twice and ether layer was removed by aspiration. The aqueous layer was heated on a boiling water bath (15 min) to remove traces of ether. The solution is made up to 25 ml with distilled water. Nitrogen (in 5 ml aliquots) was determined to calculate protein in the digest as follows:

In vitro Protein Digestibility (%) = (Protein in digest) ÷ (Protein in defatted flour) × 100

The protein digestibility corrected amino acid score (PDCAAS) of EAA was calculated based on EAA requirements for adults (FAO/WHO/UNU 1985) as follows:

PDCAAS (%) = [EAA in 100 g test protein (g)] ÷ [EAA in 100 g FAO/WHO/UNU (1985) reference pattern (g)] × D

where, D is the *in vitro* protein digestibility (%).

The *in vitro* digestibility of starch was estimated based on Bergmeyer (1984). Samples (100 mg each) of defatted test flour were incubated (37ºC, 3 hr) with diastase [(α-amylase, 1300 units/g) (Hi-Media, Mumbai, India) (2 mg/12.5 ml 0.02 M potassium phosphate buffer, pH 7.0)] followed by inactivation with NaOH (0.5 N, 1 ml). Zero-time control was maintained by inactivating the enzyme prior to addition of substrate. The inactivated reaction mixture was centrifuged and supernatants were made up to 10 ml with distilled water. Maltose liberated by the enzyme was estimated according to Nelson (1944) and percentage hydrolysis was calculated.

Statistical analysis

The differences in proximate composition, minerals, protein and carbohydrate fractions, amino acids, fatty acids, antinutritional factors of raw beans vs. cooked beans were assessed by paired t-test (Stat Soft Inc., 1995).

The paired t-test was also employed for comparison of starch digestibility between raw and cooked mangrove beans.

Results and discussion

Physical features, proximate composition and minerals

Physical features of ripened beans (n=20) showed that mean fresh weight per seed (1.88±0.39 g) is about thrice higher than dry weight (0.59±0.16 g). Cotyledon dry weight was 0.43±0.15 g (73%), while seed coat 0.16±0.02 g (27%). Length, width, thickness and hilum length were 2.31±0.22, 1.3±0.07, 0.95±0.17 and 1.23±0.14 cm respectively. Percent moisture of cooked and dried bean flours was lower than dried raw bean flours (5.4 vs. 6.3%) (Table 1) and thus low moisture of bean flours possess extended shelf life.

Table 1. Proximate composition of ripened beans and dry seed flours of *Canavalia cathartica* on dry weight basis (n=5; mean±SD).

Component	Ripened beans	
	Raw	Cooked
Moisture (%)	6.34±0.7[a]	5.43±0.7[a]
Crude protein (g/100 g)	25.9±0.48[a]	13.83±0.73[b]
Crude lipid (g/100 g)	2.74±0.71[a]	1.54±0.27[b]
Crude fiber (g/100 g)	9.06±0.59[a]	8.34±0.18[b]
Ash (g/100 g)	3.76±0.29[a]	3±0.16[b]
Crude carbohydrates (g/100 g)	58.54±0.97[a]	73.3±0.76[b]
Energy (kJ/100 g)	1516±18.99[a]	1517±5.73[a]
Vitamin C (mg/100 g)	0.256±0.02[a]	0.058±0.02[b]

Figures across the column with different letters are significantly different ($p<0.05$, paired t-test).

The crude protein of raw beans (25.9%) was comparatively higher than many conventional edible legumes (19.4-25.3%) (e.g. *Cajanus cajan, Cicer srietinum, Phaseolus aureus, P. mungo, Vigna unguiculata*, lentils, and red and white kidney beans) (Gupta and Wagle, 1978; Jambunathan and Singh, 1980; Nwokolo and Oji, 1985; Nwokolo, 1987; Rehman and Shah, 2005), some wild legumes (14%-24.8%) (e.g. *Atylosia scarbaeoides, Erythrina indica, Lablab purpureus, Neonotonia wightii, Rhynchosia filipes, Tamarindus indica, Vigna trilobata*) (Arinathan *et al*., 2003; Puglenthi *et al*., 2004), common cereals (7.7-8.6%) (wheat, parboiled rice) and egg (12.6%) (Livsmelelsverk, 1988) (Table 1). However, proteins in ripened raw beans of mangrove *C. cathartica* were comparable with seeds of *Canavalia ensiformis*, *C. gladiata* and *C. maritima*

(22.4-35.3%) (Sridhar and Seena, 2006). The crude lipid was higher in raw beans than cooked beans (2.7 vs. 1.5%), so also dry seeds of *Canavalia maritima, C. cathartica* and *C. brasiliensis* (1.3–1.9%) (Gomes *et al*., 1988; Mayworm *et al*., 1998; Arun *et al*., 2003; Seena and Sridhar, 2006). The mangrove beans of *C. cathartica* are valuable due to high protein and low fat in human diet formulations. The crude fiber falls within the range of *C. ensiformis* and *C. gladiata* (8.3-9.06 vs. 2.1-12.8%) (Sridhar and Seena, 2006). High crude fiber in diet is known to enhance the digestibility and promote health benefits such as decreasing the blood cholesterol and reduce the risk of large bowel cancers (Anderson *et al*., 1995; Salvin *et al*., 1997). The ash in raw beans declined on cooking (3.8 vs. 3%) possibly due to drainage of minerals. Total carbohydrate was higher than *C. brasiliensis, C. cathartica, C. ensiformis, C. maritima* (58.5 vs. 50.5-52.8%) (Gomes *et al*., 1988; Mayworm *et al*., 1998; Seena *et al*., 2005) and within the values recorded for *C. ensiformis* and *C. gladiata* (50.5-65.8%) (Sridhar and Seena, 2006). Cooking of beans raised the crude carbohydrates (58.5 vs. 73.3%) possibly due to breakdown of complex polysaccharides, draining of proteins and minerals. Legume carbohydrates are known to reduce the plasma cholesterol and gradually elevate the blood glucose levels (Leeds, 1982; Walker, 1982). Calorific value of *C. cathartica* beans (1516-1517 kJ/100 g) did not altered much on cooking and higher than commonly cultivated pulse crops (1358-1426 kJ/100 g) (Kuzayali *et al*., 1966). Vitamin C of beans is lower than green gram, bengal gram and horse gram (0.26 vs. 2.4-3.9 mg/100g) (Naveeda and Jamuna, 2005).

Among the minerals in raw beans, calcium was highest (380.5 mg/100g) followed by magnesium (122.7 mg/100 g) and phosphorus (107.4 mg/100 g) (Table 2). The difference in minerals in *Canavalia* spp. has been attributed by Vadivel and Janardhanan (2001) to genetic origin, geographical source, soil fertility levels and efficiency of mineral uptake by plants. Magnesium, zinc and manganese of raw as well as cooked beans meet the NRC/NAS (1989) requirement pattern for infants. Selenium (46 mg/100 g) was completely drained on pressure-cooking. Magnesium, zinc and selenium are known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal atrophy, impaired spermatogenesis, congenital malformations and bleeding disorders (Chaturvedi *et al*., 2004). Selenium also acts as antioxidant and protects cells against free radicals (Combs and Gray, 1998) and also prevents the toxic effects of heavy metals (arsenic, cadmium, mercury and tin). Alternative thermal treatments such as extrusion cooking might prevent mineral draining in ripened beans.

Table 2. Mineral compositions of ripened beans and dry seed flours of *Canavalia cathartica* on dry weight basis (mg/100 g) (n=5; mean±SD).

Mineral	Ripened beans		NRC/NAS pattern for infants*
	Raw	Cooked	
Sodium	49.83±0.6[a]	40.9±1.09[b]	120-200
Potassium	94.71±7.24[a]	85.89±2.49[b]	500-700
Calcium	380.46±1.58[a]	241.75±1.43[b]	600
Phosphorus	107.39±7.83[a]	88.15±6.64[b]	500
Magnesium	122.65±2.27[a]	103.56±5.62[b]	60
Iron	1.72±0.04[a]	0.874±0.04[b]	10
Copper	0.183±0.004[a]	0.114±0.02[b]	0.6-0.7
Zinc	18.38±0.43[a]	14.44±1.29[b]	5
Manganese	8.15±0.08[a]	7.06±0.07[b]	0.3-1
Selenium	45.98±0.78[a]	0	-

*NRC/NAS (1989)

Figures across the column with different letters are significantly different ($p<0.05$, paired t-test).

Proteins and carbohydrate fractions

True protein in raw beans drastically decreased on cooking (21.9 vs. 12.5%) (Table 3). In raw beans, globulins (12%) were the major protein fraction followed by albumins (6.1%), however, cooking decreased all protein fractions of beans. Albumins are known to be rich in sulfur-amino acids and the EAA (Baudoin and Maquet 1999). Albumins in raw beans decreased to about one-third on pressure-cooking in our study, so also non-protein nitrogen. Starch in raw beans increased (44.7 vs. 52%), while total sugars (2.27 vs. 2.25%) and non-reducing sugars (2.1 vs. 2.2%) did not severely altered on cooking (2.27 vs. 2.25%), but reducing sugars decreased (0.2 vs. 0.1%). Low digestibility of starch is known to promote slow and moderate post-prandial glucose and insulin responses (Jenkins *et al.*, 1980) and thus beneficial in management of diabetes and hyperlipidemia (Jenkins *et al*., 1998, 1994).

Table 3. Protein and carbohydrate fractions (g/100 g) of ripened beans and dry seed flours of *Canavalia cathartica* on dry weight basis (n=5; mean±SD; percent in parenthesis).

Protein/carbohydrate fraction	Ripened beans	
	Raw	Cooked
Total protein	21.9±0.46[a] (100)	12.52±0.46[b] (100)
Albumins	6.08±0.37[a] (27.76)	2.69±0.43[b] (21.47)
Globulins	11.99±0.48[a] (54.75)	8.14±0.12[b] (64.96)
Prolamins	0.8±0.05[a] (3.65)	0.45±0.05[b] (3.59)
Glutelins	3.01±0.13[a] (13.74)	1.26±0.16[b] (10.06)
Starch	44.69±2.92[a]	52.03±0.7[b]
Non-protein nitrogen	0.63±0.08[a]	0.21±0.07[b]
Total sugars	2.27±0.23[a] (100)	2.25±0.21[a] (100)
Reducing sugars	0.19±0.02[a] (8.3)	0.09±0.03[b] (4)
Non-reducing sugars	2.08±0.24[a] (91.7)	2.16±0.08[a] (96)

Figures across the column with different letters are significantly different ($p<0.05$, paired t-test).

Table 4. Amino acid composition of ripened beans and dry seed flours of *Canavalia cathartica* (g/100 g protein).

Amino acid	Ripened beans		FAO/WHO/UNU pattern for adults[a]
	Raw	Cooked	
Glutamic acid	8.22	7.03	
Aspartic acid	7.22	6.18	
Serine	3.51	3.2	
Threonine	2.9	2.59	0.9
Proline	2.59	2.55	
Alanine	2.82	2.62	
Glycine	2.55	2.36	
Valine	3.2	2.97	1.3
Cystine	0.62	0.58	
Methionine	0.5	0.46	1.7[b]
Isoleucine	2.66	2.47	1.3
Leucine	4.75	4.44	1.9
Tyrosine	2.47	2.36	
Phenylalanine	2.7	2.55	1.9[c]
Tryptophan	ND	ND	0.5
Lysine	3.67	3.47	1.6
Histidine	1.93	1.47	1.6
Arginine	2.97	2.78	

[a] Essential amino acid pattern for adults (FAO/WHO/UNU, 1985)
[b] Methionine+Cystine
[c] Phenylalanine+Tyrosine
ND, Not detectable

Amino acids and fatty acids

Amino acid profiles of raw and cooked beans were compared with EAA for adults (FAO/WHO/UNU, 1985) (Table 4). The acidic amino acids (glutamic acid and aspartic acids) were the major amino acids of raw as well as cooked beans. Except for cystine + methionine and tryptophan, the raw and cooked beans meet the FAO/WHO/UNU (1985) amino acid requirement for adults. In edible legumes, usually lysine will be higher than sulphur-amino acids (cystine and methionine) (Norton *et al*., 1985; Jansman, 1996), in our study lysine (3.5-3.7%) in beans is higher than whole egg lysine (FAO, 1970), while histidine of raw beans (1.9%) was at par with FAO/WHO (1991) pattern.

Table 5. Fatty acid composition of ripened beans and dry seed flours of *Canavalia cathartica* (mg/g lipid) (n=3, mean).

Fatty acid	Ripened beans	
	Raw	Cooked
Saturated fatty acids		
Lauric acid (C12:0)	0.487	-
Tridecanoic acid (C13:0)	0.486	1.176
Myristic acid (C14:0)	0.027	-
Pentadecanoic acid (C15:0)	0.441	0.127
Palmitic acid (C16:0)	0.077	0.028
Heptadeconoic acid (C17:0)	1.761	1.547
Stearic acid (C18:0)	0.462	1.906
Arachidic acid (C20:0)	-	0.073
Heneicosanoic acid (C21:0)	1.43	12
Behenic acid (C22:0)	-	5.954
Lignoceric acid (C24:0)	2.972	-
Pentacosanoic acid (C25:0)	0.039	-
Polyunsaturated fatty acids		
Palmitoleic acid (C16:1)	0.1	1.306
Oleic acid (C18:1)	-	8.939
Linoleic acid (C18:2)	0.157	1.895
Linolenic acid (C18:3)	6.241	-
Eicosenoic acid (C20:1)	4.398	-
Eicosadienoic acid (C20:2)	-	0.073
Arachidonic acid (C20:4)	-	2.893
Sum of essential fatty acids	6.4	4.79
Sum of saturated fatty acids	7.7	22.8
Sum of polyunsaturated fatty acids	10.9	14.6
P/S ratio[a]	1.42	0.638

-, Not detectable

[a] Ratio of polyunsaturated/saturated fatty acids

A drastic alteration was seen in FAMEs of beans after cooking (Table 5). Lignoceric (saturated) and linolenic (unsaturated) acids were higher in raw beans, while heneicosanoic and oleic acids in cooked beans. Among essential fatty acids, raw beans possessed linoleic and linolenic acids, while cooked beans linoleic and arachidonic acids. Cooking of beans slightly increased the polyunsaturated fatty acids without elevating the P/S ratio. Polyunsaturated fatty acids are known to lower the risk of cardiovascular diseases (Ezeugu *et al*., 1998).

Antinutritional features

Total phenolics as well as tannins of raw beans decreased on cooking (Table 6). Orthodihydric phenols and trypsin inhibitor activity were absent in beans. Phytohemagglutination activity of raw beans reduced to about 50% in cooked beans. The lectin, con A has been reported to be one of the most potent antinutritional factors of seeds of *C. ensiformis* (Udedibie and Carlini, 1998), which binds to carbohydrates of brush border, prevents absorption, resists digestion and depresses growth (Liener, 1980; Putsztai, 1989). Thermal treatments have been shown to destroy lectins, thereby decreasing hemagglutination (Armour *et al.*, 1998). Our study revealed that thermal processing brings down the hemagglutination to some extent and indicates that con A level might have decreased or partially denatured. The con A is known to be associated with globulin fraction of seed protein. The globulin fraction of raw beans (12%) decreased to one-third (8.1%) on pressure-cooking. This clearly reveals that other processes in addition to thermal treatment may effectively denature or inactivate con A of mangrove *Canavalia* beans.

Table 6. Antinutritional components of ripened beans and dry seed flours of *Canavalia cathartica* (g/100 g) (n=5; mean±SD).

Component	Ripened beans	
	Raw	Cooked
Total phenolics	4.65±0.05[a]	3.32±0.12[b]
Orthodihydric phenols	NP	NP
Tannins	0.048±0.008[a]	0.022±0.002[b]
Trypsin inhibition activity	NP	NP
Phytohemagglutinin activity: Rabbit RBC*	25	13

*Titre: maximum dilution where agglutination was observed
NP, Not present
Figures across the column with different letters are significantly different ($p<0.05$, paired t-test).

Protein and starch digestibility

Cystine + methionine and tryptophan were limiting in raw beans, while cystine + methionine, tryptophan and histidine in cooked beans (Table 7). The protein digestibility was higher in cooked beans than raw beans (82.3 vs. 53.7%), while PDCAAS was higher in raw than cooked beans. Except for cystine + methionine, tryptophan and histidine, the rest in raw beans PDCAAS ranged between 110 and 173.2%, while in cooked beans it was between 156.3 and 236.8%. Low protein digestibility in raw beans may be attributed to the formation of protein complexes with antinutritional factors (Cheryan, 1980; Reddy *et al*., 1985). However, pressure-cooking increased the accessibility of proteins to enzymatic attack mainly due to inactivation of proteinatious antinutritional factors (Poel *et al.*, 1991).

Table 7. Essential amino acid (EAA) score, protein digestibility corrected amino acid score (PDCAAS) and in vitro protein digestibility (IVPD) of ripened beans of *Canavalia cathartica*.

EAA score, IVPD and PDCASS	Ripened beans	
	Raw	**Cooked**
EAA score[a]		
Threonine	322.22	287.78
Valine	246.15	228.46
Cystine + Methionine	65.88	61.18
Isoleucine	204.62	190.0
Leucine	250.0	233.68
Tyrosine + Phenylalanine	272.11	258.42
Lysine	229.38	216.88
Histidine	120.63	91.88
IVPD (%)	53.744	82.268
PDCAAS (%)[a]		
Threonine	173.18	236.75
Valine	132.29	187.95
Cystine + Methionine	35.41	50.33
Isoleucine	109.97	156.31
Leucine	134.36	192.25
Tyrosine + Phenylalanine	146.24	212.6
Lysine	123.28	178.42
Histidine	64.83	75.58

[a] Calculated according to FAO/WHO/UNU (1985) pattern for adults.

The starch digestibility was significantly low ($P<0.05$) in raw beans than cooked beans (1.01 vs. 6.64 mg maltose/hr/100 g). The raw bean starch was indigestible as it forms granules and is less accessible to hydrolytic enzymes

(Colonna *et al*., 1992). High ratios of amylose/amylopectin, high dietary fiber and antinutrients in raw beans reduce the bioavailability of starch (Deshpande and Cheryan, 1984; Thompson and Yoon, 1984). The antinutritional factors are also known to inhibit α-amylase activity (Deshpande and Cheryan, 1984). Other factors influence digestibility includes: intact tissue/cell structures enclosing starch granules hinder the swelling and solubilization of starch (Tovar *et al*., 1990). On cooking, due to hydrolysis of starch through gelatinization it is easily accessible to enzymatic attack (Yu-Hui, 1991; Bishnoi and Khetrapaul, 1993; Mbofung *et al*., 1999). Enhancement of starch digestibility due to destruction of antinutrients has also been reported (Yu-Hui, 1991; Mbofung *et al*., 1999). Thus, the improved starch digestibility in cooked mangrove beans in our study can be attributed to the hydrolysis of starch and partial elimination of antinutritional factors (Siddhuraju and Becker, 2005). There is further scope to improve the nutritional qualities of ripened beans of mangrove *Canavalia cathartica* suitable for human or livestock consumption.

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