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Original Article

Effect of roasting and pressure-cooking on nutritional and protein quality of seeds of mangrove legume *Canavalia cathartica* from southwest coast of India

S. Seena[a], K.R. Sridhar[a,*], A.B. Arun[b], Chiu-Chung Young[b]

[a]*Microbiology and Biotechnology, Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore 574 199, Karnataka, India*
[b]*College of Agriculture and Natural resources, Department of Soil Environmental Science, National Chung Hsing University, 250 Kuo-Khang Road, Taichung, Taiwan 40227, People's Republic of China*

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Abstract

Raw and processed (roasted and pressure-cooked) seeds of mangrove wild legume (*Canavalia cathartica*) of southwest coast of India were evaluated for nutritional and antinutritional qualities. The seeds consist of 28–32% proteins and 1600–1630 kJ/100 g of energy. A significant difference was seen between the proximate composition of raw and pressure-cooked seeds ($P<0.05$, *t*-test). Among the minerals, potassium was highest (240–828 mg/100 g) followed by phosphorus (84–120 mg/100 g) and sodium (21–41 mg/100 g). Globulins (18.2%) constituted the bulk of the seed proteins followed by albumins (7.3%) as in most of the legumes. Unlike the pressure-cooked seeds, SDS-PAGE revealed three protein bands in roasted seeds (51.4, 39 and 33.1 kDa) indicating partial or complete denaturation. The essential amino acids (EAA): cystine + methionine of processed seeds exceeded than that of rice; cystine + methionine, tyrosine + phenylalanine and lysine of the roasted seeds and cystine + methionine of pressure-cooked seeds were higher than FAO/WHO pattern. Threonine, valine and isoleucine of roasted seeds were comparable to FAO/WHO pattern, so also valine, isoleucine and lysine of pressure-cooked seeds. The carbohydrates, polyunsaturated/saturated fatty acid ratio and sulphur-amino acids were higher than soybeans. The raw seed flours were devoid of tannins and trypsin inhibitors, in addition, thermal processing decreased total phenolics and hemagglutinins. Growth and nitrogen balance studies in rats were performed to determine food efficiency ratio, protein efficiency ratio, net protein retention (NPR), protein retention efficiency (PRE), true digestibility, biological value (BV) and net protein utilization (NPU) of roasted and pressure-cooked seeds. Pressure-cooked seeds showed better biological indices than roasted seeds. Except for NPR, PRE, BV and NPU, rest of the parameters analyzed for protein quality was significantly different between roasted and pressure-cooked seed diet ($P<0.05$, *t*-test). Our study clearly indicated that *Canavalia cathartica* seeds of mangroves possess high protein and EAA. Even though domestic roasting and pressure-cooking partially detoxified con A-like lectins or hemagglutinins, improved methods of processing are essential to maximize the quality of protein with minimum loss of seed nutrients. This is the first study on the biochemical and protein quality evaluation of mangrove bean, *Canavalia cathartica* and warrants its conservation and utilization as a future potential protein source for humans and or livestock.

Keywords: Protein quality; Essential amino acids; Lectins; Seeds; Roasting; Pressure-cooking; *Canavalia cathartica*; Legume; Mangroves

1. Introduction

Studies pertaining to the search of alternative source of nutrition and protein quality are of great importance in tropical developing countries to alleviate hunger and malnutrition particularly in children and pregnant women, as they are most vulnerable (Coulter et al., 1988; Pelletier, 1994). It is known that the cereal diets in developing countries deprive humans from indispensable amino acids and energy (Young and Pellet, 1990). Efforts are underway to exploit the wild legumes in tropics as food source (e.g., Rajaram and Janardhanan, 1992; Arinathan et al., 2003; Arun et al., 2003).

*Corresponding author. Tel.: +0091 824 2287 261; fax: +0091 824 2287 367.
E-mail address: sirikr@yahoo.com (K.R. Sridhar).

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The genus *Canavalia* accommodates 48 species distributed throughout the tropics (Udedibie and Carlini, 1998) and serious work has been conducted by researchers to tap this nutritional asset. Proximate analysis, antinutritional factors (Bressani et al., 1987; Rajaram and Janardhanan, 1992; Mohan and Janardhanan, 1994; Ekanayake et al., 1999; Arun et al., 2003), functional properties (Abbey and Ibeh, 1987; Akpapunam and Sefa-Dedeh, 1997), detoxification (Leon et al., 1998; Melcion et al., 1998: Udedibie and Carlini, 1998), protein quality of raw and processed seeds (Bressani et al., 1987; Bressani and Sosa, 1990; Ekanayake et al., 2000) of *Canavalia ensiformis* , *Canavalia gladiata* and *Canavalia maritima* has been performed extensively. The nutritive value and protein digestibility of raw seeds of legumes are poor and hence they have to be subjected to thermal treatment (Liener, 1962). Raw *Canavalia* seeds have been shown to contain high antinutritional factors, especially hemagglutinin which renders high toxicity (Rosenthal, 1977; Crine and Lemieux, 1982; Natelson, 1985). The current study aims to decipher biochemical composition, antinutritional features and animal experimentation in order to assess the nutritional quality, growth rate and nitrogen balance of thermally processed seeds of mangrove bean, *Canavalia cathartica*.

2. Materials and methods

2.1. Seed samples and processing

Seeds of *Canavalia cathartica* Thouars were obtained from the Nethravathi mangroves of southwest coast of India (12°50′ N, 74°50′ E) during February–March 2003. They were sun dried for 3 days after separation of debris, immature and damaged seeds. Mean weights and dimensions of seeds were determined and were divided into two groups. First set was roasted on sand bath at 180 °C for 20 min. After attaining room temperature, they were cut, dehulled, milled (Wiley mill, 30 mesh) and stored in glass containers. A second set of seeds was cut, dehulled, soaked in freshwater for 1 h and pressure-cooked in household pressure cooker for 30 min with 1:3 (v/v) freshwater. Pressure-cooked cotyledons were sun dried, milled (Wiley mill, 30 mesh) and stored in glass containers.

2.2. Nutritional features

2.2.1. Proximate analysis

The moisture of the seed flour was determined on drying at 100 °C in an incubator (Scientronic SBIM-25; New Delhi; India) until a constant weight was attained. The difference in initial and final weight of flour was expressed in percentage moisture. Micro-kjeldahl method was employed to determine the total nitrogen and the crude protein ($N \times 6.25$) (Humphries, 1956). Crude lipid (Soxhlet extraction), crude fiber and ash contents (gravimetric) were determined based on methods outlined in AOAC (1990).

In order to calculate energy, carbohydrate was estimated based on Müller and Tobin (1980):

Total crude carbohydrates (%)

$$= 100 - (\text{Crude protein} + \text{Crude lipid} + \text{Crude fiber} + \text{Ash}).$$

Gross energy was calculated based on formula given in Ekanayake et al. (1999):

Gross energy (kJ/100 g DM)

$$= (\text{protein} \times 16.7) + (\text{lipid} \times 37.7) + (\text{carbohydrates} \times 16.7).$$

2.2.2. Mineral composition

Mineral constituents viz., sodium, potassium, calcium, magnesium, iron, copper, zinc and manganese of the seed flour were determined by atomic absorption spectrophotometer (GBC 902; Australia) upon digestion with a mixture of concentrated nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v) (AOAC, 1990). Ascorbic acid method was employed to determine the total phosphorus as orthophosphate on measuring the absorbance at 880 nm with KH_2PO_4 as standard (Spectrophotometer, Bausch and Lomb 21; Germany) (APHA, 1995).

2.2.3. Protein isolation and separation

Total proteins of the raw seed flour were extracted according to Basha et al. (1976). To save the prolamin fraction, the ethanol treatment was omitted. Proteins were precipitated with 10% trichloroacetic acid (TCA) and estimated by the method of Lowry et al. (1951). The albumin and globulin fractions were separated according to Murray (1979). The remaining pellet was treated with 80% ethanol (1:10 w/v) overnight, centrifuged at 20,000*g*, 20 min (Remi C-24; Mumbai, India), the supernatant containing prolamin was air-dried and dissolved in 0.1 N NaOH (1:10 w/v), centrifuged (20,000*g*, 20 min), the supernatant thus obtained designated as glutelins as it is alkali soluble. The protein fractions obtained were precipitated with 10% TCA and redissolved in 0.2N NaOH to determine the protein content (Lowry et al., 1951).

For protein separation, 100 μg flour of roasted and pressure-cooked seeds was dissolved in 100 μL buffer consisting of 60 mM tris-HCl, pH 6.8, 10% (w/v) glycerol, 2% (w/v) SDS, 10% (v/v) mercaptoethanol. Processed samples were boiled for 2 min at 100 °C, cooled and 2 μL 50% (w/v) bromophenol blue solution were added (Miersch et al., 1998). Separation of soluble proteins 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 mini vertical slab gel electrophoresis unit (BROVIGA; 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 (Sigma).

2.2.4. Amino acid analysis

Amino acids of the roasted and pressure-cooked samples were assessed as outlined by Hofmann et al. (1997, 2003).

Known quantity of seed flours were hydrolyzed with 6N HCl (15 mL) for 4 h at 145 °C. After cooling, HCl was eliminated in a rotoevaporator (Büchi Laboratoriumstechnik AG RE121; Switzerland) combined with a diaphragm vacuum pump (MC2C; Vacuubrand GmbH, Germany). Internal standard, trans-4-(Aminomethyl)-cyclohexanecarboxylic acid (Aldrich, 85765-3; purity, 97%) was added to each sample. The derivatization step consisted of esterification with trifluoroacetylation (Brand et al., 1994).

Samples of standard amino acids were weighed out in reaction vials and dried using CH_2Cl_2 under a gentle stream of helium and slow heating in an oil bath (40–60 °C) to remove water traces. A 12 mL of freshly prepared acidified isoporpanol (acetyl chloride, 3 mL + 2-propanol, 12 mL) was added and the mixture was heated at 100 °C for 1 h. After cooling, the reagent was eliminated with a gentle stream of helium at 60 °C, followed by combined evaporation with three successive aliquots of CH_2Cl_2 to remove propanol and water. The dry residue was trifluoroacetylated with 200 μL trifluoroacetic anhydride overnight at room temperature. An aliquot of this solution was used without further treatment for 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^{-1} with following temperature and pressure: initial 50 °C (1 min), increased to 100 °C at 10 °C min^{-1} (10 min), increased to 175 °C at 3 °C min^{-1} (10 min), increased to 250 °C min^{-1} (10 min); head pressure, 13 psi (90 kpa).

The essential amino acid (EAA) score was determined employing the formula:

$$\text{EAA score} = \frac{\text{Mg EAA in 100 mg test protein}}{\text{Mg of EAA in 100 mg reference pattern}^{a}} \times 100.$$

2.2.5. Fatty acid analysis

Fatty acid methyl esters of the seed flour samples were determined based on the procedure outlined by Garces and Mancha (1993). Samples of 50 mg together with respective fatty acids [American Oil Chemists Society (AOCS); Merck, Germany] as the internal standard were placed in the tubes with teflon-lined caps and methylated with a mixture containing methanol, benzene, DMP and H_2SO_4 (37:20:5:2) (v/v). A 2.1 mL 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 microliter upper layer containing the fatty acid methyl esters (FAMEs) was injected to GLC (Sigma Instruments, Baroda, India) in a glass column (Silar, 10%) packed with 5% ethylene glycol succinate on Supelcoport 80/100 isothermically at 200 °C. Conditions for the analysis were as under: carrier gas, N_2; injector temperature, 225 °C; FID detector temperature, 265 °C; oven temperature, 200 °C; flow rate: N_2, 35 mL min^{-1}, H_2, 30 mL min^{-1}, O_2, 75 mL min^{-1}.

The polyunsaturated and saturated fatty acid ratio was calculated as follows:

$$\frac{P}{S}\ \text{ratio} = \frac{\text{Sum of saturated fatty acids}}{\text{Sum of polyunsaturated fatty acids}}.$$

2.3. Antinutritional features

2.3.1. Phenolics

Total phenolics of the seed flour samples were assayed after extracting twice with 50% methanol in a water bath at 95 °C for 10 min (Rosset et al., 1982). The pooled extract was made up to 10 mL, 0.5 mL extract was mixed with equal quantity of distilled water and treated with 5 mL Na_2CO_3 (in 0.1 N NaOH). After 10 min, 0.5 mL Folin-Ciocalteu's phenol reagent (diluted 1:2 with distilled water) was added and read at 725 nm. Tannic acid was used as standard.

2.3.2. Tannins

Tannins were analyzed by radial diffusion assay outlined by Hagerman (1987). A 1% (w/v) solution of agarose was prepared in buffer (50 mM acetic acid and 60 μM ascorbic acid; pH, 5) by heating to boiling while stirring. A 0.1% (w/v) bovine serum albumin was added to the above solution, cooled to 45 °C and stirred. The solution was dispersed into petriplates. Wells of 4 mm diameter were punched with a sterile cork borer. Seed samples were extracted for 1 h with 50% (w/v) aqueous ethanol (0.5 mL for 100 mg seed sample). Aliquots of 8, 16 and 32 μL seed extracts were directly loaded to wells and plates were incubated at 30 °C for 96–120 h. Two diameters of the precipitated zones were measured at right angles to another. Tannin concentration was measured by the square of the mean of two diameters using a calibration curve.

2.3.3. Trypsin inhibition activity (TIA)

The TIA of the seed flour was determined based on enzymatic assay (Kakade et al., 1974). One gram of freshly ground and processed seed flour was extracted with 50 mL 0.01 N NaOH and the suspension was made up to 2 mL with distilled water, 2 mL trypsin solution (4 mg in 200 mL 0.001 M HCl) was added to each test tube and kept in water bath at 37 °C. To each tube, 5 mL BAPNA solution (40 mg *N*-a-Benzoyl-DL-Arginine *p*-nitroanilide hydrochloride

[a] FAO-WHO, 1991

(Aldrich, 85711-4; purity, ⩾99%) in 1 mL dimethyl sulfoxide diluted to 100 mL with tris buffer at 37 °C) was added, after 10 min the reaction was terminated by adding 1 mL acetic acid (30%), mixed thoroughly, filtered and the absorbance of filtrate was measured at 410 nm against reagent blank (1 mL 30% acetic acid containing 2 mL each trypsin and distilled water + 5 mL BAPNA solution).

2.3.4. Hemagglutination activity

Hemagglutination was carried out using trypsin-treated human erythrocyte suspension (A, B, O) (Hankins et al., 1980) with slight modification. For preparation of erythrocytes, autoclaved saturated tri-sodium citrate was used as an anticoagulant. Three milliliter blood was collected from human subjects directly into a graduated tube containing 1 mL tri-sodium citrate solution and mixed. The solution containing erythrocytes was centrifuged at 1000*g* 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 1000*g* for 5 min at 4 °C. The cells were then treated with 50 μg/mL trypsin [*N*-Benzoyl-L-tyrosine ethyl ester (BTEE), 0.04 units/mg solid] (Aldrich, 85658-4; purity, 98%) 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 2% (v/v) cell suspension. For agglutination assay, trypsin treated erythrocyte suspension (2% in PBS) was used (Hankins et al., 1980). Twenty-five microliter of extract (extracted from 0.05 g seed flour) was incubated with 75 μL erythrocyte suspension in a microtitre plate for 30 min at room temperature and was examined for agglutination under a microscope.

2.4. Biological evaluation of protein quality

2.4.1. Test animals

Male Wistar rats of 21 days old weighing 30 ± 5 g were obtained for growth experiments. They were randomly divided into four groups each consisting of five rats in polypropylene metabolic cages housed in a temperature-controlled room (22 ± 1 °C) with 50% relative humidity and 12 h photoperiod. Food and water were given ad libitum.

2.4.2. Diet composition

The diet of rats consists of casein, protein-free (basal diet), roasted and pressure-cooked seed flours (test diets). All the diets except for basal diet, consists of 10% protein. The roasted and pressure-cooked seed flours were incorporated to the basal diet at the expense of corn starch to give 10% protein. The group of rat fed with casein as an active source of protein served as control. The diets were prepared and stored in airtight containers a week prior to the experiment.

2.4.3. Growth performance

Protein efficiency and net protein ratio (PER and NPR) was carried out according to the method outlined by Pellet and Young (1980) for 28 days. The food consumption and body weight of rats was assessed at weekly and 10-day interval. The PER, corrected PER, food efficiency ratio (FER) at 4 weeks and NPR for 10 days was calculated as follows:

$$\text{PER} = \frac{\text{Weight gain of the test animal (g)}}{\text{Protein consumed (g)}},$$

$$\text{Corrected PER} = \frac{\text{PER} \times 2.5}{\text{Determined PER for reference casein}},$$

where 2.5 as standard value for casein.

$$\text{FER} = \frac{\text{Weight gain of the test animal (g)}}{\text{Food consumed (g)}},$$

$$\text{NPR} = \frac{\text{Weight gain of the test animal (g)} + \text{Weight loss of the protein free test animal (g)}}{\text{Weight of test protein consumed (g)}}.$$

The protein retention efficiency (PRE) was calculated according to Bender and Doel (1957):

$$\text{PRE} = \text{NPR} \times 16.$$

2.4.4. Nitrogen balance experiments

Nitrogen balance studies were carried out by the method described by Chick et al. (1935). Twenty adult male albino rats weighing 60–68 g were distributed into four groups in polypropylene metabolic cages. First group of rats was fed with casein; Second with protein-free diet (basal diet); the rest with roasted and pressure-cooked seed diet. Food and water were given ad libitum. The experiment was performed for 14 days; 9 days for acclimatization and the remaining 5 days as collection period. Urine and feces were collected and pooled separately each day. The nitrogen content of urine and feces were estimated by micro-kjeldahl method (AOAC, 1990).

True digestibility (TD) and biological value (BV) were determined as follows:

$$\text{TD} = \frac{N_i - (NF_1 - NF_2)}{N_i} \times 100,$$

$$\text{BV} = \frac{N_i - (NF_1 - NF_2) - (NU_1 - NU_2)}{N_i - (NF_1 - NF_2)} \times 100,$$

where, N_i is the nitrogen intake of animal fed test diet, NF_1 the nitrogen excreted in feces of animals fed test diet, NF_2 the nitrogen excreted in feces of animal fed protein-free diet (basal diet), NU_1 the nitrogen excreted in urine of animals fed test diet, NU_2 the nitrogen excreted in urine of animals fed protein-free diet (basal diet).

Net protein utilization (NPU) was calculated (Platt et al., 1961):

$$\text{NPU} = \frac{\text{BV} \times \text{TD}}{100}.$$

2.5. Statistical analysis

The *t*-test was employed to ascertain the difference between raw and roasted and pressure-cooked seed flours for proximate composition, mineral components and total phenolics (Stat Soft Inc., 1995). The difference in growth and nitrogen balance in rats fed with roasted and pressure-cooked seed flours was also assessed by *t*-test.

3. Results and discussion

3.1. Seed characteristics

Assessment of whole seed, cotyledon and seed coat weight based on analysis of 20 seeds revealed 0.97 ± 0.07, 0.69 ± 0.05 (71.1%) and 0.28 ± 0.03 (28.9%) g/seed, respectively. Seed length, width, thickness and hilum length were 1.89 ± 0.03, 1.27 ± 0.06, 0.93 ± 0.04 and 1.24 ± 0.08 cm, respectively. The seed weight and dimensions of *Canavalia cathartica* were almost similar and expected to yield the same amount of dry matter as that of jack bean (Akpapunam and Sefa-Dedeh, 1997).

3.2. Proximate composition

Moisture content of pressure-cooked seeds were lesser than that of raw and roasted seeds (5.65 vs. 11.2% and 8.61%) (Table 1). Crude protein of roasted (29.6%) and pressure-cooked (28%) seeds were almost similar and falls within the protein range of most legumes (17–30%) (Reddy et al., 1984). The crude protein of raw and processed seeds surpassed whole seeds of wild legumes: winged bean (*Neonotonia wightii*, 20.6%) (Viswanathan et al., 2001), sword bean (*Canavalia gladiata*, 26.8%) (Ekanayake et al., 1999), beach bean (*Canavalia maritima*, 27.1%) (Abbey and Ibeh, 1987) and velvet bean (*Mucuna monosperma*, 23.5%) (Mohan and Janardhanan, 1995). The protein content also exceeded green gram (*Phaseolus aureus*, 22.3%), black gram (*P. mungo*, 23.3%) (Gupta and Wagle, 1978), pigeon pea (*Cajanus cajan*, 19.4%), (Nwokolo, 1987), chick pea (*Cicer arietinum*, 20.7%) (Jambunathan and Singh, 1980) and cow pea (*Vigna unguiculata*, 22.5%) (Nwokolo and Oji, 1985), so also cereals like whole wheat flour (8.55%), parboiled rice (7.7%) and egg (12.6%) (Livsmedelsverk, 1988).

Crude lipid content of raw and processed seeds (1.52–1.92%) was lesser than many wild legumes: *Atylosia scarabaeoides* (4.56%), *Canavalia gladiata* (9.3%), *Lablab purpureus* (8.33%), *N. wightii* (4.64%) and *V. trilobata* (12.3%) (Arinathan et al., 2003). Crude lipid of processed seeds was higher than most of the raw *Canavalia* spp. (1.60–1.80%) (Bressani et al., 1987). Crude fiber of the pressure-cooked seeds was comparatively lesser than raw and roasted seeds (1.68 vs. 2.56, 2.38%) and the fiber of seeds of *Canavalia cathartica* was lesser than *Canavalia ensiformis* (8.5%), *Canavalia gladiata* (12.8%), *Canavalia maritima* (17.3%) of Central America as observed by Bressani et al. (1987). Low crude fiber is nutritionally appreciated because it traps less proteins and carbohydrates (Balogun and Fetuga, 1986). Ash content of raw and roasted seeds were higher than pressure-cooked seeds (3.36, 3.32 vs. 3.15%) and was lesser than *Canavalia ensiformis* (4.64%) and *Canavalia gladiata* (3.72%) (Rajaram and Janardhanan, 1992). Crude carbohydrates of pressure-cooked seeds were greater than raw and roasted seeds (65 vs. 60.4, 62.5%). The energy value of pressure-cooked seeds exceeded than raw and roasted seeds (1630 vs. 1600 and 1620 kJ/100 g) and was higher than commonly cultivated pulses (1360–1430 kJ/100 g) (Kuzayali et al., 1966). The high-energy value is due to high protein and carbohydrate (60.4–65.0 g/100 g) content of *Canavalia cathartica* seeds.

Table 2 registers the mineral composition of raw and processed seeds. Pressure-cooking drained most of the minerals of seeds except for calcium, copper and zinc, which were not significantly different between roasted and pressure-cooked seeds ($P \geqslant 0.05$). The mineral content of raw and processed seeds does not meet the recommended dietary allowance (NRC/NAS, 1989). A decrease in the minerals was also noticed by Aletor and Ojo (1989) after cooking, which is attributed mainly by the enhanced permeability of seed coat of underutilized legumes of Nigeria.

Table 1
Proximate composition of raw and processed seed flours of *Canavalia cathartica* on dry weight basis (mean ± SD, $n = 5$)

Component	Raw seeds	Roasted seeds	Pressure-cooked seeds
Moisture (%)	11.2 ± 1.5^{a}	8.61 ± 0.5^{bc}	5.65 ± 0.2^{bd}
Crude protein (g/100 g)	32.1 ± 1.9^{a}	29.6 ± 1.3^{ac}	28.0 ± 1.9^{bc}
Crude lipid (g/100 g)	1.52 ± 0.1^{a}	1.88 ± 0.3^{bc}	1.92 ± 0.3^{bc}
Crude fiber (g/100 g)	2.56 ± 0.2^{a}	2.38 ± 0.2^{ac}	1.68 ± 0.3^{bd}
Ash (g/100 g)	3.36 ± 0.3^{a}	3.32 ± 0.1^{ac}	3.15 ± 0.1^{bc}
Energy value (kJ/100 g)	1600 ± 6^{a}	1620 ± 7^{bc}	1630 ± 9^{bd}

Figures across the columns with different letters are significantly different ($P < 0.05$, *t*-test).

Table 2
Mineral composition of raw and processed seed flours of *Canavalia cathartica* on dry weight basis (mg/100 g) (mean ± SD, $n = 5$)

Minerals	Raw seeds	Roasted seeds	Pressure-cooked seeds
Sodium	40.5 ± 2^{a}	39.5 ± 1^{ac}	20.8 ± 3^{bd}
Potassium	828 ± 33^{a}	825 ± 13^{ac}	240 ± 37^{bd}
Calcium	27.3 ± 5^{a}	28.9 ± 1^{ac}	26.2 ± 3^{ac}
Phosphorus	120 ± 3^{a}	120 ± 13^{ac}	83.8 ± 3^{bd}
Magnesium	6.83 ± 1.2^{a}	6.80 ± 0.8^{ac}	3.96 ± 0.4^{bd}
Iron	1.27 ± 0.2^{a}	1.23 ± 0.3^{ac}	0.89 ± 0.1^{bd}
Copper	0.14 ± 0.02^{a}	0.34 ± 0.5^{ac}	0.30 ± 0.5^{ac}
Zinc	1.02 ± 0.3^{a}	0.84 ± 0.2^{bc}	0.91 ± 0.2^{ac}
Manganese	0.12 ± 0.04^{a}	0.13 ± 0.01^{ac}	0.10 ± 0.003^{ad}

Figures across the columns with different letters are significantly different ($P < 0.05$, *t*-test).

Separation of proteins from roasted seeds by SDS-PAGE resulted in three bands of 51.4, 39 and 33.1 kDa, whereas a smear was observed in case of pressure-cooked seeds reveals partial or complete denaturation of proteins. True protein in 100 g raw seed flour was 28.3 ± 0.59 g. Albumin, globulin, prolamin and glutelin were: 7.32 ± 0.18 (25.8%), 18.24 ± 1.28 (64.4%), 0.74 ± 0.24 (2.6%), and 2 ± 0.63 (7.1%) g/100 g true protein, respectively. Among the seed protein fractions, albumin and globulin were found to be the highest as with many other legumes. Albumins are known to be rich in sulphur-amino acid and other EAA (Baudoin and Maquet, 1999). The true protein content exceeded than that of winged bean (15.2%) (Viswanathan et al., 2001), sword bean (20.8%) and *Cassia floribunda* (16.3–17.7%) (Vadivel and Janardhanan, 2001). The amino acid profile of roasted seeds (Table 3) was better when compared to pressure-cooked seeds which was similar to the observations of Bressani et al. (1987) within the processed seeds of *Canavalia ensiformis*. The acidic amino acids of the roasted and pressure-cooked seeds viz., glutamic acid (6.4, 5.6%) and aspartic acid (6.8, 5.8%) form a major part of amino acids of *Canavalia cathartica*. Generally, lysine of legumes is found to be higher and limiting in sulphur-amino acids (Norton et al., 1985; Jansman, 1996). Lysine of the processed seeds of *Canavalia cathartica* (5.6–6.6 g/100 g protein) is comparable with that of whole egg protein (7%) (FAO, 1970). Surprisingly, sulphur-amino acids, cystine and methionine of roasted seeds of *Canavalia cathartica* surpassed FAO/WHO pattern (FAO/WHO, 1991), rice (Livsmedelsverk, 1988) and soybean (Bau et al., 1994). Similarly, sulphur-amino acids of pressure-cooked seeds also surpassed FAO/WHO pattern (FAO/WHO, 1991), soybean (Bau et al., 1994) and was comparable with that of rice (Livsmedelsverk, 1988). Other EAA: phenylalanine + tyrosine and lysine of roasted seeds were more than FAO/WHO pattern. Threonine, valine and isoleucine of roasted and valine, isoleucine and lysine of pressure-cooked seeds were also comparable with that of FAO/WHO pattern.

Table 4 deals with the fatty acid profile of processed seeds of *Canavalia cathartica*. Processed seeds mainly consisted of polyunsaturated fatty acids. The palmitoleic acid of roasted seeds was high compared to the pressure-cooked seeds (13.2 vs. 1.0 g/100 g lipid), thereby elevating P/S ratio of roasted seeds (105.2 vs. 39.4). The P/S ratio of processed seeds was higher than soybean (39.4–105.2 vs. 3.4) and lowers the risk of cardiovascular diseases (Ezeagu et al., 1998). Processed seeds possess the essential fatty acid, linoleic acid.

3.3. Antinutritional features

Total phenolics of raw seeds were low and pressure-cooking was effective in removing phenolics than roasting (940 vs. 1440 mg/100 g) (Table 5). Tannins and trypsin inhibitors were absent as that of *Canavalia cathartica* and *Canavalia maritima* of coastal sand dunes (Arun et al., 2003). Raw seed flours of *Canavalia cathartica* exhibited

Table 3
Amino acid composition of roasted and pressure-cooked seed flours of *Canavalia cathartica* (mg/100 mg protein; mean, $n = 3$)

Amino acid	Roasted seeds		Pressure-cooked seeds		FAO/WHO pattern[b]	Whole egg protein[c]
	Content	EAA score[a]	Content	EAA score[a]		
Glutamic acid	6.4		5.6			12.7
Aspartic acid	6.8		5.8			9.6
Serine	0.9		0.6			7.6
Threonine	2.2	64.7	0.6	17.7	3.4	5.1
Proline	3.2		2.3			4.2
Alanine	2.2		1.4			5.9
Glycine	1.3		1.2			3.3
Valine	2.9	82.9	2.0	57.1	3.5	6.9
Cystine	4.0		2.9			5.9
Methionine	0.3	172	0.2	124	2.5[d]	3.4
Isoleucine	2.6	92.9	2.0	71.4	2.8	6.3
Leucine	3.8	57.6	3.4	51.5	6.6	8.8
Tyrosine	2.6		2.0			4.2
Phenylalanine	4.0	104.8	3.6	88.9	6.3[e]	5.7
Tryptophan	ND		ND		1.1	1.7
Lysine	6.6	113.8	5.6	96.6	5.8	7.0
Histidine	ND		ND		1.9	2.4
Arginine	0.8		0.6			6.1

ND: Not detected.
[a]Essential amino acid score (FAO/WHO, 1991).
[b]FAO/WHO pattern (FAO/WHO, 1991).
[c]Whole egg protein (FAO, 1970).
[d]Methionine + cystine.
[e]Phenylalanine + tyrosine.

Table 4
Fatty acid composition of roasted and pressure-cooked seed flours of *Canavalia cathartica* (g/100 g lipid) (mean, $n = 3$)

Fatty acid	Roasted seeds	Pressure-cooked seeds
Saturated fatty acids		
Tridecanoic acid ($C_{13:0}$)	0.17	0.26
Myristic acid ($C_{14:0}$)	ND	0.02
Pentadecanoic acid ($C_{15:0}$)	0.16	0.09
Palmitic acid ($C_{16:0}$)	ND	0.29
Lignoceric acid ($C_{24:0}$)	0.09	ND
Polyunsaturated fatty acids		
Myristoleic acid ($C_{14:1}$)	0.30	0.23
Palmitoleic acid ($C_{16:1}$)	13.2	1.00
Oleic acid ($C_{18:1}$)	0.08	Trace
Linoleic acid ($C_{18:2}$)	9.80	7.41
Linelaidic acid ($C_{18:2}$)	13.4	10.2
Eicosadienoic acid ($C_{20:2}$)	7.44	7.12
Eicosapentaenoic acid ($C_{20:5}$)	ND	Trace
Sum of saturated fatty acids	0.42	0.66
Sum of polyunsaturated fatty acids	44.2	26.0
P/*S* ratio[a]	105.2	39.4

ND: Not detected.
[a]Ratio of polyunsaturated/saturated fatty acids.

Table 5
Some antinutritional components of raw and processed seed flours of *Canavalia cathartica* (Phenolics, mg/100 g on dry weight basis; mean ± SD, $n = 5$)

Component/activity	Raw seeds	Roasted seeds	Pressure-cooked seeds
Total phenolics	1450 ± 0.1^{a}	1440 ± 0.1^{ac}	940 ± 0.1^{bd}
Tannins	NP	NP	NP
Trypsin inhibition activity	NP	NP	NP
Haemagglutination activity			
A[a]	++++	++	++
B[a]	++++	+++	+++
O[a]	++++	++	++

Figures across the columns with different letters are significantly different ($P < 0.05$, *t*-test).
++++: Red blood cells clumped strongly.
+++: Clumpy patches.
++: Grainy.
NP: Not present.
[a]Human blood groups.

vigorous hemagglutinin activity (Table 5), which declined on roasting and pressure-cooking. Hemagglutinin activity also varied within the blood groups, but similar type of results was observed for processed samples. The blood groups A and O revealed slightly weak activity when compared to B group. Phytohemagglutinin or con A was first isolated and crystallized by Sumner and Howell (1936). Con A was reported as one of the most important antinutritional factors in raw *Canavalia ensiformis* seeds (Udedibie and Carlini, 1998). Con A binds to carbohydrates of intestine and resists digestion (Putsztai, 1989).

3.4. Protein quality

Animal growth and nitrogen balance studies have been performed to assess the protein quality of processed seeds of *Canavalia cathartica* using casein, protein-free (basal diet) (Table 6) and test diets. Food intake of pressure-cooked seeds was better than the roasted seeds (107.7 vs. 94 g) (Table 7). Body weight gain of roasted and pressure-cooked seeds was found to be considerably lower than that of casein (4.43, 8.27 vs. 33.27). Pressure-cooked seed diet showed almost double the weight increase than the roasted seeds. Since the food and protein intake was low, its efficiency ratio also declined. Bressani and Sosa (1990) registered a decrease in weight when the rats were fed with whole roasted (5, 10, 15 min at 130, 140, 170 °C) seeds of *Canavalia ensiformis*. The net protein retention of the pressure-cooked seeds was slightly better than that of roasted seeds in our study (1.43 vs. 1.32) (Table 8). The PRE of processed seeds was only half as that of casein (21.2–22.9 vs. 42). The PER of the roasted and pressure-cooked whole seeds of *Canavalia ensiformis* was 1.12 and 1.36 indicating almost similar results (Bressani and Sosa, 1990).

The TD (53.48, 56.01), BV (46.68, 48.31), NPU (24.97, 27.06) of roasted and pressure-cooked seeds of *Canavalia cathartica* (Table 9) were found to be lower than casein and more or less similar to the results obtained on feeding the roasted and autoclaved cotyledons of *Canavalia gladiata* (Ekanayake et al., 2000). Low TD, BV and NPU may be mainly due to con A-like lectin in *Canavalia cathartica*, which accounts for about 20% of the total protein of *Canavalia ensiformis* seeds (Dalkin and Bowles, 1983). Even with the low NPU, the animals in our study showed positive nitrogen balance. The most important antinutritional factor (ANF) that limits the use of raw *Canavalia ensiformis* is lectins (Melcion et al., 1998). The other toxins of *Canavalia ensiformis* viz., urease and canatoxin exerted no toxic effect on oral administration, hence, not

Table 6
Composition of basal diet of experimental albino rats

Components	(g/100 g)
Corn starch	80
Corn oil	10
Non-nutritive cellulose	5
Salt mixture[a]	4
Vitamin mixture[b]	1

[a]Salt mixture: $CaCO_3$, 78.6 g; $Ca_3C_{12}H_{10}O_{14} \times 4H_2O$, 308.3 g; $CaHPO_4 \times 2H_2O$, 112.8 g; K_2HPO_4, 218.8 g; KCl, 124.7 g; NaCl, 77.1 g; $MgSO_4$, 38.3 g; $MgCO_3$, 35.2 g; $Fe(C_6H_{17}N_3O_7)$, 15.3 g; $MnSO_4 \times H_2O$, 0.201 g; $CuSO_4 \times 5H_2O$, 0.078 g; KI, 0.041 g; $AlNH_4(SO_4)2 \times 12H_2O$, 0.507 g.
[b]Vitamin mixture: Vitamin A, 1000 IU; Vitamin D, 100 IU; Vitamin E, 10 IU; Vitamin K, 0.5 mg; Thiamine, 0.5 mg; Riboflavin, 1 mg; Pyrodoxine, 0.4 mg; Pantothenic acid, 4 mg; Niacin, 4 mg; Choline, 200 mg; Inositol, 25 mg; Para-aminobenzoinc acid, 10 mg; Vitamin B_{12}, 2 μg; Biotin, 0.02 mg; Folic acid, 0.2 mg; added cellulose to make up to 1 g.

Table 7
Food intake, protein intake, gains in body weight, food efficiency ratio (FER) and protein efficiency ratio (PER) of casein and processed seed flours of *Canavalia cathartica* fed to albino rats (mean ± SD, $n = 5$)

Dietary group	Food intake (g)	Protein intake (g)	Gain in body weight (g)	FER	PER	Corrected PER[1]
Casein	141.14 ± 2	14.11 ± 0.2	33.27 ± 1.13	0.24 ± 0.02	2.35 ± 0.11	2.5 ± 0
Roasted seeds	94.01 ± 5.53[a]	9.4 ± 0.55[a]	4.43 ± 0.67[a]	0.05 ± 0.01[a]	0.47 ± 0.1[a]	0.5 ± 0.12[a]
Pressure-cooked seeds	107.69 ± 7.06[b]	10.77 ± 0.71[b]	8.27 ± 0.95[b]	0.073 ± 0.02[b]	0.77 ± 0.14[b]	0.82 ± 0.16[b]

Figures in same column with different letters are significantly different ($P < 0.05$, t-test).
[1]Based on values of 2.5 as standard for casein.

Table 8
Net protein retention (NPR) and protein retention efficiency (PRE) of casein and processed seed flours of *Canavalia cathartica* fed to albino rats (mean ± SD, $n = 5$)

Dietary group	Gain in weight (g)	Weight loss (g)	Protein consumed (g)	NPR	PRE
Casein	9.7 ± 0.27	3.27 ± 0.32	4.87 ± 0.05	2.62 ± 0.08	41.97 ± 1.2
Roasted seeds	1.48 ± 0.3[a]	3.27 ± 0.32	3.59 ± 0.09[a]	1.32 ± 0.04[a]	21.12 ± 0.64[a]
Pressure-cooked seeds	2.05 ± 0.44[b]	3.27 ± 0.32	3.71 ± 0.09[b]	1.43 ± 0.06[a]	22.88 ± 0.97[a]

Figures in same column with different letters are significantly different ($P < 0.05$, t-test).

Table 9
True digestibility (TD), biological value (BV) and net protein utilization (NPU of casein and processed seed flours of *Canavalia cathartica* fed to albino rats (mean ± SD, $n = 5$)

Dietary group	TD (%)	BV (%)	NPU (%)
Casein	90.46 ± 1.25	87.96 ± 0.57	79.57 ± 1.61
Roasted seeds	53.48 ± 0.87[a]	46.68 ± 2.43[a]	24.97 ± 1.61[a]
Pressure-cooked seeds	56.01 ± 0.31[b]	48.31 ± 1.58[a]	27.06 ± 0.94[a]

Figures in same column with different letters are significantly different ($P < 0.05$, t-test).

considered as ANF (Udedibie and Carlini, 1998). In our study, among the ANF, con A-like lectin seems to be the most important toxin which is highly resistant to heat treatment and digestion like that of *Canavalia ensiformis* (Udedibie and Carlini, 1998). Domestic pressure-cooking and roasting had a little nutritional upgrading effect on mangrove bean, *Canavalia cathartica* necessitatating alternate strategies for detoxification of thermally stable con A-like lectin.

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