International Journal of Agricultural Technology 2016, Vol. 12 (7.1): 1275-1295
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Nutraceutical Attributes of Ripened Split Beans of Three *Canavalia* Landraces

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K.R. Sridhar, S.J. Shreelalitha, P. Supriya and A.B. Arun (2016). Nutraceutical attributes of ripened split beans of three *Canavalia* landraces. International Journal of Agricultural Technology12 (7.1): 1275-1295.

This study addressed the nutraceutical potential of ripened split beans of three wild legume landraces of *Canavalia* of the Southwestern India. The split beans possess novel nutritional qualities like low fat, high protein-energy, high albumin/globulin ratio, low Na/K ratio, high Ca/P ratio, essential fatty acids, essential amino acids, high protein efficiency ratios (PER) and high *in vitro* protein digestibility (IVPD). The crude protein content of split beans is comparable or higher than many edible legumes. They possess ω-6 (linoleic and γ-linolenic acids) and ω-3 (α-linolenic, eicosatrienoic and docosahexaenoic acids) fatty acids. The ratio of ω-6/ω-3 fatty acids of cooked split beans of *Canavalia martima* falls within the FAO-WHO recommended pattern. Several essential amino acids of cooked split beans are comparable or higher than soybean, wheat and FAO-WHO standard. The high PER (>2) as well as increased IVPD of cooked split beans denotes their suitability for consumption. The low fat and high protein content in split beans of *Canavalia* landraces combat protein-energy malnutrition in hyperlipidemic patients. The high albumin/globulin ratio indicates low antinutritional factors. Low crude fibre in split beans traps less proteins and carbohydrates results in low risks of blood cholesterol and large bowel cancers. Increased carbohydrates in cooked split beans help to combat intestinal cancers, impart low glycemic index and facilitate management of type II diabetes. The contents of magnesium, copper and manganese in split beans are comparable or higher than NRC-NAS recommended pattern. The selenium content is adequate to protect cells from free radicals and toxic heavy metals. Split beans with low sodium are suitable in formulation of diets for those suffering from hypertension. The low Na/K ratio (<1) of split beans helps to control blood pressure, while the high Ca/P ratio (>1) prevents calcium loss as well as restores calcium in bones. The ripened split beans of three *Canavalia* landraces of Southwest coast of India serve as future potential inexpensive indigenous nutraceutical sources.

Key words: Amino acids, coastal sand dunes, fatty acids, mangroves, minerals, proteins, proximal features, traditional food, wild legumes

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Introduction

Protein-energy malnutrition is one of the major problems in developing countries owing to inaccessibility of adequate animal-derived foods and overdependence on monocarbohydrate diets (Vietmeyer 1986; Steiner 1996; FAO 2000; Pastor-Cavada *et al*. 2009; Boye *et al*. 2010). Alternatively, legumes are gaining more importance worldwide due to their versatile nutritional and therapeutic potential (Nestel *et al*. 2004). Legumes are known to lower the risk of several life threatening ailments especially cardiovascular diseases, obesity and type II diabetes (Schröder 2007; Sievenpiper *et al*. 2009). Many wild legumes (e.g. cluster, velvet and winged beans) are well known siyrces if economically viable protein-energy (Singh *et al*. 2007; Bhat and Karim 2009; Boye *et al*. 2010). Several tribal sects are dependent on 30 wild legumes occurring in different geographic locations in India (Arora *et al*. 1980; Gunjatkar and Vartak 1982; Viswanathan *et al*. 1999, 2001; Narayanan and Kumar 2007).

Vast coastal belt of the Indian Subcontinent is potential storehouse of underexplored and economically valuable wild legumes (Arun *et al*. 1999; Rao and Suresh 2001; Rao and Sherieff 2002; Sridhar and Bhagya 2007). Two landraces of *Canavalia cathartica* (in coastal sand dunes and mangroves) and another landrace of *C. maritima* (in coastal sand dunes) are known to meet requirements of human nutrition as well as health (Arun *et al*. 1999; Seena and Sridhar 2006; Sridhar and Seena 2006; Seena *et al*. 2007; Sridhar and Niveditha 2014). Moreover, *C. maritima* has wide pantropical distribution in coastal region (Nakanishi 1988; Vattanparast *et al*. 2011). Three landraces of coastal *Canavalia* possess desired agrobotanical traits like fast growth, large seeds, high seed yield, nitrogen fixation (in association with rhizobia) and tolerant to abiotic stresses (e.g. temperature, salinity and burial) (Arun *et al*. 2001; Arun and Sridhar 2004; Seena and Sridhar 2006; Sridhar and Seena 2006; Seena *et al*. 2007). In the coastal regions of southwest India, landraces of *Canavalia* serve as disaster or alternative traditional food sources during food scarcity. The coastal dwellers and fisherman communities employ many approaches to process ripened beans of *Canavalia* before cooking. The present study addresses the nutraceutical potential of ripened split beans of three coastal landraces of *Canavalia* used by the natives to propagate their importance and novelty as inexpensive indigenous future nutraceutical sources.

Materials and Methods

Seeds and processing

Greenish yellow ripened pods of *Canavalia cathartica* Thouars were collected from the coastal sand dunes (CSD) of Someshwara (12º47′N, 74º52′E) and Nethravathi mangrove (12°50′N, 74°51′E) of Southwest India (Fig. 1). Ripened pods of *Canavalia maritima* Thouars were sampled from the CSD of Someshwara (Fig. 2). After separation of ripened seeds from the pods, length (*l*), breadth (*b*), thickness (*t*), *l/b* ratio, fresh weight of seed, fresh weight of split beans and dry weight of split beans were determined.

The seed coat along with testa was removed as practiced by the local and fisherman community. The five split bean samples collected from five different locations of CSD and mangroves were divided into two groups. The first group was sun-dried for 2–3 days, powdered (Wiley Mill, mesh # 30) and preserved in air-tight containers. The second group was pressure-cooked with freshwater (1:3 v/v) followed by sun drying and milling.

Proximal and mineral analysis

Moisture content of the flours was estimated gravimetrically by drying at 100°C in ventilated oven to express moisture in percent. Crude protein (N × 6.25) of the flours was evaluated by micro-Kjeldahl method (Humphries 1956). Total lipid was determined gravimetrically by the Soxhlet extraction using petroleum ether (AOAC 1995). Crude fibre as well as ash contents were determined gravimetrically following the methods outlined in AOAC (1995).

The carbohydrates were calculated based on Müller and Tobin (1980):

$$\text{Carbohydrates (\%)} = 100 - (\text{\% Protein} + \text{\% Lipids} + \text{\% Fibre} + \text{\% Ash}) \quad (1)$$

The calorific value was calculated based on Ekanayake *et al*. (1999):

$$\text{Gross energy (kJ/100 g)} = (\text{Protein} \times 16.7) + (\text{Lipid} \times 37.7) + (\text{Carbohydrates} \times 16.7) \quad (2)$$

Minerals (sodium, potassium, calcium, magnesium, iron, copper, zinc, manganese and selenium) of the split bean flours were determined by atomic absorption spectrophotometry (GBC 902; Australia) (AOAC 1995). The vanadomolybdophosphoric acid method was employed to determine the content of total phosphorus (AOAC 1995).

Fatty acid analysis

Fatty acid methyl esters (FAMEs) of lipid extracted from split bean flours were prepared based on the method by Padua-Resurreccion and Banzon (1979). Esterified samples were injected into the gas Chromatograph (GC-2010, Shimadzu, Japan) equipped with auto injector (AOI) and capillary column (BPX-70). The elutants were detected by flame ionization detector (FID), and the amplified signals were transferred and monitored with GC-Solutions software. The analytical conditions of autosampler, injection port settings, column oven settings and column information of the gas chromato-

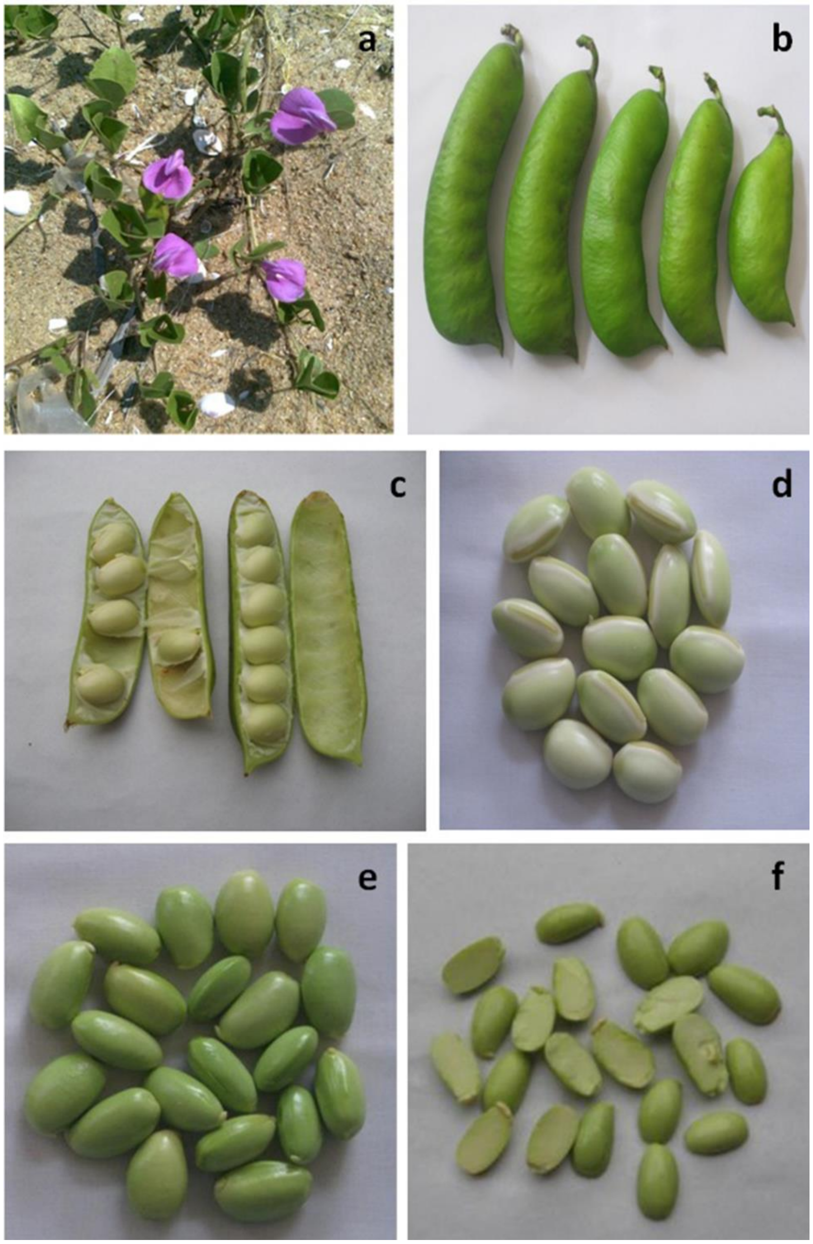

Fig. 1. Horizontal spread of *Canavalia cathartica* on the coastal sand dune of Someshwara (Mangalore, Karnataka, Southwest coast of India) showing flowers and tender pods (a), ripened pods (b), opened ripened pods (c), ripened beans (d), dehusked ripened beans (e) and fresh ripened split beans (f).

Fig. 2. Horizontal spread of *Canavalia maritima* on the coastal sand dune of Someshwara (Mangalore, Karnataka, Southwest coast of India) showing flowers and tender pods (a), ripened pods (b), opened ripened pods (c), ripened beans (d), dehusked ripened beans (e) and dried ripened split beans (f).

graph were according to Nareshkumar (2007). Quantification of the FAMEs in split bean flours was based on standard mixture (C_4–C_{24}) (Sigma, USA) run under similar conditions of analysis. The concentration and area of each peak of FAME was computed using the GC Post-run analysis software.

Five ratios of fatty acids (TUFA/TSFA; TPUFA/TMUFA, $C_{14:0}+C_{15:0}+(C_{16:0}/C_{18:0})$; $C_{18:1}/C_{18:2}$ and ω-6/ω-3) were calculated (where TUFA, total unsaturated fatty acids; TSFA, total saturated fatty acids; TPUFA, total polyunsaturated fatty acids; TMUFA, total monounsaturated fatty acids).

Protein analysis

Protein fractions. Protein fractions were extracted based on the method outlined by Gheyasuddin *et al*. (1970) and the soluble nitrogen content of protein extracts was determined by micro-Kjeldahl method (Humphries 1956).

Amino acids. Amino acid content of split bean flours was assessed based on Hofmann *et al*. (1997, 2003). The GC-C-IRMS/MS measurements were carried out using 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 and quantitative analysis of amino acids.

Protein digestibility. The *in vitro* protein digestibility (IVPD) was estimated according to Akeson and Stahmann (1964). The nitrogen content was determined by micro-Kjeldahl method (Humphries 1956) to estimate protein in the digest to express IVPD in percentage:

$$\text{IVPD (\%)} = (\text{Protein in digest / Protein in defatted flour}) \times 100 \quad (3)$$

EAA score, PDCAAS and PER. The essential amino acid (EAA) score was calculated based on FAO-WHO (1991) reference pattern:

$$\text{EAA score} = (\text{mg of EAA per g test protein}) / (\text{mg of EEA per g protein in FAO-WHO reference pattern}) \times 100 \quad (4)$$

The protein digestibility corrected amino acid score (PDCAAS) of EAA requirement for adults (FAO-WHO 1991) was estimated:

$$\text{PDCAAS} = (\text{EAA in bean protein/FAO-WHO EAA reference pattern}) \times (\text{\% IVPD}) \quad (5)$$

The protein efficiency ratios (PER) were calculated from the amino acid composition of split bean flours based on Alsmeyer *et al*. (1974):

$$PER_1 = -0.684 + 0.456 \times Leu - 0.047 \times Pro \quad (6)$$

$$PER_2 = -0.468 + 0.454 \times Leu - 0.105 \times Tyr \quad (7)$$

$$PER_3 = -1.816 + 0.435 \times Met + 0.78 \times Leu + 0.211 \times His - 0.944 \times Tyr \quad (8)$$

Data analysis

The t-test was employed to ascertain differences in proximal features, minerals, fatty acid ratios, true protein, protein fractions and IVPD between uncooked and cooked split beans (StatSoft Inc. 2008).

Results

Seed and proximal features

Characteristic features of ripened seeds and split beans of three landraces of *Canavalia* are presented in Table 1. The *l/b* ratio of seeds of all landraces was almost similar, while the dry mass of split beans per seed was highest in mangrove *C. cathartica* and least in *C. maritima* of CSD. The moisture content was significantly higher in uncooked than in cooked split bean flours (Table 2). The crude protein was significantly higher in uncooked bean flours of both landraces of *C. cathartica*, while it was opposite in total lipids and carbohydrates. The crude fibre was significantly higher in cooked split bean flours of all landraces. The calorific value was significantly higher in cooked split bean flours of only *C. cathartica* of mangrove. There was no significant difference in ash content between uncooked and cooked split bean flours.

Table 1. Characteristic features of ripened beans and split beans of three landraces of *Canavalia* (n=25; mean±SD).

	Canavalia cathartica		*Canavalia maritima*
	Coastal sand dune	Mangrove	Coastal sand dune
Length, *l* (cm)	2.14±0.15	2.31±0.16	1.78±0.13
Breadth, *b* (cm)	1.37±0.10	1.49±0.10	1.17±0.18
Thickness, *t* (cm)	1.16±0.09	1.20±0.07	1.06±0.13
l/*b* ratio	1.57±0.12	1.55±0.03	1.55±0.15
Fresh weight/seed (g)	1.45±0.26	1.71±0.32	1.12±0.23
Fresh weight of split beans/seed (g)	1.16±0.11	1.17±0.15	0.63±0.18
Dry weight of split beans/seed (g)	0.37±0.13	0.43±0.16	0.20±0.09

Minerals profile

Contents of all 10 minerals assessed were significantly higher in uncooked split beans of *C. cathartica* of CSD, while sodium, calcium, phosphorus, magnesium and manganese content were higher in *C. cathartica* of mangrove (Table 3). Potassium and selenium content were significantly higher in uncooked split beans of *C. maritima* of CSD, while sodium, calcium,

Table 2. Proximate composition of uncooked and cooked ripened split bean flours of three landraces of *Canavalia* on dry weight basis (n=5; mean±SD).

	Canavalia cathartica				*Canavalia maritima*	
	Coastal sand dune		Mangrove		Coastal sand dune	
	Uncooked	Cooked	Uncooked	Cooked	Cooked	Cooked
Moisture (%)	9.05±0.70*	7.34±0.86	8.11±031**	5.48±0.39	8.11±0.06	6.09±0.06***
Crude protein (g/100 g)	25.22±0.73***	19.61±0.48	28.55±0.48***	22.69±0.96	17.80±3.44	15.76±1.57
Total lipids (g/100 g)	2.37±0.08	3.16±0.02***	2.85±0.03	3.87±0.03***	3.27±0.82	2.68±0.68
Crude fibre (g/100 g)	1.61±0.04	2.76±0.05***	3.32±0.04	3.70±0.06***	0.76±0.15	1.28±0.29*
Ash (g/100 g)	3.24±0.32	3.17±0.37	3.46±0.30	3.42±0.20	2.79±0.12	2.70±0.29
Carbohydrates (g/100 g)	67.83±0.61	71.65±0.39***	61.92±0.28	66.47±1.26**	76.98±1.91	77.59±1.85
Calorific value (kJ/100 g)	1644±6.77	1637±7.12	1617±4.74	1632±2.26**	1679±17.5	1660±22.4

Asterisks across the uncooked and cooked columns are significantly differed (t-test: *, p<0.05, **, p<0.01, ***p<0.001).

phosphorus, magnesium and iron content were higher in cooked split beans. Magnesium, copper and manganese and selenium contents of uncooked as well as cooked split beans of all *Canavalia* spp. were almost equivalent or higher than NRC-NAS (1989) recommended pattern for infants. In addition, potassium and iron contents of uncooked as well as cooked split beans of *C. maritima* of CSD were higher than NRC-NAS pattern. Irrespective of uncooked and cooked split beans, all landraces showed favorable ratios of Na/K as well as Ca/P.

Table 3. Mineral composition (mg/100 g) of uncooked and cooked ripened split beans of three landraces of *Canavalia* on dry weight basis (n=5; mean±SD).

	Canavalia cathartica				*Canavalia maritima*		Recommended pattern
	Coastal sand dune		Mangrove		Coastal sand dune		
	Uncooked	Uncooked	Uncooked	Uncooked	Uncooked	Cooked	
Sodium	47.33±4.70**	36.61±4.21	45.62±3.98**	35.47±2.12	36.73±2.73	44.83±0.89*	120–200¥
Potassium	138.37±13.73*	117.86±13.56	133.09±11.62	124.94±7.46	972.97±5.79***	772.76±2.80	500–700¥
Calcium	161.86±16.06**	130.83±15.05	367.29±32.07*	332.78±13.90	229.51±13.49	160.92±11.2***	600¥
Phosphorus	133.42±13.23*	114.15±13.13	88.78±7.75*	78.39±4.68	149.27±0.50	154.27±0.08**	500¥
Magnesium	131.85±13.08**	98.60±11.34	160.75±14.04*	140.05±8.36	110.55±10.0	115.07±4.57	60¥
Iron	6.39±0.63**	5.32±0.61	7.30±0.64	7.40±0.44	12.56±0.22	13.80±1.0*	10¥
Copper	1.07±0.11**	0.84±0.10	1.08±0.09	1.07±0.06	0.79±0.06	0.85±0.02	0.6–0.7¥
Zinc	2.15±0.21**	1.80±0.21	2.34±0.20	2.26±0.14	2.32±0.07	2.36±0.08	5.0¥
Manganese	0.47±0.05*	0.42±0.05	0.54±0.05*	0.45±0.03	0.72±0.06	0.76±0.09	0.3–1.0¥
Selenium	1.51±0.01***	0.41±0.003	1.09±0.01***	0.61±0.01	1.02±0.01***	0.31±0.01	0.05-0.2¥
Na/K ratio	0.34	0.31	0.40	0.28	0.04	0.06	0.24–0.29
Ca/P ratio	1.21	1.15	4.14	4.25	1.54	1.04	1.2

Asterisks across the uncooked and cooked columns are significantly differed (t-test: *p<0.05, **p<0.01, ***p<0.001); ¥, NRC-NAS (1989) pattern for infants; Ψ, Pennington and Young (1990).

Fatty acids profile

The total lipids of split beans extracted by Soxhlet method consists of higher number of fatty acids in uncooked than in cooked samples of *C. cathartica* of CSD, while it was opposite for *C. maritima* of CSD (Table 4). The mangrove *C. cathartica* consists of almost similar number of fatty acids between cooked and uncooked split beans. Among the ω-6 fatty acids, except for cooked split beans of mangrove *C. cathartica*, the rest possess linoleic acid, while γ-linolenic acid was found in all except for *C. maritima*. Among ω-3 fatty acids, α-linolenic acid was present in all except for uncooked split beans of *C. maritima*. The docosahexaenoic acid was present in split beans of mangrove *C. cathartica* and uncooked split beans of CSD *C. cathartica*, while eicosatrienoic acid was confined to only uncooked split beans of mangrove *C. cathartica*.

Increased ratio of TUFA/TSFA in cooked split beans of *C. cathartica* and *C. maritima* of CSD were favorable (Table 5). Decrease in C14:0+C15:0+(C16:0/C18:0) ratio in cooked split beans of *C. maritima* of CSD was favorable, so also the C18:1/C18:2 ratio in *C. cathartica* of CSD.

Protein features

Protein fractions. Among the protein fractions, albumin content was significantly higher in uncooked split beans followed by globulin content in both *C. cathartica* landraces (Table 6). In *C. maritima*, globulin content was significantly higher in uncooked split beans, while opposite for the contents of prolamin and glutelin. Uncooked split beans of all landraces showed significantly higher true protein content than cooked split beans. Split beans of all the landraces showed increased A/G ratio on cooking.

Amino acids. Amino acids content of cooked split beans of three landraces have been compared with soybean, wheat and FAO-WHO (1991) recommended pattern (Table 7). The quantities of three EAA in *C. cathartica* of CSD (leucine, lysine and threonine), four EAA in mangrove (tyrosine + phenylalanine, threonine and valine) and eight EAA in *C. maritima* of CSD (histidine, isoleucine, leucine, lysine, tyrosine + phenylalanine, threonine and valine) surpassed the quantities in soybean, wheat and FAO-WHO stipulated standard (Bau et al. 1994; USD, 1999; FAO-WHO, 1991). The EAA/TAA ratio was higher in split beans of *C. cathartica* landraces than *C. maritima*, which was similar or higher than the ratios in soybean and wheat.

EAA score, PDCAAS, PER and IVPD. The EAA score of split beans of all landraces were favorable (>100%) except for cystine + methionine, tyrosine + phenylalanine and histidine (Table 8). The PDCAAS was higher in split beans of *C. cathartica* of mangrove than CSD, so also for *C. maritima* of CSD (Table 9). The PER_1, PER_2 and PER_3 of split beans of all landraces were favorable as they attained >2 (with one exception) (Table 10). The IVPD of split beans of all landraces was favorable as it has increased on cooking (Table 11).

Table 4. Fatty acid methyl esters of uncooked and cooked ripened split beans of three landraces of *Canavalia*.

	Canavalia cathartica				*Canavalia maritima*	
	Coastal sand dune		Mangrove		Coastal sand dune	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
Saturated fatty acids						
Caproic acid (C6:0)	+	–	–	–	–	–
Caprylic acid (C8:0)	+	–	+	+	+	+
Capric acid (C10:0)	+	+	+	+	+	+
Lauric acid (C12:0)	+	+	+	+	+	+
Tridecanoic acid (C13:0)	+	–	–	–	–	–
Myristic acid (C14:0)	+	+	+	+	+	+
Pentadecanoic acid (C15:0)	+	–	+	+	–	–
Palmitic acid (C16:0)	+	+	+	+	+	+
Heptadecanoic acid (C17:0)	+	–	–	–	–	–
Stearic acid (C18:0)	+	+	+	+	+	+
Arachidic acid (C20:0)	+	+	+	+	–	+
Behenic acid (C22:0)	+	–	+	+	–	–
Lignoceric acid (C24:0)	+	+	+	+	–	+
Total	13	07	10	10	06	08
Unsaturated fatty acids						
Palmitoleic acid (C16:1)	+	+	+	+	–	–
Cis-10-Heptadecanoic acid (C17:1)	+	–	+	+	–	+
Oleic acid (C18:1)	+	+	+	+	+	+
Linoleic acid (C18:2)	+	–	+	+	+	+
α-Linolenic acid (C18:3)	+	+	+	+	–	+
γ-Linolenic acid (C18:3)	+	+	+	+	–	–
Eicosenoic acid (C20:1)	+	+	+	+	–	–
Eicosatrienoic acid (C20.3)	–	–	+	–	–	–
Erucic acid (C 22:1)	+	–	–	–	–	–
Docosahexaenoic acid (C22:6)	+	–	+	+	–	–
Nervonic acid (C24:1)	+	–	+	+	–	+
Total	10	05	10	09	02	05

Table 5. Ratio of fatty acids of uncooked and cooked ripened split beans of three landraces of *Canavalia* (n=5; mean±SD).

	Canavalia cathartica				*Canavalia maritima*	
	Coastal sand dune		Mangrove		Coastal sand dune	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
TUFA /TSFA	0.23±0.002	0.49±0.001**	0.47±0.01**	0.42±0.01	0.10±0.10	0.14±0.20*
TPUFA/TMUFA	0.42±0.001**	0.28±0.01	0.71±0.001**	0.39±0.001	0.24±0.01*	0.23±0.01
C14:0 + C15:0 + (C16:0/C18:0)	7.87±0.16	8.36±0.01*	8.60±0.04	9.42±0.04**	8.14±1.32	7.82±1.17*
C18:1/C18:2	2.85±0.002**	2.79±0.01	1.87±0.001	2.78±0.002**	4.18±0.10*	4.78±0.03
ω-6/ω-3	2.38±0.03	3.64±0.002**	2.16±0.10	3.78±0.002**	–	5.14±0.10

Asterisks across the uncooked and cooked columns are significantly differed (t-test: *p<0.05, **p<0.01); TSFA, total saturated fatty acids; TUFA, total unsaturated fatty acids; TMUFA, total monounsaturated fatty acids; TPUFA, total polyunsaturated fatty acids.

Table 6. True proteins and protein fractions (g/100 g) of uncooked and cooked ripened split beans of three landraces of *Canavalia* (n=5; mean±SD)

	Canavalia cathartica				*Canavalia maritima*	
	Coastal sand dune		Mangrove		Coastal sand dune	
	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
True protein	19.28±0.33*	14.18±0.84	22.72±1.06*	17.32±1.07	10.33±0.43*	6.25±0.91
Albumin	10.62±0.23*	9.42±0.33	14.58±0.22**	11.44±0.26	1.53±0.31	2.80±0.78
Globulin	4.56±0.63**	1.60±0.64	3.94±0.86*	1.30±0.60	7.66±0.19*	1.56±0.10
Prolamin	1.52±0.01	1.57±0.64	0.74±0.01	1.27±0.60	0.59±0.01	0.95±0.02***
Glutelin	2.29±0.01	1.51±0.67	3.45±0.42	1.38±0.67	0.57±0.03	0.95±0.05***
A / G ratio$^{\Psi}$	2.33	5.90	3.70	8.80	0.20	1.80

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Discussion

Seed and proximal features

As the *l/b* ratio of ripened beans of all landraces is uniform, thus handling and processing like dehulling, drying, milling and cooking will be easier. The purpose of removal of seed coat and testa of ripened beans by the coastal dwellers is to eliminate antinutritional components. The seed coat with testa is easily removable and their elimination enhances the nutritional quality (Bhagya *et al*. 2006, 2007).

Table 7. Amino acid composition (mg/100 mg protein) of cooked ripened split beans of three landraces of *Canavalia* (n=5; mean±SD).

	Canavalia cathartica		*Canavalia maritima*	Soybean[a]	Wheat[b]	FAO-WHO[c]
	Coastal sand dune	Mangrove	Coastal sand dune			
Essential amino acid						
His	1.76±0.05	1.65±0.07	3.86±0.49	2.50	1.9-2.6	1.9
Ile	3.11±0.22	4.16±0.18	6.68±0.75	4.62	3.4-4.1	2.8
Leu	8.22±0.46	7.17±0.32	13.60±1.54	7.72	6.5-7.2	6.6
Lys	6.22±0.34	5.37±0.24	9.53±1.08	6.08	1.8-2.4	5.8
Cys	1.01±0.06	0.88±0.04	1.60±0.17	1.70	1.6-2.6	2.5[d]
Met	0.95±0.06	0.75±0.03	0.58±0.06	1.22	0.9-1.5	
Tyr	1.99±0.06	2.83±0.12	6.43±0.73	1.24	1.8-3.2	6.3[e]
Phe	4.01±0.16	4.28±0.19	7.49±0.93	4.84	4.5-4.9	
Trp	BDL	BDL	BDL	3.39	0.7-1	1.1
Thr	5.62±0.31	4.64±0.20	6.82±0.77	3.76	2.2-3	3.4
Val	4.11±0.31	4.87±0.21	7.62±0.86	4.59	3.7-4.5	3.5
Non-essential amino acid						
Ala	4.52±0.016	4.94±0.22	7.22±0.83	4.23	2.8-3	–
Arg	4.90±0.11	5.09±0.22	8.67±0.98	7.13	3.1-3.8	–
Asp	12.10±0.42	12.21±0.54	18.50±2.11	11.30	3.7-4.2	–
Glu	14.06±0.40	13.02±0.57	25.60±2.91	16.90	35.5-36.9	–
Gly	4.55±0.18	3.68±0.16	6.14±0.69	4.01	3.2-3.5	–
Pro	2.95±0.09	2.75±0.12	6.39±0.85	4.86	11.4-11.7	–
Ser	4.22±0.21	5.11±0.23	8.16±0.92	5.67	3.7-4.8	–
EAA / TAA ratio	0.49	0.49	0.43	0.43	0.31–0.35	–

[a], Bau *et al*. (1994); [b], USDA (1999); [c], FAO-WHO (1991); [d], Cystine+Methionine; [e], Tyrosine+Phenylalanine; EAA, Essential amino acid; TAA, Total amino acid; BDL, Below detectable level.

Table 8. Essential amino acid (EAA) score of cooked ripened split beans of three landraces of *Canavalia*.

	Canavalia cathartica		*Canavalia maritima*
	Coastal sand dune	Mangrove	Coastal sand dune
Thr	165.29	136.52	200.6
Val	117.43	139.14	217.7
Cys + Met	78.40	65.03	87.2
Ile	111.07	148.71	238.6
Leu	124.55	108.56	206.1
Tyr + Phe	95.24	112.76	221.0
Lys	107.24	92.61	164.3
His	92.63	87.26	203.2

Table 9. Protein digestibility corrected to amino acid score (PDCAAS) of cooked ripened split beans of three landraces of *Canavalia*.

	Canavalia cathartica		***Canavalia maritima***
	Coastal sand dune	Mangrove	Coastal sand dune
Thr	97.41	101.38	136.96
Val	69.20	103.33	148.53
Cys + Met	46.20	48.29	69.63
Ile	65.45	110.43	162.75
Leu	73.39	80.62	140.66
Tyr + Phe	56.12	83.73	59.34
Lys	63.20	68.77	112.17
His	54.59	64.80	138.49

Table 10. Protein efficiency ratio (PER) of cooked ripened split beans of three landraces of *Canavalia*.

	Canavalia cathartica		***Canavalia maritima***
	Coastal sand dune	Mangrove	Coastal sand dune
PER_1	2.91	2.45	4.48
PER_2	3.06	2.49	4.38
PER_3	3.50	1.78	3.36

Table 11. *In vitro* protein digestibility (IVPD) (%)of uncooked and cooked ripened split beans of three landraces of *Canavalia* (n=5; mean±SD).

Canavalia cathartica		***Canavalia cathartica***		***Canavalia maritima***	
Coastal sand dune		Mangrove		Coastal sand dune	
Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
41.86±3.50	58.93±3.09*	50.23±4.20	74.26±3.94**	64.30±40.24	66.50±25.25*

Asterisks across the uncooked and cooked columns are significantly differed (t-test: *p<0.05, **p<0.01).

The moisture level of uncooked split beans was higher than cooked split beans and comparable to uncooked and cooked intact ripened beans (Bhagya *et al*. 2006, 2007). The crude protein content of uncooked split beans (17.8–28.6%) is comparable or higher than some of the edible legumes (19.4–25.3%) (e.g. *Cajanus cajan, Cicer arietinum, Phaseolus* spp. and *Vigna unguiculata*) (Gupta and Wagle 1978; Jambunathan and Singh 1980; Nwokolo and Oji 1985; Nwokolo 1987). Cooking of split beans significantly lowered the protein content (25.2–28.6 vs. 19.6–22.7%) only in *C. cathartica* landraces.

The total lipids of uncooked split beans of *C. cathartica* landraces is generally low but significantly increased by cooking, which is almost twice higher than cooked ripened seeds (3.2–3.9 vs. 1.5–1.6%) (Bhagya *et al*. 2006, 2007). As the lipid content is low, the split beans serve as valuable weight-reducing diet (Bello *et al*. 2008).

The crude fibre content of split beans ofall landraces was significantly increased on cooking, but lower than ripened seeds (1.3–3.7 vs. 8.3–10.4%) (Bhagya *et al*. 2006, 2007). Although removal of seed coat have considerably lowered the crude fibre content of split beans, its significant increase on cooking may be due to the formation of resistant starch, products of Maillard reaction and condensed tannin (Mongeau and Brassard 1995; Perez-Hidalgo *et al*. 1997). Low quantity of crude fibre is nutritionally advantageous in trapping less proteins as well as carbohydrates (Balogun and Fetuga 1986), which helps in lowering the risks like blood cholesterol as well as large bowel cancers (Anderson *et al*. 1995; Salvin *et al*. 1997).

The carbohydrates of split beans were increased on cooking and such increase helps in combating intestinal cancers (Aranda *et al*. 2001), imparts low glycemic index and management of type II diabetes (Venn and Mann 2004). The split beans of *C. cathartica* can be considered as a potential source of carbohydrate similar to conventional cereals (≥70 g/100 g) (Adewusi *et al*. 1995) and serve as potential supplement to cereals to develop energy foods and food formulations.

Minerals profile

Magnesium, copper and manganese contents of uncooked and cooked split beans of all landraces are almost equivalent or higher than NRC-NAS (1989) recommended pattern, which is partially similar to ripened beans (Bhagya *et al*. 2006, 2007). However, in ripened beans of coastal sand dune *C. cathartica*, potassium content surpassed NRC-NAS (1989) pattern and likely the seed coat is the site of its accumulation (Bhagya *et al*. 2006). In addition, uncooked and cooked split beans of *C. maritima* of CSD are rich in potassium content, which also surpassed the NRC-NAS (1989) recommended pattern.

The ratios of Na/K (<1) and Ca/P (>1) of uncooked and cooked split beans of all landraces are desirable. Those foods possessing low Na/K ratio (<1) are known to control high blood pressure (Yusuf *et al*. 2007), while those foods with high Ca/P ratio (>1) are known to prevent calcium loss in urine and restore calcium in bones (Shills and Young 1988).

Sodium content in all landraces was low and such low quantity in diets are suitable for those suffering from hypertension. Selenium content in split beans is higher than the stipulated level as suggested by Pennington and Young (1990). The selinium content in all landraces surpassed the suggested range, which serves as prosthetic group of antioxidant enzymes, protects cells from free radicals as well as prevents the toxic effect of heavy metals (e.g. arsenic, cadmium, mercury and tin) (Combs and Gray 1998).

Fatty acids profile

The split beans possess two ω-6 (linoleic and γ-linolenic acids) and three ω-3 (α-linolenic, eicosatirenoic and docosahexaenoic acids) fatty acids. The *C. cathratica* landraces are better in composition of unsaturated fatty acids compared to *C. maritima* of CSD. Several essential fatty acids (e.g. linoleic, linolenic, arachidonic, eicosapentoaenoic and docosahexaenoic acids) were also common in ripened beans of CSD *Canavalia* spp. (Bhagya *et al*. 2006, 2007). The essential fatty acids (eicosienoic and eicosapentaenoic acids) were also seen in cooked dry seeds of CSD *C. maritima* (Seena *et al*. 2005).

Among the five fatty acid ratios (see Table 5), increase in first two ratios and decrease in the rest denotes superior nutritional qualities (Shreelalitha *et al*. 2011; Supriya *et al*. 2012). Cooking split beans significantly increased the first ratio in *C. cathartica* of mangrove and *C. maritima* of CSD. The third ratio was favorable only in *C. maritima*, so also the fourth ratio only in mangrove *C. cathartica*.

Simopoulos (2004) opined that humans are evolved on a diet with low ω-6/ω-3 ratio (~1), however, this ratio has gradually increasing in different regions of the world (4–50). Several benefits have been envisaged with low ω-6/ω-3 ratio: ratio 2.5 reduces the rectal cell proliferation in patients with colorectal cancer; ratio of 2–3 suppress the inflammation in patients with rheumatoid arthritis; ratio of 5 exhibits beneficial effect to the patients with asthma. In the present study, ω-6/ω-3 ratio in ripened split beans of *C. cathartica* landraces ranged from 2.2–3.8, which is lower than the range recommended (5–10) by the FAO-WHO (1998). However, the cooked split beans of *C. martima* possess ratio of 5.14, which falls within the FAO-WHO pattern. Low fat and high protein contents in split beans of *Canavalia* landraces of the present study are highly suitable to combat protein-energy malnutrition especially in hyperlipedemic patients (Tharanathan and Mahadevamma 2003).

Protein bioavailability

According to Baudoin and Maquet (1999), albumin consists of more sulfur amino acids as well as other EAA. High albumin in split beans usually reflected in increased sulfur amino acids (cystine + methionine), while low globulin results in low antinutritional components as many antinutritional components are related to globulin content (Bhagya *et al*. 2006, 2007). Unlike intact ripened beans (0.51–0.56 vs. 0.33–0.37) (Bhagya *et al*. 2006, 2007), the A/G ratio increased in split beans on cooking (0.2–3.7 vs. 1.8–8.8) depicting superior quality of split beans. The globulin fractions are responsible for poor protein digestibility in *Canavalia* seeds (Bressani *et al*. 1987; Bressani and Sosa 1990; Ekanayake *et al*. 2000). Due to presence of lectins in globulin fraction, seeds of *Canavalia gladiata* strongly agglutinated all human erythrocytes (Mohan and Janardhanan 1994). In the present study, on cooking substantial quantity of globulins were lost in split beans (3.9–7.7 vs. 1.3–1.6 g/100 g), which might have resulted in drastic reduction or knock off hemagglutination potential.

Usually, seeds of legumes consist of high quantity of lysine and low quantity of sulfur amino acids (Norton *et al*. 1985), which is also applicable to split beans of *Canavalia* landraces. Almost all amino acids of cooked split beans of three landraces were surpassed the uncooked and cooked ripened beans (Bhagya *et al*. 2006, 2007). Several EAA of cooked split beans of *canavalia* landraces are comparable or higher than soybean and wheat (Bau *et al*. 1994; USDA 1999). Isoleucine, leucine, threonine and valine contents of cooked split beans of all landraces surpassed the FAO-WHO (1991) stipulated standard, which has reflected in almost uniform EAA/TAA ratios (0.43–0.49) equivalent or higher than the ratios in soybean and wheat.

According to Sarwar *et al*. (1984), the EAA score serves as an alternative to protein efficiency ratio (PER). Almost all EAA of split beans fulfilled the EAA score (except for cystine + methionine and tryptophan). The PDCAAS is one of the important parameters in evaluation of nutritional quality of edible proteins. The PDCAAS was higher in *C. cathartica* of mangrove than CSD landrace, while it was highest in CSD *C. maritima*. According to Friedman's (1996) classification, the PER is poor (<1.5), moderate (1.5–2) and superior (>2). The PER_1, PER_2 and PER_3 of cooked split beans attained >2 (except for PER_3 in mangrove *C. cathartica*, 1.8) indicating their superior quality. The IVPD has been considered on par with the *in vivo* protein digestibility. The IVPD has increased in cooked split beans in all landraces shows their suitability for human consumption.

Conclusions and outlook

There is an upsurge in identifying value-added indigenous food sources to cope with teeming population in developing countries. Several plant species possess adequate protein-energy and nutraceutical potential are utilized traditionally in different geographic regions by ethnic groups. The desired traits of such plant species for popularization and domestication include fast growth, high yield and adaptability to wide geographic/agroclimatic conditions. Among the plant species distributed in the coastal regions of the southwest India, *Canavalia* landraces are of immense value. Among the studies carried out (dry seeds, sprouted seeds, ripened seeds and ripened split beans), the ripened split beans devoid of seed coat and testa showed several desired nutritional properties (e.g. low fat, high protein-energy, essential fatty acids, essential amino acids, high protein efficiency ratio and high *in vitro* protein digestibility). Such nutritional qualities provide opportunities to combat many lifestyle-dependent diseases like hyperlipidemia, high cholesterol, large bowel cancers and type II diabetes. Further studies should focus on domestication and large-scale cultivation of *Canavalia* landraces in different agroclimatic conditions to enrich the soil qualities as well as to derive nutraceutical benefits of ripened split beans.

Acknowledgements

Authors are grateful to Mangalore University for permission to carry out this study in the Department of Biosciences. K.R. Sridhar gratefully acknowledges the UGC-BSR Faculty Fellowship award by the UGC, New Delhi. S.J. Shrililitha acknowledges the UGC, New Delhi for the award of research fellowship under scheme RFSMS. P. Supriya is grateful to BRNS, Bhabha Atomic Research Centre, Mumbai for the award of research fellowship.

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