International Food Research Journal 16: 249-260 (2009)

Nutritional evaluation of germinated seeds of coastal sand dune wild legume *Canavalia cathartica*

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Abstract: Uncooked and cooked germinated seeds of the wild legume, *Canavalia cathartica* grown on the southwest coastal sand dunes of India were evaluated for proximate composition, minerals, amino acids, *in vitro* protein digestibility, fatty acid methyl esters and antinutritional features. The crude protein (24–23.2%; P = 0.009), true protein (19.8–8.4%; P = 7.86×10^{-05}) and crude fiber (1.2–1.1%; P = 0.02) of germinated seeds were significantly decreased on cooking, while the crude lipid (2.7–2%; P = 0.004), nitrogen free extractives (69.3–70.3%; P = 0.01) and calorific value (1627–1663 kJ 100 g^{-1}; P = 0.0009) raised significantly. Albumin fraction was significantly higher among the protein fractions (uncooked, 11.8%; cooked, 4.9%; P = 7.86×10^{-05}), while the rest significantly lowered on cooking (P < 0.01) except for glutelin (0.92–1.4%; P = 0.31). The SDS-PAGE of albumin and globulin fractions revealed a substantial reduction in the intensity of bands in cooked seeds. Although most of the minerals severely drained in cooked seeds, magnesium, iron, copper and manganese fulfilled the NRC/NAS standards. Many essential amino acids (threonine, valine, isoleucine, leucine, phenylalanine + tyrosine, lysine and histidine) of uncooked and cooked seeds surpassed the FAO/WHO pattern. The histidine of germinated seeds was higher than whole egg protein (2.7–3.5 vs. 2.4%). The *in vitro* protein digestibility of germinated seeds elevated significantly on cooking (59.6–71.7%; P = 0.02). Cooking also increased unsaturated fatty acids, particularly the linoleic acid (0.35–3.95 g 100 g^{-1} lipid) and also P/S ratio (0.11–0.78). The germinated seeds were devoid of tannins and trypsin inhibitors, whereas the total phenolics and hemagglutinin activities considerably decreased on cooking. The overall nutritional qualities of germinated seeds of coastal sand dune *C. cathartica* is superior to dry seeds, but demands additional suitable method to eliminate the toxins or to decrease below threshold level.

Keywords: *Canavalia cathartica*, wild legume, coastal sand dunes, germinated seeds, nutritional features

Introduction

Exploration and utilization of unconventional legumes are promising to fulfill the deficiency of proteins and essential fats in human nutrition as evidenced by African wild legumes (Apata and Olighobo, 1994; Badifu, 1994; Madubuike *et al*., 1994; Ezeagu *et al*., 1996; Petzke *et al*., 1997). *Canavalia* species are among the 30 wild legumes consumed by the tribal sects in India (Arora *et al*., 1980; Gunjatkar and Vartak, 1982; Viswanathan *et al*., 1999, 2001). *Canavalia* species occurring on the coastal sand dunes are also promising source of proteins and essential amino acids (Bressani *et al*., 1987; Sridhar and Seena, 2006; Seena and Sridhar, 2006). According to Smartt (1990), the Genus *Canavalia* encompass four subgenera with 51 species widely distributed in tropical and subtropical regions of the world. *Canavalia cathartica* Thouars (common name: Maunaloa; synonyms: *C. microcarpa* (DC.) Piper; *C. turgida* Graham *ex* A. Gray; *C. virosa* (Roxb.) Wight *et* Arn.; *Dolichos virosus* Roxb.; *Lablab microcarpus* DC.) is one of the frequent, drought tolerant and wildly distributed wild legumes on the coastal sand dunes of southwest coast of India (Arun *et al*., 1999). Another variety of *C. cathartica* grows in the mangroves of the Indian southwest coast differs from the coastal sand dune variety in growth pattern, size and color of leaves, pods and seeds (Seena and Sridhar, 2007). Purseglove (1974) considered *C. cathartica* as a wild ancestral form of *C. gladiata* distributed throughout tropical Asian and African continents. Sastrapradja *et al*. (1981) reported a natural hybrid of *C. cathartica* × *C. gladiata* and artificial hybridization of *C. cathartica* vs. *C. ensiformis* resulted in decreased pollen fertility of F1 and F2 progenies. Gamma irradiation at the doses of 4 and 6 krad has improved the germination potential

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of seeds of *C. cathartica* (Rodrigues, 1993). Tissue cultured plants of *C. cathartica* were successfully hardened and transplanted to the field by Kathiravan and Ignacimuthu (1999). Nutritional assessment of *C. cathartica* of coastal sand dune and mangrove varieties has been confined only to dry seeds, ripened beans and tender pods (Sridhar and Seena, 2006; Bhagya et al., 2006a, 2006b, 2007; Bhagya and Sridhar, 2007).Therefore, the main objective of the present study is to evaluate the nutritional potential of uncooked and cooked germinated seeds of *C. cathartica* of the southwest coastal sand dunes of India employing conventional methods to ascertain whether they are superior to dry seeds.

Materials and Methods

Seed processing

Seeds of *C. cathartica* were gathered from the dried pods of plants grown on the sand dunes of Someshwara of the southwest coast of India (12º47′N, 74º52′E). Healthy seeds were chosen and sun dried for two days. For cessation of seed dormancy, the hilum portion of seeds was scarified on grinding against a granite stone wheel. They were soaked on wet cotton overnight and allowed to germinate on the moist sand bed. Germination of scarified seeds usually occurred within 48 hr. After dehusking, they were rinsed in water and grouped into two sets. The first set was sun dried for two days, while the second, pressure-cooked in freshwater (1:3 v/v) using a household pressure-cooker prior to sun drying for two days. The dried seeds were milled (Wiley mill, 30 mesh) and stored in airtight containers in refrigerator for nutritional analysis.

Nutritional analysis

Moisture content of seed flours was estimated gravimetrically. Total nitrogen and crude protein were determined by micro-Kjeldahl method (Humphries, 1956). The crude lipid, crude fiber and ash were estimated gravimetrically based on AOAC (1990). The nitrogen-free extractives (NFE) were determined based on Müller and Tobin (1980), while calorific value by Ekanayake *et al*. (1999):

NFE (%) = 100 – (Crude protein + Crude lipid + Crude fiber + Ash)

Calorific value (kJ 100 g^{-1}) = (Protein × 16.7) + (lipid × 37.7) + (Carbohydrates × 16.7) Trueprotein and its fractions were determined according to Basha *et al*. (1976) and Lowry *et al*. (1951). The albumin and globulin fractions were separated by SDS-PAGE (Laemmli, 1970). Mineral estimation was based on atomic absorption spectroscopy (AOAC, 1990), while total phosphorus by sepectrophotometry (APHA, 1995). Amino acids were assessed using gas chromatography-combustion-isotope ratio mass spectrometer (GC-C-IRMS/MS) (Hofmann *et al*. (1997, 2003) with derivatizaton of esterification with trifluoroacetylation (Brand *et al*., 1994). The essential amino acid (EAA) score was calculated considering FAO-WHO (1991) reference pattern:

[(Mg EAA in 100 mg test protein)] ÷ [(Mg of EAA in 100 mg FAO-WHO pattern)] × 100

The *in vitro* digestibility (IVPD) was determined according to Akeson and Stahmann (1964) and the protein digestibility corrected amino acid score (PDCAAS) of EAA requirement for adults (FAO-WHO, 1991) were also estimated:

IVPD (%) = [(Protein in digest)] ÷ [(Protein in defatted flour)] × 100

PDCAAS (%) = [(EAA in test protein)] ÷ [(FAO-WHO EAA requirement)]× 100

The fatty acid methyl esters (FAMEs) of seed flours were measured using gas liquid chromatography (Garces and Mancha, 1993). The ratio of polyunsaturated and saturated fatty acid (P/S) was calculated:

P/S ratio = (Sum of polyunsaturated fatty acids) ÷ (Sum of saturated fatty acids)

Antinutritional analysis

The total phenolics was estimated according to the Rosset *et al*. (1982), while tannins by vanillin-HCl method by Burns (1971). Trypsin inhibition activity was determined based on enzymatic assay (Kakade *et al*., 1974). Hemagglutinin activity against human erythrocytes (A, B, AB, O) was evaluated according to Hankins *et al*. (1980) and titre value noted based on serial dilution.

Data analysis

The differences in proximate composition, protein fractions, IVPD and total phenolics between the flours of uncooked and cooked germinated seeds were assessed by t-test (StatSoft Inc., 1995).

Table 1. Proximate composition of flours of uncooked and cooked germinated seeds of *Canavalia cathartica* of coastal sand dunes on dry weight basis (n=5; mean±SD) [1)]

Component	Germinated seeds	
	Uncooked	Pressure-cooked
Moisture (%)	9.16±0.05[a]	5.32±0.04[b]
Crude protein (%)	23.95±0.27[a]	23.15±0.21[b]
Crude lipid (%)	2.00±0.07[a]	2.69±0.36[b]
Crude fiber (%)	1.19±0.03[a]	1.06±0.09[b]
Ash (%)	3.52±0.08[a]	2.80±0.12[b]
Nitrogen free extractives (%)	69.33±0.10[a]	70.32±0.61[b]
Calorific value (kJ 100 g^{-1})	1627±13[a]	1663±7[b]

[1)] Figures across the columns with different letters are significantly different ($P < 0.05$, t-test)

Table 2. True protein fractions of flours of uncooked and cooked germinated seeds of *Canavalia cathartica* of coastal sand dunes on dry weight basis (g 100 g^{-1}; percent in parenthesis) (n=5; mean±SD)[1)]

Protein fraction	Germinated seeds	
	Uncooked	Pressure-cooked
True protein	19.76±0.54 (100)[a]	8.42±0.51 (100) [b]
Albumins	11.77±0.51 (59.6)[a]	4.88±0.02 (58.0)[b]
Globulins	4.59±0.50 (23.2)[a]	0.80±0.51 (9.5)[b]
Prolamins	2.48±0.53 (12.6)[a]	1.37±0.01 (16.3)[b]
Glutelins	0.92±0.004 (4.7)[a]	1.37±0.02 (16.3)[a]

[1)] Figures across the columns with different letters are significantly different ($P < 0.05$, t-test)

Results and Discussion

Proximate composition

Cooking germinated seeds resulted in significant loss of flour moisture ($P < 0.001$), crude protein ($P < 0.01$), crude fiber ($P < 0.05$) and ash ($P < 0.001$), while significant increase in crude lipid ($P < 0.01$), NFE ($P < 0.05$) and calorific value ($P < 0.001$) as seen in dry seeds (Seena *et al*., 2005) (Table 1). The crude protein substantially lower than dry seeds (23.2-24% vs. 29.2-35.5%), but surpassed some of the common legumes such as winged bean (*Neonotonia wightii*) (20.6%) (Viswanathan *et al*., 2001), velvet bean (*Mucuna monosperma*) (23.5%), green gram (*Phaseolus aureus*) (22.3%), some black gram genotypes (*Phaseolus mungo*) (23.3%) (Gupta and Wagle, 1978) (*P. vulgaris*) (19.1%), (*P.lunatus*) (19.7%) (Baudoin and Maquet, 1999), pigeon pea (*Cajanus cajan*) (19.4%) (Nwokolo, 1987), chickpea (*Cicer arietinum*) (20.7%) (Jambunathan and Singh, 1980) and cowpea (*Vigna unguiculata*) (22.5%). Crude protein also higher than the whole-wheat flour (8.5%), parboiled rice (7.7%) and eggs (12.6%) (Livsmedelsverk, 1988). However, the crude protein of germinated *C. cathartica* was lower than uncooked and thermally processed (cooked, authoclaved and roasted) gila beans (*Entada scandens*) (26.6-27%) (Vadivel *et al*., 2008). The crude lipid was more than dry seeds (2-2.7% vs. 1.4%), while crude fiber was higher than cooked dry seeds (1.1-1.2 vs. 1%). According to Balogun and Fetuga (1986), the low amount of fiber in food is nutritionally valuable as it traps less protein and carbohydrates, but high fiber diet is warranted for several health benefits particularly to lower the blood cholesterol and reducing the risks of large bowel cancer (Anderson and Johnstone, 1995; Salvin *et al*., 1997). Unlike dry seeds, the ash content decreased on cooking germinated seeds (3.5% vs.

Table 3. Mineral compositions of flours of uncooked and cooked germinated seeds of *Canavalia cathartica* of coastal sand dunes on dry weight basis (mg 100 g^{-1})

Mineral	Germinated seeds		NRC/NAS1)
	Uncooked	Pressure-cooked	
Sodium	7.50	5.10	120-200
Potassium	340.00	280.00	500-700
Calcium	510.00	340.00	600
Phosphorus	340.00	290.00	500
Magnesium	400.00	300.00	60
Iron	15.60	9.80	10
Copper	1.19	1.02	0.6-0.7
Zinc	4.79	4.49	5.0
Manganese	5.36	3.44	0.3-1
Selenium	8.60	8.20	–

[1)] NRC/NAS (1989) recommended pattern for infants

2.8%) possibly due to severe loss of minerals (Seena *et al*., 2005) (see Table 3). The NFE was significantly elevated on cooking (69.3 vs. 70.3%) ($P < 0.05$) as seen in dry seeds (58.4% vs. 65.4%), which caused a significant increase in calorific value (1627 vs. 1663 kJ 100 g^{-1}) ($P < 0.001$). High carbohydrates in seeds are known to combat intestinal cancers (Aranda *et al*., 2001) and result in low glycemic index in prevention and management of type II diabetes (Venn and Mann, 2004). The calorific value of germinated seeds was higher than many cultivated legumes (1627-1663 vs. 1358-1426 kJ 100 g^{-1}) (Kuzayali *et al*., 1966) and wild legumes such as velvet beans (*Mucuna prurients* var. *utilis*) (1541 kJ 100 g^{-1}) (Vadivel and Pugalenthi, 2007) and gila beans (*Entada scandens*) (1516 kJ 100 g^{-1}) (Vadivel *et al*., 2008).

Protein fractions

True protein significantly decreased on cooking germinated seeds (19.8 vs. 8.4%) ($P < 0.001$) (Table 2). The uncooked germinated seeds consist of higher true protein than many legumes such as winged bean (*Psophocarpus*) (15.2%) (Viswanathan *et al*., 2001) and senna (*Cassia floribunda*) (16.3–17.7%) (Vadivel and Janardhanan, 2001). Uncooked germinated seeds possess low quantity of true protein compared to the uncooked dry seeds (19.8 vs. 28.6%) (Seena *et al*., 2005). All protein fractions were significantly decreased ($P < 0.05$-0.001) except for glutelins ($P = 0.31$). Uncooked and cooked germinated seeds possess highest albumin (11.8 and 4.9%), while globulin in uncooked dry seeds (18.3%). Albumins are known to possess more sulfur-amino acids as well as other EAA (Baudoin and Maquet, 1999). Although albumin decreased substantially in germinated seeds on cooking (11.8 vs. 4.9%), the EAA including sulfur-amino acids were not deprived too much (see Table 4). Separation of albumin and globulin fractions of uncooked germinated seeds by SDS-PAGE showed 5 and 3 prominent bands respectively at the range of 3 to 43.4 kDa (Figure 1). In cooked germinated seeds, the bands were either absent or smear-like unclear bands. The low intensity of bands indicates partial denaturation of albumin as well as globulin in cooked seeds and resulted in lowering the agglutination of human erythrocytes (see Table 7).

Minerals profile

The minerals drained substantially in cooked germinated seeds possibly due to increased seed permeability, which resulted in decreased ash content (Table 3). The dry seeds also showed drastic decrease in minerals on cooking (Seena *et al*., 2005). In some wild legume seeds, cooking resulted in loss of minerals (Aletor and Ojo, 1989). In our study, calcium (340-510 vs. 44-83.7 mg 100 g^{-1}), phosphorus (290-340 vs. 99.4-137 mg 100 g^{-1}), iron (9.8-15.6 vs. 2.18-2.88 mg 100 g^{-1}), magnesium (300-400 vs. 3.58-5.3 mg 100 g^{-1}) copper (1.02-1.19 vs. 0.1-0.35 mg 100 g^{-1}) and manganese (3.44-5.36 vs. 0.79-1.36 mg 100 g^{-1}) in germinated seeds were higher than dry seeds (Seena *et al*., 2005). Uncooked and cooked germinated seeds fulfilled the NRC/NAS (1989) recommended pattern for magnesium (60 mg 100 g^{-1}), iron (10 mg 100 g^{-1}), copper (0.6-0.7 mg 100 g^{-1}) and manganese (0.3-1 mg 100 g^{-1}). Sodium

Table 4. Amino acid compositions of uncooked and cooked germinated seeds of *Canavalia cathartica* of coastal sand dunes on dry weight basis (g 100 g^{-1} protein) (EAA score in parenthesis)

Amino acid	Germinated seeds		Whole egg Protein[1)]	FAO-WHO Pattern[2)]
	Uncooked	Pressure-cooked		
Glutamic acid	18.15	14.75	12.70	
Aspartic acid	18.37	12.47	9.60	
Serine	6.20	6.01	7.60	
Threonine	4.95 (145.5)	5.01 (147.4)	5.10 (150)	3.40
Proline	5.70	4.28	4.20	
Alanine	5.02	4.96	5.90	
Glycine	4.41	4.36	3.30	
Valine	5.31 (151.7)	4.94 (141.1)	6.90 (197.1)	3.50
Cystine	1.15	1.13	5.90	
Methionine	0.57 (68.8)[3)]	0.90 (81.2)[3)]	3.40 (372)[3)]	2.50[3)]
Isoleucine	4.16 (148.6)	4.76 (170)	6.30 (225)	2.80
Leucine	9.18 (139.1)	9.12 (138.2)	8.80 (133.3)	6.60
Tyrosine	4.14	4.14	4.20	
Phenylalanine	4.88 (143.2)[4)]	5.00 (145.1)[4)]	5.70 (157.1)[4)]	6.30[4)]
Tryptophan	–	–	1.70 (154.6)	1.10
Lysine	6.53 (112.6)	6.54 (112.76)	7.00 (120.7)	5.80
Histidine	3.48 (183.2)	2.66 (140)	2.40 (126.3)	1.90
Arginine	5.97	5.87	6.10	

[1)] Whole egg protein (FAO, 1970)
[2)] FAO-WHO (1991) pattern
[3)] Methionine + Cystine
[4)] Phenylalanine + Tyrosine
–, Not detectable

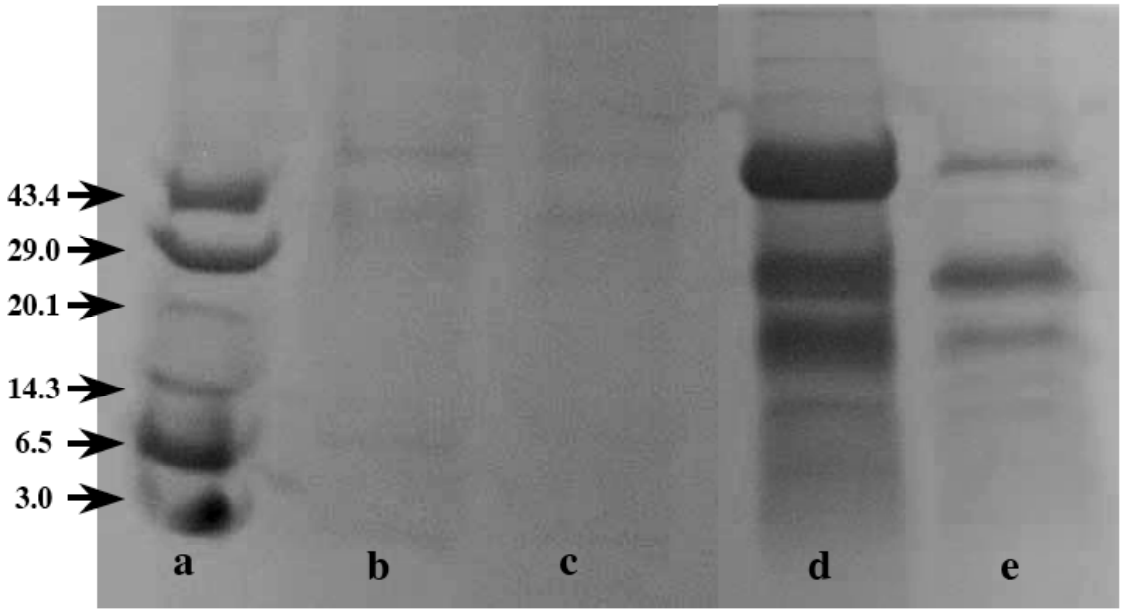

Figure 1. Albumin and globulin fractions on SDS-PAGE (lane a, ladder: 3.0 – 43.4 kDa; lane b, albumin of cooked seeds; lane, c globulin of cooked seeds; lane d, albumin of uncooked seeds; lane e, globulin of uncooked seeds)

Table 5. *In vitro* protein digestibility (IVPD) (%) (n=5, mean±SD) and protein digestibility corrected amino acid score (PDCAAS)[1)] (%, n=5, mean) of flours of germinated seeds of *Canavalia cathartica*[2)]

IVPD/PDCAAS	Germinated seeds	
	Uncooked	Pressure-cooked
IVPD	59.57±7.93[a]	71.70±1.63[b]
PDCAAS		
Threonine	86.73	105.65
Valine	90.38	101.20
Cystine + Methionine	40.98	58.22
Isoleucine	88.50	121.89
Leucine	82.86	99.08
Tryptophan	0	0
Tyrosine + Phenylalanine	85.29	104.02
Lysine	67.07	80.85
Histidine	109.11	100.38

[1)] Calculated based on FAO-WHO (1991)
[2)]Figures across the columns with different letters are significantly different ($P < 0.05$, t-test)

was very low in germinated seeds compared to dry seeds (5.1-7.5 vs. 24.1-49.2 mg 100 g^{-1}). The diet with low sodium is advantageous in alleviation of hypertension. As antioxidants, iron, zinc, manganese and selenium are known to strengthen the immune system (Talwar *et al*. 1989). Selenium as prosthetic group of antioxidant enzymes protects cells against free radicals and also prevents toxic effects of heavy metals such as arsenic, cadmium, mercury and tin (Combs and Gray, 1998). Zinc and selenium are also known to improve the sperm count as well as sperm mobility (Netter *et al*. 1981; Hunt *et al*. 1992).

Amino acids profile

With a few exceptions (e.g. threonine, methionine, isoleucine, phenylalanine), the amino acids profile of uncooked germinated seeds of *C. cathartica* was higher than cooked seeds (Table 4). Interestingly, the dry seeds lost amino acids severely on cooking (Seena *et al*., 2005). Glutamic (14.8-18.2%) and aspartic acids (12.5-18.46%) were the major amino acids in uncooked and cooked germinated seeds as seen in dry seeds. Usually, leguminous seeds consist more of lysine but deficient in sulfur-amino acids (Norton *et al*., 1985; Jansman, 1995-1996). In our study, lysine in

Table 6. Fatty acid compositions of flours of uncooked and cooked germinated seeds of *Canavalia cathartica* of coastal sand dunes on dry weight basis (g 100 g^{-1} total lipids)

Fatty acid	Germinated seeds	
	Uncooked	Pressure-cooked
Saturated fatty acids		
Caprylic acid ($C_{8:0}$)	–	6.50
Capric acid ($C_{10:0}$)	5.43	5.70
Lauric acid ($C_{12:0}$)	–	–
Tridecanoic acid ($C_{13:0}$)	–	–
Myristic acid ($C_{14:0}$)	0.01	0.18
Pentadecanoic acid ($C_{15:0}$)	0.003	0.07
Palmitic acid ($C_{16:0}$)	0.21	2.75
Stearic acid ($C_{18:0}$)	–	–
Arachidic acid ($C_{20:0}$)	0.101	–
Heneicosanoic acid ($C_{21:0}$)	–	0.61
Behenic acid ($C_{22:0}$)	0.0002	–
Tricosanoic acid ($C_{23:0}$)	–	–
Polyunsaturated fatty acids		
Myristoleic acid ($C_{14:1}$)	–	–
Palmitoleic acid ($C_{16:1}$)	–	–
Elaidic acid ($C_{18:1}$)	0.26	–
Oleic acid ($C_{18:1}$)	–	6.13
Linoleic acid ($C_{18:2}$)	0.35	3.95
Linolenic acid ($C_{18:3}$)	–	–
Eicosadienoic acid ($C_{20:2}$)	–	–
Eicosenoic acid ($C_{20:1}$)	–	2.23
Arachidonic acid ($C_{20:4}$)	–	–
Eicosapentaenoic acid ($C_{20:5}$)	–	–
Sum of saturated fatty acids	5.75	15.81
Sum of polysaturated fatty acids	0.61	12.31
Sum of essential fatty acids	0.35	3.95
P/S ratio[1)]	0.11	0.78

–, Not detectable
[1)] Ratio of polyunsaturated/saturated fatty acids

uncooked and cooked germinated seeds surpassed the FAO/WHO (1991) pattern (6.5 vs. 5.8%) and sulfur-amino acids (valine + methionine) concentration of cooked germinated seeds was superior to uncooked seeds (EAA score: 81.2 vs. 68.8). The rest of the EAA (threonine, valine, isoleucine, leucine, phenylalanine + tyrosine and histidine) surpassed the FAO/WHO (1991) pattern (see Table 4). Leucine (9.12 vs. 8.8%) and histidine (2.66 vs. 2.4%) of cooked germinated seeds also higher than the whole egg protein, while threonine (5.01 vs. 5.1%) and tyrosine (4.14 vs. 4.2%) were narrowly differed (FAO, 1970). Threonine (5.01 vs. 3.8%), Valine (4.94 vs. 4.6%), leucine (9.12 vs. 7.7%), tyrosine (4.14 vs. 3.4%), phenylalanine (5 vs. 4.8%), lysine (6.54 vs. 6.1%) and histidine (2.66 vs. 2.5%) were also surpassed the quantity present in soybean (Bau *et al*., 1994). Surprisingly, the dry seeds of *C. cathartica* devoid of histidine, while in germinated seeds it was higher than the whole egg protein (3.5-3.7 vs. 2.4%) (FAO, 1970). Except for tryptophan (0 vs. 0.5%), rest of the EAA of germinated seeds are superior to FAO/WHO/UNU (1985) standards recommended for adults.

In vitro protein digestibility

Significant increase of IVPD of cooked germinated seeds of *C. cathartica* (59.6 vs. 71.7%) (P = 0.02) (Table 5) indicates partial elimination or denaturation of antinutritional factors. Cooking might have increased the accessibility of proteins to enzymatic attack by inactivation of antinutritional factors as depicted by Poel *et al*. (1991). The high

Table 7. Antinutritional components of flours of uncooked and cooked germinated seeds of *Canavalia cathartica* of coastal sand dunes (mean observation of five independent observations) (Total phenolics: mg g^{-1} dry weight basis; n=5, mean±SD)[1)]

Component	Germinated seeds	
	Uncooked	Pressure-cooked
Total phenolics	0.32±0.02[a]	0.10±0.01[b]
Tannins	–	–
Trypsin inhibitors	–	–
Phytohemagglutinin activity against human erythrocytes[2)]		
A+ve	6	3
A–ve	6	3
B+ve	NA	NA
B–ve	NA	NA
AB+ve	5	1
AB–ve	3	NA
O+ve	1	NA
O–ve	4	2

[1)] Figures across the columns with different letters are significantly different ($P < 0.05$, t-test)
[2)] Titre value: Maximum dilution where agglutination was observed
–, Not present
NA, No agglutination

IVPD of defatted soybean flour was related to decreased protein-based anitnutrients such as trypsin and chymotrypsin (Abu-Tarboush, 1998). In our study, the germinated seeds of *C. cathartica* were devoid of trypsin inhibitors and the improvement in IVPD on cooking can be attributed to decrease of other antinutrients such as lectins and phenolics (Cheryan, 1980; Reddy *et al*., 1985). The PDCAAS of EAA (except for tryptophan, 0%) were higher in cooked seeds than uncooked seeds (58.2-121.9 vs. 41-109.1%). With exception of tryptophan (0%) and cystine + methionine (58.2%), the PDCAAS of the EAA of cooked seeds was above 80% (80.9-121.9%).

Fatty acids profile

Among saturated fatty acids, capric acid was highest in uncooked seeds, while caprylic, capric and palmitic acids in cooked seeds (Table 6). The uncooked seeds showed only two unsaturated fatty acids (elaidic and linoleic acids), while cooked seeds possess three (oleic, linoleic and eicosenoic acids). In uncooked dry seeds, the saturated and unsaturated fatty acid representatives were stearic and oleic acids respectively (Seena *et al*., 2005). Minor quantity of saturated fatty acids were seen in cooked dry seeds with appreciable quantities of unsaturated fatty acids such as linoleic, linolenic and eicosadienoic acids (Seena *et al*., 2005). Among ω–3 fatty acids, in addition to linolenic acid, eicosapentaenoic acid was also present in cooked dry seeds (Seena *et al*., 2005). The sum of essential fatty acids was also substantially higher in cooked dry seeds than uncooked dry seeds or germinated seeds (11.1 vs. 0-4 mg g^{-1} lipid). Overall, fatty acids profile of cooked dry seeds is superior to germinated seeds as they consist of more of unsaturated fatty acids including two ω–3 fatty acids. In our study, the P/S ratio of cooked germinated seeds was higher than uncooked seeds (0.78 vs. 0.11), but it was the highest in cooked dry seeds (156.3) (Seena *et al*., 2005). Ezeagu *et al*. (1998) opined that elevated P/S ratio in legumes reduces the risks of cardiovascular disease. As the germinated seeds of *C. cathartica* possess low fat protein, it seems to be suitable for hyperlipedemic patients to combat protein-energy malnutrition (Tharanathan and Mahadevamma, 2003).

Antinutritional features

The uncooked and cooked germinated seeds of *C. cathartica* were devoid of tannins and trypsin inhibitors (Table 7). The total phenolics decreased significantly in cooked seeds (0.3 vs. 0.1 mg g^{-1}) ($P < 0.001$) as seen in dry seeds (1.5 vs. 1.3 mg g^{-1}) (Seena *et al*., 2005). The protein extract of uncooked seeds exhibited higher agglutination titre values than cooked seeds (titre: 1-6 vs. 1-3) on human erythrocytes with exception of B+ve and B–ve, wherein it was not agglutinated by uncooked and cooked extracts. The cooked seeds exhibited substantially lower

agglutination titre values indicate at least partial loss of lectin potential. However, even at low quantity of hemagglutinins is not desirable as it interfere the digestion and absorption (Ichev and Ovtscharoff, 1981; Thompson *et al*., 1986). According to Bell (1960), Nakatsu *et al*. (1996) and Rosenthal (1970), the non-protein amino acid, canavanine (Cav) of *C. ensiformis* diminish during seed germination. Rosenthal (1990) opined that at the time of seed inbibition and germination, the Cav will be mobilized and released into the rhizosphere. However, Esonu (1996) has demonstrated strong effects of Cav in chicks even after seed sprouting prior to thermal treatment. A slight decrease in the quantity of Cav during germination was seen and it reached the lowest level after 24 hr of seed germination in dark (D'Mello *et al*., 1988). According to Rosenthal (1977), Obizoba and Obiano (1988) and D'Mello and Walker (1991), the Cav solubilizes in water and on heating transforms into non-toxic cyclic deaminocanavanine. Cooking germinated seeds of *C. cathartica* revealed a substantial decrease of hemagglutinin activity against human erythrocytes. To eliminate or bring down the lectin activity of germinated seeds below threshold level, alternate measures (e.g. soaking germinated seeds) prior to cooking may yield a favorable result.

Conclusion

Cooked germinated seeds of *Canavalia cathartica* of the coastal sand dunes of Southwest coat of India are adequate with some minerals, improved *in vitro* protein digestibility and promising essential amino acids requirement. The germinated seeds were devoid of tannins and trypsin inhibitors, while total phenolics and hemagglutination activity decreased substantially on pressure-cooking germinated seeds. The nutritional qualities of germinated seeds even though superior than dry seeds, a combination or additional methods are necessary to eliminate or to decrease the hemagglutination potency to below threshold level for the purpose of human consumption.

Acknowledgements

The authors are grateful to Mangalore University for granting permission to carry out this study at the Department of Biosciences. This study was carried out by MDC during the tenure of Faculty Improvement Programme (University Grants Commission, New Delhi). Authors are thankful to Dr. N. Yadukumar, National Research Centre for Cashew Research, Puttur, Karnataka and Dr. Rajeev Bhat, Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Malaysia for technical advice. MDC is thankful to the Principal and Fr. Leo D'Souza, Director, Applied Biology, St. Aloysius College, Mangalore for support.

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