

Effects of processing technique on the nutritional composition and antinutrients content of under –utilized food legume *Canavalia ensiformis* L.DC.

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Abstract: Jack Bean (JB) (*Canavalia ensiformis*) is an indigenous legume promoted in Tamilnadu as a green manure cover crop. It contains high protein but it is under - utilized due to the presence of 3, 4-dihydroxy-L-phenylalanine (L-Dopa) and other anti nutritional compounds. To improve its nutritional potential as a protein source, Jack bean was processed and evaluated for nutritional composition. Effects of processing at different methods like soaking, cooking and autoclaving on the contents of anti-nutritional compounds and crude protein were investigated. Raw beans contained high crude protein (29.8 g/100⁻¹). Contents of ether extract; crude fibre and ash were 4.2, 7.37 and 4.48 g/100⁻¹, respectively. Raw whole Jack bean contained L-Dopa (1.7 g/100⁻¹) content. Other anti-nutritional compounds included total phenols 3.83 g/100-1, trypsin inhibitor activity (TIA) 378.3 TIU and tannins 82.5 mg/100⁻¹. The present study also evaluated the changes of anti-nutritional factors of Jack bean by subjecting to soaking, autoclaving and cooking. Maximum reduction was observed in TIA, L-Dopa and Total free phenols with autoclaving, while the soaking and cooking decreases the levels of tannins. Autoclaving was more effective method in reducing trypsin inhibitor activity, polyphenols and L-Dopa than the various cooking treatments.

Keywords: Jack bean, anti-nutritional compounds, L-Dopa, processing methods.

Introduction

Legumes are good sources of cheap and widely available proteins for human consumption. They are staple foods for many people in different parts of the world (Youseff *et al.*, 1989). Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high (Vijayakumari *et al.*, 1997). They range between the highly utilized legumes such as soybeans, cowpeas to the lesser known ones like African yam beans (*Sphenostylis stenocarpa*), *Mucuna conchinchinesis* and *Mucuna flagellipes* (“ukpo”). Studies have shown that the lesser known legumes together with other conventional legumes can be used for combating protein malnutrition prevalent in the third world. This can be achieved by the consumption of the legumes whole and in various processed forms (condiments) (Arisa and Aworh, 2007).

Jack bean (*Canavalia ensiformis*) is one of the under exploited tropical dry beans. It is, however, fairly widely distributed, being cultivated in Africa, Asia, the West Indies, Latin America and India. The jack bean can be grown in marginal soils and arid to semi arid regions not suitable for common legumes such as *Phaseolus* and *Vigna* species. It has, therefore,

great potential in most tropical and subtropical parts of the world (Akpapunam and Sefa-Dedeh, 1997). The seed of jack bean, the highly produced large seeded tropical legume, contains about 300 g/kg crude proteins and 600 g/kg carbohydrates (Udedibie and Nkwocha, 1990). *Canavalia ensiformis* ranks among the underutilized legumes that could ameliorate protein deficiency in human nutrition, particularly in developing countries. The mature seeds are consumed by the Indian tribal sects, Kurumba, Malayali, Erula and other Dravidian groups, after cooking (Mittre, 1991).

In western countries this legume is used as a cover crop and the roasted seeds are ground to prepare coffee-like drink (Bressani *et al.*, 1987). It can be grown relatively easily and produce high yields in the region of low altitude; high temperature and relative humidity. The environment of different locations plays an important role in the determination of quality and quantity of seed proteins. Location effect is relatively more important than that of cultivar of effect of protein content (Dodd and Pushpamma, 1980). The objective of this study was to process jack bean by different methods in order to remove anti-nutritional compounds and assess effects of treatments on nutritional composition.

Materials and Methods

Status of the germplasm collection

Mature seeds of jack bean were collected from Kuppanatham, Madurai (Dt), Tamilnadu, South India.

Nutritional value

The dried seeds were cleaned thoroughly and any foreign materials, broken seeds and immature seeds were removed. The moisture content of the seeds was estimate by taking 25 transversely cut seeds at a time the weight was taken before and after incubation in a hot air oven at 80°C for 24 hours, followed by cooling in a desicator. Oven dried seeds were weight and ground in a Willey Mill (Scientific equipment) and passed through a 60-mess size screen. The proximate composition such as moisture, crude protein, crude lipid and ash content of raw and processed JB seeds were determined by following AOAC (1990) method by employing the micro-kjeldahl method for crude protein, and soxhlet extraction method for crude lipid analysis. The total carbohydrate was estimated by following the method of Hedge and Hofreiter (1962).

Soaking

Whole seeds were soaked in distilled water for 24 h at room temperature in the bean: water ration of 1:10(w/v). After soaked, the water was drained off and the seeds were dried at 55°C for 6 h in a hot air oven.

Cooking

Another set of seeds were cooked in distilled water (100°C) in a bean: water ratio of 1:10 (w/v) for 20 min. The cooked seeds were rinsed with distilled water and dried at 55°C for 6 h in a hot air oven.

Autoclaving

Separated batches of seeds were autoclaved at 15 psi (121°C) in distilled water in the bean: water ratio of 1:10 (w/v) for 30 min. After treatment, the seeds were rinsed with distilled water and dried at 55°C for 6 h in a hot air oven.

Antinutritional compounds

The anti-nutritional compounds such as tannins, L-Dopa, oligosaccharides such as raffinose, verbascose, stachyose, haemagglutinating activity, trypsin inhibitor activity were analyzed according to the methodology described by Vadivel and Pugalenti (2008).

Estimation of oligosaccharides

The oligosaccharides composition of raw and processed JB seed samples was determined by taking 5 g of the seed flour was extracted with 50 ml of 70% (v/v) aqueous ethanol. Separation of oligosaccharides was done by TLC. The separated spots were compared with standard (raffinose, stachyose and verbascose obtained from Sigma Chemicals) and the sugar spots were scrapped, eluted in 2 ml of distilled water and then filtered. 1ml of the extract was treated with 1 ml of 0.2 M thiobarbituric acid and 1 ml of conc. HCL and kept on boiling water bath for 6 min. After cooling, the absorbency was measured at 432 nm and the oligosaccharide contents were quantified.

Determination of haemagglutinating activity

The haemagglutinating activity of raw and processed samples was analyzed by haemagglutinating assay in the presence of 10 mM Mn²⁺ in a round bottomed micro-titer plate using 2% (v/v) trypsinized cattle blood erythrocyte suspension in saline phosphate buffer (pH 7.0). One haemagglutinating unit (HU) is defined as the least amount of the extract per ml of the last dilution, which giving positive agglutination.

Determination of trypsin inhibitor activity

The trypsin inhibitory activity was determined by taking 2 g of the seed sample was stirred with 30 ml of 0.1 M sodium phosphate buffer (pH 7.6) for 4 h. The contents were centrifuged at 12,000 rpm for 20 min at 0°C. The clear supernatant obtained was dialyzed against 0.05 M sodium phosphate buffer (pH 7.6). The dialyzed extract obtained was used for trypsin inhibitor assay procedure, which consisted of reaction mixture of suitable amount of enzyme, inhibitor extract and buffer (0.1 M sodium phosphate buffer, pH 7.6). The mixture was incubated for 10 min at 37°C and the reaction was initiated by adding 1 ml of 2% casein solution (substrate). The reaction was stopped exactly after 20 min by adding 3 ml of 5% trichloro acetic acid solution. After standing for 20 min at room temperature the solution was centrifuged at 2,000 x g for 10 min. The clear supernatant obtained was analyzed for residual enzyme activity. One trypsin inhibitor unit (TIU) is defined as the number of trypsin units inhibited by 1 ml of the extract and expressed in TIU/kg DM.

Estimation of tannins

The tannins content of the raw and processed JB seed samples were estimated by taking 1 g of air dried seed flour was extracted with 50 ml of 1% (v/v) HCL in methanol. The samples were shaken on a

reciprocating shaker for 24 h at room temperature. Then the contents were centrifuged at 10,000 x g for 5 min and the supernatant was collected and used for further analysis. The tannins content was quantified by using Vanillin-HCL method. The vanillin reagent reacts with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a purple coloured product, which is measured at 500 nm. Average values of three separate determinations were expressed in g/kg DM basis.

Estimation of L-Dopa

The L-dopa content was quantified by taking 1 g of seed flour with 5 ml of 0.1 NHCL in a test tube and kept on a boiling water bath for 5 min. After cooling, 5 ml of ethanol was added and shaken for 10 min. Then the contents were centrifuged at 5000 x g for 10 min and the supernatant was collected and made up to a known volume. From this extract, the L-dopa content was determined by measuring the ultra violet light absorption at 282 nm in a spectrophotometer (Make: Elico; Model: SL-177) using L-dopa (Sigma chemicals) as a standard.

Estimation of poly phenols

Polyphenol substances were estimated by Folin-Denis method. About 200 mg defatted material was taken in a 250 ml round bottomed flask and 100ml of 1% HCL in methanol was added. The contents were refluxed for 2 h, cooled, filtered and the volume was made up to 100 ml with acid-methanol after few types of washing. 0.2 ml of extract was taken and 7.5 ml of water and 0.5 ml Folin-Denis reagent were added and mixed. To this, 1 ml of saturated sodium carbonate solution was added and volume made up to 10 ml with water, mixed and the absorbance was measured at 760 nm after 30 min. The results were calculated as mg tannic acid equivalent/g sample and expressed as mg/100 d dry wt.

Results and Discussion

Nutritional value

The crude protein content of raw JB seeds (29.8 g/kg DM) (Table 1) were found to be higher when compared to an earlier report on certain common legume grains such as *Mucuna pruriens* var. *pruriens* (24.9 g/kg DM) (Udedibie and Carlini, 1998); *Canavalia gladiata* (29.3 & 2.46 g/kg DM) (Siddhuraju and Becker, 2001) and *Entada scandens* (26.82 g/kg DM) (Vadivel *et al.*, 2008). According to Bressani (2002), higher level of protein content of seed materials of *Canavalia ensiformis* has nutritional significance, since moderate intake of these seeds will

greatly increased the total dietary protein intake of the consumers. Its utilization as a protein ingredient in the animal feed will reduce the over-dependence on the conventional protein supplements notably soybean and other common legumes. The crude lipid content of raw JB seeds (4.2 g/kg DM) were found to be higher than *Cassia floribunda* (2.1-3.1%) (Vadivel and Janardhanan, 2001); *Canavalia gladiata* (2.46%) (Siddhuraju and Becker, 2001) and *Canavalia gladiata* (2.8-3.8%) (Pugalenthi and Vadivel, 2005), but lower than the value reported earlier on *Mucuna pruriens* var. *pruriens* (9.6%) (Adebowale *et al.*, 2005) and *Entada scandens* (9.53%) (Vadivel *et al.*, 2008). The crude fiber content of raw JB seeds (7.37 g/kg DM) was found to be comparable with that of earlier reports on the same Jack bean (4.71-11.4%) (Sridhar and Seena, 2006); *Canavalia gladiata* (9.32%) and *C. virosa* (10.47%) (Siddhuraju and Becker, 2001); *Mucuna monosperma* (8.9-9.2%) (Pugalenthi *et al.*, 2003). The Nitrogen Free Extractive (NFE) or crude carbohydrate raw JB seeds (54.15%) were found to be lower when compared to previous reports on certain under-utilized food legumes such as *Cassia floribunda* (58-60.5%) (Vadivel and Janardhanan, 2001); *Mucuna monosperma* (59.60%) (Pugalenthi *et al.*, 2003) and *Tamrindus indica* (58.8%) (Pugalenthi *et al.*, 2004). When considering the effect of various common processing methods on the nutritional profiles of JB, all the presently studied processing methods have not exhibited any significant reduction of proximate composition of JB seeds.

Table 1. Proximate composition of raw and differentially processed Jack bean seeds

Nutritional analysis	Treatments			
	Raw	Soaking	Cooking	Autoclaving
Moisture ¹	8.41 ± 0.01	6.51 ± 0.011	6.45 ± 0.05	5.97 ± 0.05
Ash ¹	4.48 ± 0.05	4.16 ± 0.01	4.20 ± 0.01	4.01 ± 0.05
Crude lipid ¹	4.2 ± 0.11	4.19 ± 0.011	4.06 ± 0.015	3.74 ± 0.05
Crude fibre ¹	7.37 ± 0.06	6.36 ± 0.15	6.73 ± 0.20	6.86 ± 0.15
Total carbohydrate ¹	50.8 ± 1.0	44.9 ± 0.1	47.13 ± 0.15	49.1 ± 0.1
Protein ¹	29.8 ± 0.47	31 ± 0.1	30.3 ± 0.15	29.9 ± 0.152
NFE ²	54.15	54.29	54.71	55.49
Calorific value ³	1560.3	1582.30	1572.71	1567.0

¹Values are mean of three replicates, ± Standard error

²Values expressed on g/100 g sample

³NFE – Nitrogen Free Extractives expressed on Percentage (%) basis

⁴Values expressed on KJ/100 g DM

Anti-nutritional compounds

The effect of various processing methods such as soaking pressure cooking and autoclaving on the levels of anti-nutritional compounds of JB seeds were given in the table 2 & 3. The level of total free phenolics (3.83%) of JB seeds was found to be lower when compared to earlier reports on *Sesbania sesban* (5.95%) (Hossain and Becker, 2001); *Mucuna pruriens* var. *utilis* (9.7%) (Vadivel and Pugalenthi, 2007); *M. pruriens* (7.75%), *M. cochichinensis* (6.53%) (Adebowale *et al.*, 2005) and *Entada scandens* (6.23%) (Vadivel *et al.*, 2008).

Table 2. Effect of various processing methods on the anti-nutritional compounds of Jack bean seeds

Processing methods	¹ Total free phenols	² Tannins	³ Trypsin Inhibitors	⁴ L-Dopa
Raw seeds	3.83 ± 0.28 (0.00%)	82.5 ± 8.6 (0.00%)	378.3 ± 2.88 (0.00%)	1.7 ± 0.3 (0.00%)
Soaking	1.8 ± 0.01 (-53%)	45.3 ± 4.73 (-45%)	264.83 ± 0.152 (-30%)	1.1 ± 0.01 (-35%)
Cooking	1.23 ± 0.05 (-67%)	28.9 ± 0.1 (-64%)	141.93 ± 0.05 (-62%)	0.81 ± 0.01 (-52%)
Autoclaving	0.84 ± 0.01 (-78%)	13.9 ± 0.1 (-83%)	97.9 ± 0.15 (-74%)	0.49 ± 0.05 (-71%)

Values are mean of three replicates, ± Standard error

¹Values expressed on g/100 g sample

²Values expressed on mg/100 g sample

³Values expressed on trypsin inhibitor unit (TIU)/ g sample

Table 3. Effect of various processing methods on the anti-nutritional compounds of Jack bean seeds

Processing methods	Oligosaccharides			Hae.aggluti. activity
	¹ Raffinose	² Stachyose	³ Verbascose	
Raw seeds	1.51 ± 0.015 (0.00%)	1.80 ± 0.011 (0.00%)	4.86 ± 0.01 (0.00%)	+
Soaking	0.63 ± 0.05 (-58%)	0.85 ± 0.05 (-52%)	2.64 ± 0.05 (-45%)	-
Cooking	0.50 ± 0.01 (-66%)	0.54 ± 0.05 (-70%)	1.49 ± 0.02 (-69%)	-
Autoclaving	0.36 ± 0.015 (-76%)	0.31 ± 0.05 (-82%)	0.97 ± 0.011 (-80%)	-

Values are mean of three replicates, ± Standard error

¹Values expressed on g/100 g sample

+ denotes agglutination

- denotes no agglutination

The tannin content of JB (0.825%) was also found to be lower when compared to the tannin content of certain common legume seeds such as *Pisum sativum* (0.92%) (Nikolopoulou *et al.*, 2007); *Phaseolus vulgaris* (1.7%) and *Cajanus cajan* (1.4%) (Sangronis and Machado, 2007). In nutritional point of view, the content of both total free phenolics and tannins are not desirable for human consumption. Phenolic compounds were reported to decrease the digestibility of proteins, carbohydrates and the availability of vitamins and minerals (Liener, 1994). They also have decreased the activity of digestive enzymes such as α – amylase, trypsin, chymotrypsin and lipase and may cause damage to the mucosa of digestive tract and also reduced the absorption of nutrients such as vitamin B12.

The L-Dopa content of JB seeds (1.7%) (Table 2) was comparable with that of *Cassia floribunda* (1.57%) and *C. obtusifolia* (1.34%) (Vadivel and Janardhanan, 2005) and appears to be lower when compared to *Tamrindus indica* (2.64%), *Erythrina indica* (2.96%) and *Sesbania bispinosa* (2.01%) (Pugalenti *et al.*, 2004); *Arbus preicatorius* (2.03%) (Pugalenti *et al.*, 2007) and *Entada scandens* (3.95%) (Vadivel *et al.*, 2008). L-Dopa is a pharmaceutically active compound, used in the treatment of Parkinson's disease, but potentially toxic in nutritional point of view (Pugalenti *et al.*, 2007). The raw JB seeds were found to exhibit the oligosaccharides level of 1.51% raffinose, 1.80% stachyose and 4.86% verbascose (Table 3). These values were found to be comparable with that of an earlier report on *Mucuna pruriens* var *utilis* (0.95 - 1.20% of raffinose, 1.05 - 1.45% of stachyose and 2.49 - 4.34% of verbascose) (Vadivel and Pugalenti, 2007) and higher when compared to

the oligosaccharides content of *Vigna mungo* (0.27 - 0.76 % of raffinose, 0.25 - 0.80 % of stachyose and 1.12 - 3.32% verbascose) (Girigowda *et al.*, 2005). The trypsin inhibitor activity of the raw JB seeds (378.3 TIU) (Table 2) was found to be higher when compared with that of previous results on *Mucuna pruriens* var *utilis* (94 TIU) (Vadivel and Pugalenti, 2007); *Mucuna monosperma* (65.4 TIU); *Vigna unguiculata* (75.2 TIU) (Rivas-Vega *et al.*, 2006).

The soaking and cooking processing methods employed in the present study were found to reduce significant levels of various antinutritional compounds such as total free phenolics (53 and 67% respectively), tannin (45 and 64% respectively), L-Dopa (35 and 52% respectively), trypsin inhibitor activity (30 and 62% respectively), oligosaccharides such as raffinose (58 and 66% respectively), stachyose (52 and 70% respectively) and verbascose (45 and 69% respectively) (Table 2). Among the various processing methods employed, the autoclaving was found to be more effective in reducing the maximum levels of total free phenolics (78%), tannin (83%), L-Dopa (71%), oligosaccharides such as raffinose (76%), stachyose (82%) and verbascose (80%) and trypsin inhibitor activity (74%) of presently investigated JB seeds. Similarly, a maximum level of reduction of various antinutritional compounds under autoclaving treatment was reported in some previous studies on *Vigna aconitifolia* and *V. sinensis* (Vijayakumari *et al.*, 1998); *Mucuna pruriens* var *utilis* (Vadivel and Pugalenti, 2007); *Bauhinia purpurea* (Vijayakumari *et al.*, 2007); *Phaseolus vulgaris* (Shimelis and Rakshit, 2007) and *Entada scandens* (Vadivel *et al.*, 2008).

The results of the present study indicated that the JB seeds have good nutritional profile with high level protein, lipid and other nutrients comparable with that of other common legume grains. Among the various common processing methods employed, the autoclaving treatment was found to be more effective in reducing various antinutritional compounds of JB seeds without affecting the nutritional quality.

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