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BIOLOGICAL EVALUATION OF PROTEIN QUALITY OF RAW AND PROCESSED SEEDS OF GILA BEAN (*Entada scandens* BENTH.)

[EVALUACIÓN BIOLÓGICA DE LA CALIDAD DE LA PROTEÍNA DE SEMILLAS CRUDAS Y PROCESADAS DEL FRIJÓL GILA (*Entada scandens* BENTH.)]

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SUMMARY

The effect of various processing methods on the proximate composition, antinutritional compounds and protein quality of gila bean seeds (*Entada scandens* Benth.), collected from South India was investigated. The mature seeds were found to contain higher level of protein (26.8%); lipid (9.5%); fiber (15.7%); ash (5.4%) and carbohydrates (42.5%). Even though, various antinutritional substances were present in the raw gila bean seeds, the autoclaving treatment effectively reduced their maximum levels without affecting the nutritional profiles. The rats fed with autoclaved seeds included in the diet for 28 days exhibited better growth performance such as feed intake (239 g) and body weight gain (67.4 g). Moreover, the protein quality parameters such as protein efficiency ratio, true digestibility, biological value, net protein utilization and utilizable proteins of gila bean seeds were also significantly improved by autoclaving treatment when compared to other processing methods such as soaking, cooking and roasting.

Key words: Antinutritional compounds, biological value, *Entada scandens*, gila beans, processing methods, protein quality.

RESUMEN

Se estudió el efecto de varios meétodos de procesamiento sobre la composición química, factores antinutricionales y calidad de la proteína del frijol gila (*Entada scandens* Benth.) colectada en la región Sur de la India. Las semillas maduras tienen mayores contenidos de proteína (26.8%); lípidos (9.5%); fibra (15.7%); cenizas (5.4%) y carbohidratos (42.5%). Aún cuando varios factores antinutricionales fueron encontrados en la semilla cruda, el proceso por autoclave redujo sus niveles sin afectar el perfil nutricional. Ratas alimentadas por 28 días con dietas que contenían semillas sometidas a proceso de autoclave exhibieron mejores indicadores de crecimiento tales como consumo de alimento (239 g) y ganancia de peso (67.4 g). Más aún los parámetros de calidad de la proteína tales como digestibilidad verdadera, valor biológico, eficiencia de conversión de la proteína, utilización neta de proteína y proteína utilizable fue mejor en semillas tratadas por autoclave en comparación con otros métodos como remojado, cocinado o rostizado.

Palabras clave: Compuestos antinutricionales, valor biológico, *Entada scandens*, frijol gila, métodos de procesamiento, calidad de la proteína.

INTRODUCTION

Most of the developing tropical countries have depended upon soybean and other common legume grains as a protein source for both human beings and animals. But, their production is not sufficient to meet the protein requirements of increasing population and expanding livestock industries (Vijayakumari *et al*., 2007). The heavy demand for these common legumes has given rise to a disproportionate increase in their prices, and consequently, the cost of the food and feedstuffs. Hence, recent research efforts have been directed to identify and evaluate under-utilized legume seeds as alternative/additional protein sources (Janardhanan *et al*., 2003).

In this context, the seeds of *Entada scandens* Benth. (= *Entada phaseoloides* (L.) Merill) (gila bean), receives more attention as a protein source. It is widespread through out the tropics and in India, it is distributed in Sub-Himalayan tracts, Sikkim, Assam, West Bengal, Western and Eastern Ghats of South India and Andaman and Nicobar Islands (Janardhanan *et al*., 2003). The seeds are hollowed out and filled with snuff and often called as 'snuffbox sea beans' (Siddhuraju *et al*., 2001). The gila bean seeds were

reported to contain high level of protein (17.4-27.7%) with major proportion of albumin proteins, has a balanced amino acid composition, more nitrogen solubility, high content of lipid (3.1-10.8%) with predominant unsaturated fatty acids and high content of digestible starch (Janardhanan and Nalini, 1991; Mohan and Janardhanan, 1993; Vijayakumari *et al*., 1993; Banerjee and Dixit, 1998; Siddhuraju *et al*., 2001).

In India, the boiled seeds of gila bean are being consumed by Karib tribes of Assam and Oceanic group of tribes such as Onges and Great Andamanese. The soaked seed kernels are roasted/boiled and eaten by Northeast tribal sects such as Garo, Khasi, Naga and Kanikkars of Tamil Nadu and Kerala (Mohan and Janardhanan, 1993; Siddhuraju *et al*., 2001; Janardhanan *et al*., 2003). The half-ripened seeds are used as a coffee substitute in South America (Janardhanan and Nalini, 1991; Siddhuraju *et al*., 2001). A paste prepared from the gila bean seeds is known to cure inflammatory glandular swellings (Janardhanan and Nalini, 1991). In India, the ground seeds are taken internally for a variety of remedies including contraception, snakebites and aphrodisiac. Occasionally, Indian village people use the seed powder of gila bean as natural shampoo to wash their hairs (Siddhuraju *et al*., 2001).

The exploitation and development of such potential non-conventional legume seeds as protein source for human beings/animals may offer a good scope to meet the increasing protein requirements at large, particularly in the developing countries. However, before recommending such indigenous foodstuffs, their nutritional properties and biological value should be thoroughly investigated. Although reports are available on the biochemical composition and nutritional value of gila bean seeds (Janardhanan and Nalini, 1991; Mohan and Janardhanan, 1993; Siddhuraju *et al*., 2001), information regarding the biological value of seed proteins appears to be meager. Hence, in the present study, an attempt has been made to evaluate the biological value and protein quality of raw and differentially processed gila bean seeds collected from South India.

MATERIALS AND METHODS

Seed sample

Entada scandens Benth. seeds were collected from Anaimalai Hills, Pollachi, Coimbatore District, Tamil Nadu, South India on 15.11.2006. The seed materials were collected from three separate *E. scandens* plants and pooled together. Soon after collection, the immature and damaged seeds were removed and the mature seeds were dried in the sun light for 24 h and stored in plastic containers until further use.

Processing methods

The whole seeds of gila beans were randomly divided in to five separate batches each of 100 g weight and the first batch of seeds were soaked in distilled water for 6 h at room temperature (28 ± 2^{o}C) in the bean to water ratio of 1:10 (w/v). The second batch of seeds were cooked at 90-95^{o}C for 1 h in the bean to water ratio of 1:10 (w/v). The third batch of seeds were taken in the bean to water ratio of 1:10 (w/v) in a metal container and autoclaved at 15 lb pressure (121^{o}C) for 30 min. The fourth batch seeds were roasted for 30 min at 100-110^{o}C in an iron pot along with clean fine sand to prevent the burning of the seed coat and to ensure the uniform distribution of heat. After each treatment, the treated seeds were rinsed with distilled water separately and then dried at 55^{o}C for 6 h in a hot air oven (Make: Kemi Equipements, Model: KOA-3). The fifth batch of raw seeds were stored as such with out any treatment.

Analytical methods

All the processed as well as raw seeds were powdered in a Willey Mill to 60-mesh size and the powdered samples were used for further analysis. The proximate composition such as moisture, crude protein, crude lipid, crude fiber and ash content of raw as well as processed seed samples were determined by following AOAC (1990) method. Nitrogen free extractives (NFE) and calorific value were calculated by following the method of Siddhuraju *et al*. (1992).

The antinutritional compounds such as total free phenolics and tannin content of raw and processed seed samples were extracted and estimated by following the method of Sadasivam and Manickam (1992) and Burns (1971), respectively. The L-Dopa (L-3,4-Dihydroxyphenylalanine), a non-protein toxic amino acid, was quantified according to the method of Brain (1976), whereas, the phytic acid content was determined by following the method of Wheeler and Ferrel (1971) and the oligosaccharides content was estimated by following Pugalenthi *et al*. (2006) method. The haemagglutinating activity was analyzed according to the method of Makkar *et al*. (1997) and trypsin inhibitor activity was determined by casein digestion method (Mulimani and Vadiraj, 1993) while α-amylase inhibitor activity was measured according to the Mulimani and Rudrappa (1994) method.

Biological evaluation of protein quality

Fifty numbers of 23 days old male albino rats with an initial body weight of 40 ± 5 g were equally divided

into five groups with 10 animals in each group and housed individually in cages. The animals were maintained at 22°C with 12 h light and 12 h dark at Karpagam Animal House (Approved by Animal Ethical Committee, Government of India). The experimental diets were prepared according to the method of Chapman *et al*. (1959) by including corn starch (80%), corn oil (10%), non-nutritive cellulose (5%), mineral mixture (4%) and vitamin mixture (1%). The test diets were prepared by incorporating raw and differentially processed seeds as protein source at 10% level in the diet and the control diet was prepared by incorporating casein as a protein source. The control and test diets were fed to respective animal groups along with water *ad libitum* for 28 days and the growth performance of the experimental animals were analyzed.

Feed intake (FI): Dry feed fed (g) / animal / 28 days

BWG: FBW (g) – IBW (g)

Where:
BWG = Body weight gain
FBW = Final body weight
IBW = Initial body weight

The protein content of the diet was determined by micro-kjeldahl method (AOAC, 1990) and the feed efficiency ratio (FER) and protein efficiency ratio (PER) were calculated according to the method of Chapman *et al*. (1959).

$$\text{FER} : \frac{\text{Body weight gain (g)}}{\text{Dry feed consumed (g)}}$$

$$\text{PER} : \frac{\text{Body weight gain (g)}}{\text{Protein consumed (g)}}$$

The nitrogen balance studies were conducted for 14 days with 60 numbers of male albino rats of 50 ± 5 g body weight. The rats were randomly separated into six groups with 10 animals in each group and individually housed in polypropylene metabolic cages. The animal groups were fed with control casein diet, raw and differentially processed gila beans included test diets separately and one batch was also fed with protein free basal diet for the determination of endogenous and metabolic nitrogen loss in faeces and urine. After 9 days of acclimatization period, the urine and faeces of the experimental animals were collected for five days and pooled separately. The nitrogen content of the urine and faeces were estimated by micro-kjeldahl method (AOAC, 1990) and the values of true digestibility (TD) and biological value (BV) of seed proteins were determined by following the method of Chick *et al*., 1935) and net protein utilization (NPU) by Platt *et al*. (1961), whereas, the level of utilizable proteins (UP) was calculated by Gupta *et al*. (1979) method.

$$\text{TD}: \frac{\text{NI} - (\text{NF1} - \text{NF2})}{\text{NI}} * 100$$

$$\text{BV}: \frac{\text{NI} - (\text{NF1} - \text{NF2}) - (\text{NU1} - \text{NU2})}{\text{NI} - (\text{NF1} - \text{NF2})} * 100$$

$$\text{NPU}: \frac{\text{NI} - (\text{NF1} - \text{NF2}) - (\text{NU1} - \text{NU2})}{\text{NI}} * 100$$

Where,
TD = True digestibility
BV = Biological value
NI = Nitrogen intake of the animals.
NF1= Nitrogen excreted in the faeces of the animals fed with test diet.
NF2= Nitrogen excreted in the faeces of the animals fed with protein free diet.
NU1= Nitrogen excreted in the urine of the animals fed with test diet.
NU2= Nitrogen excreted in the urine of the animals fed with protein free diet.

Statistical analysis

Results were expressed as mean values ± standard deviations of three separate determinations. The data was subjected to one-way analysis of variance (ANOVA) and the significance of difference between the means at 5% was determined by Duncan's Multiple Range Test (DMRT) using Irristat software (version 3/93).

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of raw and processed seeds of gila bean was shown in Table 1. The crude protein and lipid content of raw seeds (26.8% & 9.5%) were found to be higher when compared to certain common legumes such as *Cicer arietinum* (20.7% & 4.16%); *Vigna mungo* (23.6% & 0.45%); *V. radiata* (24.5% & 0.71%); *V. aconitifolia* (25.3% & 0.69%) and *Phaseolus vulgaris* (25.1% & 0.9%) as reported by Bravo *et al*. (1999). The protein and lipid content of autoclaved seed samples (26.9% & 9.5%) were higher than the raw and other processed seeds, which was in consonance with the earlier report on *Mucuna pruriens* var. *utilis* (Siddhuraju and Becker, 2005). Reduction in the ash content of soaked seeds (10%) when compared to raw seed samples might be due to the leaching of both micro and macro minerals into the soaking medium through the enhanced permeability of the seed coat during soaking treatment.

Antinutritional compounds

The effect of various processing methods such as soaking, cooking, autoclaving and roasting on the levels of antinutritional compounds of gila bean seeds were given in Table 2. Among the various processing methods employed, the autoclaving was found to significantly (p<0.05) reduce the maximum levels of various antinutritional substances such as total free phenolics (73%), tannins (70%), L-Dopa (76%), phytic acid (79%), oligosaccharides such as raffinose (72%), stachyose (66%) and verbascose (71%), haemagglutinating activity (80%), trypsin inhibitor activity (82%) and amylase inhibitor activity (68%). Similarly, significant reduction of various antinutritional compounds during autoclaving treatment was reported for several under-utilized legumes such as *Bauhinia purpurea* (Vijayakumari *et al*., 1997a); *Dolichos lablab* (Vijayakumari *et al*., 1995); *Mucuna monosperma* (Vijayakumari *et al*., 1996); *Prosopis chilensis* (Vijayakumari *et al*., 1997b); *Vigna aconitifolia* and *V. sinensis* (Vijayakumari *et al*., 1998).

Growth performance of the animals

The growth performance of the experimental animals fed with diets containing raw and processed gila bean seeds were illustrated in Table 3. The feed intake (FI) value of the animals fed with diet containing raw gila bean seeds (135 g) was significantly lower (p<0.05) as compared to casein (322 g) and treated seeds (182-239 g) and earlier reports on *Canavalia ensiformis* (225 g) and *C. gladiata* (244 g) (Bressani *et al*., 1987), but higher than the vegetable peas (105-120 g) (Saharan and Khetarpaul, 1994) and *C. maritima* (93 g) (Seena *et al*., 2005). The notable difference in the FI value of rats fed with diet containing raw and differentially processed gila bean seeds was probably due to the difference between the diets in protein quality and levels of antinutritional compounds of incorporated gila bean seeds. The maximum reduction on the levels of various antinutritional substances under autoclaving treatment might be related to larger FI value (239 g) of animals fed with autoclaved gila bean seeds inclusive diet.

The animal group fed with raw gila bean included diet showed lowest body weight gain (BWG) value (24.6 g) when compared to the control (106 g) and test animals fed with diet included with treated seeds (42-67 g). The BWG value of animals fed with diet containing raw seeds of the present study was in agreement with an earlier report on vegetable peas (23-28 g) (Saharan and Khetarpaul, 1994). Among the different treated seeds, the autoclaved seeds significantly improve the BWG of rats (67 g), which was higher than the BWG values for pressure-cooked seeds of *C. ensiformis* (28 g) and *C. gladiata* (32 g) (Bressani *et al*., 1987). Soaking and cooking processing methods were not demonstrated to have a beneficial effect on the growth rate of the animals. It might be due to the presence of heat resistant antinutritional compounds in the gila beans, which are not destroyed completely by soaking and cooking treatments.

Table 1. Proximate composition of raw and differentially processed seeds of *Entada scandens* (g/100 gDM except where stated).

Proximate composition	Raw seeds	Processed seeds			
		Soaked Seeds	Cooked seeds	Autoclaved seeds	Roasted seeds
Moisture	$8.26^{b} \pm 0.15$	$8.85^{e} \pm 0.25$	$8.74^{c} \pm 0.15$	$8.76^{d} \pm 0.10$	$7.98^{a} \pm 0.30$
Crude protein	$26.82^{d} \pm 0.02$	$26.41^{a} \pm 0.25$	$26.63^{c} \pm 0.20$	$26.95^{e} \pm 0.75$	$26.57^{b} \pm 0.20$
Crude lipid	$9.53^{d} \pm 0.12$	$9.46^{a} \pm 0.20$	$9.52^{c} \pm 0.15$	$9.54^{e} \pm 0.02$	$9.48^{b} \pm 0.20$
Crude fiber	$15.71^{e} \pm 0.20$	$14.96^{a} \pm 0.02$	$15.26^{c} \pm 0.25$	$15.48^{d} \pm 0.15$	$15.03^{b} \pm 0.15$
Ash	$5.45^{e} \pm 0.15$	$4.91^{a} \pm 0.10$	$5.24^{b} \pm 0.20$	$5.39^{c} \pm 0.04$	$5.43^{d} \pm 0.25$
NFE	$42.49^{a} \pm 0.02$	$44.26^{e} \pm 0.25$	$43.35^{c} \pm 0.12$	$42.64^{b} \pm 0.10$	$43.49^{d} \pm 0.17$
Calorific value (kJ / 100 g DM)	$1516^{a} \pm 0.14$	$1536^{e} \pm 0.05$	$1527^{d} \pm 0.13$	$1521^{b} \pm 0.06$	$1526^{c} \pm 0.22$

[1]Values expressed on g/100g sample.
NFE = Nitrogen Free Extractives
Values are mean and standard deviation of three separate determinations.
Values in the same row with different roman superscript are significantly different (p<0.05).

Table 2. Effect of various processing methods on the antinutritional compounds of *Entada scandens* seeds.

Antinutritional compounds	Raw seeds	Processed seeds			
		Soaked Seeds	Cooked seeds	Autoclaved seeds	Roasted seeds
Total free phenolics[1]	$6.23^{e} \pm 0.74$	$4.85^{c} \pm 0.14$	$3.47^{b} \pm 0.08$	$1.68^{a} \pm 0.14$	$5.08^{d} \pm 0.21$
Tannins[1]	$5.65^{e} \pm 0.03$	$4.12^{c} \pm 0.02$	$4.67^{d} \pm 0.02$	$1.71^{a} \pm 0.01$	$2.44^{b} \pm 0.01$
L- Dopa[1]	$3.92^{e} \pm 0.03$	$2.13^{b} \pm 0.25$	$2.75^{c} \pm 0.14$	$0.94^{a} \pm 0.05$	$2.84^{d} \pm 0.16$
Phytic acid[1]	$1.16^{e} \pm 0.01$	$0.82^{c} \pm 0.23$	$0.85^{d} \pm 0.07$	$0.24^{a} \pm 0.06$	$0.56^{b} \pm 0.01$
Raffinose[1]	$1.23^{e} \pm 0.14$	$1.08^{d} \pm 0.12$	$0.82^{c} \pm 0.17$	$0.34^{a} \pm 0.01$	$0.61^{b} \pm 0.08$
Stachyose[1]	$2.49^{e} \pm 0.19$	$1.14^{b} \pm 0.15$	$1.36^{c} \pm 0.02$	$0.83^{a} \pm 0.04$	$1.91^{d} \pm 0.13$
Verbascose[1]	$4.33^{e} \pm 0.15$	$3.57^{d} \pm 0.43$	$2.72^{b} \pm 0.32$	$1.26^{a} \pm 0.12$	$3.18^{c} \pm 0.15$
Haemagglutinating activity[2]	$94.86^{e} \pm 0.41$	$75.41^{d} \pm 0.25$	$42.76^{c} \pm 0.07$	$18.64^{a} \pm 0.15$	$25.19^{b} \pm 0.18$
Trypsin inhibitor activity[3]	$58.20^{e} \pm 0.19$	$17.46^{b} \pm 0.10$	$22.83^{c} \pm 0.12$	$10.35^{a} \pm 0.04$	$47.65^{d} \pm 0.13$
Amylase inhibitors activity[4]	$16.23^{e} \pm 0.24$	$12.51^{d} \pm 0.14$	$9.77^{c} \pm 0.14$	$5.14^{a} \pm 0.02$	$8.36^{b} \pm 0.16$

Values are mean and standard deviation of three separate determinations.
Values in the same row with different roman superscript are significantly different (p<0.05).
[1]Values expressed on g/100g sample dry matter basis.
[2]HU- Haemagglutinating unit / g sample
[3]TIU- Trypsin inhibitor unit / g sample
[4]AIU- Amylase inhibitor unit / g sample

Table 3. Growth performance of experimental animals fed with diets containing raw and processed seeds of *Entada scandens*.

Experimental diets	Growth Performance of the experimental animals			
	Feed Intake (g/28 days)	Body Weight Gain (g/28 days)	Feed Efficiency Ratio	Protein Efficiency Ratio
Control (Casein)[1]	$322.54^{f} \pm 0.23$	$106.32^{f} \pm 0.21$	$0.33^{f} \pm 0.01$	$3.32^{f} \pm 0.12$
Raw GB[2]	$135.21^{a} \pm 1.34$	$24.65^{a} \pm 0.15$	$0.18^{a} \pm 0.05$	$1.89^{a} \pm 0.24$
Soaked GB[3]	$182.83^{b} \pm 0.34$	$42.13^{b} \pm 0.13$	$0.23^{b} \pm 0.03$	$2.34^{b} \pm 0.17$
Cooked GB[4]	$198.46^{d} \pm 3.12$	$48.39^{d} \pm 0.15$	$0.24^{c} \pm 0.05$	$2.54^{c} \pm 0.02$
Autoclaved GB[5]	$239.34^{e} \pm 0.41$	$67.44^{e} \pm 0.21$	$0.28^{e} \pm 0.02$	$2.93^{e} \pm 0.30$
Roasted GB[6]	$183.58^{c} \pm 1.53$	$46.52^{c} \pm 0.14$	$0.25^{d} \pm 0.02$	$2.58^{d} \pm 0.04$

Values are mean and standard deviation of three separate determinations.
Values in the same column with different roman superscript are significantly different (p<0.05).

The raw gila bean seeds exhibited poor feed efficiency ratio (FER) (0.18) and protein efficiency ratio (PER) (1.89) values when compared to control and processed seeds (Table 3), which might be due to the high concentration of antinutritional substances and poor protein quality of the raw gila bean seeds. However, the FER and PER values of raw gila bean seeds were higher than the faba beans (0.032 & 0.32) (Gupta *et al*., 2005), but lower than vegetable peas (0.22 & 2.17) (Saharan and Khetarpaul, 1994). Among the different treatments, the autoclaving resulted in significant improvement (p<0.05) of FER (0.28) and PER (2.93) of gila bean seeds. The PER value of autoclaved gila beans was higher when compared to *C. ensiformis* (1.24) and *C. gladiata (*1.24) (Bressani *et al*., 1987). The results observed from the present study showed that the higher the FI, the higher the PER values obtained, which was coincided with an earlier report given by Bender (1956), in which he has pointed out that the PER determination is depends upon feed consumption.

The raw as well as processed seeds exhibited lower PER levels when compared to control casein. Usually much of the sulphur containing amino acids such as cysteine and methionine supplied in the normal diet were used to synthesize pancreatic enzymes (Fernandez *et al*., 1996). This exacerbated the deficiency of sulphur containing amino acids in legume seeds, were manifested as a lower production of body tissues. Rerouting of retained nitrogen in the animal group fed gila bean seeds included diets may explain why PER in these groups were significantly lower than the control, despite the fact that protein content was similar among these groups.

Among the processed seeds, the soaked and cooked seeds exhibited lower PER values (2.34 & 2.54, respectively). It might be due to the soaking and cooking treatments are not effective in reducing the antinutritional compounds, which interfere with the protein quality of gila bean seeds. The lower PER value of roasted (2.58) seeds compared to casein (3.32) and autoclaved seeds (2.93) could be due to the fact that dry heat accelerates the millard reaction and makes the protein unavailable for digestion (Sagarbieri, 1989). According to Friedman (1996), PER value below 1.5 describes a protein of poor quality; between 1.5 and 2.0 an intermediate quality and above 2.0 good quality. Hence, the seed proteins of raw gila beans was considered as intermediate quality proteins, whereas, the processed gila bean seeds possess proteins with good quality.

Protein quality

The protein quality such as true digestibility (TD), biological value (BV), net protein utilization (NPU) and utilizable proteins (UP) of raw and processed gila bean seeds were presented in Table - 4. The TD value of raw gila bean seeds was found to be lower (69%) when compared to control (91%) and treated seeds (74.1-80.3%) of the present study, but higher when compared to TD value of *C. maritima* (42.2 %) (Seena *et al*., 2005); *Vicia faba* (63.4%) (Gupta *et al*., 2005); *Bauhinia purpurea* (46.4%) (Vijayakumari *et al*., 1997a) and comparable with vegetable peas (65.8-66.7%) (Saharan and Khetarpaul, 1994). The autoclaving treatment significantly improved the TD level of gila beans (80.3%), which was higher than the values reported for *C. ensiformis* (76.4%) (Bressani *et al*., 1987) and *V. faba* (71.4%) (Gupta *et al*., 2005). The consumption of raw legume seed proteins was reported to increases the endogenous nitrogen loss through the shedding of intestinal mucosa (Sanoja and Bender, 1983; Fairweather-Tain *et al*., 1983), an effect that reduces the biological value of raw legume seed proteins. Further, the presence of various antinutritional substances, including trypsin inhibitors, which inhibits the complete digestion of protein and increases the excretion of endogenous faecal nitrogen (Nestares *et al*., 1996) was also partly responsible for the decrease in the level of TD value of seed proteins of raw gila bean seeds.

Among the differentially treated seeds, lowest BV was recorded for raw gila bean seeds (62%), which was comparable with that of BV level of vegetable peas (62.9-63.1%) (Saharan and Khetarpaul, 1994) and faba bean (60.4%) (Gupta *et al*., 2005), but found to be higher than *B. purpurea* (57.2%) (Vijayakumari *et al*., 1997a). The NPU value of raw gila bean seeds was also lower (42.8%) when compared to casein (73.6%) and treated seeds (47.2-56.4%), but similar with that of an earlier report on vegetable peas (41-42%) (Saharan and Khetarpaul, 1994) and higher than *C. maritima* (16.8%) (Seena *et al*., 2005) and *V. faba* (38.3%) (Gupta *et al*., 2005). The autoclaved gila beans exhibited highest level of NPU (56.4%) among the different treated seeds, which was also higher than the previous report on *B. purpurea* (46.4%) (Vijayakumari *et al*., 1997a). The level of UP of raw gila bean seeds (10.2%) was higher than that of vegetable peas (8.4-8.6%) (Saharan and Khetarpaul, 1994) and *B. purpurea* (7.2%) (Vijayakumari *et al*., 1997a). The highest level of UP (19.1%) was registered by autoclaved gila bean seeds when compared to raw and other processed seeds (10.2-15.4%).

Rats that received dietary casein as a protein source were able to take full advantage of the nitrogen that they retained to favour growth, probably as a result of more balanced supply of amino acids provided by this diet. In rats fed with raw gila bean seeds, part of the nitrogen retained may have been rerouted for the synthesis of digestive enzymes such as trypsin and chymotrypsin (Liener, 1994) to offset the effect of various antimetabolic substances present in the raw gila bean seeds, hence the seed proteins of raw gila beans obtain poor TD, BV, NPU and UP values.

Table 4. Protein quality of raw and processed seeds of *Entada scandens*.

Experimental diets	Protein quality of gila bean seeds			
	True Digestibility (%)	Biological Value (%)	Net Protein Utilization (%)	Utilizable Proteins (%)
Control (Casein)[1]	$91.47^{f} \pm 0.18$	$82.53^{f} \pm 0.21$	$73.65^{f} \pm 0.21$	$60.38^{f} \pm 0.19$
Raw GB[2]	$69.24^{a} \pm 0.32$	$62.27^{a} \pm 0.23$	$42.83^{a} \pm 0.15$	$10.27^{a} \pm 0.16$
Soaked GB[3]	$74.56^{c} \pm 0.31$	$67.44^{b} \pm 0.15$	$48.29^{c} \pm 0.13$	$14.65^{b} \pm 0.14$
Cooked GB[4]	$76.81^{d} \pm 0.13$	$68.26^{c} \pm 0.02$	$48.56^{d} \pm 0.15$	$15.42^{d} \pm 0.16$
Autoclaved GB[5]	$80.35^{e} \pm 0.12$	$73.51^{e} \pm 0.31$	$56.48^{e} \pm 0.21$	$19.16^{e} \pm 0.62$
Roasted GB[6]	$74.13^{b} \pm 0.24$	$68.49^{d} \pm 0.12$	$47.24^{b} \pm 0.14$	$15.08^{c} \pm 0.15$

Values are mean and standard deviation of three separate determinations.
Values in the same column with different roman superscript are significantly different ($p<0.05$).

The protein quality parameters such as TD, BV, NPU and UP of the autoclaved gila bean seeds were higher than the raw and other processed seeds, which was in consonance with the previous study on *B. purpurea* (Vijayakumari *et al*., 1997a) and *Vigna unguiculata* (Dario and Salgado, 1994). The decrease in the trypsin inhibitor activity and other antinutritional constituents as a consequence of autoclaving treatment would have been reducing the faecal nitrogen excretion in rats fed with autoclaved seeds included diet. Improvement in the protein quality after autoclaving treatment might be attributed to reduction on the levels of various antinutrients in addition to some other factors such as disruption of protein structure and increased accessibility of the seed proteins to enzymatic attack (Nielson, 1991).

The fact that no such decrease in faecal nitrogen excretion was occurred in the rats fed with diet containing roasted seeds may have been due to the fact that dry heat treatment cause isopeptide formation (Dutson and Orcutt, 1984; Kirk, 1984). This reduces the protein quality, as isopeptides are not hydrolyzed in the intestine, are resistant to proteolytic enzymes and are thus excreted in faeces. As a result, digestibility and availability of some amino acids are reduced (Kirk, 1984) and thus gave poor values of BV, NPU and UP for roasted gila bean seeds. Geervani and Theophilus (1980) also observed that wet heat processing method improves the protein quality of *Cicer arietinum* and *Vigna radiata* to a greater extent than dry heat methods.

CONCLUSIONS

The results of the present study indicated that the autoclaving treatment was suitable and more effective in reducing various antinutritional compounds of gila bean seeds without affecting its nutritional quality. When considering the biological value, the autoclaved gila bean seeds exhibited better animal growth performance and protein quality than the raw and other processed seeds. Hence, such economic and potential processing method could be adapted for the versatile utilization of gila bean seeds as an alternative protein source in the diets of human beings or animals, which will clearly reduce the over-dependence on common legumes for increasing protein requirements, especially in the developing countries.

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