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COMPARATIVE NUTRITIONAL EVALUATION OF LITTLE KNOWN LEGUMES, *Tamarindus indica, Erythrina indica* AND *Sesbania bispinosa*

[EVALUACION NUTRICIONAL DE *Tamarindus indica, Erythrina indica* Y *Sesbania bispinosa*]

M. Pugalenthi[1*], V. Vadivel[1], P. Gurumoorthi[2] and K. Janardhanan[2]
[1] Department of Biotechnology
Karpagam Arts and Science College, Coimbatore – 641 021, India
e – mail: pugalmuthiah@rediffmail.com
[2] Seed Technology Laboratory, Botany Department
Bharathiar University, Coimbatore – 641 046. India
**Corresponding author*

SUMMARY

The seed samples of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa* were collected and analyzed for their chemical composition with a view to evaluate their nutritional potential. The proximate composition reveals that except *T. indica* both the seed samples of *E. indica* and *S. bispinosa* are found to contain high content of crude protein. All the three seed samples contain relatively high content of crude lipid. The seed protein fractionation exhibits that except *T. indica* other two seed samples contain globulin as the predominant protein fraction. Though pulses are deficient in sulphur containing amino acids, seed proteins of *T. indica,* are found to possess sulphur containing amino acids comparable with that of FAO / WHO (1991) requirement pattern. Mineral profiles were also analyzed in all the three seed samples. The IVPD values range from 62.01 to 65.82%. The antinutritional factors such as total free phenolics, tannins, non-protein amino acid L- Dopa, oligosaccharides and haemagglutinating activity were also analyzed. Various processing methods such as soaking followed by cooking and enzymatic treatment to reduce/ eliminate the levels of oligosaccharides were also employed. The presently studied tribal pulses exhibit high level of nutrients, besides *in vitro* protein digestibility and low level of antinutritional factors. After conducting toxicological / animal feeding experiments, these little known tribal pulses may be recommended for large scale consumption as an alternative potential source of protein.

Key words: Proximate composition, amino acid composition, flatulence factors, total free phenolics, processing methods.

RESUMEN

Se colectaron y analizaron semillas de *Tamarindus indica, Erythrina indica* y *Sesbania bispinosa* para evaluar su potencial nutricional. Excepto *T. indica,* las semilas de *E. indica* y *S. bispinosa* tuvieron un elevado contenido de proteína cruda. Las tres especies contenien niveles relativamente altos de lípidos. Excepto por *T. indica* las otras dos especies contienen globulinas como fracción proteica predominante. La proteína de *T. indica* contiene amino ácidos azufrados equiparables a la norma FAO / WHO (1991). La digestibilidad in vitro de la proteína fluctió de 62 a 65%. Se analizó también el perfil de minerales y los contenidos de factores antinutricionales como fenoles totales, L-dopa y actividad hemaglutinante. Se evaluó el efecto de métodos de procesamiento sobre los niveles de oligosacaridos. Las especies estudiadas presentan un alto contenido de nutrients, alta digestibilidad in vitro de su proteína y un bajo nivel de factores antinutricionales. Estas especies poco conocidas pudieran ser recomendadas como fuente alternativa de proteina después de efectuar los estudios toxicológicos y de alimentación animal.

Palabras clave: Composición química, amino ácidos, factores de flatulencia, fenoles totales, métodos de procesamiento.

INTRODUCTION

In India, legumes constitute an important foodstuff and are an economic source of protein in the diets of economically weaker sections of population (Kumar *et al*., 1991). Some of the wild nuts and seeds used as food in several parts of the world have considerable promise as protein source (Amubode and Fetuga, 1983). The proteins are an essential component of the diet, needed for survival of animals and humans. Proteins basic function in nutrition is to supply

adequate amounts of required amino acids (Friedman, 1996).

Large segments of human population and animals in developing countries suffer from protein malnutrition (Conway and Toenniessen, 1999). Although grain legumes have been identified as cheap potential source of protein, the per capita availability is meager. The availability and consumption of protein foods in India will remain inadequate due to population explosion and urbanization and results in Protein Energy Malnutrition (PEM). The PEM problem can be alleviated by finding alternative cost effective sources of proteins (Prakash and Misra, 1988; Waterlow, 1994).

With an increasing interest in new food sources, the seeds of wild plants including the tribal pulses receive more attention, because they are highly resistant to disease and pests and exhibit good nutritional qualities (Janardhanan and Vadivel, 1994). The underutilized legumes / wild tribal pulses have tremendous potential for commercial exploitation but remain ignored. They offer a good scope to meet the ever-increasing demands for vegetable protein. Although they have high protein content and possess good nutritional value, their utilization is limited by the presence of some antinutritional / antiphysiological / toxic substances.

Hence, the present study deals with the nutritional and antinutritional aspects of three indigenous wild / little known / underutilized tribal pulses viz., *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa*.. An attempt has been made to employ certain viable processing methods to reduce / eliminate the oligosaccharides.

Tamarindus indica

Tamarind is an arboreal fruit. The fruit pulp is most acidic and has a uncommon plant acid, tartaric acid. The pulp of the fruit is used in the preparation of beverage and to flavor confections, curries and sauces (Siddhuraju *et al*., 1995a). The seeds are used as feed for cattle and pigs, as a valuable remedy in diarrhoea and dysentery, as a base in cosmetics, in pharmaceutical industry, as a curative against rheumatism and as a soil stabilizer (Anon, 1955). India is the main producer and consumer of tamarind in the world. (Shankaracharya, 1998). Tamarind kernel powder is used in developing food products such as jelly and marmalades (Bhattacharya *et al.,* 1983). Rao and Subramanian (1984) and Marangoni *et al* (1988) have attempted to produce protein concentrates or meals from kernel proteins. The presence of tannins and colouring matter in testa makes the whole seed unsuitable for human consumption. The testa, which produces some side effects such as depression, constipation and gastro-intestinal inflammation has to be completely removed before using the kernels for food purposes (The Wealth of India, 1976). The seed kernels have been used as food either alone or mixed with cereal flours. Certain hill tribes eat kernels mixed with flowers of mahua (*Madhuca latifolia*) (The Wealth of India, 1976).

Erythrina indica

It is a deciduous tree with orange red flowers and grows to a height of 15 m with rough bark. The trifoliate leaves are employed in the treatment of venereal buboes, inflammatory swellings of lymph nodes especially in the groins and armpits. Information on chemical composition and nutritional value is very scanty. The seeds have a total protein content of 32.6 – 37.6 % (Prakash and Misra, 1987). It has relatively low levels of antinutritional factors.

Sesbania bispinosa

It is an erect, low annual shrub with thick stems. The stems provide a strong durable fiber, which is used in paper industry. It is grown as a green manure (adding 150 kg N / ha), leaves used for forage and for poultry feed in South Africa. It has the capacity to suppress the weeds like *Impacta cylindrica* (Duke, 1981; NAS, 1980). Seed flour is used in the treatment of ringworm, skin diseases and wounds (Duke, 1981). The mature seeds of this species are known to be cooked and eaten by the Indian tribals, Katkharis and Ghonds (Siddhuraju *et al*., 1995b). Meager information is available on the nutritional potential and chemical nature of this underutilized legume.

MATERIALS AND METHODS

Samples

The seed samples of the three tribal pulses were collected from Western Ghats and Eastern Ghats, Tamil Nadu, South India from natural stands. *Tamarindus indica.* L. seeds were collected from Karukkathi, Ramanathapuram district, *Erythrina indica* Lam. seeds were collected from Bharathiar University Campus, Coimbatore district, and *Sesbania bispinosa* (Jacq.) W.F.Wight seeds were collected from Dharmapuri, Theni district in Tamil Nadu, India.

Preparation of raw seed samples

Collected seed samples were dried in the sunlight for 24 h. After removing immature and damaged seeds, the dried mature seeds were powdered in a Wiley Mill to 60-mesh size with suitable precaution to avoid contamination of samples. The powders were stored in plastic containers at room temperature (25°C) until further use.

Proximate analysis

The moisture content of seeds was determined by taking 50 transversely cut mature seeds weighing before and after drying in an oven at 80°C for 24h (Janardhanan, 1982). The Nitrogen content was determined by micro-kjeldahl method (Humphries, 1956) and crude protein content was calculated by multiplying the N value with constant 6.25. The crude lipid content was estimated by extracting the samples with ether in a sohxlet for 16h and the ash content of the three seed samples was estimated by following the method of AOAC (1970). The Total Dietary Fiber (TDF) content was determined by using non-enzymatic gravimetric method proposed by Li and Cardozo (1994). The Nitrogen Free Extractives (NFE) or crude carbohydrate content was calculated by the method of Muller and Tobin (1980). The Calorific value of the seeds were calculated by multiplying the crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7 respectively following the method of Siddhuraju *et al* (1992a).

Total protein content and fractionation of proteins

Extraction of albumins and globulins were done by the method of Murray (1979). The prolamins and glutelins were extracted by the method of Rajaram and Janardhanan, (1990). The protein contents of total protein and different solubility classes of proteins were determined by following the method of Lowry *et al* (1951).

Analysis of amino acid profiles of seed flour

The amino acid profiles were analyzed in a Hitachi Perkin Elmer (Model KLA 3B) automated amino acid analyzer. Sulphur containing amino acids were oxidized by using performic acid before the acid hydrolysis.

Lipid extraction and fatty acid analysis

The total lipids were extracted from the seed flour according to the method of Folch *et al* (1957). Fatty acid analysis was performed by Gas Chromatography (Shimadzu, Model RIA, Shimadzu Corporation, Tokyo, Japan) using an instrument equipped with a flame ionization detector and a glass column packed with 1 % diethylene glycol succinate on chromosorb W (silanised 80 / 100 mesh).

Mineral analysis

After the triple acid digestion, the minerals in the seed samples like sodium and potassium were estimated by using Flame Photometer Model- EEL. Calcium and magnesium were estimated by using the method of Jackson (1967). The phosphorous was estimated by using the method of Dickman and Bray (1940). The micronutrients viz., iron, copper, zinc and manganese were estimated by using Atomic Absorption Spectrophotometer (PERKIN – ELMER Model 5000) according to Issac and Johnson (1975) method.

***In vitro* protein digestibility (IVPD)**

The IVPD of seed samples was measured according to the multienzyme technique (Ekpenyong and Borchers, 1979). Fifty ml of glass distilled water was added to the seed flour (amount of sample was adjusted so as to contain 6.25 mg/ml). The sample was allowed to hydrate for 1h. at 5°C. The sample suspension was adjusted to pH 8.0 with 0.1N HCl and / or 0.1N NaOH while stirring in a water bath maintained at 37°C for 15 min. The multienzyme solution, consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase / ml was maintained in an ice bath and adjusted to pH 8.0 with 0.1 N HCl and / or NaOH. Five ml of this solution was added to the protein suspension while stirring at a constant temperature of 37°C. The pH of the hydrolysate was measured exactly 10 min. after the addition of multienzyme solution. The percentage of *in vitro* protein digestibility was calculated following the formula given below (Hsu *et al.*, 1977).

$$Y = 210.464 – 18.103\ X$$

Where:
X = pH of protein suspension after 10 min. digestion with multienzyme solution and,
Y = Percentage of digestibility.

Analysis of antinutritional factors of the seed samples.

Extraction and estimation of total free phenolics and tannins

Total free phenolics were extracted by the method of Maxon and Rooney (1972). One gram of air-dried seed flour was placed in a 100-ml flask, with 50 ml of 1 % (v/v) HCl in methanol. The samples were shaken on a reciprocating shaker for 24 h at room temperature. The contents were centrifuged at 10,000 x g for 5 min and the supernatant was used for further analysis. The extracted free phenolics were estimated following the method of Sadasivam and Manickam (1992). One-ml aliquots of the above extract were pipetted into different test tubes to which 1-ml of folin-phenol reagent and 2 ml of 20 % (w/v) Na_2CO_3 solution were added. The tubes were shaken and placed in a boiling water bath for exactly 1 min and then were cooled under running tap water. The resulting blue solution was diluted to 25 ml with distilled water and the absorbance was measured at 650 nm with a Spectronic 20D spectrophotometer. If precipitation occurred, it

was removed by centrifugation at 5000 x g for 10 min. before measuring the absorbance. The amount of phenolics present in the sample was determined from a standard curve prepared with catechol. A blank containing all the reagents minus plant extract was used to adjust the absorbance to zero. Average values of triplicate estimations were expressed as g 100 g^{-1} of the seed flour on a dry weight basis. The tannin content of seed samples were estimated by the method of Burns (1971). From suitable aliquots of the above extract, tannin content was quantified by the Vanillin-HCl method using phloroglucinol as a standard at 500 nm with a Spectronic 20 D spectrophotometer. The average values of triplicate estimations of all samples were expressed as g 100 g^{-1} seed flour on dry weight basis.

Extraction and estimation of L-Dopa

The non-protein amino acid, L-Dopa (3,4-dihydroxyphenylalanine) was extracted and quantified in the seed flour following the method of Brain (1976).

Assay for haemagglutinating activity

Albumin and globulin protein fractions obtained under fractionation of different solubility classes of seed proteins were employed as protein samples for determining haemagglutinating activity (Liener, 1976). Human blood was procured from Blood Bank of ReVijay Clinical Laboratory, Coimbatore. Blood erythrocyte suspension was prepared by washing the blood samples (A, B and O) separately with phosphate-buffered-saline and centrifuged at 1000 rpm for 30 min and supernatants were removed. The washed cells were diluted with phosphate-buffered-saline. 5 drops of protein fractions were mixed with different blood group and allowed to stand for 20 min and centrifuged at 1000 rpm for 3 min. After centrifugation, the tubes were shaken and the presence or absence of agglutination activity was noticed.

Extraction and estimation of oligosaccharides

Extraction of oligosaccharides was done by following the method of Somiari and Balogh (1993). Five grams each of both raw and processed seed flours were extracted with 50 ml of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 h and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25 ml of 70% (v/v) ethanol. The filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in 5 ml of double-distilled water. Separation of oligosaccharides was done by TLC. 30 g of cellulose-G powder were dissolved in 45 ml of double distilled water and shaken well until the slurry was homogeneous. TLC plates were coated with the slurry and air-dried. Spotting of the sugar samples was done by using micropipettes. 5 µl aliquots of each sample were spotted thrice separately. The plates were developed by using a solvent system of n-propanol, ethyl acetate and distilled water (6:1:3), and dried (Tanaka *et al.,*1975).The plates were sprayed with α-naphthol reagent (1%, w/v). Plates were dried in a hot-air oven. The separated spots were compared with standard sugar spots. Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped, eluted in 2 ml of distilled water kept overnight and filtered through Whatman No. 1 filter paper. The filtrates were subjected to quantitative estimation. The eluted individual oligosaccharides were estimated by the method of Tanaka *et al.* (1975). 1 ml of the eluted and filtered sugar solution was treated with 1 ml of 0.2 M thiobarbituric acid and one ml of concentrated HCl. The tubes were boiled in a water bath for exactly 6 min. After cooling, the oligosaccharide contents were quantified in a Spectronic 20 D spectrophotometer at 432 nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was expressed on dry weight basis.

Processing methods

Soaking followed by cooking

Whole seeds were soaked in distilled water for 16 h at room temperature in the bean: water ratio of 1:10 (w/v). After 16 h, the water was drained off and the seeds were cooked in distilled water for 10 min with bean: water ratio of 1:10 (w/v).

Crude α-galactosidase treatment

Partial purification of α-galactosidase from locally available guar seeds (*Cassia sericea*) was done by following the method of Shivanna *et al*. (1989). The seeds were surface sterilized by treating with a 0.1% (w/v) mercuric chloride solution for 15 min and then were washed with distilled water. Washed seeds were arranged at the bottom of moist filter paper, rolled and allowed to germinate at 27°C for 3 days. After germination, seeds were homogenized with 0.2 M acetate buffer (pH 5) in a homogenizer for 10 min at full speed. The homogenate was filtered through muslin cloth and allowed to settle down for few hours. The supernatant was decanted and centrifuged at 12,000 rpm for 30 min using a High Speed Refrigerated Centrifuge. The supernatant was precipitated with ammonium sulphate. Precipitate was subjected to centrifugation. After centrifugation, the residue was employed as crude enzyme and dissolved in acetate buffer (pH 5). The extracted crude α-galactosidase enzyme activity was determined as described by Mulimani *et al*. (1997). Treatment of 5 g of seed flour was done with 40 ml of crude α-galactosidase (0.45 units min^{-1}) at 50°C for 4 h with

occasional shaking. For control, the volume of enzyme was replaced with 50 mM of acetate buffer (pH 5). After 4 h of incubation, the contents were filtered through Whatman No. 1 filter paper. The residue was dried at 60°C for 24 h The dried samples were subjected to separation by thin layer chromatography, and estimation of oligosaccharides

Statistical analysis

All the analyses were estimated in triplicate determinations. Estimates of mean and standard error for the aforesaid parameters were calculated.

RESULTS AND DISCUSSION

Crude protein

Legume seeds are valuable source of protein, oil, carbohydrates, minerals and vitamins. They are playing an important role in human nutrition mainly in developing countries (Mohamed and Rangappa, 1992; Yanez *et al.,* 1995). In the present study, *Sesbania bispinosa* shows high content of crude protein (31.08 %) than the other two species (Table 1). *T. indica* contain high levels of crude protein than the levels reported earlier (Ishola *et al.,* 1990; Bhattacharya *et al*., 1994; Siddhuraju *et al.,* 1995a). Information on the levels of crude protein in *Tamarindus indica* seems to be meager. The crude protein content in *Erythrina indica* is lower when compared with earlier reports (Pant *et al.,* 1974; Banerjii and Dixit, 1988; Prakash and Misra, 1987) and *Sesbania bispinosa* exhibits higher level when compared to earlier reports in the same species (Siddhuraju *et al.,* 1995b; Banerji and Dixit, 1988).

Crude lipid

T. indica contained a high level of crude lipid content (7.84%) (Table 1). This value is found to be higher than that of earlier reports in the same species (Bhattacharya *et al.,* 1994; Siddhuraju *et al.,* 1995a). Crude lipid content of *Erythrina indica* is found to be more or less equal to that of *Prosopis gladulosa* (Harden and Zolfaghari, 1988) whereas the crude lipid content of *Sesbania bispinosa* is comparable to that of earlier reports in the same species (Siddhuraju *et al.,* 1995b).

Total Dietary Fiber (TDF) and ash content

T. indica contains the highest percentage of TDF (Table 1) compared to other legumes of this study. However, the TDF level of *T. Indica* seems to be low compared to certain cultivated legumes like lentil, green gram, pigeonpea and chick- pea (Ramulu and Udhayasekara Rao, 1997); cowpea and kidney bean (Singh *et al.,* 2000).

The seeds of *Erythrina indica* exhibit the highest level of ash (Table 1) of all the three seed samples. This value is found to be higher than that of earlier reports in *T. Indica (*Ishola *et al.,* 1990; Bhattacharya *et al.,* 1994; Siddhuraju *et al.,* 1995a) and *Sesbania bispinosa* (Siddhuraju *et al.,* 1995b).

Nitrogen free extractive and Calorific value

Among the presently investigated three species, *Erythrina indica* exhibits higher levels of Nitrogen Free Extractives (NFE) than *T. Indica* and *S. bispinosa* (Table 1). These values are found to be higher than that of some of the earlier investigated wild pulses like *Afzelia africana* (Madubuike *et al.,* 1994); *Lonchocarpus longystilus* (Sotelo *et al.,* 1995); *Pachyrhizus erosus* (Santos *et al.,* 1996); *Parkia filicoidea* (Fetuga *et al.,* 1974); *Prosopis juliflora* (Del valle *et al.,* 1983) and *Tylosema esculentum* (Bower *et al.,* 1988). In the present study, *S. bispinosa* exhibit the highest calorific value when compared to *E. indica* and *T. indica.*

Total protein and protein fractionation.

Among the studied wild pulses, *E. indica* showed highest level of total proteins than other two species (Table 2). *E. indica* and *S. bispinosa* are found to contain more total protein than that of *Cassia floribunda* (Janardhanan, 1993); *C. laevigata* (Siddhuraju *et al.,* 1995a); *C. obtusifolia* (Vijayakumari *et al.,* 1993); *Entada scandens* (Mohan and Janardhanan, 1993). The total protein content of *T. Indica* is found to be lower when compared to an earlier report in the same species (Siddhuraju *et al.,* 1995a). *E. indica* and *S. bispinosa* are found to contain higher levels of protein content than that of cultivated legumes like black gram and green gram (Gupta and Wagle, 1978) and field pea and vegetable pea (Saharan and Khetarpaul, 1994).

In general the globulin constitutes the major seed storage protein in legumes. Except *T. Indica*, in the other two species globulins constitute the major storage protein fraction (Table 2). This is in consonance with some earlier reports in *Cassia obtusifolia* and *Entada scandens* (Vijayakumari *et al.,* 1993) and *Mucuna monosperma* (Arulmozhi and Janardhanan, 1992). In *T. Indica,* albumin fraction forms the major seed protein followed by globulins. This also is in agreement with that of an earlier report in *Phaseolus lunatus* (Vijayakumari *et al.,* 1993).

Table 1. Proximate Composition of each germplasm of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa.* (The data are means and standard errors of triplicate determinations).

Component	g $100g^{-1}$ seed flour		
	T. indica	*E. indica*	*S. bispinosa*
Moisture	7.24 ± 1.12	6.86 ± 0.28	11.06 ± 0.82
Crude protein	14.0 ± 1.16	21.45 ± 0.72	31.08 ± 0.21
Crude lipid	7.84 ± 0.64	4.24 ± 0.36	6.23 ± 0.12
Total Dietary fiber	14.75 ± 2.16	7.9 ± 0.72	6.81 ± 0.09
Ash	4.58 ± 0.42	4.26 ± 0.21	3.27 ± 0.11
Nitrogen Free Extractives	58.83	62.15	52.61
Calorific value (kj/100g DM)	1511.83	1555.97	1632.49

Table 2. Total protein and protein fractionation of each germplasm of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa*. (The data are means and standard errors of triplicate determinations).

Component	*T. indica*		*E. indica*		*S. bispinosa*	
	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein	g/100g seed flour	g/100g seed protein
Total protein	6.9 ± 0.27	100.00	24.38 ±0.21	100.00	21.81±0.24	100.00
Albumins	2.6 ± 0.18	37.68	5.44 ± 0.8	22.31	5.42 ± 0.16	24.86
Globulins	2.4 ± 0.35	34.78	15.47 ±0.15	63.45	13.22±0.09	60.64
Prolamins	0.6 ± 0.15	8.69	1.34 ± 0.11	5.49	1.24 ± 0.04	5.68
Glutelins	1.3 ± 0.12	18.84	2.13 ± 0.15	8.74	1.92 ± 0.02	8.81

Amino acid composition

In *T. Indica,* except threonine, all the essential amino acid like valine, cysteine, methionine, phenylalanine, tyrosine, isoleucine, leucine, histidine and lysine are present in more than the adequate levels (Table 3) when compared with FAO / WHO (1991) requirement pattern. In *E. indica* the essential amino acids cysteine, methionine, threonine and isoleucine are deficient and in *S. bispinosa* the essential amino acids such as cysteine, methionine and threonine were found to be deficient when compared with FAO / WHO (1991) requirement pattern.

Fatty acid composition

Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance. The linoleic acid is found to be predominant in all the three investigated species (Table 4). Its concentration is comparable to some wild legumes like *Adenanthera pavonica, Parkia clappertonaie, Bauhinia monandra, Cassia nodosa* (Balogun and Fetuga, 1985); *T. indica* (Siddhuraju *et al.,* 1995a); *Indigofera linifolia* and *Sesbania bispinosa* (Siddhuraju *et al.,* 1995b); *Lens esculenta, Cajanus indicus* and *Lathyrus sativus* (Choudhury and Rahman, 1973). *Cassia obtusifolia, Entada scandens, Phaseolus lunatus* (Vijayakumari *et al.,* 1993); *Vigna trilobata* (Siddhuraju *et al.,* 1992a) and *Entada phaseoloides* (Sengupta and Basu, 1978) and *Glycine max* and *Vigna unguiculata* (Ologhobo and Fetuga, 1983; Omogbai, 1990).

Presence of high levels of unsaturated fatty acids in all the presently studied tribal pulses are nutritionally desirable and also are comparable with some edible legumes like Goa bean and Soybean (Rao and Belavady, 1979); *Phaseolus vulgaris* and *Vigna unguiculata* (Omogbai, 1990) and certain tribal pulses like *Alysicarpus rugosus* (Siddhuraju *et al.,* 1992b); *Cassia obtusifolia* and *Phaseolus lunatus* (Vijayakumari *et al.,* 1993); *Cassia laevigata* and *T. indica* (Siddhuraju *et al.,* 1995a) and *Indigofera linifolia* and *S. bispinosa* ((Siddhuraju *et al.,* 1995b).

The detected levels of antinutritional fatty acid, behenic acid in *T. indica* (5.03 %) is in agreement with earlier reports in the same species (Siddhuraju *et al.,* 1995a); *Mucuna monosperma* (3.52 %) and *M. pruriens* var. *utilis* (2.26 – 3.97 %) (Mohan and Janardhanan, 1995).

Table 3. Amino acid composition of the total seed proteins of each germplasm of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa* (g $100g^{-1}$ protein)

Amino Acid	*T. indica*	Essential amino acid score	*E. indica*	Essential amino acid score	*S. bispinosa*	Essential amino acid score	FAO /WHO requirement pattern
Aspartic acid	12.14	-	10.10	-	9.96	-	-
Glutamic acid	13.5	-	15.17	-	14.05	-	-
Alanine	3.7	-	3.96	-	3.51	-	-
Valine	6.1	174.29	4.36	124.57	4.11	117.43	3.5
Glycine	5.8	-	4.01	-	3.85	-	-
Arginine	6.3	-	6.07	-	4.48	-	-
Serine	3.6	-	5.26	-	4.31	-	-
Cystine	1.9	-	0.56	-	0.83	-	2.5
Methionine	2.12	-	0.81	-	1.01	-	-
Theronine	3.10	91.18	3.21	94.41	3.04	89.41	3.4
Phenylalanine	3.8	109.52	4.91	136.34	4.32	117.14	6.3
Tyrosine	3.10	-	3.68	-	3.06	-	-
Isoleucine	4.34	155.00	2.14	76.43	3.23	115.36	2.8
Leucine	8.7	131.82	7.01	106.21	7.22	109.40	6.6
Histidine	3.2	168.42	3.61	190.00	3.21	168.95	1.9
Lysine	6.5	112.07	6.01	103.62	5.34	92.07	5.8
Tryptophan	ND	-	ND	-	ND	-	1.1
Proline	1.12	-	2.80	-	4.91	-	-

ND- Not determined

Table 4. Fatty acid composition of each germplasm of *Tamarindus indica*[a], *Erythrina indica*[a] and *Sesbania bispinosa*[a]

Fatty acid (%)	*T. indica*	*E. indica*	*S. bispinosa*
Lauric acid (C12 : 0)	NP	1.95	0.35
Myristic acid (C14 : 0)	NP	3.03	3.1
Palmitic acid (C16 : 0)	14.67	13.53	18.27
Stearic acid (C18 : 0)	5.27	11.47	11.06
Oleic acid (C18 :1)	23.67	26.52	14.67
Linoleic acid (C18 : 2)	49.13	35.87	43.68
Linolenic acid (C18 : 3)	2.23	7.63	8.87
Behenic acid (C22 :0)	5.03	NP	NP

[a] - Average values of two determinations.
NP - Not Present

Mineral composition

Among the presently investigated three legume seeds, *T. indica* registers the lowest level of sodium content (Table 5), but it is seems to be higher compared to an earlier report in the same species (Ishola *et al.,* 1990; Siddhuraju *et al.,* 1995a). But when compared to Recommended Dietary Allowances (RDA) of NRC / NAS (1980), all the three species were deficient in sodium content.

Among the three species *S. bispinosa* registers the lowest level of potassium. However this values seem to be higher compared to an earlier report in the same species (Siddhuraju *et al.,* 1995b) and other legumes like *Vigna unguiculata* (Akinyele, 1989). *T. indica* is found to contain more than adequate level of potassium compared to RDA's of NRC / NAS (1980).

All the three species contain more calcium content compared to *Prosopis juliflora* (Marangoni and Alli, 1988); *Sesbania grandiflora* (Pant and Bishnoi, 1984) and *T. indica* (Siddhuraju *et al.,* 1995a). But, all the three legumes were deficient in calcium content compared to RDA's of infants (NRC / NAS, 1980).

All the presently studied pulses are found to contain more magnesium content (Table 5) compared to some tribal pulses like *Canavalia ensiformis* and *C. gladiata* (Rajaram and Janardhanan, 1992; Mohan and

Janardhanan, 1994; Rodriques and Torne, 1991); *C. virosa* (Rodriques and Torne, 1991); *Mucuna monosperma* (Mohan and Janardhanan, 1995) and *Mucuna pruriens* var. *utilis* (Siddhuraju *et al.,* 1996a). All the three species were found to contain high magnesium content compared to RDA's NRC / NAS (1980).

Among the three species, *T. Indica* registers the highest level of phosphorous content. It appears to be higher than that of earlier report in the same species (Ishola *et al.,* 1990; Siddhuraju *et al.,* 1995a). But the phosphorous content of presently studied species is deficient according to RDA's of infants (NRC / NAS, 1980).

Among the three wild legumes, *S. bispinosa* registers the high level of iron(Table 5) and this value seems to be higher than that of an earlier report in the same species (Siddhuraju *et al.,* 1995b).

Among the three underutilized pulses, *T. Indica* exhibits the highest level of zinc and manganese. This also seems to be higher than that of earlier report in the same species (Siddhuraju *et al*., 1995a) and the copper level is low in all the three species. The Zn and Cu levels of all the presently studied species are comparable with *Phaseolus vulgaris* (Apata and Ologhobo, 1994) and Mn content was comparable to *Phaseolus* v*ulgaris, Vigna unguiculata, Cicer arietinum* and *Pisum sativum* (Meiners *et al*., 1976). But the presently screened three pulses were deficient in Fe, Cu, Zn and Mn content when compared to children RDA's of Indians.

Table 5. Mineral Composition of each germplasm of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa* (mg $100g^{-1}$ seed flour). (The data are means and standard errors of triplicate determinations).

Mineral	*T. indica*	*E. indica*	*S. bispinosa*
Sodium	28.83 ± 1.34	54.16 ± 2.0	112.32 ± 0.68
Potassium	1315.28 ± 5.74	920.8 ± 4.64	827.42 ± 0.24
Calcium	248.56 ± 1.3	235.06 ± 2.16	268.67 ± 0.48
Magnesium	285.14 ± 2.82	304.08 ± 2.74	208.26 ± 0.52
Phosphorus	369.47 ± 2.14	281.17 ± 4.13	336.07 ± 0.28
Iron	7.14 ± 0.92	6.57 ± 0.86	7.42 ± 0.06
Copper	0.59 ± 0.16	0.81 ± 0.09	0.96 ± 0.08
Zinc	6.94 ± 0.51	5.94 ± 0.36	4.38 ± 0.21
Manganese	0.81 ± 0.12	0.64 ± 0.71	0.76 ± 0.04

In vitro protein digestibility (IVPD)

The IVPD values of *T. Indica* and *S. bispinosa* are found to be lower (Table 6) than that of an earlier report in the same species (Siddhuraju *et al.,* 1995 a, b). The IVPD level of *E. Indica* is comparable to that of *Cassia laevigata* (Siddhuraju *et al.,* 1995a). The IVPD values of the presently studied three pulses seem to be higher than that of *Cajanus cajan* (Singh and Eggum, 1984).

Table 6. *In vitro* protein digestibility (IVPD) of mature raw seeds of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa*.

Germplasm	**IVPD (%)**
Tamarindus indica	62.01
Erythrina indica	63.83
Sesbania bispinosa	65.82

Antinutritional factors

Total free phenolics and tannins.

Phenolic compounds inhibit the activity of digestive enzymes like α -amylase, trypsin, chymotrypsin and lipase (Salunkhe *et al.,* 1982) and decreases the digestibility of proteins, carbohydrates and availability of vitamins and minerals (Udayasekhara Rao and Deosthale, 1982).

E. Indica exhibits lower level of phenolics and tannins (Table 7) compared to *Acacia leucophlea* (Vijayakumari *et al.,* 1994) and *A. nilotica* (Siddhuraju *et al.,* 1996b). *T. Indica* is found to contain lower level of phenolics and higher level of tannins compared to earlier studies in the same species (Siddhuraju *et al.,* 1995a). The levels of both phenolics and tannins in *S. bispinosa* appear to be higher than an earlier report in the same species (Siddhuraju *et al.,* 1995b).

Tannins and phenols can be eliminated by decortication, soaking and heat treatment or cooking

process (Singh, 1988; 1993; Kataria *et al.,* 1989; Singh and Singh, 1992). Soaking followed by cooking before consumption is suggested as a mean of removing of harmful effects of polyphenolic compounds when the pulses are consumed as whole seed (Udayasekhara Rao and Deosthale, 1982).

L-Dopa

L – Dopa (3,4 – dihydroxyphenylalanine) is a non-protein amino acid which causes skin eruptions and increases body temperature in the consuming people when present in high concentrations (Jebadhas, 1980).

All the three species contain low level of L-Dopa (Table 7) when compared with *Cassia obtusifolia* (Mohan and Janardhanan, 1995) and *Entada phaseoloides* (Mohan and Janardhanan, 1993). Among three seed samples, *E. Indica* exhibits the highest percentage of L-Dopa.

The level of L-Dopa is significantly reduced by repeated soaking and boiling of seeds (Jebadhas, 1980). It is also observed that drying effects substantial lose in content of L – Dopa (Longo *et al.,* 1974; Larher *et al.,* 1984). Repeated boiling seeds in water and decanting of the water for seven times resulted in significant reduction in the level of L-Dopa (Janardhanan, 1982). Dry heat treatment also has been found to be more effective in reducing the L – Dopa content (Siddhuraju *et al.,* 1996b).

Haemagglutinins (lectins)

Lectins are toxic glycoproteins that have the ability to bind with carbohydrate moieties on the surface of the human red blood cells (RBC) and cause them to agglutinate. Lectins can combine with intestinal mucosal cells and cause interference with the absorption of available nutrients (Liener, 1994).

Table 7. Content (%) of total free phenolics (TP), tannins and L-Dopa in raw seeds of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa*. (The data are means and standard errors of triplicate determinations).

	T. indica	*E. indica*	*S. bispinosa*
TP	2.71 ± 0.08	0.70 ± 0.06	1.02 ± 0.007
Tannins	7.1 ± 0.31	0.55 ± 0.02	1.36 ± 0.05
L-Dopa	2.64 ± 0.84	2.96 ± 0.13	2.01 ± 0.40

The globulin fraction of *T. indica* exhibits weak agglutinating activity without any specificity against A, B and O human blood groups (Table 8). Nonetheless, albumin protein specifically agglutinates the human B blood group. This is in agreement with an earlier report in the same species (Siddhuraju *et al.,* 1995a). The globulin fraction of *E. indica* shows strong agglutinating activity against human blood group A but weak activity against B and O human blood groups. Nonetheless, albumin protein specifically agglutinates blood group A. The albumin of *S. bispinosa* exhibits weak agglutinating activity; whereas, the globulin protein specifically agglutinates the erythrocytes of A and O blood groups. This is in agreement with an earlier report in the same species (Siddhuraju *et al.,* 1995b).

Lectins are highly sensitive to heat treatment (Singh, 1988). Haemagglutinating activity decreases during germination in *Glycine max, Phaseolus vulgaris, Vicia faba* and *Vigna radiata* (Valdebouze *et al.,* 1980). A significant reduction in lectin activity has been noticed when the seeds of certain pulses were subjected to dry heat treatment and autoclaving (Siddhuraju *et al.,* 1996b; Vijayakumari *et al.,* 1997) and cooking and autoclaving (Vijayakumari *et al.,* 1995; 1996).

Table 8. Data on Haemagglutinating activity in raw seeds of *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa*

Protein fraction	Erythrocytes from the human blood group	*T. indica*	*E. indica*	*S. bispinosa*
Albumins	A	-	+	+
Albumins	B	+	-	+
Albumins	O	-	-	+
Globulins	A	+	++	++
Globulins	B	+	+	-
Globulins	O	+	+	++

\+ Clumping, pellet partially dispersed.
++ Clumping, no dispersion of pellet.
\- No clumping, pellet dispersed easily.

Oligosaccharides

Ingestion of large quantities of beans is known to cause flatulence in humans and animals. The raffinose family sugars (raffinose, stachyose and verbascose) are important contributors of flatus. These are not digested by man due to the lack of α -galactosidase enzyme (Gitzelmann and Aurricctuo, 1965). The microflora in the lower intestine metabolizes these oligosaccharides and produce flatus gases.

Among the presently investigated three wild species, the seed samples of *S.bispinosa* and *T. Indica* are found to contain the highest level of total oligosaccharieds followed by *E. Indica* (Figures 1-3). In the present study, all three species contain verbascose as the major oligosaccharide. This is in agreement with earlier reports in *Vigna mungo* (Navikul and D' Appolonia, 1978); *Cajanus cajan* and *Phaseolus munjgo* (Reddy *et al.,* 1984); *Cajanus cajan, Cicer arietinum, Phaseolus mungo, P. vulgaris and Vicia faba* (Jood *et al.,* 1985) and *Cajanus cajan* (Mulimani and Devendra, 2000). All the three species are found to contain lower levels of stachyose than Pinto bean (Navikul and D' Appolonia, 1978); winged bean, *Phaseolus vulgaris,* red gram (Reddy *et al.,* 1984) and cowpea (Somiari and Balough, 1993).

Effect of processing methods.

Soaking followed by cooking

A substantial reduction in levels of raffinose (66.9%) and stachyose (59.5%) in *Sesbania bispinosa* (Fig 3) and significant reduction in verbascose (24.8%) in *T. indica* (Fig -1) and *S. bispinosa* has been observed during 16h of soaking followed by 10 min of cooking in water. It is in agreement with an earlier report in *Cicer arietinum, Cajanus cajan, Phaseolus mungo* and *P. vulgaris* (Jood *et al.,* 1985). But, soaking followed by cooking is found to be ineffective in reducing verbascose content in *E. indica* (Fig 2).

Decrease in contents of raffinose, stachyose and verbascose due to cooking might be attributed to heat hydrolysis of the oligosaccharides to simple disaccharides and monosaccharides or to the formation of other compounds (Onigbinde and Akinyele, 1983).

Enzymatic treatment

The level of oligosaccharides is very much reduced by enzymatic treatment. Marked reduction in content of raffinose in the seed sample of *E. Indica* (94.5%) and *S. bispinosa* (90.0%) is obtained when samples are treated with crude α–galactosidase enzyme (Fig 2 and 3). This is in agreement with an earlier report in soybean (Mulimani *et al.,* 1997). All the three species register reduction of verbascose ranging from 64.3 % to 95.6%.

The reduction of raffinose family of oligosaccharides by crude α-galactosidase enzyme may be due to conversion of oligosaccharides in to di and monosaccharides by means of cleaving the α -galactosidic linkages. In conclusion, the enzyme treatment is found to be more effective in eliminating oligosaccharides.

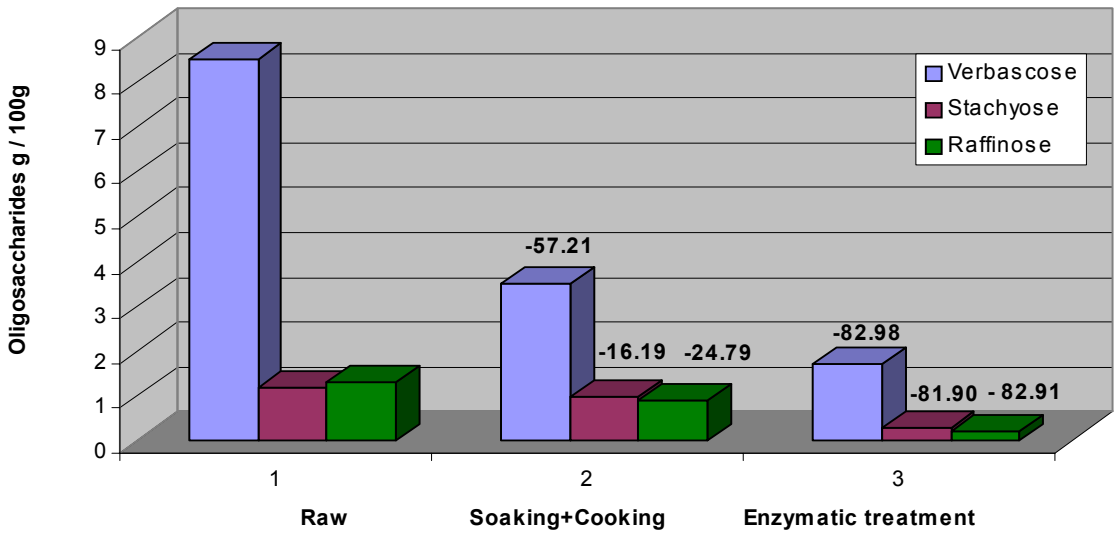

Figure- 1. Effect of various treatment on the levels of oligosaccharides in seed samples of *Tamarindus indica* (The values on top of each bar represent the percentage reduction of sugars with respect to particular treatment).

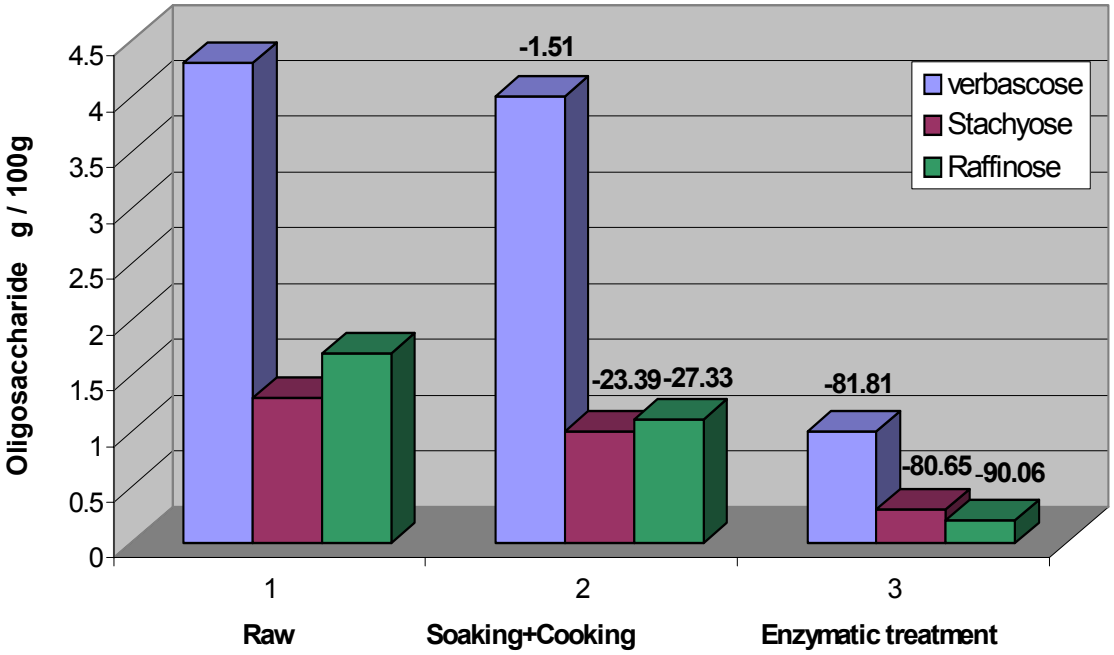

Figure- 2. Effect of various treatment on the levels of oligosaccharides in seed samples of *Erythrina indica* (The values on top of each bar represent the percentage reduction of sugars with respect to particular treatment).

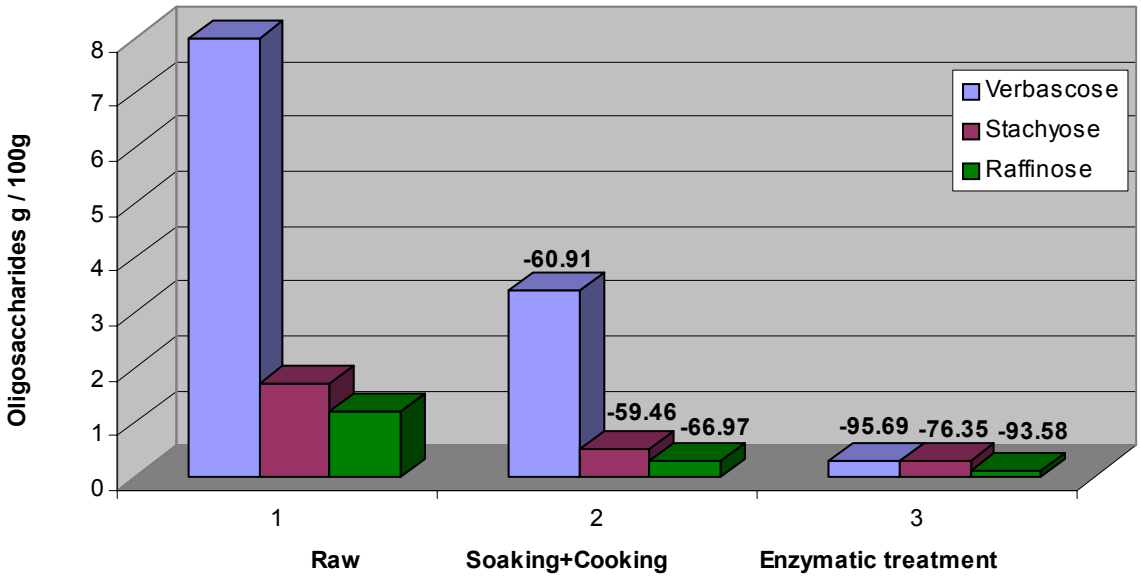

Figure- 3. Effect of various treatment on the levels of oligosaccharides in seed samples of *Sesbania bispinosa* (The values on top of each bar represent the percentage reduction of sugars with respect to particular treatment).

CONCLUSION

Among the presently investigated pulses, except, *T. Indica* other two pulses, *E. indica* and *S. bispinosa,* have been identified as rich source of crude protein. All the three species contain high content of crude lipid, total dietary fiber and calorific value and possess good amino acid composition with essential fatty acids. *T. Indica* contains higher levels of sulphur containing amino acids cystein and methionine (4.02%) when compared with that of FAO / WHO (1991) (2.50%) requirement pattern. Among the three species, *S. bispinosa* exhibits the highest IVPD value. Except *T. Indica* other two contain less amount of antinutritional factors like total free phenolics, tannins and L-Dopa. In *E. indica* and *S. bispinosa,* the globulin exhibits strong agglutinating activity against human erythrocyte 'A' whereas, the globulin fraction of *S. bispinosa* exhibits strong agglutinating activity against 'O' blood group. Among the presently studied tribal pulses, *E. indica* contains lower level of oligosaccharides. Of the two processing methods employed enzymatic treatment is found to be more effective in reducing the levels of flatulence factors. The presently studied tribal pulses exhibit high level of nutrients, besides *in vitro* protein digestibility and low level of antinutritional factors. After conducting toxicological / animal feeding experiments, these little known tribal pulses may be recommended for large scale consumption as an alternative potential source of protein.

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