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Chemical composition and nutritional evaluation of an underexploited legume, *Acacia nilotica* (L.) Del.

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Nutritional and antinutritional characteristics and biological value of *Acacia nilotica* (L.) Del. seeds were investigated. The mature seeds contained 234 g kg^{-1} crude protein, 126 g kg^{-1} crude fibre, 66.6 g kg^{-1} crude fat, 39.7 g kg^{-1} ash and 534 g kg^{-1} carbohydrates. Potassium, phosphorus, magnesium, iron and manganese occurred in high concentrations. The essential amino acid profile compared well with the FAO/WHO recommended pattern except for cystine, methionine and threonine. Cystine and methionine were the first limiting amino acids. When compared with the globulin fraction, albumins appeared to be a richer source of cystine, methionine, threonine, lysine and tryptophan. Oleic and linoleic acids constituted the predominant fatty acids (66.9%). Both dry-heating and autoclaving reduced the antinutritional components significantly. The *in vitro* protein digestibilities of raw, dry heat-treated and autoclaved seeds were 61.2%, 77.4% and 80.2%, respectively. Biological value, true digestibility and net protein utilization were significantly higher in processed seed than in raw seeds. The utilizable protein difference was insignificant between raw and processed seed samples. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Large segments of the population in the developing countries suffer from protein malnutrition. Projections based on current trends indicate a gap between human population and protein supply. Hence research efforts are being directed to this area to identify and evaluate underexploited sources as alternative protein crops for the world of tomorrow (Egbe & Akinyele, 1990). In this regard, various studies are being carried out to assess the potential of indigenous legumes that are not widely used as food, as a dietary source of protein, as well as a genetic resource for the improvement of traditional legume crops.

In India, young pods and mature seeds of *Acacia nilotica* are known to be cooked and eaten by tribal people living in Western Rajasthan (Singhvi & Bhandari, 1992). However, information on the nutritional characteristics of *A. nilotica* is scanty (Pant *et al.*, 1974; Prakash & Misra, 1987). Hence this study was undertaken to evaluate the nutritional and antinutritional properties and biological value of *A. nilotica* seeds. In addition, elimination/reduction in contents of antinutritional components has been studied after subjecting the seeds to both moist and dry heat treatments.

MATERIALS AND METHODS

Dry mature seeds of *Acacia nilotica* (L.) Del. were collected from Bharathiar University campus, Coimbatore, Tamil Nadu, India.

Proximate composition

The moisture content was determined by following the method of Rajaram & Janardhanan (1990). The seeds were powdered separately in a Wiley mill to 60 mesh size. The fine seed powder so obtained was stored in screw-cap bottles at room temperature and used for further analysis. Nitrogen content was estimated by the micro-Kjeldahl method (Humphries, 1956) and crude protein content was calculated (%N×6.25). The contents of crude lipid, crude fibre and ash were determined in accordance with the standard methods of AOAC (1970). Carbohydrate was obtained by difference. The energy value of the seeds was estimated in kilojoules by multiplying the percentages of crude protein, crude fat and carbohydrates by the factors 16.7, 37.7 and 16.7, respectively.

Fibre analysis

The method of Goering & Van Soest (1970) was used to determine neutral detergent fibre (NDF) as well as acid detergent fibre (ADF). Insoluble hemicellulose was calculated as the loss in the weight of ADF residue after treatment with sulphuric acid. The loss in weight of the above residue upon ashing was used to calculate the lignin content.

Mineral analysis

From the triple acid-digested sample, calcium, magnesium, iron, zinc, manganese, copper were determined using a Perkin Elmer atomic absorption spectrophotometer (Model 5000; Perkin Elmer, USA), while a flame photometer (Elico, India) was used for the determination of potassium and sodium. Total phosphorus was assayed colorimetrically (APHA, 1980) at 630 nm using a spectrophotometer (Model Spectronic 20D; Milton Roy, USA).

Extraction and estimation of total seed proteins and seed protein fractionation

The total (true) proteins were extracted following the method of Rajaram & Janardhanan (1990). The extracted proteins were purified by precipitation with cold (200 g litre^{-1}) trichloracetic acid (TCA) and estimated by the method of Lowry *et al.* (1951). The albumin and globulin fractions of seed proteins were extracted and separated according to the method of Murray (1979). From the residual pellet, the prolamin protein fraction was extracted by treating the pellet with ethanol (800 ml litre^{-1}, 1:10, w/v) overnight. After centrifugation (20 000*g* for 20 min at room temperature) the supernatant containing prolamins was air-dried and dissolved in 0.1 M NaOH. The resulting pellet was extracted with NaOH (0.4 g litre^{-1}, 1:10, w/v) overnight and centrifuged at 20 000*g* for 20 min at room temperature. The supernatant thus obtained was designated as glutelins. All four fractions so obtained were precipitated and washed with cold TCA (100 g litre^{-1}). All samples were redissolved in 0.2 M NaOH and protein content was determined by the method of Lowry *et al.* (1951). A portion of precipitated proteins was centrifuged and freeze-dried prior to amino acid analysis.

Amino acid analysis

Known amounts of precipitated total seed proteins and protein fractions, albumins and globulins, were acid-hydrolysed with 6 M HCl for 24 h at 110°C in a sealed tube under vacuum. The amino acid analysis was performed using an automated precolumn derivatization with *o*-phthaldialdehyde using reverse-phase high-performance liquid chromatography (Model 23250). The cystine content of protein samples was obtained separately by the method of Liddle & Saville (1959). For the determination of tryptophan content of proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together with 0.75 ml of 5 M NaOH. The ampoules were flame-sealed and incubated at 110°C for 18 h. The tryptophan content of the alkaline hydrolysates was determined colorimetrically by the method of Spies & Chambers (1949). The contents of the various amino acids recovered were presented as grams per 100 grams of protein and are compared with the FAO/WHO (1990) reference pattern. The essential amino acid score was calculated as follows:

$$\text{Essential amino acid score} = \frac{\text{grams of essential amino acid in 100 grams of test protein}}{\text{grams of essential amino acid in 100 grams of FAO/WHO reference pattern}} \times 100$$

Lipid extraction and fatty acid analysis

The total lipids were extracted from the seeds according to the method of Folch *et al.* (1957) using chloroform and methanol mixture in the ratio of 2:1 (v/v). Methyl esters were prepared from the total lipids by the method of Metcalfe *et al.* (1966). Fatty acid analysis was performed by gas chromatography (Model R1 A; Shimadzu, Japan) using an instrument equipped with a flame ionization detector and a glass column (2 m×3 mm) packed with 1% diethylene glycol succinate on Chromosorb W (silanized 80/100 mesh). The carrier gas was nitrogen, at a flow rate of 32 ml min^{-1}. The column temperature gradient was 4°C min^{-1} from 190°C to 240°C. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters; the relative weight percentage of each fatty acid was determined from integrated peak areas.

Heat treatments

About 1 kg of seeds was autoclaved at 15 lb pressure for 30 min in water using a 10:1 water:seed ratio. The autoclaving liquid was separated and autoclaved seeds were dehydrated with a hot air oven at 60°C to a constant weight and powdered to 60 mesh size. Approximately the same amount of seeds was roasted at 150°C for 30 min, allowed to cool, and then ground for analysis of antinutritional compounds, *in vitro* protein digestibility and feeding tests.

Analysis of antinutritional compounds

The antinutritional components, total free phenolics (Sadasivam & Manickam, 1992), tannins (Burns, 1971) and hydrogen cyanide (HCN) (Jackson, 1967) were quantified in raw as well as in moist and dry heat-treated seeds. The colorimetric procedure of Wheeler & Ferrel (1971) was followed to estimate phytic acid. Trypsin inhibitor activity was determined by the enzymic assay of Kakade *et al.* (1974). One trypsin unit is

expressed as an increase of 0.01 absorbance unit per 10 ml of reaction mixture at 410 nm. Trypsin inhibitor activity is defined in terms of trypsin units inhibited per milligram of protein. Chymotrypsin inhibitor activity was assayed in a 0.1 M phosphate buffer, pH 7.6, extract of acetone-treated meal by the procedure of Kakade *et al.* (1970).

α-Amylase activity was determined according to the procedure outlined by Moneam (1990). One unit of enzyme activity was defined as that which liberates 1 μmole of reducing groups (calculated as maltose) min^{-1} at 37°C and pH 7.0 under the specified conditions (Deshpande *et al.*, 1982) from soluble starch.

α-Amylase inhibitor activity was evaluated according to the method of Deshpande *et al.* (1982). One gram of sample was extracted with 10 ml of distilled water for 12 h at 4°C and centrifuged at 5000*g* for 20 min and the supernatants were tested for α-amylase inhibitory activity. Extract containing inhibitor (0.25 ml) was incubated with 0.25 ml of enzyme solution for 15 min at 37°C. To this mixture, after preincubation, was added 0.5 ml of 1% starch solution. At the end of 30 min, the reaction was stopped by addition of 2 ml of dinitrosalicylic acid reagent and heating in a boiling water bath for 10 min. The test tubes were then cooled under running cold tap water and made to a final volume of 13 ml with distilled water. The absorbance was recorded at 540 nm in a Spectronic 20D spectrophotometer. The liberated reducing sugars were expressed as maltose. One unit of α-amylase activity inhibited was defined as one α-amylase inhibitory unit.

The total crude lectins were extracted by the method of Almeida *et al.* (1991). The protein content of the extracts was estimated by the method of Lowry *et al.* (1951). The phytohaemagglutinating activity of raw and treated seed samples was determined by the method of Tan *et al.* (1983) and expressed as haemagglutinating units (HU mg^{-1} protein).

Determination of *in vitro* protein digestibility (IVPD)

The IVPD of raw, dry heat-treated and autoclaved seed samples was determined following the multienzyme method of Hsu *et al.* (1977).

Biological evaluation of protein quality

Biological evaluation of protein quality of raw and treated seed samples was conducted according to the method of Eggum (1973). Groups of five Wistar male rats, each weighing about 60 g, were fed a daily diet of 10 g (dry weight basis) containing 150 mg of nitrogen. The experiments consisted of a 4-day preliminary period and a 5-day balance period during which faeces and urine were quantitatively collected. At the end of 5 days, unconsumed diet weight was recorded and total nitrogen intake was calculated. Another group of rats of the same weight and age were fed on a nitrogen-free diet to calculate the endogenous and metabolic nitrogen losses. True protein digestibility (TD), biological value (BV), net protein utilization (NPU) and utilizable protein (UP) were determined for all samples.

Statistical analysis

The data were statistically analysed using Duncan's multiple range test by the method of Alder & Roessler (1977).

RESULTS AND DISCUSSION

The chemical, fibre and mineral composition of *A. nilotica* seeds is given in Table 1. The contents of crude protein and crude fat are found to be higher than some other *Acacia* species (Kapoor *et al.*, 1973; Brand & Maggiore, 1992). The relatively high level of crude fat in *A. nilotica* seeds indicates a good source of energy.

The fibre composition is determined because of the recent interest in the potential role of dietary fibre in human nutrition. Fibre helps to maintain the health of the gastrointestinal tract, but in excess may bind trace elements, leading to deficiencies of iron and zinc. The NDF is known to give a reliable estimate of the total fibre in a vegetable foodstuff which is available for microbial fermentation in the gut (Garcia & Palmer, 1980). The fibre analysis reveals that *A. nilotica* seeds contain less detergent fibre than *Mucuna utilis* (Ravindran & Ravindran, 1988).

Table 1 reveals that potassium, as in most legumes (Meiners *et al.*, 1976), is the predominant mineral.

Table 1. Chemical, fibre and mineral composition of *Acacia nilotica* seeds

Chemical composition (g kg^{-1} DM)	
Moisture (g kg^{-1} FW)	55.2 ± 0.5
Crude protein (N×6.25)	233.7 ± 1.2
Crude fibre	126 ± 2.4
Crude fat	66.6 ± 0.6
Ash	39.7 ± 0.4
Carbohydrates (by difference)	534
Energy content (kJ kg^{-1} DM)	15 325
Fibre (g kg^{-1} DM)	
ADF	81.0 ± 3.6
NDF	153 ± 2.5
Hemicellulose	72.0 ± 4.1
Cellulose	69.0 ± 1.7
Lignin	10.0 ± 0.4
Mineral composition (mg kg^{-1} DM)	
Sodium	276.3 ± 2.1
Potassium	11 082 ± 11.8
Calcium	2375 ± 2.6
Magnesium	2618 ± 4.3
Phosphorus	5287 ± 8.2
Manganese	37.3 ± 1.3
Iron	245 ± 1.7
Copper	25.1 ± 0.3
Zinc	27.0 ± 0.6

Results are the average values of three determinations ± standard error.
DM, dry matter; FW, fresh weight.

Table 2. Amino acid composition and essential amino acid score of total seed proteins and protein fractions (albumins and globulins) of *Acacia nilotica* (g per 100 g protein)

Amino acid	Total (true) seed proteins	Essential amino acid score	Albumins	Essential amino acid score	Globulins	Essential amino acid score	FAO/WHO (1990) values
Aspartic acid	14.7		12.5		11.2		
Glutamic acid	18.8		14.2		17.0		
Alanine	7.11		6.51		8.16		
Valine	5.62	161	3.86	110	5.24	150	3.50
Glycine	8.30		5.71		6.77		
Arginine	5.40		4.19		5.89		
Serine	3.86		5.16		5.24		
Cystine	1.20	84.0	1.54	131	1.05	92.8	2.50
Methionine	0.90		1.73		1.27		
Threonine	3.02	88.8	5.35	157	3.86	114	3.40
Phenylalanine	4.10	99.0	3.81	110	4.72	119	6.30
Tyrosine	2.14		3.10		2.75		
Isoleucine	2.88	103	3.16	113	4.18	149	2.80
Histidine	4.10		2.81		2.93		
Lysine	6.42	111	8.40	145	6.30	109	5.80
Tryptophan	1.87	170	2.09	190	0.82	74.6	1.10
Proline	ND		ND		ND		
Leucine	8.16	124	6.21	94.1	9.70	147	6.60

ND, not determined.

When compared to Recommended Dietary Allowances (NRC/NAS, 1980) the seeds of *A. nilotica* contain more than adequate levels of Mg, Fe and Mn, as in the case of *Acacia leucophloea* (Vijayakumari *et al.*, 1994).

From the results of seed protein fractionation, it is evident that globulins and albumins together form the major (81%) seed storage proteins, whereas glutelins and prolamins account for only a small fraction (147 and 42.0 g kg^{-1}, respectively) of the total protein. These results are in close agreement with those obtained for *Dolichos biflours* and *Phaseolus aconitifolius* (Pant *et al.*, 1974).

Amino acid composition and essential amino acid score of total seed proteins, albumins and globulins of *A. nilotica* are shown in Table 2. The contents of valine, isoleucine, leucine, lysine and tryptophan in *A. nilotica* seem to be higher than FAO/WHO (1990) values.

The tryptophan content appears to be higher compared to some other species of *Acacia* (Rivett *et al.*, 1983; Shamanthaka Sastry & Murray, 1986). The amino acid profiles of protein fractions, albumins and globulins, of *A. nilotica* show that leucine in the former fraction and tryptophan, cystine and methionine in the latter fraction appear to be the limiting amino acids. Albumins of *A. nilotica* seem to be a good source of threonine. The amino acids threonine, phenylalanine, tyrosine, isoleucine, leucine and lysine of the globulin fraction in the present study are comparable to the globulins of *Acacia alata* (Pettigrew & Watson, 1975).

Table 3. Fatty acid composition of *Acacia nilotica* seed lipids (% of total fatty acids)

Fatty acid	%
Myristic acid ($C_{14:0}$)	0.15
Palmitic acid ($C_{16:0}$)	18.1
Stearic acid ($C_{18:0}$)	7.80
Oleic acid ($C_{18:1}$)	28.8
Linoleic acid ($C_{18:2}$)	38.2
Linolenic acid ($C_{18:3}$)	2.62
Arachidic acid ($C_{20:0}$)	1.30
Others (unidentified)	3.03

The fatty acid profile of the total lipids is shown in Table 3 and it reveals that the seed lipids of *A. nilotica* exhibit a high proportion of linoleic acid (38.2%), oleic acid (28.8%) and palmitic acid (18.1%) and have a high ratio of unsaturated:saturated fatty acids (greater than 1:1, which is a desirable feature in human food). Earlier investigations established similar trends in several *Acacia* species (Rivett *et al.*, 1983; Brand & Maggiore, 1992).

Although the legume seeds are rich in protein, the protein digestibility and utilization are poor due to the presence of certain antinutritional components (Liener, 1994).

The contents of both total free phenolics and tannins (Table 4) are higher than some of the commonly consumed legumes (Udayasekhara Rao & Deosthale, 1982). The application of dry heat, in the present study, significantly reduced the levels of tannins and total free phenolics which is in conformity with an earlier investigation of this laboratory in *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al.*, 1995). Nonetheless, autoclaving is very effective in reducing the contents of both total free phenolics and tannins.

Phytic acid level of *A. nilotica* is comparable with that of a white variety of *Cicer arietinum* species and lower than that of another brown variety of *C. arietinum* (Khan *et al.*, 1988). Significant reduction in phytic acid content has been observed during dry heat treatment and autoclaving in *A. nilotica*. The same percentage loss has been observed in other Indian tribal pulses (Vijayakumari, 1994; Vijayakumari *et al.*, 1996).

HCN content seems to be negligible in raw seeds of *A. nilotica*. Dry heat treatment significantly reduces the

Table 4. Some antinutritional compounds present in raw and processed seeds of *Acacia nilotica*

Antinutrient	Raw	Dry-heated	Autoclaved
Total free phenolics (g kg^{-1} DM)	23.6^a	13.7^b (42)	8.0^c (66)
Tannins (g kg^{-1} DM)	16.7^a	10.4^b (38)	7.2^c (57)
Phytic acid (g kg^{-1} DM)	9.2^a	6.6^b (28)	5.4^c (41)
Hydrogen cyanide (mg kg^{-1} DM)	38.3^a	16.2^b (58)	9.4^c (75)
Trypsin inhibitor activity (TIU mg^{-1} protein)	51.2^a	11.3^b (78)	6.8^c (87)
Chymotrypsin inhibitor activity (CIU mg^{-1} protein)	18.5^a	6.6^b (64)	Nil^c (100)
α-Amylase inhibitor activity (units g^{-1} sample)	64.6^a	23.6^b (63)	12.1^b (81)
Phytohaemagglutinating activity (HU mg^{-1} protein) of erythrocytes from human blood group:			
A	104^a	46^b (56)	23^c (78)
B	162^a	73^b (55)	36^c (78)
O	10^a	Nil^b (100)	Nil^b (100)
In vitro protein digestibility (%)	61.2^a	77.4^b	80.2^b

Values represent the mean of triplicate determinations expressed on a dry weight basis. Values in parentheses indicate the percentage loss from raw seeds.
CIU, chymotrypsin inhibitor unit; TIU, trypsin inhibitor unit; HU, haemagglutinating unit.
Values with the same superscript in each row do not differ significantly from each other ($P < 0.05$).

content of HCN. This gains support from the previous report in *Prosopis chilensis* (Vijayakumari, 1994). When subjected to autoclaving, HCN content was reduced significantly compared to raw seeds of *A. nilotica*. Similar observations have been made in several other tribal pulses (Vijayakumari, 1994; Vijayakumari *et al.*, 1995, 1996).

The trypsin inhibitor activity of *A. nilotica* is comparable with that of various edible legumes such as *C. arietinum*, *Vigna unguiculata* and *Lens esculenta* (Al-Bakir *et al.*, 1982). Significant reduction of trypsin inhibitor activity has been noticed under both dry heat and autoclaving processes. Hildrand *et al.* (1981) observed a loss of proteinase inhibitory activity by heating aqueous extracts of winged beans. Autoclaved seeds exhibit complete reduction in chymotrypsin inhibitor activity.

Between dry-heating and autoclaving in the present investigation, autoclaving significantly eliminates the activity of α-amylase inhibitor. This accords with an earlier report in six different varieties of *C. arietinum* (Mulimani & Rudrappa, 1994).

Lectin activity was markedly reduced when subjected to both dry heat treatment and autoclaving in respect of A and B blood groups. The presence of residual lectin activity after dry heat treatment has been reported in an earlier study in *Phaseolus vulgaris* (Almeida *et al.*, 1991). Complete loss in lectin activity was observed in blood group O, as has been reported earlier in *Phaseolus mungo* (Kaul & Bajwa, 1987) and different cultivars of *C. arietinum* (Bansal *et al.*, 1988).

The elimination/reduction of lectin activity may be due to the breakdown of haemagglutinins (proteins) into their subunits or undergoing some other unknown conformational changes in their native structure which might be required for their haemagglutinating activity (Batra, 1987).

The raw seeds of *A. nilotica* exhibit 61.2% IVPD. Dry-heating for 30 min increased the IVPD value to 77.4% (27% improvement) whereas autoclaving improved the IVPD further (80.2%). The increase in the protein digestibility of legumes on autoclaving may be attributed, not only to the removal of the polyphenols in the seed hull (Moneam, 1990), but also to the structural disintegration of the native protein including enzyme inhibitors.

In addition to chemical analysis, biological evaluation of protein provides useful information with regard to overall quality. The biological value (BV), true digestibility (TD), net protein utilization (NPU) and utilizable protein (UP) of raw, dry-heated and autoclaved seed samples of *A. nilotica* along with casein (control) are presented in Table 5. Autoclaving significantly influences the protein quality parameter when compared with raw seeds. The BV, TD and NPU values of

Table 5. Biological evaluation of raw and processed seeds of *Acacia nilotica*

Measurement	Raw	Dry-heated	Autoclaved	Casein (control)
Biological value (%)	54.2^b	57.5^{bc}	60.4^c	74.8^a
True digestibility (%)	68.5^b	74.7^{bc}	78.3^c	93.2^a
Net protein utilization (%)	37.1^b	43.0^{bc}	47.3^c	69.7^a
Utilizable protein (%)	8.6^b	9.8^b	10.5^b	58.1^a
Protein (%)	23.3	22.8	22.2	83.3

Results are based on five determinations for each treatment.
Net protein utilization = [(true protein digestibility) × (biological value)]/100.
Utilizable protein = [(protein) × (net protein utilization)]/100.
Protein = N × 6.25 (dry weight basis).
Values with the same superscript in each row do not differ significantly from each other ($P < 0.05$).

autoclaved *A. nilotica* seeds are comparable to cooked mungbeans (Khan *et al.*, 1979). However, insignificant differences between dry-heated and autoclaved samples have been observed in respect of BV, TD, NPU and UP.

When compared with casein and cultivated legumes such as peanut (Jambunathan *et al.*, 1992) and pigeonpea (Singh *et al.*, 1990), both dry-heated and autoclaved seeds of *A. nilotica* exhibit low levels of BV, TD and NPU. This may be attributed to the presence of high levels of polyphenols and fibre since most of these compounds are concentrated in the seed coat. Nonetheless, decrease in BV of both dry-heated and autoclaved seeds might be due to heat treatment, which causes considerable nutritional damage to methionine, the most important amino acid in grain legumes (Shemer & Perkins, 1975).

The present report on the nutritional value of the seeds of *A. nilotica* suggests that they may be useful as a food source and merit wider use, especially in the preparation of protein isolates and food formulations which could be used as cereal supplements.

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