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Fractionation of leaves and biochemical composition of the fractions

(Pecahan daun dan komposisi biokimianya)

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Key words: leaf protein concentrates, pressed cake, in vitro digestibility, polyphenol (free and bound)

Abstrak

Konsentrat protein daun disediakan daripada pokok spesies *Albizzia procera*, *Moringa oleifera* dan *Sesbania grandiflora*. Kandungan protein, lemak kasar, abu, serabut kasar, kalsium, fosforus, ferus, natrium, kalium dan asid amino di dalam konsentrat tersebut dianalisis. Nilai faktor antipemakanan seperti polifenol (bebas dan terikat) dan alkaloid juga dibincangkan. Analisis menunjukkan konsentrat protein daun daripada daun pokok adalah sumber protein yang baik (48–60% mengikut berat kering). Protein daun *A. procera* mengandungi abu yang terendah (4%) manakala protein daun *M. oleifera* mengandungi kecernaan in vitro yang tertinggi (50%) antara ketiga-tiga konsentrat protein. Kedua-dua konsentrat protein dan daun segar kaya dengan sumber kalsium, kalium, fosforus dan ferus tetapi kurang dalam natrium. Konsentrat protein juga adalah sumber yang baik dalam asid amino melainkan *A. procera* yang sulfurnya mengandungi asid amino yang terendah. Daun segar (9%) dan konsentrat protein (8%) *S. grandiflora* mengandungi ferus yang tertinggi dibandingkan dengan dua spesies lagi. Kehadiran alkohol dapat dikesan pada daun segar ketiga-tiga spesies.

Abstract

Leaf protein concentrates were prepared from tree species *Albizzia procera*, *Moringa oleifera* and *Sesbania grandiflora*. Leaf protein concentrates were analyzed for protein, crude fat, ash, crude fibre, calcium, phosphorous, iron, sodium, potassium and amino acids. Calculated values for the antinutritional factors like polyphenols (free and bound) and alkaloids were also included. Analysis showed that the leaf protein concentrate from tree leaves was a good source of protein (48–60% on dry weight basis) compared with other fractions. Leaf protein of *A. procera* had the lowest amount of ash (4%), while leaf protein of *M. oleifera* had the highest in vitro digestibility (50%) among the three protein concentrates. Both fresh leaves and protein concentrates were rich sources of calcium, potassium, phosphorous and iron but poor source of sodium. The protein concentrates were good sources of essential amino acids except *A. procera* where sulphur containing amino acids were limiting. The fresh leaves (9%) and protein concentrate (8%) of *S. grandiflora* had the highest polyphenol content compared with two other species. The presence of alkaloids was observed in the fresh leaves of the three species.

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Introduction

Food consumed by a great majority of people in developing countries is deficient in protein and also quality, causing widespread malnutrition and under nutrition. In view of the world wide need for additional sources of food, research work has been undertaken to utilize the tree leaves either as a source of fodder (Nag and Matai 1992a; Ahn et al. 1997) or as a raw material for leaf protein extraction. Leaf protein extracted from tree leaves is a good source of crude protein (Nag and Matai 1994). Though tree leaves are a potential source of extracted leaf protein, up to this stage, the chemical composition and total nutritive value of only a few trees have been done (Devi et al. 1965; Srivastava and Mohan 1989; Farinu et al.1992). Little information is available on the chemical composition and antinutritional factors of the tree leaves namely *Albizzia procera*, *Sesbania grandiflora* and *Moringa oleifera.*

Therefore this study was carried out with the following objectives:

(1) To fractionate the tree leaves of *A. procera***,** *M. oleifera* and *S. grandiflora* in order to obtain leaf protein,
(2) To determine the chemical composition of each fraction of these tree leaves (fresh leaves, leaf protein concentrate, pressed cake) and
(3) To evaluate the utilization of each fraction of tree leaves for nutritional purposes.

Materials and methods

Preparation of leaf protein concentrate

Fresh leaves of each species were collected from different sites in and around Calcutta. The harvested plant material was transported to the laboratory, washed thoroughly and drained free of water. A portion of the plant material was dried in an oven for analytical studies. The washed plant material was pulped in an International Biological Programme type pulper (Davy and Pirie 1969) and pressed using a manual bench press to extract the juice. Samples of juice were heated to 80 °C to precipitate the proteinous mass and separated by centrifugation. The protein coagulum obtained was known as leaf protein concentrate, which was washed twice with water, freeze dried and stored at 4 °C. The fibre (pressed cake) residue left after extraction of protein concentrate was dried in an oven at 105 °C for 48 h.

Proximate analysis

Crude protein content (N x 6.25) was determined by the Microkjeldahl procedure (AOAC 1984) using automatic Kjel – Foss equipment (Model 1026). Crude fat was determined by refluxing for 8 h with (2:1) v/v chloroform and methanol in soxhlet apparatus (AOAC 1984). Ash was determined by the incineration of a sample (0.5 g) in oven at 550 °C for 3 h. Dietary fibre was determined by the neutral detergent fibre method (Goering and Van Soest 1970). In vitro digestibility of leaf protein was measured by the method of Akeson and Stahmann (1964) as modified by Saunders et al. (1973). In vitro dry matter digestibility of pressed cake was measured by the method of Tilley and Terry (1963).

Mineral analysis

For analysis of several mineral contents, the tree leaves and their fractions (0.5–1.0 g) were digested with triacid mixture (HNO_3: H_2SO_4: $HClO_4$) for 4–5 h till the white fumes ceased.

Sodium, potassium and calcium were analyzed by using Systonic 121 flame photometer. Phosphorous was analyzed by the Molybdate vanadate method in conjunction with spectrophotometric measurement (Kitson and Mellon 1944). Iron was analyzed by Orthrophenanthroline method in conjunction with spectrophotometric measurement (Jackson 1958).

Antinutritive substances

Total polyphenols (free and bound) were extracted following the method of Singh and Venkataraman (1982) and estimated by the method of Swain and Hillis (1959). Alkaloids were tested qualitatively by the method of Hultin and Torsell (1965).

Amino acids analysis

The amino acid compositions of the protein samples (100 mg) were estimated with HPLC (Japan Spectroscopic Co. Ltd.) after hydrolysis in 25 mL of 6N HCl for 22 h at 110 °C in the refluxing flasks (Reddy et al. 1990). The sulphur amino acids were determined in the same manner on the samples treated with performic acid (Moore 1963).

Statistical analysis

For the results of each assay at least three replicates were made from each sample. To test differences between tree species, the mean values of chemical, mineral and antinutritional factors were subjected to analysis of variance (ANOVA) following the method of Snedecor and Cochran (1967). In ANOVA when significance was observed at 5% level, the least significant difference (L.S.D.) for the same significance level was determined.

Results and discussion

Proximate composition

Leaf protein concentrate prepared from tree leaves contained an unusually high amount of protein which ranged from 48.18% *(S. grandiflora)* to 59.96% *(A. procera) (Table 1).* It was already reported that the protein value of leaf protein concentrate from leucerne and red clover were lower than the values for leaf protein from tree leaves (Maciejewicz-Rye and Hanczakowski 1990; Nag and Matai 1992b). The crude fat of pressed cake of tree leaves varied from 16.00% to 16.88% and these values were lower compared to pressed cake of *Ailanthus excelsa* which was reported by Nag and

Table 1. Proximate composition and in vitro digestibility of tree leaves and its fractions (dry weight basis)

Name of tree species	Crude protien (%)			Total ash (%)			Crude fat (%)			Crude fibre (%)			In vitro digestibility			In vitro dry matter digestibility		
	FL	LP	PC	FL	LP	PC	FL	LP	PC	FL	LP	PC	FL	LP	PC	FL	LP	PC
Albizzia procera	26.65	59.96	21.24	8.24	3.95	5.50	25.26	29.36	16.88	13.35	0.81	25.35	–	32.81	–	–	–	26.80
Moringa oleifera	25.72	51.67	21.87	13.12	4.17	10.00	20.64	32.26	16.01	5.95	0.88	20.93	–	50.35	–	–	–	36.44
Sesbania grandiflora	33.78	48.18	20.67	8.33	5.08	8.03	18.28	25.48	16.00	12.07	0.77	20.09	–	37.59	–	–	–	18.72
L.S.D. $p = 0.05$	0.147	0.163	0.154	0.140	0.15	0.163	0.127	0.140	0.154	0.137	N.S.	0.153	–	–	–	–	–	–

FL = Fresh leaves, LP = Leaf protein concentrate, PC = Pressed cake, N.S. = not significant

Matai (1994). The ash content in leaf protein was generally lower in the other two fractions.

Total ash content varied widely among the leaf proteins of tropical aquatic plants (Dewanji et al. 1997). For animal feeding, the protein and ash contents of leaf protein are recommended to be 55% and 11% respectively (Fiorentinni and Galloppini 1983) while for human consumption the range is reported to be 55–65% for protein and 2–5% for ash (Bray 1977). The results of in vitro digestibility on leaf protein fraction showed that leaf protein of *M. oleifera* had the highest digestibility (50.35%). The in vitro dry matter digestibility of the three pressed cakes showed that pressed cakes of *S. grandiflora* were poorly digested (18.72%).

Mineral composition

A number of minerals exist in animal body either in combination with each other or with organic constituents. Some of them are essential as they perform important functions while others are present only because of their presence in food. The mineral content of each fraction of tree leaves was studied and recorded in *Table 2*. The major mineral in this study was calcium which ranged from 0.96% to 2.98%. Among the three species, leaves of *M. oleifera* and its fractions were rich in phosphorous (0.25% to 0.48%) and iron (0.11% to 0.40%). Potassium was found within a range of 0.18% to 4.02% in the three species.

Antinutritive substances

Antinutritive substances are mainly digestive, inhibiting toxins and deleterious substances which limit biological utilization of food and feed. Alkaloids which comprise the largest single class of secondary plant substances was studied in view of their poisonous properties. The absence of alkaloids in the leaves of *A. procera*, *M. oleifera* and *S. grandiflora* was observed in all leaf protein samples *(Table 3)*. The phenolics contents of leaf protein samples

Table 2. Mineral content of the tree leaves and its fractions (dry weight basis)

Name of tree species	Calcium (%)			Phosphorous (%)			Iron (%)			Sodium (%)			Potassium (%)		
	FL	LP	PC	FL	LP	PC	FL	LP	PC	FL	LP	PC	FL	LP	PC
Albizzia procera	1.64	2.25	0.96	0.16	0.26	0.25	0.12	0.27	0.17	0.26	–	–	3.57	–	–
Moringa oleifera	2.98	2.13	1.73	0.48	0.27	0.25	0.11	0.22	0.40	0.32	–	0.11	3.43	0.18	4.02
Sesbania grandiflora	2.57	2.15	1.83	0.17	0.27	0.24	0.06	0.09	0.13	0.27	0.17	0.03	1.96	0.64	4.00
L.S.D. $p = 0.05$	0.083	0.087	0.083	0.097	N.S.	N.S.	N.S	0.08	N.S.	N.S.	–	0.075	0.079	0.093	N.S.

FL = Fresh leaves, LP = Leaf protein concentrate, PC = Pressed cake, N.S. = not significant

Table 3. Antinutritive substances (dry weight basis) of tree leaves and its fractions

Name of tree species	Polyphenolic content (%)											
	Free			Bound			Total			Alkaloids (%)		
	FL	LP	PC	FL	LP	PC	FL	LP	PC	FL	LP	PC
Albizzia procere	6.31	2.26	–	1.88	1.22	–	8.18	3.48	–	+	–	–
Moringa oleifera	6.59	4.04	–	0.78	3.08	–	7.38	7.12	–	+	–	–
Sesbania grandiflora	2.55	6.05	–	6.81	1.86	–	9.36	7.91	–	+	–	–
L.S.D. $p = 0.05$	0.08	0.093	–	0.104	0.118	–	0.212	0.201	–			

FL = Fresh leaves, LP = Leaf protein concentrate, PC = Pressed cake

were studied in view of their adverse effects on growth due to their interference with their protein digestibility and utilization (Liener 1994). The fresh leaves (9.36%) as well as protein concentrate (7.91%) of *S. grandiflora* had the highest polyphenol content when compared with the fractions of the other two species *(Table 3)*. Phenolics content of leaf protein concentrates extracted from leafy vegetables and legume crops was reported to range from 1.4% to 2.2% (Subba Rau et al. 1972). It is believed that the process of leaf protein extraction removes toxic or unpalatable components from the material and thus makes the pressed cake a new source of ruminant's feed (Pirie 1987).

Amino acids

The amino acid composition of the protein fractions prepared from tree leaves and the minimal requirements established for children of 2–5 years and 10–12 years old are presented in *Table 4*. The interesting feature is that the leaf protein concentrates have good amount of essential amino acids and these data may have nutritional interest. Based on the FAO\WHO\UNU (1985) pattern, the leaf protein concentrate of *M. oleifera* is slightly deficient in lysine for children aged 2–5 years. Of nutritional importance, protein fraction of *M. oleifera* and *S. grandiflora* have an exceptionally high content of methionine + cystine close to that reported for cow's milk, chicken egg and human milk which varies from 3.3 g to 5.7g FAO/WHO/UNU (1985). The amino acid composition of these protein fractions was similar to those leaf protein concentrate prepared from tropical leaf species (Nag and Matai 1994). Comparison of the amino acids content of leaf protein concentrate with FAO/WHO/UNU (1985) pattern of amino acid requirement for children suggests that leaf protein concentrates contain more essential amino acids than the standard.

Conclusion

On the basis of overall composition of tree leaves and its fractions, the following can be concluded:

- Tree leaves and its fractions are good source of protein. Leaf protein of *M. oleifera* is nutritionally superior in terms of high protein, low ash and with good digestibility.
- The protein concentrates in this study are good source of lysine which is the limiting amino acid in the cereals. This study indicates that these protein concentrates could be a good protein supplement to cereal based diets.
- These protein concentrates have good balance of essential amino acid composition compared with those of recommended standard for children (2–5 year old).

Table 4. Comparison of amino acid composition (g per 16 g N) of leaf protein concentrates with FAO/WHO/UNU (1985) pattern of amino acid requirements for children

Amino acids	Leaf protein concentrates				
	A. procera	*M. oleifera*	*S. grandiflora*	Child 2–5 yrs	Child 10–12 yrs
Isoleucine	5.61	5.97	5.55	2.80	2.80
Leucine	9.57	10.30	10.00	6.60	4.40
Lysine	5.81	5.73	5.39	5.80	4.40
Cystine	0.89	2.52	1.26	–	–
Methionine	1.35	2.00	2.08	–	–
Total sulphur containing	2.24	4.52	3.34	2.50	2.20
Tyrosine	5.43	6.13	5.51	–	–
Phenylalanine	7.18	8.29	7.61	–	–
Total aromatic	12.61	14.42	13.12	6.30	2.20
Threonine	4.53	4.70	4.50	3.40	2.80
Valine	6.08	6.97	6.59	3.50	2.50
Histidine	2.34	2.34	2.69	1.90	1.90
Total essential amino acids	48.79	54.77	51.18	33.90	24.10
Arginine	6.60	6.54	6.28	–	–
Aspartic acid	9.86	10.50	9.54	–	–
Glutamic acid	11.20	11.60	11.06	–	–
Serine	4.43	4.40	4.25	–	–
Glycine	6.43	6.85	6.15	–	–
Alanine	6.10	6.86	6.12	–	–
Total non essential amino acids	44.62	46.75	43.4	–	–

- The tree leaves and its fractions are rich in calcium, potassium, phosphorous and iron except sodium.
- The pressed cake left after extraction of leaf protein contained good amount of protein comparable to original ones. The extraction technology may reduce the levels of antinutrients of pressed cake and enable them to be utilized as a new source of feed.

From the above studies it is evident that efforts should be directed to improving feed/food situation in the world based on utilization of forest resources.

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References

Ahn, Jong-H, Elliot, R. and Norton, B. W. (1997). Oven drying improves the nutritional value of *Callindra calothyrons* and *Gliricidia sepium* as supplements for sheep given quality straw. *J. Sci. Food Agric*. ***75:*** 503–10

Akeson, W. R. and Stahmann, M. A. (1964). A pepsin - pancreatin digest index of protein quality. *J. Nutr*. ***83:*** 257–61

AOAC (1984). *Official Methods Of Analysis*. 14th ed., (Willams, S. ed.) Assoc. Off. Anal. Chemistry

Bray, W. J. (1977). The processing of leaf protein to obtain food grade products. *Green crop fractionation, Proc. Occasional Sym. No.9*. (Wilkins, R. J., ed.) British Grassland Society

Davys, M. N. G. and Pirie, N. W. (1969). A laboratory scale pulper for leafy plant material . *Biotechnol. Bioeng*. ***11:*** 517–28

Devi, A. V. Rao, N. A. N. and Vijayaraghavan, P. K. (1965). Isolation and composition of leaf protein from certain species of Indian flora. *J. Sci. Food Agric.* ***16:*** 116–20

Dewanji, A., Chanda, S., Si, L., Barik, S. and Matai, S. (1997). Extractability and nutritional value of leaf protein from aquatic plants. *Plants Food For Hum. Nutr.* ***50:*** 349–57

FAO\WHO\UNU (1985). *Energy and protein requirements*. Report of a Joint FAO\WHO\UNU meeting series No.724, WHO, Geneva, Switzerland

Farinu, G. O., Ajiboye, S. O. and Sakiru, A. (1992). Chemical composition and nutritive value of leaf protein from *Leucaena leucocephala*. *J. Sci. Food Agric.* ***59:*** 127–9

Fiorentini, R. and Gallopinni, C. (1983). The protein from leaves. *Qual. Plant Foods Hum. Nutr.* ***32:*** 335–0

Hultin, E. and Torsell, K. (1965). Alkaloids screening of Swedish plants. *Phytochem.* ***4:*** 425–33

Jackson, M. L. (1958). *Soil chemical analysis*. Prentice Hall Inc., Englehood Cliffs, N. J.

Kitson, R. E. and Mellon, M. G. (1944). Colorimetric determination of phosphorous as Molybdo-phosphoric acid. *Industry and Engineering Chemistry. Analyst.* ***16:*** 379–8

Liener, I. E. (1994). Implications of nutritional components in soyabean foods. *Crit. Rev. Food Sci. Nutr*. ***34*****:** 31–67

Maciejewicz- Rye, J. and Hanczakowski, P. (1990). Improvement of the nutritive value of cereals by leaf protein supplementation. *J. Sci. Food Agric.* ***5*****:** 99–104

Moore, S. (1963). On the determination of cystine as cysteic acid. *J. Bio. Chem.* ***283:*** 235–50

Nag, A. and Matai, S. (1992a). Chemical composition of some fodder trees in and around Calcutta. *Indian. Vet. J.* ***69:*** 411–4

—— (1992b). Preliminary studies on protein extraction from tree leaves. *Indian J. Agric. Biochem.* ***5 (1& 2)*****:** 77–81

—— (1994). *Ailanthus excelsa* Roxb. (Simaroubacae), a promising source of leaf protein. *J. Agric. Food Chem.* ***42:*** 1115–7

Pirie, N. W. (1987). *Leaf protein and its by products in human and animal nutrition,* 2nd ed., p. 209 Cambridge University Press

Reddy, G. U., Ohshima, M. and Nishimura, T. (1990). Effect of Ribonucleic Acid (RNA) removal from the yeast propagated on Italian ryegrass brown juice on the nutritive value of rats. *Jpn. J. Zootech. Sci.* ***61(10):*** 945–61

Saunders, R. M., Conor, M. A., Booth, A. N., Bicckoff, E. M. and Kohler, G. O. (1973). Measurement of digestibility of alfalfa concentrates by in vivo and in vitro methods. *J. Nutr.* ***4:*** 530–5

Singh, N. and Venkatraman, L.V. (1982). *Status of research on leaf protein and microalgae in India*; p. 86 Central Food Technological Research Institute; Mysore; India

Snedecor, G. W. and Cochran, W. G. (1967). *Statistical Methods*, 6th ed, p. 593. New Delhi: Oxford and IBH Publishing

Srivastava, G. P. and Mohan, M. (1989). Leaf protein concentrate from mulberry (*Morus alba* L.) tree leaves. In *Extended abstracts 3rd. International Conference on Leaf Protein Research* p. 190–2, 1–7 Oct. Society For Green Vegetation Research, Pisa, Perugia , Viterbo, Italy

Subba Rau, B. H., Ramana, K. V. and Singh, N. (1972). Studies on nutritive value of leaf proteins and factors effecting their quality. *J. Sci. Food Agric*. ***23*****:** 233–45

Swain, T. and Hillis, W. E. (1959). The phenolics constituents of Prunus domestica I. The quantitive analysis of phenolic constituents. *J. Sci. Food Agric*. ***10:*** 63–8

Tilley, J. M. A. and Terry, R. A. (1963). A two stage technique for the in-vitro digestion of forage. *J. Br. Grassland Soc*. ***18*****:** 104–11

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