

Ailanthus excelsa Roxb. (Simaroubaceae), a Promising Source of Leaf Protein

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Fresh leaves of *Ailanthus excelsa* and three protein fractions, unfractionated, chloroplastic, and cytoplasmic, as well as pressed cake left after extraction of protein were subjected to proximate analysis. The cytoplasmic protein fraction contained 62.71% crude protein, while whole leaf contained 20.86%. The unfractionated and chloroplastic protein fractions contained more crude fat than the whole leaf and pressed cake. Compared to whole leaf and pressed cake, protein fractions were low in crude fiber. The presence of polyphenols was also studied. The amino acid composition of the cytoplasmic fraction also showed an excellent balance of essential amino acids. Results indicated that the leaf protein fractions from *A. excelsa* were nutritionally superior to the whole leaf, pressed cake, and soyabean protein.

INTRODUCTION

Tree leaves contain considerable amounts of protein, and it has been suggested that they may be suitable for leaf protein extraction (Siren, 1970). Mohan and Srivastava (1981) reported quite satisfactory recoveries of leaf protein (34-44% crude protein) from certain tree leaves, and the fiber residue remaining after the protein extraction had more than 7-10% crude protein.

The leaves of an amazingly fast growing tree, *Ailanthus excelsa* Roxb. (Simaroubaceae), commonly known as ardu, are found almost throughout the tropical and subtropical parts of India, especially the dry tracts of Gujarat, Punjab, Haryana, and the Deccan plateau. The leaves are rated as highly palatable and nutritious fodder for sheep and goats, and an average tree yields about 500-700 kg of green leaves twice a year (Bhandari and Gupta, 1972). Singh and Patyanak (1977) noted a high percentage of crude protein and calcium in *A. excelsa*. This plant, which has not been studied in detail, could be utilized for the commercial production of leaf protein.

The present study was therefore undertaken to use the leaf protein concentrate prepared from the leaves of *A. excelsa* as a nonconventional protein-rich food (cytoplasmic fraction) and feed source (unfractionated fraction).

MATERIALS AND METHODS

The plants were washed twice in washing tanks with a continuous flow of water and spread uniformly on an iron net to drain off the excess water. The washed material was fed into a specially designed pulper (Davys and Pirie, 1969), where the crop was macerated, which resulted in the mechanical disintegration of the cell walls. Nine hundred grams of pulp was pressed in a laboratory press (Davys et al., 1969) which separated the juice and fiber or pressed cake. Samples of leaf protein were precipitated by heating the green juice to 80 °C. The protein coagulum obtained is known as *unfractionated leaf protein concentrate*, which is centrifuged twice and finally freeze-dried. To obtain the other fractions, the required amount of juice is heated to 50-55 °C. The protein coagulum is prepared, which is known as *chloroplastic leaf protein concentrate*. The supernatant liquid after centrifugation of the protein coagulum is again heated to 80-85 °C to obtain *cytoplasmic leaf protein concentrate*.

The nitrogen, total ash, and crude fat were analyzed by using standard procedures (AOAC, 1984). The protein contents were calculated by multiplying the nitrogen values by 6.25. Polyphenols (free and bound) were extracted from leaf protein concentrate

following the method of Singh and Venkatraman (1982). The Swain and Hills (1959) method of estimation was used, which is based on the oxidation of Folin-Denis reagent containing sodium tungstate phosphomolybdic and orthophosphoric acid (AOAC, 1984). The amino acid compositions of protein samples (100 mg) were estimated with HPLC (Japan Spectroscopic Co. Ltd.) after hydrolysis in 25 mL of 6 N HCl for 22 h at 110 °C in refluxing flasks (Reddy et al., 1990). The sulfur amino acids were determined in the same manner on the samples treated with performic acid (Moore, 1963).

The data on nitrogen extractability and percent proximate principles of different heat-fractionated leaf protein concentrates of *A. excelsa* were subjected to analysis of variance (ANOVA) (Snedecor and Cochran, 1967). In ANOVA, when significance was observed at the 5% level, the *least significant difference* (LSD) for the same significance level was determined.

RESULTS AND DISCUSSION

Extractability of Protein. Observations on the extractability of protein from *A. excelsa* leaves are presented in Table 1. The range of protein nitrogen extractability during the three collections varied from 35.23% to 42.10%. A study done on 65 tree species (Singh, 1983) showed that protein nitrogen extractability ranged from 1.01% in *Ficus virens* and 28.4% in *Barringtonia acutangula*.

Proximate Principles. The average values (three sets of observations) on chemical composition are recorded in Table 2 with the data of soybean protein as reference (Indian Council of Medical Research, 1982). The values revealed that the cytoplasmic fraction had maximum protein (62.71%) followed by the unfractionated and chloroplastic fractions, which is similar to the results of other studies (Mohan and Srivastava, 1982). In the pressed cake 15% of crude protein remained on a dry matter basis. Whole leaf contained the highest ash content (9.55%) compared to the protein fractions. However, the values of this study were high compared to those recommended for human consumption. The unfractionated and chloroplastic protein fractions contained about 3 times more crude fat than the cytoplasmic protein fraction, which is similar to the findings of others (Chakraborty et al., 1984). Crude fiber contents of three protein fractions, unfractionated, chloroplastic, and cytoplasmic, were 0.74%, 1.08%, 0.59%, respectively, and these values were quite low compared to 30.81% present in pressed cake. Nitrogen-free extract (NFE) of these protein fractions was

Table 1. Data on Nitrogen Extractability of *A. excelsa*

	first collection, ^{d,e} dated Feb 2, 1988	second collection, ^{d,f} dated Aug 9, 1988	third collection, ^{d,e} dated Feb 20, 1989
pulp DM % ^a	22.55	18.86	20.53
pulp N % ^b	3.21	4.07	3.46
fiber DM %	35.13	28.50	33.46
fiber N %	2.80	3.18	2.76
protein nitrogen extractability	41.06	35.23	42.10
total nitrogen extractability	47.42	45.47	49.70
lsd, ^c ($P = 0.05$)	0.36	0.21	0.83

^a DM, dry matter. ^b N, nitrogen. ^c lsd, least significance difference. ^d Collections were not from the same tree. ^e Preflowering stage. ^f Postflowering stage.

Table 2. Percent Proximate Principles (on DM Basis) of the Heat-Fractionated Leaf Protein Concentrate

	crude protein	crude fat	crude fiber	total ash	NFE ^c	polyphenols		
						free	bound	total
fresh leaves	20.86	18.58	13.55	9.55	37.46	3.39	4.12	7.51
pressed cake	15.50	17.44	30.81	7.47	28.78	2.22	0.56	2.78
unfractionated LPC ^a	47.02	33.42	0.74	3.86	14.96	5.20	1.49	6.69
chloroplastic LPC	39.07	35.61	1.08	6.83	17.41	2.55	1.02	3.59
cytoplasmic LPC	62.71	12.66	0.59	5.64	18.4	4.42	0.73	4.97
soybean protein ^b	43.20	19.50	3.70	4.60				
LSD ($P = 0.05$)	0.59	0.61	0.64	0.58		0.51	0.50	0.95

^a LPC, leaf protein concentrate. ^b Indian Council of Medical Research (1982). ^c NFE, nitrogen-free extract.

Table 3. Amino Acid Composition of the Heat-Fractionated Leaf Protein Concentrate (Grams per 16 g of Nitrogen)

amino acid	unfractionated LPC	chloroplastic LPC	cytoplasmic LPC	soybean protein ^a
lysine	6.17	5.99	7.75	6.40
threonine	4.72	4.69	4.85	4.10
serine	4.87	4.71	4.38	5.60
glutamic acid	12.27	12.26	12.53	19.10
glycine	6.57	7.19	6.89	4.20
alanine	6.67	6.65	6.79	4.30
valine	7.20	7.08	7.20	5.00
isoleucine	6.18	6.09	6.16	4.00
leucine	10.75	11.27	10.10	7.80
tyrosine	5.98	5.90	6.01	3.80
phenylalanine	7.87	8.25	7.65	5.20
methionine	1.79	1.65	2.11	1.40
cystine	1.00	0.96	1.58	1.80
aspartic acid	10.80	10.80	11.13	11.60
arginine	6.37	6.21	8.01	7.70
histidine	2.43	2.41	2.98	2.80

^a Brulé and Savoie (1988).

about half that of the whole leaf and pressed cake. The phenolic content of the leaf protein samples were studied in view of the adverse effects of these compounds on growth due to their interference with protein digestibility (Syngé, 1975). In the present study the polyphenols were found to be highest in the whole plant followed by the unfractionated protein fraction. This is contrary to the findings of Mohan and Srivastava (1984), who observed slightly higher content of polyphenols in the cytoplasmic LPC than in the chloroplastic and unfractionated leaf protein concentrate from leaves of *Sesbania grandiflora*.

The present study on proximate principle showed that fiber residue is low in ash and polyphenol content, for this it may be utilized as a feed source like whole leaf. As shown in Table 2, considerable amounts of crude protein and crude fat and a lower amount of total ash were contained in the unfractionated and cytoplasmic fractions and thus were highly comparable to soybean protein.

Amino Acid Composition. The amino acid composition of the three protein fractions prepared from *A. excelsa* was studied, and the results are given in Table 3 with the data of soybean protein as a reference (Brulé and Savoie, 1988). Leucine and phenylalanine were higher in the

unfractionated and chloroplastic fractions than in the cytoplasmic fraction. The cytoplasmic fraction was very high in lysine and tyrosine when compared to soybean protein. In this study, the difference in methionine content among the three fractions was small, but it seemed to be of importance from a nutritional standpoint because methionine was the first limiting amino acid in the leaf protein (Woodham, 1971). Compared to soybean protein the reference standard, the three fractions of *A. excelsa* were found to be low in cystine and high in methionine, glycine, alanine, and valine.

The present study showed that the cytoplasmic protein fraction from *A. excelsa* was a fairly good source of protein. The content of amino acids observed in the cytoplasmic protein fraction was nutritionally superior to that of the other two fractions.

Conclusion. This study conclusively indicated that the cytoplasmic protein fraction can be used for human consumption, whereas the other two fractions could be utilized as a nutritious feed for ruminants and nonruminants.

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LITERATURE CITED

- AOAC. *Official Methods of Analysis*, 14th ed.; Horwitz, W., Ed.; Association of Official Analytical Chemists: Washington, DC, 1984; p 1141.
- Bhandari, D. S.; Gupta, M. L. Studies on the digestibility and nutritive value of Ardu (*Ailanthus excelsa* Roxb.). *Indian Vet. J.* 1972, 49 (5), 512-516.
- Brulé, D.; Savoie, L. In vitro digestibility of Protein and Amino acids in protein mixture. *J. Sci. Food Agric.* 1988, 43, 361-372.
- Chakraborty, S.; Bagchi, D. K.; Matai, S. Use of by product leaves of some vegetable crops as source of protein based on some nutritional parameters. In *Current Trends in Life Sciences Vol. XI, Progress in Leaf Protein Research*; Narendra Singh, Ed.; Today and Tomorrow's Printers and Publishers: New Delhi, 1984; pp 233-238.

- Davys, M. N. G.; Pirie, N. W. A laboratory scale pulper for leafy plant material. *Biotechnol. Bioeng.* **1969**, *11*, 517-528.
- Davys, M. N. G.; Pirie, N. W.; Street, G. A laboratory scale press for extracting juice from leaf pulp. *Biotechnol. Bioeng.* **1969**, *11*, 529-538.
- Indian Council of Medical Research. *Nutritive Values of Indian Foods*; Gopalan, C., Ramasastri, B. V., Balasubramanian, Eds.; National Institute of Nutrition: Hyderabad, 1982; p 63.
- Mohan, M.; Srivastava, G. P. Studies on the extractability and chemical composition of leaf protein from certain trees. *J. Food Sci. Technol.* **1981**, *18*, 48-51.
- Mohan, M.; Srivastava, G. P. Biochemical composition and nutritive value of unfractionated and fractionated, chloroplast and cytoplasmic leaf protein from *Gliricidia maculata*. *Proceedings of the 1st International Conference on Leaf Protein Research*, Aurangabad, India; 1982; pp 257-262.
- Mohan, M.; Srivastava, G. P. Biochemical composition and nutritive value of unfractionated and fractionated chloroplast and cytoplasmic leaf proteins from *Sesbania grandiflora* (August tree). *Proceedings of the 2nd International Conference on Leaf Protein Research*; Iwao Tasaki, Ed.; 1984; pp 245-247.
- Moore, S. On the determination of cystine as cysteic acid. *J. Biol. Chem.* **1963**, *238*, 235-237.
- Reddy, G. U.; Ohshima, M.; Nishimura, T. 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.* **1990**, *61* (10), 945-961.
- Singh, A. K. Screening of some common Indian trees for leaf protein. *Acta Bot. Hung.* **1983**, *29*, 281-292.
- Singh, N. P.; Patnayak, B. C. Nutritive value of *Ailanthus excelsa* Roxb. (Ardu) leaves for sheep. *Indian Vet. J.* **1977**, *54* (3), 198-201.
- Singh, N.; Venkatraman, L. V. *Status of Research on Leaf Protein and Microalgae in India*; Central Food Technological Research Institute: Mysore, India, 1982; p 86.
- Siren, G.; Blomback, B.; Alden, T. Proteins in forest tree leaves. *Inst. Skogsforuering Rapp Och uppsatser* **1970**, No. 28, 22.
- Snedecor, G. W.; Cochran, W. G. *Statistical Methods*, 6th ed.; Oxford and IBH Publishing: New Delhi, 1967; p 593.
- Swain, T.; Hillis, W. E. The phenolic constituents of *Prunus domestica* L. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* **1959**, *10*, 63-68.
- Synge, R. L. M. Interactions of Polyphenols with proteins in plant and plant products. *Qual. Plant Plant Foods Hum. Nutr.* **1975**, *24*, 337-350.
- Woodham, A. A. The use of animal tests for the evaluation of leaf protein concentrates. In *Leaf Protein: Its Agronomy, Preparation, Quality and Use*; Pirie, N. W., Ed.; Blackwell Scientific Publications: Oxford, U.K., 1971; pp 115-130.

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