Amino Acid Composition of Leaf Proteins Extracted from Some Aquatic Weeds

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The chemical composition and amino acid profile of leaf protein extracted from five aquatic weeds, namely *Alternanthera philoxeroides*, *Azolla pinnata*, *Lemna minor*, *Limnanthemum cristatum*, and *Pistia stratiotes*, were investigated. The nitrogen content in the leaf proteins ranged from 6.1 to 8.7%, β-carotene levels varied from 462.5 to 674.7 μg/g, and the total polyphenols ranged from 1.3 to 2.9%. There were no large differences in the amino acid composition of the five samples, suggesting that protein of a uniform composition could be extracted from aquatic plants. Levels of essential amino acids in the leaf proteins compared favorably with FAO reference pattern and chick requirements, indicating that leaf protein extracted from unwanted aquatic plants could be used for food/feed purposes. Even the fibrous byproduct, left after the extraction of aquatic leaf proteins, having 2.1–3.2% nitrogen and low nitrate contents (0.23–0.65%) could be utilized as an additional feed for ruminants.

INTRODUCTION

In developing countries rapid population growth coupled with limited resources of cultivable land creates serious problems in the steady supply of food and feed. Aquatic weeds which produce dense stands in numerous bodies of water represent an abundant natural resource. Not all aquatic plants can be consumed directly because of the presence of secondary constituents in them (McClure, 1970), but the extraction of edible protein from their leaves could prove to be promising.

Information on the nutritional composition of such potential sources of protein being inadequate, the possibility of using common fresh water plants as food/feedstuffs was investigated in our laboratory (Banerjee and Matai, 1990; Dewanji, 1991, 1993). On the basis of leaf protein extraction data, chemical analysis of dry samples, and standing crop estimations, *Alternanthera philoxeroides*, *Azolla pinnata*, *Lemna minor*, *Limnanthemum cristatum*, and *Pistia stratiotes* showed great potential for protein extraction.

Since the biological value of a protein is dependent upon its constituent amino acids, studies on protein nutrition in natural ecosystems will require data on amino acid composition of that protein source. There have been some studies on the amino acid composition of aquatic plants (Boyd, 1969, 1970; Buckingham et al., 1978; Muztar et al., 1978) but just a few on leaf protein extracted from them (Taylor, 1971; Rusoff et al., 1980). The distribution of amino acids within a leaf is also relevant to the work on extracted leaf protein because it is often stated that the leaf protein composition changes with age or with the nutritional status of the plant (Garcha et al., 1970; Stabursvik and Heide, 1974).

Thus, information on nutritive and amino acid composition of the aquatic leaf protein is essential if utilization prospects are to be considered. This study was therefore undertaken to evaluate the nutrient content and amino acid composition of leaf protein prepared from five aquatic plants. Besides leaf protein, the process of leaf protein extraction also produces a fibrous residue from which part of the protein has been extracted. The chemical composition of this leftover byproduct from aquatic plants was also analyzed in an attempt to study its scope for utilization as a feed for ruminants.

MATERIALS AND METHODS

Collection of Samples. Samples of *A. philoxeroides*, *A. pinnata*, *L. minor*, *L. cristatum*, and *P. stratiotes* were collected from ponds in and around Calcutta, which lies between 22° 20′ and 22° 40′ N latitude and 88° 10′ and 88° 40′ E longitude. Each sample was drained free of water and transported to the laboratory where they were used for leaf protein extraction.

Protein Extraction. The washed plant material was hand-fed into a specially designed pulper (Davys and Pirie, 1969). The pulper was set with its outflow discharge falling on the belt of a belt press (Davys and Pirie, 1965), where the juice from the pulped material was separated through a perforated roller and the fibrous residue was collected as a byproduct. The protein was precipitated from the juice by steam injection. The protein coagulum was then filtered, washed, pressed, freeze-dried, and stored at 4 °C.

Chemical Analysis. The fiber samples were oven-dried at 100 °C to constant weight for dry matter determinations. Freeze-dried material was used for leaf protein analyses, while oven-dried samples were used for fiber analyses. Nitrogen was estimated by micro-Kjeldahl method, while crude fat was extracted with chloroform/methanol (2:1) in a Soxhlet apparatus. Ash values were obtained by heating the samples at 550 °C for 4 h in a muffle furnace. Crude fiber and β-carotene were estimated according to the AOAC (1984) procedure. Calorific value was determined using the Toshniwal oxygen bomb calorimeter. Polyphenols were extracted following the method of Singh and Venkataraman (1982) and estimated by the method of Swain and Hillis (1959). *In vitro* digestibility was measured according to the method of Saunders et al. (1973). The fiber samples were analyzed for their nitrate content using the method of Humphries (1956).

Amino Acid Composition. Lyophilized samples (10 mg) of leaf protein were weighed into Pyrex tubes (14 × 20 mm) and mixed with 10 mL of constant-boiling HCl. After replacement of air above the mixture with N_2, the tube was sealed under reduced pressure. Hydrolysis was carried out in an autoclave at 110 °C for 24 h. Duplicate samples were hydrolyzed, and the hydrolysate was analyzed using a Nihondenshi JLC-6AH automatic amino acid analyzer (Horigome and Uchida, 1980).

Statistical Analysis. Data on the composition of leaf protein and fibrous residue from five aquatic plants are presented as mean values of three replicates and were subjected to analysis of variance (Snedecor and Cochran, 1967). When significance was observed at 5% level, the least significant difference (LSD) for the same significance level was determined.

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Table I. Composition of Leaf Protein Prepared from Five Aquatic Plants (Mean of Three Samples)

	leaf protein (dry weight basis)					
	A. philoxeroides	*A. pinnata*	*L. minor*	*L. cristatum*	*P. stratiotes*	LSD[a] ($P = 0.05$)
nitrogen, %	7.7	6.3	6.1	8.7	8.2	0.28
crude fat, %	11.0	9.9	11.4	8.4	14.4	1.98
crude fiber, %	4.6	2.8	2.7	3.4	1.5	0.57
ash, %	7.3	4.1	6.0	4.4	5.8	0.94
calorific value, kcal/g	2.5	4.2	2.8	4.3	3.6	0.08
β-carotene, μg/g	462.5	632.8	627.2	674.7	653.7	27.64
total polyphenols, %	2.9	1.7	2.1	2.7	1.3	0.53
in vitro digestibility, %	71.5	77.7	77.9	78.1	80.7	2.95

[a] LSD, least significant difference.

Table II. Amino Acid Composition[a] of Leaf Proteins from Five Aquatic Plants and Alfalfa

	leaf protein					
amino acid	*A. philoxeroides*	*A. pinnata*	*L. minor*	*L. cristatum*	*P. stratiotes*	alfalfa[b]
lysine	6.93	6.10	5.93	4.64	7.04	6.7
histidine	2.62	2.27	2.65	2.14	2.88	2.5
arginine	5.47	6.22	5.99	5.98	6.31	6.5
aspartic acid	10.20	10.34	10.56	10.14	9.62	10.2
threonine	4.91	4.97	5.13	4.88	4.76	5.2
serine	4.97	5.32	5.54	4.86	4.84	4.3
glutamic acid	14.01	13.83	13.60	14.37	13.44	11.1
proline	5.78	4.73	4.53	5.59	5.04	4.8
glycine	5.55	5.76	5.62	5.83	5.74	5.3
alanine	6.13	7.00	7.11	6.43	6.34	6.0
valine	6.77	6.76	6.43	7.01	6.73	6.8
methionine	1.24	1.22	1.38	1.43	1.10	2.3
isoleucine	6.01	5.95	5.67	5.99	5.96	5.3
leucine	9.39	9.44	9.60	9.82	9.62	8.9
tyrosine	4.15	4.17	4.24	4.57	4.60	4.4
phenylalanine	5.86	5.92	6.01	6.31	5.96	5.7
crude protein (N × 6.25)	48.20	39.10	38.30	54.20	51.10	

[a] Mean of duplicate determinations. Grams of amino acid per 100 g of recovered amino acid. [b] Bickoff et al. (1975) (results converted from grams of amino acid per 16 g of nitrogen).

RESULTS AND DISCUSSION

Chemical Composition. The composition of leaf proteins prepared from five aquatic plants is presented in Table I. Nitrogen percent of leaf protein was found to be maximum in *L. cristatum* (8.7%) followed by *P. stratiotes* (8.2%). All leaf protein had above 8% crude fat, while crude fiber values were below 5%. Ash values ranged from 4.1% in *A. pinnata* to 7.3% in *A. philoxeroides* leaf protein. When compared to leaf proteins prepared from terrestrial plants, aquatics had lower fat content but higher ash values (NAS, 1984). The calorific value of leaf proteins ranged from 2.5 kcal/g in *A. philoxeroides* to 4.3 kcal/g in *L. cristatum*. Except for ash and calorific values, there appears to be no significant difference between the two small floating varieties, *A. pinnata* and *L. minor*, for all other parameters listed in Table I.

The β-carotene content of leaf protein ranged from 462.5 μg/g in *A. philoxeroides* to 674.7 μg/g in *L. cristatum*, with four of the five leaf proteins having values above 600 μg/g. Apart from being a source of provitamin A, β-carotene has use both in anticancer treatment and for respiratory diseases (Colditz et al., 1985).

The total phenolic content of the leaf protein was studied in view of the adverse effects of these compounds on growth due to their interference with protein digestibility (Synge, 1975). For all five leaf proteins studied, the total polyphenolic content was below 3%. Fafunso and Byers (1977) found total phenolic contents of 1.3 and 1.6% for grass and lucerne leaf protein preparations, respectively, while that of leaf proteins extracted from leafy tops of different vegetable and legume crops was reported to be in the range 1.4–2.2% (Subba Rau et al., 1972). A wider range of polyphenolics (0.8–4.0%) was noted in leaf proteins by Maliwal (1983).

In a previous study on these aquatic plants (Banerjee and Matai, 1990), the total polyphenolic content was estimated to be 6.7% for *A. philoxeroides*, 5.2% for *A. pinnata*, 7.2% for *L. minor*, 3.2% for *L. cristatum*, and 2.2% for *P. stratiotes*. It is interesting to note that extracted leaf proteins had a lower polyphenolic content than the original plant, indicating that the process of extraction probably helps to reduce the total polyphenolic content of the plant. Rambourg and Monties (1983) also found a significant reduction in the concentration of polyphenolics in lucerne leaf protein washed with solvents and water.

Amino Acid Composition. The amino acid compositions (in grams of amino acid per 100 g of recovered amino acid) of the five aquatic leaf protein samples along with alfalfa leaf protein are reported in Table II. Among the five leaf proteins studied, no large differences were observed in their comparative amino acid compositions. The crude protein content of leaf protein varied from 38.3 to 54.2%, a range of 15.9%, but the amino acid content of the proteins did not show this large a variation, suggesting that protein of a uniform composition could be extracted from aquatic plants. The largest differences ranged from 4.64 to 7.04 in lysine and from 4.53 to 5.78 in proline. Even using comparable preparations and standard hydrolysis conditions, the variation in amino acid content of extracted leaf protein was reported to be greater for some amino acids than others, the largest differences being observed in the *S*-amino acids proline and lysine (Byers, 1983).

Various analyses have shown a close similarity in the amino acid patterns of different leaf proteins prepared from terrestrial plants (Gerloff et al., 1965; Byers, 1971; Cheeke et al., 1980). Even on comparison with alfalfa leaf

Table III. Amino Acid Composition[a] of Three Aquatic Plants

amino acid	*A. pinnata*[b]	*L. minor*[c]	*P. stratiotes*[d]
lysine	6.45	6.37	6.92
histidine	2.31	1.78	2.19
arginine	6.62	5.99	4.60
aspartic acid	9.39	11.28	11.86
threonine	4.70	4.97	5.02
serine	4.10	4.84	5.02
glutamic acid	12.72	12.30	13.39
proline	4.48	4.97	4.94
glycine	5.72	6.56	6.21
alanine	6.45	7.65	7.06
valine	6.75	6.56	6.31
cystine	2.26	0.64	0.37
methionine	1.88	1.72	1.74
isoleucine	5.38	5.35	5.21
leucine	9.05	9.56	9.21
tyrosine	4.10	3.19	4.17
phenylalanine	5.64	4.97	5.97
tryptophan	2.01	1.27	
crude protein	27.94	17.70	23.03
true protein[e]	23.42	20.00	17.63

[a] Grams of amino acid per 100 g of recovered amino acid. [b] Buckingham et al. (1978) (average of four samples). [c] Muzter et al. (1978) (results converted from grams of amino acid per 16 g of nitrogen). [d] Boyd (1969) (average of two samples; results converted from percent dry weight). [e] Sum of amino acids.

protein, the amino acid composition of leaf proteins extracted from aquatic plants did not show much variation, as can be seen from Table II.

Although species differences in leaf protein amino acid composition have also been reported (Oelshlegel et al., 1969), considering the contribution of ribulose 1,5-biphosphate (RuBP) carboxylase/oxygenase to leaf protein and the consistency of amino acid composition of that enzyme among species, it is not surprising that considerable consistency is found in leaf protein from different species (Byers, 1983).

Literature reports on amino acid composition of aquatic plants were scanned in an attempt to compare the differences between the amino acid composition of a plant and that of the protein extracted from it. Studies on three plants, namely *A. pinnata*, *L. minor*, and *P. stratiotes*, which are common to this study, were found, and their amino acid compositions are presented in Table III. All values were converted to grams of amino acid per 100 g of recovered amino acid to facilitate ease of comparison. The crude protein contents of the plants used in this study were 21.9% for *A. pinnata*, 20.4% for *L. minor*, and 20.5% for *P. stratiotes* (Banerjee and Matai, 1990).

Although the protein content of the leaf protein increased considerably in comparison to that of the plant, the variation in amino acid content of the plants and that of the leaf proteins extracted from it was almost nil. A maximum difference of only 2.24 was noted in the aspartic acid content of the *P. stratiotes* plant (11.86) and that of its leaf protein (9.62). Thus, an almost uniform amino acid composition could be obtained in leaf proteins extracted from different aquatic plants (Table II) and, given the same plant, in that of the plant and leaf protein extracted from it (Table III). Even varying levels of polyphenolics in the leaf protein (Table I) did not contribute to any difference in the amino acid composition, as has also been reported by Horigome and Uchida (1981). Fafunso and Byers (1977) found that treatment with poly(vinylpyrrolidone) (PVP) during the extraction process resulted in a large decrease in the phenolic content of leaf protein preparations, but no difference was noted in the amino acid composition of the treated and untreated

Table IV. Essential Amino Acid Content[a] of Aquatic Leaf Proteins Compared to FAO/WHO Reference Standards and NRC Requirements for Chicks

amino acid	FAO/WHO reference pattern[b] preschool child	adult	range values of five aquatic leaf proteins	NRC[c] requirement for chicks
threonine	3.4	0.9	4.81–5.30	3.5
valine	3.5	1.3	6.42–7.62	4.3
methionine[d]	2.5	1.7	1.13–1.55[e]	3.8
isoleucine	2.8	1.3	5.66–6.51	3.8
leucine	6.6	1.9	9.14–10.67	7.0
phenylalanine[f]	6.3	1.9	9.77–11.82	6.5
lysine	5.8	1.6	5.04–7.20	5.5
histidine	1.9	1.6	2.20–2.95	2.0
arginine			5.50–6.50	6.0
glycine			5.58–6.33	5.0
tryptophan	1.1	0.5		1.0

[a] Grams of amino acid per 100 g of crude protein. [b] FAO/WHO (1985). [c] NRC (1984). [d] Requirements for methionine plus cystine. [e] Values for methionine only. [f] Requirements for phenylalanine plus tyrosine.

samples. This is probably because native polyphenols and their derivatives form protein–polyphenol complexes with the reactive groups of lysine and methionine which inhibit the rate of attack by proteolytic enzymes (Horigome and Kandatsu, 1968; Free and Satterlee, 1975), thereby reducing *in vivo* digestibility.

Although the nutritional quality must be finally established with feeding trials, *in vitro* tests using proteolytic enzymes are useful for rapid screening because only a few milligrams of material is needed as opposed to larger quantities and longer times needed for animal experiments. *In vitro* digestibility tests using pepsin–pancreatin revealed that all of the aquatic leaf proteins have digestibility above 70% (Table I).

A comparison with standards was done to provide a means of predicting the contribution of these aquatic leaf proteins toward meeting human/animal amino acid requirements. The numbers and types of amino acids which are essential for a particular species are not exactly the same for other species. The requirement varies depending on the age and species, as is evident from Table IV. The requirement of essential amino acids is critical in the nutrition of nonruminants such as humans and chicks, since, unlike the ruminants, they do not have the ability to synthesize certain essential amino acids (Banerjee, 1988).

Table IV gives the range values of essential amino acids present in the leaf proteins compared to the requirement for humans (FAO/WHO, 1985) and for chicks (NRC, 1984). All leaf proteins contained well above the suggested pattern of requirement for adults and satisfied the required amounts for preschool children and chicks, thereby indicating that they could be used as a food/feed source. In this context, the high lysine content of leaf protein could make it an important source of supplemental protein to cereal-based diets, which are commonly limiting in this amino acid. To make possible the effective utilization of these plants as a source of amino acids, feeding trials should be conducted to evaluate the acceptability and nutritional effectiveness of the protein as a supplement.

Composition of Fibrous Residue. The chemical composition of the fibrous matter remaining after the juice has been expressed from the original plant is reported in Table V. Nitrogen content of the fiber samples ranged from 2.1% in *L. cristatum* to 3.2% in *P. stratiotes*. Byers and Sturrock (1965) found 0.74–3.3% nitrogen in the fibers from 17 crops. On comparison with locally used animal feeds (Banerjee, 1988), the fibers contained sufficient protein to meet the requirements of ruminants since the

Table V. Composition of Fibrous Residues from Five Aquatic Plants (Mean of Three Samples)

	fibrous residue					
	A. philoxeroides	*A. pinnata*	*L. minor*	*L. cristatum*	*P. stratiotes*	LSD[a] ($P = 0.05$)
dry matter, %	17.4	16.3	14.2	15.1	17.5	1.29
nitrogen,[b] %	2.5	2.5	2.8	2.1	3.2	0.16
crude fat,[b] %	5.3	4.1	3.4	3.9	4.3	0.53
crude fiber,[b] %	32.6	28.6	29.7	34.7	29.7	NS[c]
ash,[b] %	11.3	8.3	16.6	10.2	11.8	1.19
nitrate,[b] %	0.36	0.55	0.35	0.23	0.65	0.08

[a] LSD, least significant difference. [b] Dry weight basis. [c] NS, not significant.

ruminants need only 9% protein for maintenance and about 15% for milk production (Arkcoll and Davys, 1971). Except for the crude fat content which was higher than that of conventional roughages, the other constituents in the fiber samples had comparable values. The nitrate content in the fiber fractions were well below levels of toxicity for ruminants as given by Bondi and Alumot (1987). Studies have been conducted on the use of the dried fiber residue from other plant sources as a feed for cattle (Ohshima and Sogo, 1984) and sheep (Fujihara and Ohshima, 1984).

Conclusions. On the basis of their overall nutrient composition, with special emphasis on nitrogen, β-carotene, and amino acid composition, the leaf proteins extracted from aquatic plants could be used for ruminant feeding and, depending on the plant species, could be used in monogastric livestock/human diets. Harvesting these plants would not pose any additional problem since most of them are floating macrophytes which have properties that make them suitable for relatively economical repeated harvesting. The only exception, *A. philoxeroides*, is an emergent weed which produces dense stands at the edges of shallow ponds and ditches. Hence, they can also be easily removed. Thus, in countries where low availability of good quality foods and feeds is a serious problem, leaf protein extracted from unwanted aquatic plants could offer an excellent relief if the technology could be used to reduce the levels of antinutrients and extract the protein as a concentrate. The leaves of *A. philoxeroides* originally showed a positive reaction to alkaloids (Banerjee and Matai, 1990), but its complete absence was noted when leaf protein was extracted from the same plant (Dewanji, 1991). Similarly, levels of polyphenolic compounds were also reduced.

It would be advantageous if the byproducts of the leaf protein extraction process, the fiber and the deproteinized juice, were put to some use so as to avoid local pollution as well as for economic reasons. It is evident that the fibrous residue from aquatic plants has potential for use as ruminant feed, and if destined for a dehydrated meal, then the loss of water content caused by the pressing process doubles the efficiency of the dehydrated plant. Studies in our laboratory on the use of the deproteinized juice from two aquatic plants have also shown that these could be used successfully by yeast as a medium for microbial protein production, simultaneously reducing BOD levels which otherwise could cause pollution problems (Chanda, 1992).

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