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EXTRACTION AND CHEMICAL EVALUATION OF HYDRILLA PROTEIN CONCENTRATE

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The proximate composition of hydrilla plant, hydrilla protein concentrate and hydrilla protein residue was determined. The amino acid profile of hydrilla protein concentrate was estimated on the amino acid analyser. The result showed that all the fractions were quite rich in protein and mineral contents. The amino acid analysis of HPC (hydrilla protein concentrate) compared favourably with the FAO (1965) provisional recommendations.

INTRODUCTION

Interest in the study of leaf protein production increases every year, in view of the low protein production in contrast to a growing requirement. The general aim of this study was to develop protein concentrate from water plants for human consumption to combat the protein shortage. Hydrilla a wildly grown water plant was selected for the present study due to its abundance. The area being comparatively new needs extensive investigations before the protein concentrate thus prepared can be served. The study was undertaken (a) to prepare the hydrilla protein concentrate and (b) to analyse the different fractions during operation.

MATERIALS AND METHODS

Collection of the Material

A survey was conducted in the viccinity of Faisalabad City to locate Hydrilla species and to assess its availability. The identification of the plant was based on botanical characteristics. The plant was collected from the railway ponds either manually or with the help of a hook tied to a rope.

Preparation of hydrilla protein concentrate (HPC)

(a) *Extraction*. Hydrilla plants were thoroughly washed and their

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protein extracted by the method of Morrison and Pirie (1961) by pulping and belt pressing at PCSIR Laboratories, Lahore.

(b) *Precipitation of the protein.* The juice thus obtained was passed through a 60 mesh sieve to remove fibrous material. The pH of the juice was adjusted to 5.5 and steam heated to 80° ± 2°C for efficient precipitation of the protein. The precipitate was separated from the juice in a basket centrifuge.

(c) *Drying.* The precipitate was dried at 80° ± 2°C to eliminate moisture. The dried product was designated as HPC. The HPC was ground, passed through a 60 mesh sieve and preserved in air tight jars for further investigation.

(d) *Leaf protein residue.* The fibrous portion left after extraction of the plant was sun dried and packed.

Chemical Analysis

(a) *Proximate Analysis.* The samples of hydrilla plants, hydrilla protein concentrate and the residue left after extraction were analysed for proximate composition (A.O.A.C., 1971). Nitrogen free extract was calculated by difference.

(b) *Amino acid analysis.* Hydrilla protein concentrate was analysed for amino acid composition (Spackman *et al.*, 1958) using EEL Automatic Amino Acid Analyser. The dried HPC was hydrolysed with 6N HCl in a sealed glass tube for 22 hours at 110°C under vacuum. The hydrolysate was dried to remove the acid at 40°C under vacuum using rotary evaporator. The dry residue was dissolved in a known quantity of 2.2 pH buffer and filtered to get a clear solution of the hydrolysate and an aliquot was loaded on the ion exchange columns of the EEL amino acid analyser. Acidic, neutral and basic amino acids were eluted at pH 3.25, 4.25 and 5.28, respectively, using sodium citrate buffers. The elution was done with a flow rate of 15 and 30 ml per hour for ninhydrin and the buffer, respectively. Tryptophan was measured colorimetrically by the method of Miller (1967).

RESULTS AND DISCUSSION

Proximate Composition

The data on the moisture, crude protein, ether extract, total ash, crude fibre and nitrogen free extract contents for dried hydrilla plant, hydrilla protein concentrate and hydrilla plant residue are given in Table 1.

Table 1. *Proximate Composition*

Description	Moisture %	Crude protein %	Ether extract %	Total ash %	Crude fibre %	Nitrogen free extract %
Hydrilla plant (dried)	3.05	13.13	1.50	43.60	10.10	28.62
HPC	4.28	30.62	5.55	24.04	1.22	34.29
Hydrilla plant residue	8.56	10.94	0.54	40.95	23.70	15.31

Protein content of the hydrilla plant was somewhat lower and ash content higher than that earlier reported (Protein 15% and ash 17 to 27.6%). The protein content was close to that reported by Boyd (1969) whereas a wide difference was observed in mineral contents. This difference might be due to the higher mineral content of water which led to higher uptake of minerals in the plants under study.

Hydrilla protein concentrate had quite similar protein content as *Alternanthera philoxoroides* protein concentrate which was reported to have 31.4 per cent but was lower than other equatic plant protein concentrates (Boyd, 1968). Contradictory reports regarding the protein contents of various plant species appear in literature (Valli Devi *et al.*, 1965 and Shurpalekar *et al.*, 1970). Species differences may be the cause of such variations.

The fibrous residue had a protein content of 10.94 per cent on as such basis which is more or less similar to the original plant but with a relative rise in crude fibre. Seeing the composition of the residue, its use in ruminant rations may be recommended.

Amino acid composition

The data on the amino acid profile of the Hydrilla protein concentrate and FAO (Anonymous, 1965) provisional recommendations are shown in Table 2.

Table 2. *Amino acid composition of HPC (g/16 g N).*

Amino acid	HPC	FAO (1965) provisional recommendations for LPC
Aspartic acid	10.67	—
Threonine	4.99	2.8
Serine	5.35	—
Glutamic acid	12.17	—
Proline	2.64	—
Glycine	7.63	—
Alanine	6.06	—
Valine	5.15	4.2
Methionine	1.85	2.2
Iso-leucine	4.55	4.2
Leucine	9.90	4.8
Tyrosine	4.40	2.8
Phenyl alanine	5.71	2.8
Lysine	5.97	4.2
Arginine	6.86	—
Tryptophan	2.16	1.4

The amino acid profile of HPC compared favourably with the findings of Hodgson (1964) for two grass species, Wilson and Tilley (1965) for lucerne protein, Boyd (1968) for equatic plant proteins and Buchanan (1969) for wheat leaf protein. The lysine content of HPC was more than the lysine contents of 18 plant proteins which fell in the range of 3-4 per cent (Valli Devi, 1965). However, methionine content was within the range of these species. The iso-leucine, leucine, lysine, phenyl alanine, tryptophan threonine, tryosine and valine contents were more than the FAO (Anonymous, 1965) provisional recommendations for LPC with an exception of methionine which was somewhat less in HPC.

LITERATURE CITED

Anonymous. 1965. F.A.O. Nutrition report Ser. No. 37 (cited by Pirie, N.W. Proc. Nut. Soc. 1969, 28: 85–90).

Boyd, C.E. 1968. Fresh water plants: A potential source of protein. Econ. Bot., 22: 359–368.

Boyd, C.E. 1969. The nutritive value of three species of water weeds. Econ. Bot., 23: 123.

Buchanan, R.A. 1969. In vivo and in-vitro methods of measuring nutritive value of leaf protein preparations. Brit. J. Nut. 23: 533–545.

Hodgson, H.C. 1964. The protein amino acid composition and N distribution in two tropical grasses. J. Sci. Fd. Agric., 15: 721–724.

A.O.A.C. 1971. Official methods of analysis of the association of official Agricultural chemists, Washington, 4 D.C.

Miller, E.L. 1967. Determination of tryptophan contents of feeding stuffs with particular reference to cereals. J. Sc. Fd. Agric., 18: 381.

Morrison, J.E. and N.W. Pirie. 1961. The large scale production of protein from leaf extracts. J. Sci. Fd. Agric., 12: 1.

Shurpalekar, K.S., N. Singh and O.E. Sundra Valli. 1970. Nutritive value of leaf protein from lucerne (Medicago sativa) growth responses in rats at different levels and to supplementation with lysine and or methionine. Nut. Abs. & Rev. 40: 5051.

Spackman, D.H., H. Stein and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem., 30: 1190.

Valli Devi, A., N.A.N. Rao and P.K. Vijayaraghavan. 1965. Isolation and composition of leaf protein from certain species of Indian flora. J. Sci. Fd. Agric. 16: 116–120.

Wilson, R.F. and J.M.A. Tilley. 1965. Amino acid composition of lucerne and grass protein preparations. J. Sci. Fd. Agric., 16: 173–178.