Extraction Of Protein From Water Hyacinth[1]

K. G. TAYLOR, R. P. BATES and R. C. ROBBINS

Efforts are being made to clear water hyacinth (Eichhornia crassipes) from Florida waterways. Research is being conducted to find commercial uses for the plant and to help defray removal and disposal costs. Some work has been done to determine the nutritional value of aquatic plants, including hyacinth (1). An earlier paper from this laboratory reported on the protein and amino acid composition of the whole hyacinth plant (10). This study indicated that the protein might be nutritionally useful for man or monogastric animals, if it could be extracted from the fiberous plant.

Extraction of leaf protein has been championed by Pirie in England (7) and techniques for extracting crude protein from field crops has been accomplished on a pilot scale (8, 6). Byers (4) and Dalta et al. (5) have conducted studies on the preparation of protein concentrate from the leaves of water hyacinth. The extraction of protein from the leaves only is not practical under the present system for removal of the hyacinth from waterways. Thus the present investigation was initiated to determine the feasibility of extracting protein from the whole plant.

MATERIALS AND METHODS

This investigation consisted of three phases: (1) crude protein was determined in chopped plant material and press juice collected from a mechanical harvester, (2) entire plants were harvested by hand and subjected to different extraction procedures and (3) the most effective protein extraction procedure from the above trials was evaluated in terms of recoverability of essential amino acids from the whole plant.

PHASE 1

The press juice collected from the Hiller machine operating in a phosphate pond in Lakeland, Florida was obtained in January 1969. The machine chops the plants into coarse particles which are passed through a screw press, the press juice is expelled and the residue is dehydrated by a gas fired rotary dryer. The juice obtained from the machine process was transported to the Food Science Laboratory at the University of Florida and refrigerated at 1°C until processed. Prior to protein precipitation the press juice was clarified in an Electro E-500 Centrifuge Clarifier. Two kg aliquots were acidified with concentrated HCl to pH 3.8 and then heated to 80°C. The juice was then refrigerated 36 hours at 1°C. The sedimented precipitate was separated from the supernatant by filtration through a Buchner funnel. The whole plant, acid supernatant, and residue were analyzed for crude protein in order to follow the efficiency of the extraction procedures.

PHASE 2

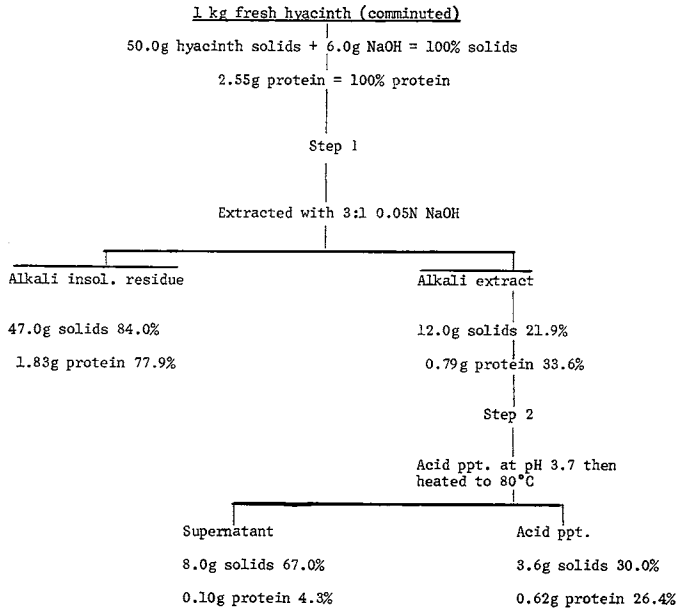
Whole hyacinth plants were collected in March from Lake Alice on the University of Florida campus. Various techniques to determine the most effective method of extraction of the protein from the water hyacinth solids were attempted on chopped hyacinth samples which had been refrigerated overnight. These procedures are outlined in Figure 1.

PHASE 3

Whole hyacinth plants were harvested from Lake Alice in June. Replicate one-kg samples of fresh chopped hyacinth (whole plant) were comminuted in a commercial 4-liter capacity Waring blender with 3 kg of 0.05*N* NaOH at low, medium, and high speed for 20 seconds each. The slurry was passed through a screw press fitted with a 0.020 inch screen. The juice was then clarified, acidified to pH 3.7 and heated to 80°C. The acidified solution was refrigerated overnight at 1°C and the precipitate separated from the supernatant on a Buchner funnel. The sediment from the clarified was added to the extracted residue.

Florida Agricultural Experiment Station Journal Series No. *3450.*

Figure 1. Mass and Crude Protein Balance During Various Steps of the Alkali Extraction Procedure on Hyacinth from Lake Alice, University of Florida Campus, June 1969.



Samples were taken during the various steps in the procedure for dry matter, total nitrogen and amino acid analysis. The determinations were performed as previously reported (10).

RESULTS AND DISCUSSION

Press juice from the Hiller machine was dark green in color and appeared unacceptable for human consumption without extensive cleanup. More serious the press juice was too low in protein content (0.15%) for the cleanup to be economically feasible. The low recovery of protein may be due to lack of sufficient crushing to release the protoplasma from greater proportion of cells. This is indicated by laboratory results in which comminuation as contrasted to chopping improved the quantity of protein extracted from 23% to 37%. However, 2 and 3 minute comminutions were not any more successful than 1 minute. Also, the pH (6.6) of the press juice from the Hiller machine does not appear to be conducive to maximum yield as evidenced by the high pH (9-12) of the extraction fluids that were most effective in the laboratory.

Hyacinth collected from Lake Alice in March and used in subsequent extraction evaluation continued 54g (5.4%) dry matter and 4.32g (0.43%) crude protein per kg fresh weight. The most effective procedure (Figure 1,C) gave a yield of 49.4% of the crude protein of the whole plant. This was followed by procedure A with 43.0% and procedure B with 34.5%. Procedure D, which tested the effect of communition time on protein yield, showed a lower yield with 3 minute comminution than with 1 or 2 minutes. An analysis of the effectiveness of these procedures revealed that a 1:3 ratio by weight of hyacinth to 0.05*N* NaOH gave best results. The larger volume of the 1:8 ratio of hyacinth to extraction solution represented excesssisve dilution of protein and gave no higher yield.

The whole hyacinth plant harvested in June from Lake Alice contained 50g dry matter (5.0% solids) and 2.35g crude protein per kg fresh weight. The extraction procedure removed 33.6% of the crude protein. The yield of protein for the whole hyacinth plant is low compared to yields obtained from leaf (legumes) by other workers (6,7) which generally ranged from 50 to 80%. It is possible that greater proportion of the protein of the whole plant is associated with fiberous structural material, rather than chloroplasts as in leaves.

The amino acid analysis of the isolates are presented in Table 1. The amino acids in the whole hyacinth plant represented 81% of the crude protein accounted for by Kjeldahl nitrogen. The 19% unaccounted for would represent tryptophan destroyed by acid hydrolysis, non-protein nitrogen and recovery losses. There does not appear to be any marked difference between the amino acid pattern in the alkali extract and extract of the fiberous residue but a definite trend in the distribution of amino acids between the supernatant and acid precipitate fraction. The first seven amino acids are highest in the supernatant while the last ten are highest in the acid precipitate fraction. The reason for this phenomena is not apparent.

The quantity of essential amino acids of hyacinth is similar to the values reported by Boyd (1) and the ratios compare favorably with those of the reference protein used by the Food and Agricultural Organization of the United Nations (Table 3) to evaluate quality of protein and of milk, indicating that hyacinth protein is balanced nutritionally. The problem with hyacinth protein extracts for human use does not appear to be one of quality but one of quantity and acceptability. However, more effective extraction procedures combined with feeding trials to evaluate the acceptability and nutritional effectiveness of hyacinth protein as a supplement might make possible effective utilization of the plant as a source of amino acids for monogastric animals.

TABLE 1. AMINO ACIDS IN WATER HYACINTH (g per 100g of Total Protein)[1]

Amino Acid	Alkali Extraction[2]			Acid Precipitation[3]	
	Whole Plant	Fiber	Extracts	Supernatant	Acid Pp
D.W. % of isolate	100.0	67.6	37.1	12.5	85.3
Aspartic acid	10.2	9.8	11.9	16.0	9.7
Threonine	5.0	4.8	3.7	5.3	4.4
Serine	4.8	4.6	3.0	5.3	3.3
Glutamic acid	9.8	9.4	10.6	13.1	8.8
Proline	4.8	4.5	4.6	3.5	4.9
Glycine	6.4	6.3	6.4	6.5	5.6
Alanine	7.1	6.8	7.3	9.5	6.7
Cystine	0.7	0.5	0.0	0.0	1.2
Valine	11.4	13.7	13.5	13.0	13.9
Methionine	2.3	2.3	2.7	2.2	2.5
Isoleucine	5.6	5.1	5.1	4.0	5.1
Leucine	8.7	8.8	8.2	5.0	8.6
Tyrosine	3.4	3.5	4.4	3.4	4.3
Phenylalanine	5.2	5.3	5.5	4.2	5.5
Histidine	2.3	2.0	2.0	1.6	2.3
Lysine	7.6	6.3	6.5	4.9	7.9
Arginine	5.2	5.8	4.4	2.2	5.5
Percent recovery	100.5	99.5	99.8	99.7	100.2

1. All values represent the average of three replicates.
2. Extracted one time with one part by weight hyacinth to three ports 0.05*N* NaOH, comminuted 1 minute. See Figure 2, step 1.
3. Extract adjusted to pH 3.7 with concentrated HCl and heated to 80°C. See Figure 2, step two.

TABLE 2. COMPARISON OF THE EFFECTIVENESS OF ALKALI EXTRACTION METHODS FOR REMOVING CRUDE PROTEIN FROM WATER HYACINTH COLLECTED FROM LAKE ALICE, UNIVERSITY OF FLORIDA CAMPUS, MARCH 1969.

Alkali Extract Sequence	Alkali Extract Procedure					
				D[4]		
				Comminution (min)		
	A[1]	B[2]	C[3]	1	2	3
First						
Crude protein (g)[5]	1.59	1.22	1.83	1.34	1.34	1.08
Recovery (% of total)[6]	37.0	28.0	42.0	31.0	31.0	25.0
Second						
Crude protein (g)	0.13	0.16	0.32			
Recovery (%of total)	3.0	3.7	7.4			
Third						
Crude protein (g)	0.13	0.12	0			
Recovery (% of total)	3.0	2.8	0			
Total						
Crude protein (g)	1.85	1.50	2.15	1.34	1.34	1.08
Recovery (% of total)	43.0	34.5	49.4	31.0	31.0	25.0

1) A. Extracted three times with one part by weight hyacinth to three parts 2% Na_2CO_3 comminuted 1 minute.
2) B. Extracted three times with one part by weight hyacinth to eight parts 0.05*N* NaOH comminuted 1 minute.
3) C. Extracted three times with one part by weight hyacinth to three parts 0.05*N* NaOH comminuted 1 minute.
4) D. Extracted once with one part by weight hyacinth to three parts 0.05*N* NaOH comminuted 1 minute or 2 minutes or 3 minutes.
5) Grams of crude protein per kg fresh hyacinth
6) 4.32 grams crude protein = 100%

TABLE 3. ESSENTIAL AMINO ACIDS PER 100g OF TOTAL AMINO ACIDS RECOVERED IN WATER HYACINTH COMPARED WITH FAO REFERENCE PATTERN, LEAF PROTEIN CONCENTRATE, CORN, AND MILK; TRYPTOPHAN NOT DETERMINED.

	Met	Cyr*	Phe	Tyr*	Thr	Lys	Ileu	Val	Leu
FAO[1]	2.2	2.0	2.8	2.8	2.8	4.2	4.2	4.2	4.8
Cow's Milk[1]	2.4	0.9	4.9	5.1	4.6	7.8	6.4	6.9	9.9
Corn Grits[2]	2.5	1.1	6.4	6.7	4.1	0.8	6.4	5.3	15.0
L. P. C.[3]	2.1	0.7	6.0	4.2	5.2	6.3	5.3	6.3	9.8
W. H.[4]	1.8	0.3	5.5	3.9	5.0	6.2	5.2	6.0	9.1
W. H. Lot II	0.8	0.3	5.2	3.3	4.8	5.9	4.8	12.8	8.0
W. H. Lot III	1.2	0.6	4.8	3.5	4.5	11.1	4.9	11.7	8.2
W. H. Lot V	2.3	0.7	5.2	3.5	5.0	7.6	5.6	11.4	8.7
W. H. Pro. Conc.	2.5	1.2	5.5	4.3	4.4	7.9	5.1	13.9	8.6

* non-essential
1. Burton 1965:144
2. Block 1956:176
3. Stahman, 1965
4. Boyd, 1969

SUMMARY

Crude protein content of water hyacinth was found to range from a low of 4.7% (dry basis) in summer, through 5.8% in winter to a high of 9.2% in the spring. The crude fiber content (dry basis) was 5.0%, 5.4% and 5.8% respectively for the three seasons. The protein was difficult to extract from the whole plant as only 33.6% was extracted by 0.05*N* NaOH and only 26.4% of the total crude protein recovered by precipitation. Although the quantity of the protein extracted was low, it appeared to be of good nutritional quality as evidenced by the proportions of essential amino acids. The pattern of amino acids remaining with the alkali insoluble was similar to that in the extract; thus, the protein quality has not been enhanced by alkali extraction. It has, however, been removed from nondigestable fiber.

LITERATURE CITED

1. Boyd, C. E. 1969. The nutritive value of three species of water seed. Econ. Bot. 23:123.
2. Block, R. S. and K. W. Weiss. 1956. *Amino Acid Handbook.* Thomas, Springfield, Ill.
3. Burton, B. T. 1965. *The Heinz Handbook of Nutrition.* McGraw-Hill, New York, N. Y.
4. Byers, M. 1961. Extraction of protein from the leaves of some plants growing in Ghana. J. Fd. Sci. and Agr. 12:20.
5. Dalta, R. K., P. R. Chakrabarty, B. C. Guba, and J. J. Ghosh. 1966. Studies on leaf proteins—preparation of protein concentrate from leaves of water hyacinth. Sci. and Cult. 32:247.
6. Morrison, J. C. and M. W. Pirie. 1961. The large scale production of protein from leaf extracts. J. Sci. Fd. and Agr. 12:1.
7. Pirie, N. W. 1966. Leaf Protein as human food. Science 152:24 June, p. 1701.
8. Pirie, N. W. 1952. Protein production from green leaves. World Crops 4:374.
9. Stahmann, M. A., I.Lima and E. D. Gerloff. 1965. Amino acid composition of leaf protein concentrate. Agr. and Fd. Chem. 13:139.
10. Taylor, K. G. and R. C. Robbins. 1968. Hyacinth Control J. 7: June, 24-25.