

# Leaf Protein Concentrate from Water Hyacinth

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## ABSTRACT

Leaf protein was extracted from water hyacinth and from 16 other aquatic weeds in Thailand. The water hyacinth leaves showed 22.6%, those of morning glory had 29.4% and water chestnut contained only 4.3% protein. Aqueous extraction of the leaves at pH 8.5 was judged suitable for water hyacinth. The water hyacinth protein was further processed into Leaf Protein Concentrate (LPC) using acid and thermal precipitation at pH 4.0 and 82C. The precipitated LPC was rinsed and dried at 60C. Chemical analysis of the water hyacinth LPC indicated 55.4% protein, 3.1% fatty acids, 1.0% fiber, 5.0% ash, and 35.5% carbohydrate. The protein fraction contained most essential amino acids and was particularly rich in leucine (5.1%) and phenylalanine (3.4%).

**Key words:** *Eichhornia crassipes*, aquatic macrophyte, green protein.

## INTRODUCTION

Leaf Protein Concentrate (LPC) contains proteins prepared from disrupted plant cells. The proteins in leaf juice were heat coagulated into green chloroplastic LPC or white cytoplasmic LPC (Telek and Graham 1983).

LPC may be used in vegetarian dishes or as supplementary food. It is likely to be nutritious because of the high protein content, and the content of unsaturated fats, carotenes, xanthophyll, starch, and minerals such as iron, calcium and phosphorus. LPC may also be used in animal feeds for swine, calves, chickens and fish (Telek and Graham 1983).

In a tropical region like Thailand where aquatic weeds are abundant, it would be of interest to determine the possibility of using these plants for the production of LPC. This investigation describes the use of water hyacinth for the preparation of LPC and determines its value as a food or feed.

## MATERIAL AND METHODS

Seventeen aquatic weeds commonly found in Thailand including *Wolffia globosa*, *Pistia stratiotes*, *Potamogeton malaiyanus*, *Typha angustifolia*, *Nelumbo nucifera*, *Eichhornia crassipes*, *Ipomoea aquatica*, *Alternanthera*

*philoxeroides*, *Mimosa pigra*, *Coix aquatica*, *Sesbania javanica*, *Ceratophyllum demersum*, *Chara zeylanica*, *Hydrilla verticillata*, *Eleocharis dulcis*, *Polygonum tomentosum* and *Brachiaria mutica* were collected from a swampy area in a suburb of Bangkok. The leaves were dried at 80C for 48 hr and analyzed for protein content using the Kjeldahl method. *Leucaena leucocephala*, a nonaquatic forage commonly used as feed in Southeast Asia for its high protein value was used for comparison. Water hyacinth was further processed for the preparation of LPC. The leaves, carefully selected from the apex down to the fifth leaf, were washed with water, cut into pieces, and crushed, with water at pH 8.5, having a solid-to-liquid ratio of 1:3, in a blender for 3 min. The slurry was filtered through cheesecloth and the juice separated. The residue was repeatedly ground and all filtered juice combined. The green juice fraction was obtained by centrifugation of the filtered juice at 1465 g for 3 min. It was acidified with 1.0 N HCl to pH 4.0 and heated at 82C for 5 min. LPC precipitated by the heat treatment was separated by centrifugation at 23,500 g for 3 min and washed with 95% ethanol or acetone or water. It was dried at 60C and ground into LPC powder.

Chemical constituents of the LPC were analyzed for protein, fat, ash, fiber, carbohydrate and amino acids.

Data, collected in 1992, on the nutrient concentrations of water in the collection area were obtained from the Royal Irrigation Department, Nonthaburi, Thailand.

Data on LPC were statistically analyzed by the Statistical Analysis System (SAS).

## RESULTS AND DISCUSSION

Data on the leaf protein content of 17 aquatic weeds and *L. leucocephala* are summarized in Table 1. Morning glory (*Ipomoea aquatica*) showed the highest leaf protein content (29.4%) followed by the nonaquatic *L. leucocephala* (26.8%). Water hyacinth (*Eichhornia crassipes*) had a somewhat lower leaf protein content of 22.6%, which is similar to data reported earlier (24.9%; Meksongsee 1984). It is generally recommended that a leaf suitable for the preparation of LPC should contain at least 20% protein (Nagy *et al.* 1978). Many of the other aquatic weeds tested here did not pass this initial criterion. However, the ranking of leaf protein content could well change under different growth conditions, where the nutrient concentrations in the water were different.

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TABLE 1. LEAF PROTEIN CONTENT (% dry wt.) OF AQUATIC WEEDS COMMONLY FOUND IN THAILAND<sup>1</sup> IN CONTRAST TO A NONAQUATIC *L. leucocephala*.

Plant types	Protein content
<i>Ipomoea aquatica</i> Forsk.	29.4 ± 0.4
<i>Nelumbo nucifera</i> Gaertn	25.3 ± 0.1
<i>Sesbania javanica</i> Miq.	25.3 ± 1.8
<i>Pistia stratiotes</i> Linn.	25.2 ± 1.6
<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	24.4 ± 2.8
<i>Eichhornia crassipes</i> (Mart.) Solms	22.6 ± 1.4
<i>Hydrilla verticillata</i> Presl	22.3 ± 1.8
<i>Mimosa pigra</i> Linn.	21.7 ± 2.1
<i>Wolffia globosa</i> Hartog & Plas	20.1 ± 0.4
<i>Polygonum tomentosum</i> Wild	19.8 ± 3.0
<i>Chara zealanica</i> Kl. ex Wild	15.5 ± 0.7
<i>Ceratophyllum demersum</i> Linn.	14.2 ± 1.1
<i>Brachiaria mutica</i> Stapf	14.1 ± 2.5
<i>Potamogeton malaiianus</i> Miquel	13.7 ± 0.6
<i>Coix aquatica</i> Roxb.	11.7 ± 1.6
<i>Typha angustifolia</i> Linn.	8.3 ± 0.9
<i>Eleocharis dulcis</i> (Burm.f.) Henschel	4.3 ± 0.3
<i>Leucaena leucocephala</i> de Wit	26.8 ± 2.6

<sup>1</sup>Nutrient concentrations of water in the collection area, reported in ppm, are 0.31 (ammonia nitrogen), 0.80 (organic nitrogen), 0.24 (nitrite), 0.38 (nitrate), 0.67 (phosphate), 0.01 (dissolved iron), 0.012 (copper) and 0.099 (manganese).

The preparation of LPC from water hyacinth by aqueous extraction of the leaves at pH 8.5, acidification (pH 4.0) and thermal precipitation at 82C were found to be satisfactory. Final rinsing with an organic solvent, such as 95% ethanol or acetone, gave higher protein yields than rinsing with water (Table 2). The higher protein yields (obtained using acetone or ethanol) were, respectively, 4.3 and 4.6 100 g<sup>-1</sup> dry leaves. The LPC produced with either ethanol or acetone treatment contained 57% protein, as opposed to the 50.7% protein by

TABLE 2. PROTEIN YIELDS FOR THE LPC PREPARATION FROM WATER HYACINTH. DATA SHOW THE YIELD FOR VARIOUS RINSING AGENTS.

Rinsing agent	LPC yield (g)	Protein (% dry wt LPC)	Protein yield (g/100 g fresh leaves)	Protein yield (g/100 g dry leaves)
Water	1.1 <sup>a</sup>	50.7 <sup>b</sup>	0.56 <sup>b</sup>	3.98 <sup>b</sup>
Acetone	1.1 <sup>a</sup>	57.3 <sup>b</sup>	0.61 <sup>a</sup>	4.34 <sup>a</sup>
95% Ethyl alc.	1.1 <sup>a</sup>	57.0 <sup>b</sup>	0.65 <sup>a</sup>	4.64 <sup>a</sup>

<sup>a,b</sup>Data statistically analyzed by the SAS and are significantly different between a and b.

rinsing with water. It is likely that the use of an organic solvent decreases some of the contaminants such as fat from the LPC as indicated by the chemical analysis of the LPC products (Table 3). The water hyacinth LPC was found to contain not only protein but also fat, ash, fiber and carbohydrates. The fat content can be made lower with the ethanol rinsing or made fat-free with the soxhlet extraction. The reduction in fat content helped reduce the rancidity of the products.

TABLE 3. CHEMICAL ANALYSIS (% dry wt) OF THE WATER HYACINTH LPC TREATED WITH WATER, 95% ETHYL ALCOHOL OR SOXHLET EXTRACTION.

Treatment	Protein	Fat	Ash	Fiber	Carbo-hydrate
Water rinsing	49.52 <sup>a</sup>	10.21 <sup>a</sup>	5.63 <sup>a</sup>	1.15 <sup>a</sup>	33.49 <sup>a</sup>
95% ethyl alc. rinsing	55.39 <sup>b</sup>	3.08 <sup>b</sup>	5.02 <sup>a</sup>	0.97 <sup>a</sup>	35.54 <sup>a</sup>
Soxhlet extraction	61.04 <sup>c</sup>	—	4.98 <sup>a</sup>	1.02 <sup>a</sup>	32.96 <sup>a</sup>

<sup>a,b,c</sup>Data statistically analyzed by the SAS and are significantly different between a, b and c.

The protein fraction of LPC was found to contain most essential amino acids (Table 4). It was particularly rich in

TABLE 4. AMINO ACID CONTENT (g/100 g<sup>-1</sup>) OF THE WATER HYACINTH LPC COMPARED TO THE FAO (1965) PROVISIONAL RECOMMENDATION.

Amino acid	LPC	FAO (1965) provisional recommendation
Ala	3.40	NA
Arg	3.56	NA
Asp	5.05	NA
Cys	0.42	NA
Cys	0.84	NA
Glu	5.90	NA
Gly	3.02	NA
His	1.10	NA
Ile	2.31	4.2
Leu	5.06	4.8
Lys	2.69	4.2
Met	1.27	2.2
Phe	3.39	2.8
Pro	2.72	NA
Ser	2.56	NA
Thr	2.63	2.8
Trp	NA	1.4
Tyr	2.16	2.8
Val	2.79	4.2

leucine (5.1%) and phenylalanine (3.4%), which exceeded the FAO (1965) provisional recommendation (Pirie 1971).

In conclusion, we have found that water hyacinth can be successfully used for the preparation of the LPC. The product appeared to have nutritional values for it contained protein, fat, carbohydrate, fiber and ash. The fat content of the LPC may be reduced after the ethanol or soxhlet treatments. The protein fraction contained most essential amino acids at appreciable concentrations. Therefore, the water hyacinth LPC has a potential of being a good source of protein in food or feed in regions where this aquatic weed is widespread. Further research concerning the animal feeding experiments are recommended in order to determine if antinutritional factors are present.

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#### LITERATURE CITED

- Meksongsee, L. 1984. Determination of protein, fat and nucleic acids in water hyacinth. Proceedings of the International Conference on Water Hyacinth, Feb. 7-11, 1983. Hyderabad India. United Nations Environment Programme, Nairobi. pp. 374-378.
- Nagy, S., L. Telek, N. T. Hall and R. E. Berry. 1978. Potential food used for protein from tropical and subtropical plant leaves. *J. Agric. Food Chem.* 26:1016-1028.
- Pirie, N. W. 1971. Leaf Protein: Its Agronomy, Preparation, Quality and Use. IBP Handbook No. 20. Blackwell Scientific Publications, Oxford, U.K. 192 pp.
- Telek, L. and H. D. Graham. 1983. Leaf Protein Concentrate. AVI Publishing Company. 844 pp.