Duckweeds (Lemnaceae Family): A Potential Source of Protein and Amino Acids

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A method for extracting the protein from four species of duckweed, an aquatic plant, is described. The crude protein of solar-dried duckweed ranged from 25.2 to 36.5% and that of the protein concentrate from 37.5 to 44.7%. The essential amino acid profile of the protein concentrate compared favorably with the FAO reference pattern with the exception of methionine. Average values (g/100 g of protein) were as follows: lysine, 4.0; methionine, 0.9; isoleucine, 3.6; leucine, 6.7; phenylalanine, 4.2; threonine, 3.1; and valine, 0.9. It is suggested that duckweed protein concentrate could be used as an effective protein supplement in diets low in lysine such as those based on corn or rice.

It is now evident that shortages of foodstuff in the world to feed a rapidly expanding world population will become more critical in the near future. In the developing countries food production has not kept pace with population growth (FAO, 1975; USDA, 1974). Therefore, it is imperative that new sources of potential foodstuffs from currently unexploited resources be investigated.

It has been established by the Food and Agriculture Organization of the United Nations (FAO, 1970; FAO, 1974) that at least 500 million people suffer from severe protein-calorie malnutrition. Protein deficiency is common among large sections of the world population due to a shortage of protein, and, therefore, various protein concentrates have been developed and recommended for alleviating protein malnutrition. Some of these protein concentrates are from single cell cultures, leaves, fish, and soybeans. An unexplored potential source of protein concentrate should be available from duckweed, an aquatic plant, since it contains a high percentage of crude protein in certain aquatic environments (Culley and Epps, 1973; Hillman and Culley, 1978; Rusoff et al., 1977, 1979).

Duckweeds (Lemnaceae family) are tiny free-floating vascular plants with world-wide distribution. There are four common genera, *Spirodela*, *Lemna*, *Wolffia*, and *Wolffiella*, and about 40 species. The plants are of relatively simple morphology as they have no stems or true leaves and usually consist of a single or a few flat oval-shaped "fronds", seldom exceeding 5 mm long. Each frond may or may not have roots and the plants rarely flower. Duckweeds reproduce by vegetative reproduction and are characterized by rapid clonal growth. The plants cluster in colonies and form green blankets or a type of mat on the surface of the water (Hillman, 1961). Many duckweeds can double their weight every 2 or 3 days under proper environmental conditions. Yields equivalent to 10–13 metric tons dry weight ha^{-1} $year^{-1}$ have been demonstrated on a small lagoon system, and in outdoor tanks maximum yields approached 20 metric tons dry weight ha^{-1} $year^{-1}$ (Said et al., 1979).

In many parts of the world, duckweeds are consumed by domestic and wild fowl, fish, herbivorous animals, and humans (Boyd, 1968; Chang et al., 1977; Culley and Epps, 1973; NAS, 1976; Rusoff et al., 1977, 1979; and Tan, 1970). The smallest (pinsize) of duckweeds (*Wolffia arrhiza*) has been used as a nutritious vegetable by Burmese, Loatians, and the people of northern Thailand for generations (Bhanthumnavin and McGarry, 1971).

The chemical composition of duckweeds as reported in the literature varies considerably due to the age of the plant, environmental temperature, and nutrient-aqueous environment. The crude protein content of duckweeds obtained from natural waters (ponds, streams, lakes, paddy fields, and ditches) has been reported to range from 7 to 20% (Maciejewska-Potapzh et al., 1970, 1975; Bhanthumnavin and McGarry, 1971; Tan, 1970). Grown in enriched waters containing mineral media or effluents from agricultural and municipal waste lagoons, the protein content (30–40%) is greatly increased over that from natural waters low in nutrients (Tulaganov, 1973; Chang et al., 1977; Hillman and Culley, 1978; Culley and Epps, 1973; Rusoff et al., 1977, 1979).

The amino acid composition of a few species of duckweed (*Lemna minor* L.) collected from the natural waters of several countries—Poland (Maciejewska-Potapzh et al., 1970, 1975), USSR (Tulaganov, 1973), Canada (Muztar et al., 1978)—and *L. polyrhiza*, *L. paucicostata*, and *Wolffia arrhiza* in Taiwan (Chang et al., 1977) has been reported to contain all of the essential amino acids in varying percentages.

Research is being conducted at the Louisiana State University Dairy Science Research Center on the use of duckweeds in waste management, nutrient recycling, and efficient energy utilization. Thus, samples of duckweed were available for this study which investigated the isolation of a protein concentrate and its amino acid composition from four species of duckweed (*Lemna gibba*, *Spirodela polyrhiza*, *Spirodela punctata*, and *Wolffia columbiana*) grown in an enriched aqueous environment (cattle waste lagoon).

MATERIALS AND METHODS

Collection of Samples. All samples of duckweed were collected from anaerobic dairy waste lagoons on the LSU campus. The lagoons contained pure stands of *L. gibba*, *S. polyrhiza*, *S. punctata* (formally *S. oligorhiza*), and *Wolffia columbiana* as identified by Culley (1978).

Samples were collected during August–November of 1978. The duckweeds harvested from the lagoons were washed on a screen with fresh tap water within 30 min after collection to remove debris associated with the plant and then sun-dried on a screened rack. Each sample was ground to a powder in a micro Wiley mill. The lagoons contained from 20 to 40 mg/L of TKN during the collection period.

Analytical Methods. Chemical analysis of the dry duckweed was determined according to standard AOAC methods (AOAC, 1975).

Extraction of Protein Concentrate. Solar-dried duckweed was saturated with 12 times its weight of 0.5 N NaOH so that the pH was above 8.5. The mixture was placed in a 4-L Waring blender and homogenized for 60 s. The juice was then squeezed out of the homogenate through a double layer of cheesecloth and clarified by

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0021-8561/80/1428-0848$01.00/0

Table I. Proximate Analysis of Duckweeds (% Dry Matter)

species	dry matter	crude protein (N × 6.25)	fat	crude fiber	ash
L. gibba	4.6	25.2	4.7	9.4	14.1
S. punctata	5.2	28.7	5.5	9.2	13.7
S. polyrhiza	5.1	29.1	4.5	8.8	15.2
Wolffia columbiana	4.8	36.5	6.6	11.0	17.1

centrifuging at 2000 rpm, and the protein was precipitated from the supernatant by acidifying to pH 3.65 with 0.1 N HCl.

The acidified suspension was heated to 75 °C to coagulate the soft gelatinous protein which was then refrigerated overnight. The supernatant was siphoned off and the precipitated protein was separated from the liquid portion by centrifugation at 2000 rpm. It was subsequently frozen in thin layers in pans and dried in a Virtis freeze-dryer at −40 °C.

The chlorophylls, other pigments, and lipids were removed from the concentrate with boiling acetone in a Soxhlet apparatus. The concentrate was dried in a desiccator.

The protein concentrate can also be obtained from washed fresh duckweed. Instead of alkalinizing with NaOH, anhydrous ammonia was bubbled through the biomass to a pH of over 8.5. The alkalinized duckweed is then treated as described above.

Amino Acid Analysis of Protein Concentrate. Samples of about 1.5 g were weighed analytically into 18 × 150 mm Pyrex test tubes. Ten milliliters of 6 N HCl was added, and evacuation was performed until evolution of gas ceased. The sealed tubes were hydrolyzed at 100 °C for 24 h. After hydrolysis, the tubes were opened and taken to dryness in a vacuum desiccator over NaOH and H_2SO_4. Ten milliliters of 0.2 N sodium citrate buffer, pH 2.2, was added. Each tube was mixed thoroughly by prolonged use of a Vortex mixer to ensure solution of the amino acids. Insoluble material was removed by passage through a Millipore filter (0.22 μm). To obtain samples of suitable concentration for amino acid analysis, 100 μL was diluted to 10.0 mL. Two hundred microliters of this diluted material was used for each amino acid analysis.

Amino acid analyses were performed on a Beckman 119 amino acid analyzer equipped for automatic sample injection. Single column methodology, as described in Beckman Technical Bulletin A-TB-116, July, 1974, was used on a 0.9 × 48 cm column of AA-15 resin. Three replicate analyses were made on each hydrolyzate.

Nucleic Acid Determinations. Nucleic acid determinations were made by the method of Monroe and Heck (1966), using 206-nm absorbance.

Composition. The average chemical composition of each of the four species of solar-dried duckweed is shown in Table I. The duckweeds had a high percentage of water (94–95%), which was apparently related to the age of the culture. Actively growing stands of duckweed which are harvested daily remove older plants, leaving predominately new growth which contains 92–93% water.

The crude protein content (N × 6.25) of the dry duckweeds in this study ranged from 25.2 to 36.5%, although duckweeds harvested daily in July from lagoons containing about 50 mL/L of TKN averaged 40% crude protein.

Crude fiber (Table I) ranged from 8.8 to 11.0%, which is much lower than that of land forages, while the ash content of 13.7 to 17.1% is much higher than that of land forages (NAS–NRC, 1958). However, frequent harvesting results in a much cleaner plant and ash values frequently do not exceed 13%.

Amino Acid Composition. Table II shows the amino acid profile, nucleic acid, and protein contents of the four duckweed protein concentrates. The crude protein values of the protein concentrates ranged from 37.5 to 44.7%, an increase of 22–48% in crude protein over that of the dry plants. The amino acid composition of the four species of protein concentrates showed very little variation. The absence of cysteine does not preclude the presence of this amino acid in the concentrate, but does reflect that levels were below the limits of detection (>0.05 g/100 g of protein). Any tryptophan present would be destroyed by the acid hydrolysis.

The essential amino acid content of the duckweed protein compared to that of the FAO reference pattern (FAO, 1973) and to that of corn and rice (NAS–NRC, 1958) is shown in Table III. It is evident that the levels of the essential amino acids with the exception of methionine in duckweed protein concentrate meet the recommendations of the FAO reference pattern. Duckweed protein is a good

Table II. Amino Acid, Nucleic Acid, and Protein Composition of Duckweed Protein Concentrate

	L. gibba	*S. polyrhiza*	*S. punctata*	*Wolffia columbiana*	mean
			g/100 g of Protein		
aspartic	7.12	7.55	7.38	5.63	6.92 ± 0.88[a]
threonine	3.20	3.45	3.31	2.55	3.12 ± 0.40
serine	2.61	2.80	2.83	2.28	2.63 ± 0.25
glutamic	7.60	8.00	7.69	5.76	7.26 ± 1.01
proline	2.93	3.28	2.95	2.41	2.89 ± 0.36
glycine	3.79	3.95	3.93	3.04	3.68 ± 0.43
alanine	4.59	4.48	4.79	3.75	4.40 ± 0.45
valine	4.96	4.40	4.71	3.49	4.39 ± 0.64
methionine	0.83	0.83	1.07	0.87	0.90 ± 0.15
isoleucine	3.87	3.75	3.76	3.06	3.61 ± 0.37
leucine	7.15	6.85	6.88	5.83	6.68 ± 0.58
tyrosine	2.91	3.05	3.14	2.17	2.82 ± 0.44
phenylalanine	4.45	4.2	4.38	3.60	4.16 ± 0.39
histidine	1.89	2.15	1.90	1.18	1.78 ± 0.42
lysine	4.13	4.3	4.26	3.37	4.01 ± 0.43
arginine	4.29	5.25	4.86	3.78	4.54 ± 0.64
true protein[b]	66.32	68.29	67.8	52.77	63.80 ± 7.40
			g/100 g of Dry Matter		
nucleic acid	6.0	6.2	6.3	6.4	6.25
crude protein (N × 6.25)	37.5	40.0	42.0	44.7	41.05

[a] Standard deviation. [b] Sum of amino acids.

Table III. Essential Amino Acids in Duckweed Protein Concentrate Compared to FAO Reference Pattern, Corn, and Rice (g/100 g of Protein)

amino acid	duckweed[a]	FAO	corn	rice
Lys	4.0	4.2	2.3	3.2
Ile	3.6	4.2	6.2	5.2
Leu	6.7	4.8	15.0	8.2
Met	0.9	2.2	3.1	3.4
Phe	4.2	2.8	5.1	5.0
Thr	3.13	2.8	3.7	3.8
Val	4.4	4.2	5.3	6.2
Trp		1.4	0.6	1.3

[a] Mean of four species.

source of lysine, which is present in low amounts in grains. In many of the underdeveloped countries, corn and rice are the main foods consumed by the population. These foods are deficient in lysine, containing only 0.2–0.3% of the amino acid on a dry matter basis (FAO, 1954; NAS-NRC, 1958), whereas duckweed protein concentrate contains 1.6–2.0% on a dry matter basis. It is evident that duckweed protein concentrate has potential as an effective supplement to grains for animal and human consumption.

ACKNOWLEDGMENT

The authors are grateful to R. S. Myers for the production and harvest of the duckweeds used in this study.

LITERATURE CITED

AOAC, "Official Methods of Analysis", 12th ed., Washington, DC, 1975.
Bhanthumnavin, K., McGarry, M. G., *Nature (London)* **232**, 495 (1971).
Boyd, C. E., *Econ. Bot.* **22**, 359 (1968).
Chang, S.-M., Yang, C.-C., Sung, S.-C., *Bull. Inst. Chem., Acad. Sin.* **24**, 19 (1977).
Culley, D. D., Jr., personal communication, School of Forestry and Wildlife Management, Louisiana State University, Baton Rouge, LA, 1978.
Culley, D. D., Jr., Epps, E. A., *J. Water Pollut. Control Fed.* **45**, 337 (1973).
FAO, "Nutrition Studies. Rice and Rice Diets", Rome, 1954.
FAO, "Lives in Peril: Protein and the Child", FAO, Rome, 1970.
FAO, "Assessment of World Food Situation—Present and Future", UN World Food Conference Documents, Rome, Nov 1974.
FAO, "Population, Food Supply, and Agriculture Development", FAO, Rome, 1975.
FAO/Who, "Energy Requirements and Protein Requirements", FAO Nutrition Meeting Report Series 52, Rome, 1973, p 63.
Hillman, W. S., *Bot. Rev.* **27**, 221 (1961).
Hillman, W. S., Culley, D. D., Jr., *Am. Sci.* **66**, 442 (1978).
Maciejewska-Potapczk, W., Konopska, L., Narzumska, E., *Acta Soc. Bot. Pol.* **39**(2), 251 (1970); *Chem. Abstr.* **74**, 97 (1971).
Maciejewska-Potapczk, W., Konopska, L., Olechnowicz, K., *Biochem. Physiol. Pflanz.* **167**, 105 (1975); *Chem. Abstr.* **82**, 279 (1975).
Monroe, H. N., Heck, A., *Analyst (London)* **91**, 78 (1966).
Muztar, A. J., Slinger, S. J., Burton, J. H., *Can. J. Plant Sci.* **58**, 843 (1978).
NAS, "Making Aquatic Plants Useful: Some Perspectives for Developing Countries", Washington, DC, 1976.
NAS-NRC, "Composition of Cereal Grains and Forages", Publ. 585, Washington, DC, June 1958.
Rusoff, L. L., Gantt, D. T., Williams, D. M., Gholson, J. H., *J. Dairy Sci.* **60**, Suppl. 1, 161 (1977).
Rusoff, L. L., Zeringue, S. P., Achacoso, A. S., Culley, D. D., Jr., *J. Dairy Sci.* **61**, Suppl. 1, 186 (1978).
Said, Z. M., Culley, D. D., Jr., Standifer, L. C., Epps, E. A., Myers, R. W., Boney, S. A., *Proc. Annu. Meet.—World Maric. Soc.*, in press (1979).
Tan, Y. T., *J. Fish Biol.* **2**, 253 (1970).
Tulaganov, A., *Dokl. Akad. Nauk Uzb. SSR* **29**(7), 56 (1972); *Chem. Abstr.* **78**, 240 (26440p) (1973).
USDA, "The World Food Situation and Prospects to 1985", Foreign Agr., Econ. Rpts. No. 98, E.R.S., USDA, Washington, DC, 1974.

Received for review October 9, 1979. Accepted April 1, 1980. This work was supported by the Louisiana Agricultural Experiment Station and by Grant No. EM 78-G-01-5261 from the Department of Energy, Division of Industrial Energy Conservation.

Molecular Weight Distribution in the Lignin Sol

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The mechanism of delignification proposed by Szabo and Goring and that by Bolker and Brenner are compared and discussed on the basis of the Flory–Stockmayer distribution. A recently derived univariate Stockmayer distribution is used to illustrate the change of lignin molecular weight with delignification. A continuous distribution is proposed for application to the gel permeation chromatography (GPC) characterization. It is demonstrated that the initial chain size distribution is important in determining the shape of GPC elution curves as well as the post gel properties.

Chemical pulping, or delignification, is an important industrial process by which the lignin in wood is dissolved and separated from the cellulose fibers. Although this process has been practiced for a long time, the detailed chemistry involved is still poorly understood. Nevertheless, it appears that a fundamental principle in the physical chemistry of nonlinear polymers may emerge as a useful tool in elucidating the mechanism of delignification.

One of the proposed mechanisms of delignification involves the application of the Flory–Stockmayer (F–S) theory of condensation of polyfunctional polymers (Szabo and Goring, 1968; Goring, 1971; Bolker and Brenner, 1970; Bolker et al., 1977). In this application delignification is treated as a reverse process of the condensation beyond

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0021-8561/80/1428-0850$01.00/0