a similar trend, as 600 ppm SO_2 pretreatment for the S.D. samples gave reflectance values approaching those of the 200 ppm SO_2 pretreated A.D. sample.

The Cl_2 pretreatment usually reduced bacteria counts, but left an unpleasant flavor in the dried mushrooms except at the lowest concentration tested (100 ppm). Flavor preferences for A.D. mushrooms with varying concentrations of Cl_2 and SO_2 showed the following: 1) the water dipped control was preferred over all Cl_2 pretreated samples at a 99% or better C.L. 2) Flavor of samples pretreated with all concentrations of SO_2 studied, was preferred over untreated controls at a 95% or better C.L.

Reduction in bacteria counts (98 to 99%) for A.D. mushrooms were obtained when Cl_2 and SO_2 pretreatments were used. These results are shown as tests 1 and 2 in Table 3. S.D. mushrooms, following these pretreatments, had bacteria counts greater than their original values. This increase in bacteria count indicated a need for additional antimicrobial treatment. Second dips in potassium sorbate (600 ppm), methyl paraben (600 ppm) and sorbic acid (200 ppm) resulted in 1% or less remaining bacteria for A.D. samples. However only sorbic acid (200 ppm) gave comparable results for S.D. samples. Final plate counts in this test were 39,000/g for A.D. and 23,000/g for S.D. Moisture content for these samples were 5.8% for A.D. and 9.5% for S.D. These values and others, when compared with bacterial reduction in the dried product did not indicate an optimum moisture content (water activity) for bacterial reduction in mushrooms as has been found with other products (9).

Table 3. Percent remaining total aerobic plate count of pretreated dried mushrooms.

Test	Pretreatment[z]	Hot-air	Solar
1.	200 ppm SO_2	1.6	+[y]
2.	600 ppm SO_2	0.6	+
3.	600 ppm potassium sorbate	0.1	+
4.	600 ppm methyl paraben	0.7	+
5.	600 ppm sodium benzoate	—	+
6.	200 ppm sorbic acid	0.1	0.1

[z]All pretreatments included a 100 ppm Cl_2 dip; tests 3, 4 and 5 had 600 ppm SO_2 dip; test 6 had 200 ppm SO_2 dip.

[y]+ indicates plate counts of dried samples were higher than original values before drying.

In conclusion Cl_2 and SO_2 pretreatments can be used to produce A.D. mushrooms with reduced bacteria concentrations. Equivalent bacterial reductions in S.D. mushrooms required sorbic acid (200 ppm) combined with SO_2 (200 ppm) pretreatments. Although SO_2 pretreatment preserved color better than ascorbic acid, additional pretreatments are necessary to obtain the desirable lighter color of imported dried mushrooms.

References

1. Association of Official Analytical Chemists. 1975. Official Methods of Analysis. Twelfth Edition. Washington, D.C. Chpt. 46.
2. Brunell, H. J., W. B. Esselen, Jr. and F. P. Griffiths. 1943. Methods for quick freezing and dehydrating mushrooms. *Fd. Ind.* 15(11):74.
3. Bryan, W. L., C. J. Wagner, Jr. and R. E. Berry. 1978. Food drying with direct solar augmented by fossil energy. *Trans. ASAE* 21(6):1232-1236 + 1241.
4. Cruess, W. V. and E. M. Mrak. 1942. The dehydration of vegetables. *Fruit Prod. J.* 21:302.
5. Instruction Manual No. 4000 for Signature Model D-1 Color-Eye. 1969. Instrument Development Laboratories, Attleboro, Mass.
6. Komanosky, M., F. B. Talley, and R. K. Eskew. 1970. Air drying of cultivated mushrooms. *Fd. Technol.* 24:1020-1024.
7. Quality Control Spice Co., Inc. 1978. Specifications for freeze dried mushrooms.
8. Standard methods for the examination of water and waste water. 1965. Twelfth Edition. Amer. Pub. Health Assn., Inc., New York, NY. Pg. 91.
9. Troller, J. A. 1979. Food spoilage by microorganisms tolerating low-a_w environments. *Fd. Technol.* 33:72-75.
10. Wagner, C. J., Jr., W. L. Bryan and R. E. Berry. 1977. Preliminary solar drying studies of some Florida fruits and vegetables. *Proc. Fla. State Hortic. Soc.* 90:158-161.
11. ————, R. L. Coleman, W. L. Bryan and R. E. Berry. 1978. Preliminary studies on SO_2 absorption in mangos, nectarines and peaches prepared for drying. *Proc. Fla. State Hortic. Soc.* 91:117-119.

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PROTEIN EXTRACTION FROM AQUATIC WEEDS

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Abstract. **Five aquatic weeds** (Potamogeton illinoensis, Eichhornia crassipes, Pistia stratioties, Hydrilla verticillata, **and** Typha spp) **were harvested from Central Florida lakes and extracted with hot dimethylsulfoxide (DMSO). The DMSO extracts were analyzed for ethanol-precipitable proteins. Pondweed** (P. illinoensis) **contained the highest amount of extractable protein (5% of dry matter). Water hyacinth** (E. crassipes) **contained about 2%, and water lettuce** (P. stratioties), **hydrilla** (H. verticillata) **and cattails** (Typha spp) **contained less than 1% extractable protein. About 2½ times as much protein was extracted from pondweed leaves with DMSO as with an aqueous buffer. Acid hydrolysates of aqueous extracted proteins from pondweed leaves and water spinach** (Ipomea aquatica) **had similar amino acid levels which were comparable to levels in leaf proteins reported from other sources. Acid hydrolysates of DMSO-extracted proteins had high levels of most of the amino acids. However, methionine and cystine were barely detected in these hydrolysates. Loss of these amino acids was attributed to oxidation by contaminating DMSO during acid hydrolysis. Lysine, arginine and tyrosine were lower in hydrolysates of DMSO-extracted proteins than of aqueous-extracted proteins.**

Aquatic weeds are presently controlled in Florida by three techniques: chemical, biological and mechanical (7).

[1]Southern Region, Science and Education Administration, U. S. Department of Agriculture. For metric conversions see table at the front of this volume. Mention of a trademark of proprietary product is for identification only and does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture and does not imply its approval of the product to the exclusion of others which may also be suitable.

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Chemical herbicides in various liquid and solid formulations are dispersed into the lake or stream to kill submerged plants. One disadvantage of this procedure is the polluting effects of the herbicide and decaying treated plants. Biological control is the cleanest, safest and least expensive. Alligator weed is presently being controlled by the flea beetle (*Agasiches hygrophila*). Research is underway to control other weeds biologically. Insects and pathogens that kill aquatic weeds are being sought in habitats where weeds appear to be controlled naturally. Mechanical harvesting is an expensive control procedure when the harvest is not utilized. However, when utilized for feed or fodder, mechanical harvesting of renewable resources becomes an attractive method of aquatic weed control.

Water hyacinth (*Eichhornia crassipes*) is the only aquatic weed in Florida that is presently utilized after harvesting. It is processed commercially as a soil additive (11) and on a limited basis as a silage for animals (2, 3). Other aquatic weeds may be more amenable for processing (less water) and may be a better feed source. The nitrogen content (crude protein) of several aquatic weeds is higher than that of water hyacinth (5), and they may be a source of extractable protein for food or feed formulations. About 8% of the dry matter (DM) of water spinach (*Ipomoea aquatica*) was extracted as protein by dimethylsulfoxide (DMSO) (6). High protein yields from nuisance water weeds would be an additional incentive for developing food and feed utilization processes for harvested plants. This paper reports the results of examining of 5 Florida water weeds for protein extractability and the amino acid content of proteins extracted from one of them.

Materials and Methods

Water spinach was harvested from a small pond maintained at the laboratory. Aquatic weeds were obtained from local lakes. Leaves were washed, blotted with paper towels to remove adherent water, weighed and used immediately. DMSO was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, other chemicals and reagents were from Fisher Scientific Company, Pittsburgh, Pennsylvania.

Moisture content was determined on 100-g samples of aquatic weeds. These were dried at 60°C in vacuum to constant weight.

Protein was determined by the biuret method (10), by Potty's method (14), and also calculated from Kjeldahl-N (6.25 x N) determined as described (15).

Protein Extraction

Protein was extracted from aquatic weeds by hot (160°C) DMSO and precipitated with ethanol by the method described for water spinach (6) but with the following modifications: Fresh leaves were homogenized with 15 vol (v/w) of DMSO at 28°C in a Model 91-263 commercial blender (Waring Products Div. DCA, New Hartford, Cn.) set at high speed for 30 sec. The homogenate was heated with stirring in an appropriate size beaker (3 x vol of homogenate) on a Thermomix (Fisher Scientific Co., Pittsburgh, Pa.) with maximum heat setting so that the homogenate reached 160°C in 8 to 9 min. The beaker was then removed from the heat and the contents cooled, centrifuged and processed as described (6). Water soluble protein was extracted from water spinach and Illinois pondweed (*Potamogeton illinoensis*) and precipitated with trichloroacetic acid (6).

Amino Acid Analysis

Lyophilized samples of protein extracted from pondweed and water spinach were analyzed for amino acids as described by Wilkinson, et al. (15).

Results and Discussion

DMSO-Extractable Protein

Illinois pondweed contained the highest and cattails the lowest amount of extractable protein (Table 1). About 2% of the DM of water hyacinth was extractable protein. Water lettuce and hydrilla had low DM and less than 1% extractable protein. The value for pondweed (5.4% protein) is slightly less than the amount (6.7% of DM) extracted from water spinach under the same conditions (6). These conditions, solvent to leaf ratio of 15 to 1 (v/w) and 100% DMSO, were optimum for protein extraction from water spinach (6). Extractability of pondweed was not affected when the ratio of solvent to fresh-leaf-weight was varied from 3 to 1 to 20 to 1. Therefore, a unit weight of pondweed leaves could be extracted effectively with as little as 3 volumes of DMSO. However, extractability was better with 100% DMSO than with mixtures of DMSO and water. Even 10% H_2O in DMSO decreased protein extraction by about 20%. Thus, in a commercial scale extraction procedure, DMSO (B.P. 189°C) would have to be separated from leaf water (B.P. 100°C) in a solvent recovery step for efficient extraction with 100% DMSO. "Because of the relative volatilities of a DMSO-water system, distillation losses in a well designed system should not exceed 1% of the feed. The overall recovery of DMSO will depend on the character and quantity of impurities present but should exceed 95-99% of the feed" (1).

Table 1. Extractability of protein from aquatic weeds.[z]

	Dry matter (DM)	Extractable protein[y]
	%	% of DM
Illinois pondweed *(Potamogeton illinoensis)*	13.3 ± 0.8	5.4 ± 0.3
Water hyacinth *(Eichhornia crassipes)*	10.6 ± 0.8	1.8 ± 0.2
Water lettuce *(Pistia stratioties)*	6.9 ± 0.4	0.9 ± 0.1
Hydrilla *(Hydrilla verticillata)*	7.8 ± 0.4	0.6 ± 0.1
Cattails *(Typha spp.)*	27.5 ± 1.8	0.3 ± 0.1

[z]Values are means ± SD of 3 analyses of fresh leaves collected on 9/4/79.
[y]Protein analyzed by both biuret and Potty's methods.

Protein from Illinois Pondweed

Illinois pondweed is classified as a submerged plant with both submerged and floating portions. In the summer it grows rapidly and has dark green leaves. In the spring some of the leaves are brown and the stems constitute a large portion of the total. We found plants harvested in May to contain 36% leaf, 46% stem and 18% seed stalk. Only about 3.9% of the leaf (dry wt) was extracted as protein with DMSO. Plants harvested in June, July and August contained about 70% leaf and no seed stalk. About 5% of the leaf (dry wt) was extracted as protein with DMSO. These results suggest that any process for maximum utilization of pondweed for protein should use leaves harvested during the mid-summer months, when extractable protein

is highest. Also, extraction of leaves separated from the stems would result in the highest yield of protein.

DMSO was more effective than aqueous buffer in extracting protein from pondweed and water spinach (Table 2). DMSO extracted more leaf mass (column A) and the DMSO extracts contained more protein (column B). About 2 and 3 times as much protein was extracted by DMSO as by the aqueous buffer (column C). Since about 50% of leaf protein is chlorophyll- or membrane-bound and not extracted by aqueous buffers (13), some of the proteins extracted by DMSO are probably from these sources.

Table 2. Comparison between DMSO and aqueous buffer as protein extractants.

Extract	A Dry matter (DM) % of leaf DM	B Protein (N x 6.25) % of A	C (AxB) Protein % of leaf DM
Pondweed			
DMSO	17.2	28.1	4.8
Aqueous buffer	13.0	11.5	1.5
Water spinach			
DMSO	18.7	31.5	5.9
Aqueous buffer	13.6	16.6	2.3

Amino Acid Patterns of DMSO and Aqueous Extracts

The amino acid patterns of acid hydrolyzed extracts obtained from pondweed and water spinach are shown in Table 3. The amino acid compositions of the aqueous extracts were similar, and the levels were comparable to those reported for leaf protein from other sources (8). As compared to aqueous extracts, the DMSO extracts contained higher amounts of most of the amino acids. However, lysine, arginine, cystine, methionine and tyrosine were lower in the DMSO extracts. The low values for all but lysine can be explained by the oxidative loss during acid hydrolysis of the sample. Bates and Deyoe (4) noted during acid hydrolysis of ground sorghum grain and maize pollen that the presence of DMSO destroyed tyrosine, histidine and arginine. Lipton and Bodwell (12) found that as little as 0.01% DMSO in the hydrolytic mixture caused loss of methionine, cystine and tyrosine. They also reported the difficulty in completely removing DMSO from protein samples. Our samples for amino acid analysis were precipitated from DMSO with 4 volumes of ethanol (4:1 v/v) and then lyophilized. Although the samples were redried under reduced pressure before hydrolysis, sufficient DMSO was probably retained to cause the loss. Tyrosine was probably protected from excessive loss by the presence of phenolic compounds extracted from the leaves with the protein. Phenol protected tyrosine but not methionine and cystine from oxidation in 6N HCl (12).

We have no ready explanation for lower values of the basic amino acid, lysine. Lysine content of wool keratin was not affected when wool was heated in DMSO at 230°C for 24 hr (9). Also, this amino acid was not susceptible to oxidative loss during acid hydrolysis of several proteins (4, 12). However, DMSO extracts of leaves contain many non-protein substances (protein fractions from DMSO extracts are less than 1/3 protein; see Table 2), some of which might have catalyzed the degradation of this amino acid during acid hydrolysis.

Several amino acid values for the protein fractions of DMSO extracts are unreliable because of the probable contamination of the acid hydrolysis with DMSO. However, the similarity of the amino acid levels in the aqueous extract of pondweed to the levels in water spinach (Table 3) and in other leaf proteins (8) suggests that pondweed leaves would probably have similar nutritional value. Feeding trials with dry leaf meal, the protein fraction from DMSO extracts and the leaf residue after DMSO extraction would show whether pondweed could be considered a source of protein for animal diets. Commercialization of DMSO extraction to obtain a high protein concentrate would be feasible if it can be demonstrated that amino acids are not destroyed in the process, that the use and recovery of DMSO is economically feasible and that the leaf residue would also be useful in animal diets.

Table 3. Amino acid compositions of acid hydrolyzed protein fractions from DMSO and aqueous extracts of pondweed and water spinach.

Amino acid	Amino acid content: g/100 g recovered			
	Pondweed		Water spinach	
	DMSO	Aqueous	DMSO	Aqueous
Lysine	3.40	7.03	2.89	6.63
Histidine	3.17	2.59	5.06	2.43
Arginine	2.43	5.33	2.67	5.88
Aspartic	10.27	10.29	10.08	10.12
Threonine	5.99	5.33	6.44	5.38
Serine	6.22	5.50	5.42	4.99
Glutamic	11.77	10.77	12.33	11.11
Proline	5.09	4.61	5.12	4.70
Glycine	7.35	5.24	6.44	5.45
Alanine	7.33	5.87	7.22	5.77
Cystine (1/2)	0.30	3.54	0.29	3.64
Valine	7.21	6.32	7.36	6.25
Methionine	0.22	2.10	00	2.05
Isoleucine	6.16	5.18	6.34	5.00
Leucine	11.25	8.71	10.97	8.56
Tyrosine	2.63	4.60	2.53	4.86
Phenylalanine	7.28	5.40	7.07	5.78

References

1. Anon. 1974 (September). Dimethyl sulfoxide recovery and environmental engineering. Crown Zellerback Corp. Chem. Products Div., Camas, Wa.
2. Bagnall, L. O., R. L. Shirley and J. F. Hentges. 1973. Processing, Chemical Composition and Nutritive Value of Aquatic Weeds. Publ. #25, Univ. of Florida, Gainesville.
3. Bagnall, L. O., J. H. Balvin and J. F. Hentges. 1974. Processing and storage of water hyacinth silage. *Hyacinth Control J.* 12:73-9.
4. Bates, L. W., and C. W. Deyoe. 1973. Dimethyl sulfoxide and amino acid analyses. *Cereal Chemistry* 50:309-311.
5. Boyd, C. E. 1968. Fresh water plants: a potential source of protein. *Economic Botany* 22:359-368.
6. Bruemmer, J. H. and B. Roe. 1979. Protein extraction from water spinach (*Ipomoea aquatica*). *Proc. Fla. State Hortic. Soc.* 92:140-143.
7. Burkhalter, A. P., L. M. Curtis, R. L. Lazor, M. L. Beach and J. C. Hudson. 1976. Aquatic weed identification and control manual. Bureau of Aquatic Plant Research and Control, Florida Department of Natural Resources, Tallahassee, Florida.
8. Byers, M. 1971. The amino acid composition of some leaf protein preparations. Leaf protein IBP Handbook No. 20. Ed. by N.W. Pirie, Blackwell Sci. Publ., Oxford. p 25.
9. Friedman, M. and N. H. Koenig. 1971. Effect of dimethylsulfoxide on chemical and physical properties of wool. *Textile Res. J.* 41:605-609.
10. Gornall, A. G., C. S. Bardawill and M. M. David. 1949. Determination of serum proteins. *J. Biol. Chem.* 177:751-766.
11. Leach, J. R. 1976. Sarasota Weed and Feed, Sarasota, Florida. Personal communication.
12. Lipton, S. H. and C. E. Bodwell. 1973. Oxidation of amino acids by dimethylsulfoxide. *J. Agric. Food Chem.* 21:235-237.
13. Lundborg, T. 1980. Fractionation of leaf proteins by differential centrifugation and gel filtration. *Physiol. Plant.* 48:175-185.
14. Potty, V. H. 1969. Determination of proteins in the presence of phenols and pectins. *Anal. Biochem.* 29:535-539.
15. Wilkinson, W. S., C. Barber and F. E. Knox. 1968. Nutrient content of dehydrated coastal Bermuda grass and Pearl millet. *J. Agric. Food Chem.* 16:665-668.