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Chemical Characterization of Protein Contentrates of Duckweed (Family Lemnaceae)

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ABSTRAK

Kandungan protein di dalam supernatan dan hampas pulpa tiga spesis rumpaitek, *Spirodela polyrrhiza, Spirodela oligorrhiza, Wolffia columbiana* dan kultur campuran telah ditentukan. Kandungan protein di dalam supernatan dan hampas pulpa tersebut adalah lebih kurang sama kecuali bagi *S.oligorrhiza* yang mengandungi tiga kali ganda nilainya. Supernatan yang dimendakkan dengan kaedah asid dan pelarut organik (aseton, isobutanol dan isopropanol) menunjukkan bahawa aras protein yang termendak berbeza mengikut jenis pelarut. Secara purata cuma lebih kurang 20% protein yang dimendakkan dan dari jumlah ini lebih banyak protein dimendakkan oleh asid (pH 5.8) dan isopropanol (5% v/v). Analisis asid amino menunjukkan bahawa kandungan asid amino perlu di dalam pelepah, supernatan dan pulpa adalah setanding dengan yang terdapat pada susu ibu dan lembu, telur dan aras rujukan FAO kecuali bagi metionina. Pemecahan bahan terlarut kepada komponen-komponen tertentu dengan menggunakan *Sephacryl* S-200 dan *Sephadex* G-50 menghasilkan enam komponen kecuali *S.oligorrhiza* yang menghasilkan tujuh. Komponen pertama mempunyai saiz >250,000 dalton manakala yang dua terkecil <10,000. Antara keduanya saiz komponen yang terpencil mempunyai saiz 10,000 hingga 70,000. Pemecahan seterusnya dengan kaedah kromatografi pertukaran anion, *DEAE Sepharose* CL-6B telah menghasilkan 12 pecahan kecuali *W.columbiana* yang menghasilkan 9 komponen sahaja. Tiga komponen pertama bercas positif dan selebihnya bercas negatif. Kandungan faktor-faktor anti-pemakanan (tanin, perencat tripsin, nitrit dan nitrat) dalam dua spesis rumpaitek, *S.polyrrhiza* dan *Lemna perpusilla* serta protein pekat masing-masing didapati rendah. Secara keseluruhannya kajian ini mendapati bahawa rumpaitek berpotensi untuk dieksploitasi sebagai bahan makanan bagi kegunaan manusia atau haiwan memandangkan kandungan faktor-faktor anti-pemakanannya yang rendah sekurang-kurangnya dalam dua species rumpaitek yang telah dikaji, beserta dengan aras protein yang tinggi diikuti oleh profail asid amino perlu yang baik samada di dalam pelepah, pulpa dan supernatan atau protein pekatnya.

ABSTRACT

The amount of protein was determined in supernatant and residual pulp of three species of duckweed, *Spirodela polyrrhiza, oligorrhiza, Wolffia columbiana* and a mixed culture. Protein content of the supernatant and the residual pulp was similar except *S. oligorrhiza* in which the supernatant had triple the value.

Precipitation studies with acid and organic solvents (acetone, isobutanol and isopropanol) showed that the amount of protein precipitated varied with the solvent used. On average, only about 20% protein was precipitated from the supernatant. More protein was precipitated by acid (pH 5.8) and isopropanol (5% v/v). Amino acid analysis showed that the essential amino acid content of the whole frond, supernatant and pulp compared favourably with human and cow's milk, egg and FAO reference pattern, with the exception of methionine. Fractionation of the soluble material using *Sephacryl* S-200 and Sephadex G-50 yielded six components except *S. oligorrhiza*, which yielded seven components. The first component was > 250,000 daltons in size and the last two < 10,000. Further fractionation by anion exchanger, *DEAE Sepharose* CL-6B yielded 12 components, except *W. columbiana*, which had 9. The first three components had net positive charge while the rest had net negative change. Anti-nutritional factors were also determined in two duckweed species (*Spirodela polyrrhiza* and *Lemna perpusilla Torrey*) and their protein concentrates. Anti-nutritional factors were found to be low; *Spirodela polyrrhiza* had higher values than *Lemna perpusilla* except for trypsin inhibitor. However analysis of variance showed no significant difference ($p \leq 0.05$) in the levels of these anti-nutritional factors between the two species.

Keywords: Duckweed, protein concentrates, precipitation, fractionation by size, fractionation by charge, anti-nutritional factors, amino acid pattern

INTRODUCTION

The vascular aquatic plants known as duckweed which belong to the family Lemnaceae have been the subject of great interest during the past decade as a potential source of protein for feed supplement for both aquatic and terrestrial animal stocks, and also possibly for human consumption either directly or indirectly. The interest is due to the following characteristics of duckweed (Hillman and Culley 1979):

i. high protein content and favourable amino acid pattern (Amado et al., 1980; Rusoff et al. 1980; Culley et al. 1981; Maznah and Ahmad Hariza and Adeniji 1986; Mbagwee 1988)

ii. rapid growth and high turnover rates (Said *et al.* 1979)

iii. response to nutrient enrichment and hence converts various materials into high quality edible tissues (Harvey and Fox, 1973; Sutton and Orness, 1975; Culley *et al.* 1978)

iv. cloning is the dominant pattern in duckweed and therefore plants with desirable characteristics can be maintained

v. the plant does not have many pests and therefore can be easily and cheaply maintained.

Various studies have indicated that the plant can be used favourably as animal feed (Rusoff *et al.* 1977; Muztar *et al.* 1977; Truax *et al.* 1978; Rusoff *et al.* 1980). One species of duckweed, *Wolffia arrhiza*, has even been traditionally

consumed by villagers in Burma and Northern Thailand as part of their diet. If duckweed is to be considered an aquacultural crop, research should be carried out to study whether the plant can be grown, managed, harvested and processed economically. In terms of protein production per unit area, duckweed is shown to produce much more protein per hectare than soybean. Estimates by Culley and Myers (1980) showed that soybean yields about 672 kg crude protein per hectare or only about 8% of the crude protein produced by duckweed. But this estimate was based on small systems (0.04 ha). However, one major problem which must be addressed in developing commercial exploitation of the plant is the high water content. Water content of Lemna and Spirodela has been shown to be 92 – 97%. Therefore in order to commercially exploit the plant, an efficient and an inexpensive method of isolation and concentration of the proteins is needed. Studies which measure the physicochemical properties of the various proteins of duckweed and correlate these properties with potential nutritive value should be useful in designing methods and apparatus to extract useable proteins from this source. The present work attempts to provide an initial view of the physicochemical properties of various duckweed protein fractions which include determination of total soluble and insoluble protein, precipitation studies by pH changes and organic solvents on the soluble fraction, fractionation by size and charge and determination of anti-nutritional factors.

MATERIALS AND METHODS

Collection of Samples

Three species of duckweed, *Spirodela polyrrhiza, Spirodela oligorrhiza, Wolffia columbiana* and a mixed culture were grown in metal tanks (1.5 m 3 0.75 m 3 0.5 m) coated with epoxy paint in a greenhouse. For the determination of antinutritional factors, *Spirodela polyrrhiza* and *Lemna perpusilla Torrey* were collected from fish ponds within the campus of Universiti Pertanian Malaysia. Samples were cleaned and freeze-dried prior to analysis. Freeze-dried samples were then homogenized and the insoluble material was spun down at 10,400 g for 20 minutes.

Precipitation Studies

The soluble material was precipitated using acid and organic solvents - acetone, isopropanol and isobutanol (Sigma, Poole, U.K.). The precipitate was spun down at 2420 g for 15 minutes and later freeze-dried. The protein content of the precipitates was estimated using the Lowry method and amino acid content was determined by amino acid analyser (Beckman Model 119B) equipped for automatic sample injection. Single column methodology as described in Beckman Technical Bulletin A-TB-116 was used on a 0.9 3 48 cm column of W-1 resin.

Samples of about 5 mg were weighed into hydrolysis tubes. Two millilitres of 6N hydrochloric acid were added and evacuation was performed until evolution of gas ceased. The sealed tubes were heated at 110°C for a 24-hr period. After hydrolysis 0.2N, pH 2.2 sodium citrate buffer was added. For the best detection, the amount of protein applied to the analyser column was about 0.1 mg.

Fractionation by Size

The soluble material was characterized by passage through Sephadex G-50 and Sephacryl S-200 columns which had been calibrated using 3 mg/ml standard including cytochromee c (12,270), myoglobin (16.900), trypsin (23.700), pepsin (34.000), BSA (66,000), b-globulin (156,000) and g-glucoronidase (280,000) (Sigma). The column (2.5 3 85 cm), was packed and equilibrated for two bed volumes of 0.5 M, pH 7 phosphate buffer. The flow rate was set at 2.25 ml/min.

Fractionation by Charge

The soluble material was further characterized by anion-exchanger, DEAE-Sepharose CL-6B (Pharmacia, Uppsala, Sweden). The size of column was 1.5 cm 3 28.0 cm with a flow rate of 40.8 ml/hr. An ionic gradient of 0-0.5 M NaCl was applied. The elution profile of the effluent was recorded at 280 nm using an ISCO UA-5 monitor.

Determination of Anti-nutritional Factors

The method used for determination of tannin was as in AOAC (1980). To measure the trypsin inhibitor content the method used was by Kakade *et al.* (1974). The method of Berry *et al* (1982) was used to determine the content of nitrite and nitrate.

RESULTS

Protein Content of Duckweed

The amount of protein, based on the amount of recovered amino acids (excluding tryptophan) in four samples of unfractionated duckweed fronds, varied from 11.5 to 25% of dry matter (Tables 1-4). The protein content of the soluble material in the supernatant and the residual pulp also varied within species. With the exception of *S. oligorrhiza*, the amount of protein in the pulp was greater than the supernatant. Results of this study indicate that the residual pulp remaining after juice expression contained an appreciable amount of protein.

Amino Acid Pattern of Duckweed

Levels of amino acids in the whole fronds, pulp and supernatant showed similar distribution patterns in all samples studied. The major contributors of essential

TABLE 1
Protein and amino acid composition of whole fronds, supernatant and pulp of *Spirodela oligorrhiza*

Amino Acids	Whole Fronds		Pulp		Supernatant	
	g/100 Protein	g/100g Duckweed	g/100 Protein	g/100 Duckweed	g/100g Protein	g/100g Duckweed
Aspartic	12.50	2.87	10.06	0.68	16.83	1.92
Threonine	5.94	1.46	5.15	0.35	4.74	0.56
Serine	4.27	1.08	3.00	0.20	5.60	0.67
Glutamic	12.41	2.84	10.81	0.73	10.27	1.22
Proline	5.46	1.25	6.23	0.42	2.80	0.33
Glycine	5.32	1.82	6.05	0.41	4.84	0.55
Alanine	6.15	1.61	6.73	0.45	7.05	0.80
Valine	6.20	1.62	7.28	0.49	5.39	0.61
Methionine	1.20	0.27	0.63	0.04	0.82	0.09
Isoleucine	4.76	1.39	5.50	0.37	3.69	0.42
Leucine	7.19	1.65	10.45	0.70	7.57	0.86
Tyrosine	3.24	0.75	3.04	0.21	6.14	0.70
Phenylalanine	6.20	1.62	60.93	0.47	6.65	0.76
Histidine	1.34	0.31	2.40	0.16	2.04	0.23
Lysine	8.02	1.94	8.41	0.56	5.53	0.63
Arginine	9.50	2.24	7.31	0.49	9.97	1.13
	% of Dry matter					
True protein	25.0		6.7		18.3	

[1]Lyophilized samples of duckweed were used

TABLE 2
Protein and amino acid composition of whole fronds, supernatant and pulp of *Spirodela oligorrhiza*

Amino Acids	Whole Fronds		Pulp		Supernatant	
	g/100 Protein	g/100g Duckweed	g/100 Protein	g/100 Duckweed	g/100g Protein	g/100g Duckweed
Aspartic	11.50	2.27	13.39	6.81	11.48	1.68
Threonine	5.47	1.08	5.31	2.70	6.30	0.92
Serine	3.81	0.75	5.53	2.81	5.96	0.87
Glutamic	12.50	2.47	11.89	6.05	11.24	1.65
Proline	6.02	1.19	4.62	2.35	6.78	1.00
Glycine	5.60	1.41	5.05	2.57	5.94	0.87
Alanine	6.40	1.36	6.63	2.37	6.81	1.00
Valine	6.69	1.42	5.86	2.98	6.20	0.91
Methionine	1.14	0.23	1.23	0.63	0.81	0.12
Isoleucine	5.21	1.33	4.32	2.20	4.29	0.63
Leucine	9.31	1.94	7.76	3.95	9.09	1.33
Tyrosine	3.79	0.85	6.02	3.07	4.15	0.61
Phenylalanine	6.44	1.77	6.17	3.14	6.67	0.98
Histidine	1.30	0.26	2.38	1.21	0.13	0.02
Lysine	7.78	1.74	5.97	3.04	7.49	1.10
Arginine	7.04	1.69	7.88	4.01	6.65	0.98
	% of Dry matter					
True protein	22.80		8.46		14.64	

[1]Lyophilized samples of duckweed were used

TABLE 3
Protein and amino acid composition of whole fronds, supernatant and pulp of *Wolffia columbiana*

Amino Acids	Whole Fronds		Pulp		Supernatant	
	g/100 Protein	g/100g Duckweed	g/100 Protein	g/100 Duckweed	g/100g Protein	g/100g Duckweed
Aspartic	14.31	1.57	18.06	1.97	11.20	1.15
Threonine	5.26	0.58	5.67	0.62	6.41	0.66
Serine	4.82	0.53	4.91	0.54	5.20	0.54
Glutamic	11.41	2.21	11.08	1.21	11.50	1.18
Proline	5.89	0.65	4.00	0.44	7.22	0.74
Glycine	5.29	0.58	5.30	0.59	5.82	0.60
Alanine	6.28	0.70	7.00	0.76	6.21	0.64
Valine	5.98	0.66	5.80	0.63	5.93	0.61
Methionine	0.95	0.11	0.76	0.08	Trace	Trace
Isoleucine	4.62	0.51	3.82	0.42	4.54	0.46
Leucine	9.24	1.02	7.42	0.81	10.21	1.05
Tyrosine	2.88	0.32	6.48	0.71	3.12	0.32
Phenylalanine	6.99	0.77	6.26	0.68	7.14	0.73
Histidine	1.09	0.12	2.07	0.23	1.72	0.17
Lysine	7.32	0.80	5.47	0.60	7.75	0.79
Arginine	7.67	0.84	5.85	0.64	6.26	0.64
			% of Dry Matter			
True protein		12.47		2.27		10.20

[1]Lyophilized samples of duckweed were used

TABLE 4
Protein and amino acid composition of whole fronds, supernatant and pulp of mixed culture

Amino Acids	Whole Fronds		Pulp		Supernatant	
	g/100 Protein	g/100g Duckweed	g/100 Protein	g/100 Duckweed	g/100g Protein	g/100g Duckweed
Aspartic	12.41	1.33	15.81	5.30	12.34	0.72
Threonine	6.10	0.59	5.37	1.82	5.78	0.34
Serine	4.27	0.51	4.37	1.48	4.47	0.36
Glutamic	12.92	1.23	14.83	5.03	12.66	0.74
Proline	4.74	0.46	3.64	1.23	5.93	0.34
Glycine	5.00	0.49	4.35	1.48	5.46	0.32
Alanine	6.68	0.79	7.25	2.46	7.12	0.41
Valine	5.86	0.97	5.02	1.70	5.75	0.39
Methionine	0.97	0.04	1.32	0.45	0.78	0.05
Isoleucine	0.37	0.53	3.53	1.20	4.92	0.29
Leucine	8.16	0.76	6.56	2.23	7.34	0.43
Tyrosine	6.33	0.51	7.06	2.40	3.52	0.20
Phenylalanine	5.96	0.70	5.06	1.72	6.36	0.37
Histidine	2.35	0.35	2.07	0.70	2.42	0.14
Lysine	6.43	0.48	5.63	1.91	7.06	0.41
Arginine	7.48	0.76	8.12	2.76	7.10	0.41
			% of Dry Matter			
True protein		10.90		5.70		5.20

[1]Lyophilized samples of duckweed were used

amino acids were Leu, Lys, Val and Arg. Methionine content was low and most was available in the soluble material (Table 1 - 4). The essential amino acid content of the whole frond, supernatant and pulp compared favourably with those of human and cow's milk, egg and FAO reference pattern, with the exception of methionine (Table 5).

Precipitation Studies on the Soluble Material

The percentage of protein precipitated by acid, acetone, isopropanol and isobutanol for each species is presented in Table 6. Results indicat that the higher percentage of protein precipitates of *S. polyrrhiza* and mixed culture were obtained by isopropanol precipitation being 25 and 26% respectively. On the other hand, for the other two species *W. columbiana and S. oligorrhiza*, a higher percentage of protein precipitate was obtained from acid precipitation than by isopropanol. The amino acid composition of the protein precipitates was found to be similar for all samples. The amounts of aspartic acid and glutamic acid were high. The amount of essential amino acids was favourable, with the exception of methionine.

Fractionation by Size

The elution profiles through Sephacryl S-200 and Sephadex G-50 of the four samples of duckweed (Fig. 1- 4) showed six distinct peaks for three samples (*S. polyrrhiza, W. columbiana and mixed culture*) with the chromatogram of *S. oligorrhiza* displaying an extra peak. However, the elution profiles of the four samples were more or less similar in pattern. Rough estimates of the size of duckweed proteins are presented in Table 7. The first peak of all samples was eluted at the void volume, suggesting that the component could constitute a large protein with a molecular weight >250,000 daltons. The last two peaks probably

TABLE 5
Essential amino acids compositionof duckweed whole fronds supernatant and pulp compared to FAO reference pattern[1], corn and rice

	Duckweed[2]						
Amino acid	Whole frond	Supernatant	Pulp	FAO	Rice	Human milk	Egg
Thr	5.6	5.4	5.8	4.2	3.2	4.3	4.7
Val	6.3	6.3	5.9	5.0	5.2	5.5	6.6
Met	1.1	0.9	0.8	2.2	3.4	4.2[3]	5.7[3]
Ile	3.3	3.5	3.2	4.2	5.2	4.6	5.4
Leu	8.6	8.5	8.9	7.0	8.2	9.3	8.6
Phe + Tyr	9.8	9.5	10.2	6.0	5.0	7.2	9.3
Lys	7.7	6.6	6.9	5.5	3.2	6.6	7.0

1. From Joint FAO/WHO Ad hoc expert committee, Energy and Protein Requirement, WHO Tech. Rep. No. 552, Geneva, Switzerland, 1973
2. Mean of three species
3. Methionine + cystein

TABLE 6
Specific precipitation studies of duckweed supernatant

Precipitate	% Soluble Protein Precipitates			
	Spirodela oligorrhiza	Spirodela polyrrhiza	Mixed Culture	Wolffia columbiana
Acid –1 pH	13.1	5.6	0.8	6.8
Acid –2 pH	6.8	7.7	3.5	0.1
Total	19.97	13.40	14.3	15.9
5% Isopropanol	6.6	20.1	14.0	2.4
10% Isopropanol	6.3	4.6	11.0	1.0
15% Isopropanol	–	–	–	6.6
Total	12.9	24.7	26.0	10.0
5% Acetone	2.0	13.1	4.1	1.1
10% Acetone	0.4	5.0	2.8	1.1
15% Acetone	4.0	2.2	1.6	4.8
Total	6.4	21.3	8.5	7.0
5% Isobutanol	–	6.2	5.1	4.0
10%Isobutanol	–	1.6	0.4	4.4
15% Isobutanol	–	0.9	3.4	3.6
Total	–	8.7	8.9	12.0

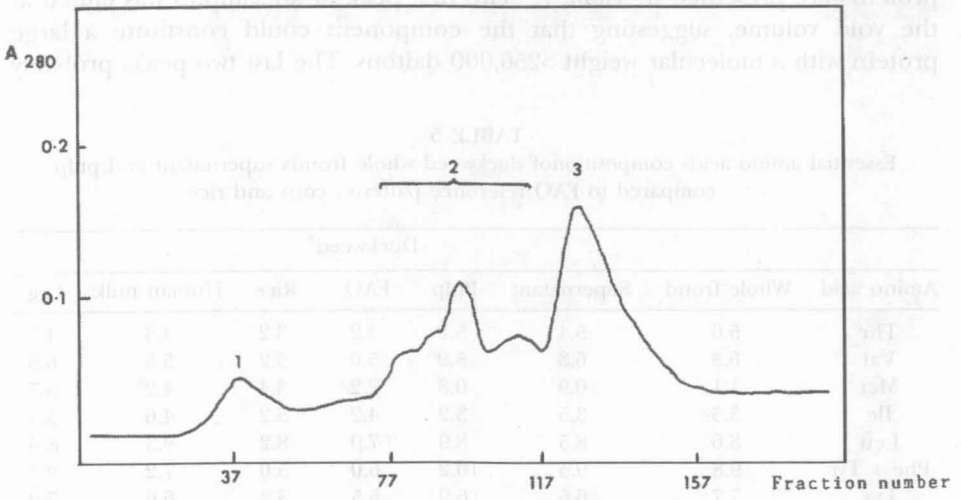

Fig 1. The elution profile of the soluble material of Spirodela polyrrhiza on Sephacryl S-200 column (2.5 x 70 cm). The flow rate was 2.3 ml/ min. Eluant: 0.5M Phosphate buffer, pH 7.

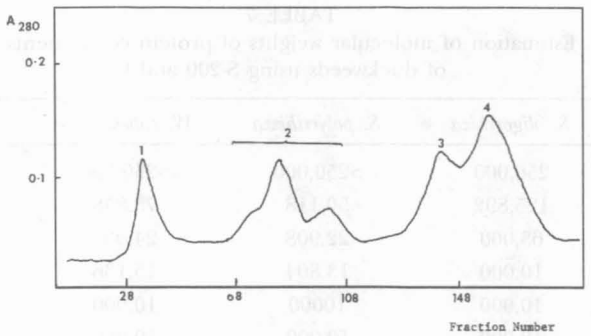

Fig 2. The elution profile of the soluble of Spirodela oligorrhiza on Sephacryl S-200 column (70 x 2.5 cm). The flow rate was 2.3 ml/min. Eluant: 0.5 Phosphate buffer, buffer, pH 7.

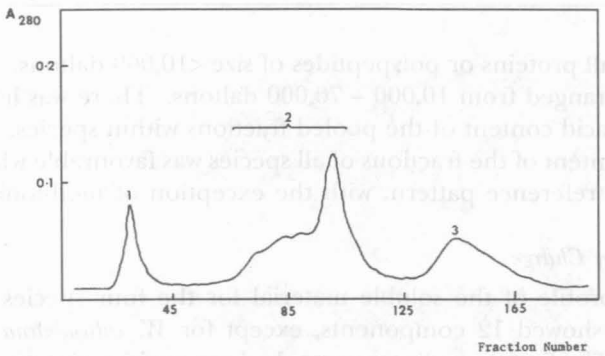

Fig 3. The elution profile of the soluble material of Wolffia columbiana on Sephacryl S-200 column (2.5 x 70 cm). The flow rate was 2.3 ml/min Eluant : 0.5M phosphate buffer, pH 7.

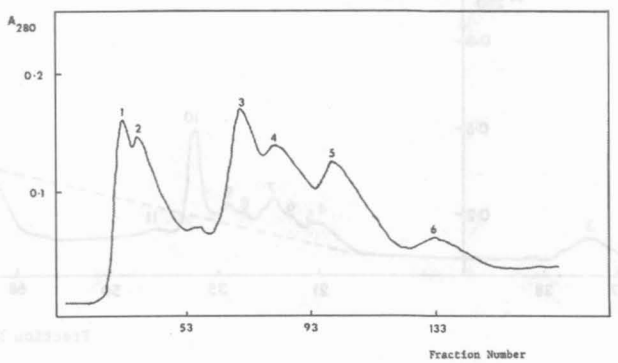

Fig 4. The elution profile of the soluble material of wild duckweed on ephacryl S-200 column (70 x 2.5). Flow rate was 2.3 ml/ min. Eluant, 0.5M Phosphate buffer, pH 7.

TABLE 7
Estimation of molecular weights of protein components of duckweeds using S-200 and F-50

Peaks	*S. oligorrhiza*	*S. polyrrihizza*	*W. columbiana*	Mixed Cultere
1	250,000	>250,000	>250,000	>250,000
2	125,892	50,118	75,858	250,000
3	63,000	22,908	24,000	100,000
4	10,000	13,804	15,136	38,019
5	10,000	10000	10,000	10,000
6	10,000	10,000	10,000	10,000
2-1	> 30.000	> 30,000	> 30,000	
2-2	> 30,000	24,000	> 30,000	
2-3	16,596	4,800	11,482	
2-4	5,754		2,240	

constitute small proteins or polypeptides of size <10,000 daltons. For the other proteins, size ranged from 10,000 – 70,000 daltons. There was little difference in the amino acid content of the pooled fractions within species. The essential amino acid content of the fractions of all species was favourable when compared with the FAO reference pattern, with the exception of methionine.

Fractionation by Charge

The elution profile of the soluble material for the four species of duckweed (Fig. 5 – 8) showed 12 components, except for *W. columbiana* which had 9 components. The first three components had net positive charge, while the rest had net negative charge.

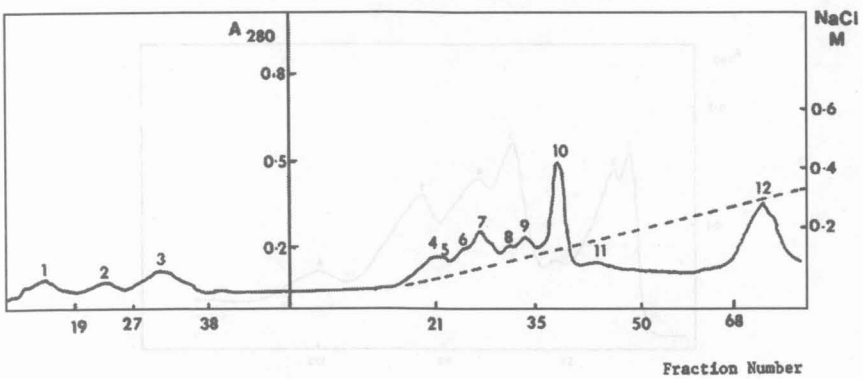

Fig 5. Elution profile of the soluble material of Spirodela polyrrhiza on DEAE-Sepharose Cl-6B. Column : 15 x 28.0 cm. Flow rate: 40.8 ml/h. Eluant: 0.01M trishydrochloride buffer, pH8. Continuous NaCl gradient.

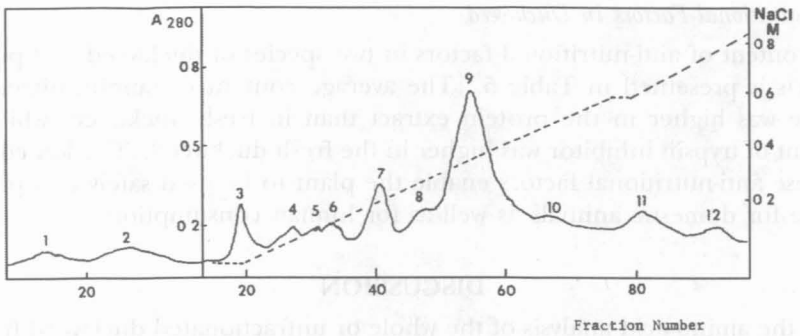

Fig 6. Elution profile of the soluble material of Spirodela oligorrhiza on DEAE-Sepharose Cl-6B. Column : 15 x 28.0 cm. Flow rate: 40.8 ml/h. Eluant: 0.01M trishydrochloride buffer, pH8. Continuous NaCl gradient.

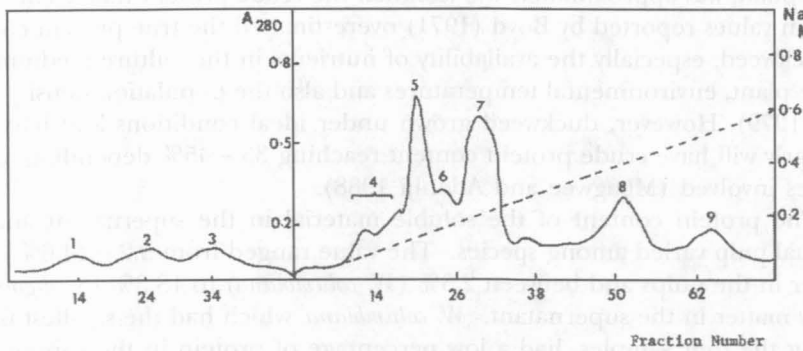

Fig 7. Elution profile of the soluble material of Wolffia columbiana on DEAE-Sepharose Cl-6B. Column : 15 x 28.0 cm. Flow rate: 40.8 ml/h. Eluant: 0.01M trishydrochloride buffer, pH8. Continuous NaCl gradient.

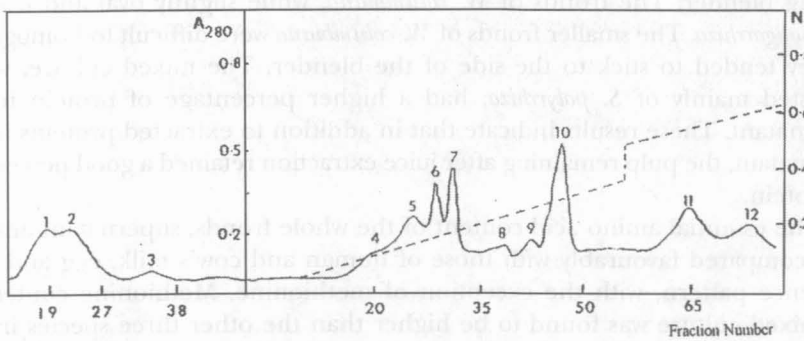

Fig 8. Elution profile of the soluble material of the mixed culture on DEAE-Sepharose Cl-6B. Column : 1.5 x 28.0 cm. Flow rate: 40.8 ml/h. Eluant: 0.01M trishydrochloride buffer, pH8. Continuous NaCl gradient.

Anti-nutritional Factors in Duckweed

The content of anti-nutritional factors in two species of duckweed and protein extracts is presented in Table 5. The average content of tannin, nitrite and nitrate was higher in the protein extract than in fresh duckweed, while the content of trypsin inhibitor was higher in the fresh duckweed. The low content of these anti-nutritional factors enable the plant to be used safely as a protein source for domestic animals as well as for human consumption.

DISCUSSION

From the amino acid analysis of the whole or unfractionated duckweed fronds, the amount of protein was found to vary slightly between species and the mixed culture. This finding is consistent with the results reported earlier by Amado *et al.* (1980), that the crude protein values ranged from 14 – 37% of the dry weight. The true protein based on the sum of recovered amino acids excluding tryptophan, was approximately 6% less than the crude protein value. The crude protein values reported by Boyd (1971) overestimated the true protein content of duckweed, especially the availability of nutrients in the culture medium, age of the plant, environmental temperatures and also the population density (Said *et al.* 1979). However, duckweed grown under ideal conditions and harvested regularly will have crude protein content reaching 35 – 45% depending on the species involved (Mbagwee and Adeniji 1988).

The protein content of the soluble material in the supernatant and the residual pulp varied among species. The value ranged from 5.2 – 14.6% of dry matter in the pulps and between 2.3% (*W. columbiana*) to 18.3% (*S. oligorrhiza*) of dry matter in the supernatant. *W. columbiana*, which had the smallest fronds among the four samples, had a low percentage of protein in the supernatant, whereas the amount of protein in *S. polyrrhiza* was divided equally between the pulp and the supernatant. In the case of *S. oligorrhiza*, more protein was found in the supernatant than in the pulp. The difference in the protein content of the supernatants is probably due to the extend of homogenization by the Waring blender. The fronds of *W. columbiana*, while slightly oval and leaf-like for *S. oligorrhiza*. The smaller fronds of *W. columbiana* were difficult to homogenize as they tended to stick to the side of the blender. The mixed culture, which consisted mainly of *S. polyrrhiza*, had a higher percentage of protein in the supernatant. These results indicate that in addition to extracted proteins in the supernatant, the pulp remaining after juice extraction retained a good percentage of protein.

The essential amino acid content of the whole fronds, supernatant and the pulp compared favourably with those of human and cow's milk, egg and FAO reference pattern, with the exception of methionine. Methionine content of the mixed culture was found to be higher than the other three species in this study, but the value was still lower than the FAO reference pattern which gave the total value of S-containing amino acids. In this study, cysteine content of the duckweed was not determined. However the amount of lysine was about 1.5

TABLE 8
Anti-nutritional factors of two species of duckweed and protein extracts

	Tannin (g/100g)	Trypsin Inhibitor (TIU/ml)	Nitrite (μg/g)	Nitrate (μg/g)
S. polyrrhiza				
Fresh	0.02 + 0.00	4.75 + 0.29	0.77 + 0.08	0.84 + 0.14
acid extract	0.21 + 0.01	3.37 + 0.60	7.08 + 0.63	1.51 + 0.75
Acetone extract	0.26 + 0.05	1.52 + 0.21	7.13 + 1.84	3.70 + 0.18
L. perpusilla				
Fresh	0.02 + 0.01	4.90 + 0.17	0.73 + 0.01	0.65 + 0.03
Acid extract	0.17 + 0.02	3.10 + 0.15	6.04 + 0.31	1.77 + 0.09
Acetone extract	0.22 + 0.03	2.05 + 0.13	6.38 + 0.45	2.52 + 0.19

times the value recommended by FAO. This shows that duckweed protein could be a good source of lysine, which is present in low amounts in grains. Apart from protein, a study by Houstein *et al.* (1990) had demostrated that cultured duckweed had high concentration of trace minerals and pigments, particularly b-carotene and also xanthophyll. The total content of carotenoids in duckweed is 10 times higher than that in terrestrial plants.

The problem associated with duckweed for commercial exploitation is the high water content of the crude plant. High water content and the inability to remove the water economically remain the main hindrance to the development of aquatic plants such as duckweed as agricultural crops. All drying technologies consume a large amount of energy, which is expensive, except for waste heat and solar energy. Sun drying would probably be the most economical method, provided drying time is not of importance. Environmental factors, such as wind and rain may present problems when drying duckweed in an open area. Utilization of presses at 780 – 7810 psi may reduce the crude protein by approximately 66 – 71%; hence, pressing is not an acceptable procedure. Therefore, a method of protein concentration is needed. One way this can be achieved is by precipitating the proteins from the supernatant either by acid or organic solvents while selecting conditions in which a good percentage of the protein is retained in the pulp. An advantage of duckweed is that it has a much lower percentage of crude fibre, (about 10% of dry weight) Then land forages. However, if the soluble material is preferred, another species of duckweed should be chosen for this purpose, e.g. *S. oligorrhiza* is preferred to *W. columbiana.*

Specific precipitation studies with acid and organic solvents on the soluble material indicated that the maximum value of protein precipitated was only 26% of the total protein in the supernatant. Hydrochloric acid and isopropanol precipitated more protein than either acetone or isobutanol. Other solvents (such as other alcohols and chloroform) could also be used to precipitate the

proteins. Fractionation of the soluble material by gel filtration using Sephacryl S-200 and Sephadex G-50 resins and ion-exchange chromatography on DEAE Sepharose CL-6B showed a mixture of proteins in the soluble material. The elution profiles of the four samples were more or less similar in pattern. However, the relative amount of the components between samples was different.

In conclusion, this study indicated that the four samples studied had relatively high percentages of protein which could be further increased by proper management of the duckweed culture system. The essential amino acid composition of the whole fronds, pulp, the supernatant and protein concentrate compared favourably with the FAO reference pattern. This factor, coupled with the low contents of anti-nutritional factors, can be used as a basis to enrich foods with individual amino acids, e.g.: lysine, and to combine protein sources for complementation and supplementation leading to an enrichment in nutritional value.

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