Potential for Protein Concentrates from Alfalfa and Waste Green Plant Material

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Leaf protein concentrates were extracted from green plant wastes (beet tops, potato vines, lima bean vines, carrot tops, and lakeweeds). Yields of solids, nitrogen, and proximate analyses are given for all fractions of the extraction process. The amino acid composition indicated that the patterns of essential amino acids compared well with recommended patterns except for methionine. Green plants are the largest unexploited source of protein and large scale protein production from forage species such as alfalfa and from plant wastes could supply the protein requirements of increasing world populations.

Malnutrition is a major international problem. Estimates are that one third to one half of the world's population suffers from nutritional deficiencies caused mostly by lack of adequate good quality protein. Altschul (1967) has estimated that the world will be 10,000,000 tons short of its protein needs by 1970. Disastrous famines have been predicted by 1975 (Paddock and Paddock, 1967).

Many ways of filling the protein gap have been proposed. Possible measures include production of fish protein concentrates, and production of protein from petroleum, seed protein, and plant cell culture, along with conventional attempts to increase normal agricultural production (Altschul, 1967; Brown, 1967; Hildebrant, 1966; Johnson, 1967; Pirie, 1967; United Nations, 1968).

Photosynthesis provides the primary source of food and energy for the world. For practical purposes it is also the only nondepletable source. Most of the suggested sources for protein include either the concentration or conversion of protein initially formed from green plants. Plant proteins (especially leaf proteins) should seriously be considered as a major future food source (Pirie, 1966; Stahmann, 1968). The major trouble with green plants as a source of protein is that their protein concentration is too low and their fiber too high to be used as a major source. Therefore, man must rely upon secondary sources in which the protein is higher in concentration and undigestible fiber low. The use of such secondary sources is usually very inefficient, since most of the original plant nutrients are lost in the concentration of protein and removal of fiber from plant material—for example, the cow is only about 10 to 15% efficient in converting plant protein to animal protein.

The solution to this problem of inefficiency would seem to be the development of mechanical means of extracting and concentrating protein from green leaves. Much work has been done in this area by Pirie and his associates (Morrison and Pirie, 1961; Pirie, 1966). Pirie's method consists basically of pulping the green material, expressing the juice, and precipitating the protein by heat. However, Hartman *et al.* (1967) thought that good amounts of nutrients were lost in the whey after precipitating the protein and suggested spray-drying the expressed juice.

Both in vitro and in vivo studies suggest that leaf protein concentrates would be a satisfactory protein food. Amino acid composition analyses have shown that leaf protein concentrates contain satisfactory amounts of all the eight essential amino acids except methionine (Gerloff *et al.*, 1965; Hartman *et al.*, 1967). An in vitro enzymatic digestion study suggested that leaf protein has a high biological value, somewhat less than milk and close to beef (Akeson and Stahmann, 1965). Studies with chicks, rats, pigs, and even infants recovering from malnutrition suggest that leaf protein could be a valuable protein source or supplement (Duckworth and Woodham, 1961; Duckworth *et al.*, 1961; Waterlow, 1962). Henry and Ford (1965) studied protein concentrates prepared from 14 different species and found that half had a biological value over 70. Singh (1967) showed that alfalfa protein was a good supplement for low-lysine and low-protein diets in children. He also states that preliminary results of a study with children on a diet consisting of 33 to 36 grams of alfalfa protein indicate a true digestibility of 85% and a biological value of 65. As a supplement to protein-deficient diets, he reported that alfalfa protein was better than skimmed milk powder. Sur (1967) has done a thorough study on water hyacinth leaf protein. Again indications were that methionine was somewhat limiting, since methionine supplementation increased the biological value and digestibility. He also demonstrated that leaf protein and rice protein have complementary amino acid patterns and good results are obtained in rat-feeding studies. Pirie and associates have given recipes for the use of leaf protein concentrates in food for humans (Byers *et al.*, 1965; Morrison and Pirie, 1960). Singh (1967) reported that such concentrates could be blended into Indian foods that were readily eaten.

This report summarizes work done in the preparation and analysis of leaf protein concentrates from several plant waste sources.

MATERIALS AND METHODS

Plant starting materials used in these studies were lakeweed (272 pounds) dredged from nearby Lake Mendota, the bulk of which was a *Myriophylum* species along with a small amount of a *Cladophora* species, beet tops (*Beta vulgaris*, 232 pounds), potato vines (*Solanum tuberosum*, 211 pounds), lima bean vines (*Phaseolus limensis*, 264 pounds) which consisted of the whole part of the plant above the ground (including the beans, for vines alone were not available), and carrot tops (*Daucus carota*, 173 pounds). Pea vine data were obtained from a previous report (Hartman *et al.*, 1967). The carrot tops and lima bean vines were harvested late in the season and

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Table I. Yields of Expressed Juices (EJ) and Protein Precipitates (PPT) from Plant Waste Materials[a]

Sample	% Yield	
	Total solids basis	Nitrogen basis
Lakeweeds		
EJ	8.12	13.6
PPT	0.64	1.46
Beet tops		
EJ	37.5	39.8
PPT	11.4	16.1
Potato vines		
EJ	32.9	48.2
PPT	12.1	26.1
Lima bean vines		
EJ	10.1	17.5
PPT	4.50	9.36
Carrot top		
EJ	10.4	21.3
PPT	7.41	14.1
Alfalfa		
EJ	35.1	44.2
PPT	16.5	26.5
Spray-dried	27.2	43.0
Pea vine, spray-dried	29.1	43.9

[a] Yield data relative only and not maximum possible. See text.

were very tough and dry. All of the material was processed within 3 to 4 hours after being obtained from the field.

The machine used for pulping and extracting was described by Hartman *et al.* (1967) and is smaller but similar in principle to that used by Morrison and Pirie (1961). The expressed juice was heated by injection of steam until its temperature reached 80° C. It was then allowed to stand overnight to allow the protein precipitate to form. The precipitates from beet tops and lakeweed were collected by filtration through a double layer of cheesecloth. The lakeweed juice contained many fine particles which passed through the cheesecloth, so the supernatant whey was centrifuged and solids were placed with the precipitate collected in the cheesecloth. The remaining precipitates were collected by a Sharples continuous flow centrifuge.

Moistures were determined in duplicate by azeotropic distillation with toluene, using about 25 grams of sample for each determination. There was a sampling error of about ±5% with potato vine and lima bean due to the heterogeneous nature of the material.

Protein (N × 6.25), fat, and crude fiber analyses were made by the General Laboratory Division, Wisconsin State Department of Agriculture. Ash was determined by the AOAC standard method (Association of Official Agricultural Chemists, 1965). All samples were freeze-dried before analysis.

Amino acid analyses were performed by the method of Spackman *et al.* (1958) using a Beckman-Spinco Model 120 amino acid analyzer. For cysteine and methionine determinations the protein was first oxidized by performic acid (16 hours at 0° C.). The protein was then hydrolyzed and the amount of cysteic acid and methionine sulfone determined with the amino acid analyzer (Bailey, 1967).

Tryptophan was determined by a process involving hydrolysis by barium hydroxide in the presence of starch (Bailey, 1967). An ionic exchange column of the amino acid analyzer was replaced with a potato starch column (60 cm.) and the hydrolyzate was eluted through this column with 0.1*M* HCl.

Table II. Composition of Starting Material (SM), Residue (R), Expressed Juice (EJ), and Protein Precipitate (PPT) of Waste Plant Material

	% Moisture	% Crude Protein	% Crude Fat	% Crude Fiber	% Ash	N-Free Extract
Lakeweed						
SM	83	7.82	1.40	8.38	37.6	44.8
R	76.5	9.69	1.60	10.42	23.9	54.4
EJ	96	13.11	3.44	1.81	34.4	47.2
PPT	91	17.68	3.90	0.95	39.3	38.2
Beet tops						
SM	90.7	26.76	4.35	8.35	13.6	46.9
R	82.5	21.88	2.32	13.12	13.3	49.4
EJ	94.1	28.37	3.89	0.44	20.8	46.5
PPT	75.8	37.93	8.33	0.64	22.2	30.9
Potato vines						
SM	91	19.04	4.85	17.12	16.0	43.0
R	85	14.72	3.49	25.01	11.9	44.9
EJ	93.9	27.88	4.78	0.55	26.4	40.4
PPT	74.5	41.26	11.98	1.83	14.9	30.0
Lima bean vines						
SM	61	16.53	1.70	17.48	8.2	56.1
R	61	12.80	1.29	19.33	11.3	55.3
EJ	79	28.41	0.61	0.45	16.6	53.9
PPT	62	34.36	4.23	0.65	33.4	27.4
Carrot tops						
SM	79.4	15.13	2.09	11.50	14.8	56.5
R	82.3[a]	15.75	2.03	14.65	12.4	55.2
EJ	97.9[a]	20.59	0.33	1.94	24.8	52.3
PPT	72[a]	43.39	10.99	2.87	8.8	34.0
Pea vines						
SM	—	12.91	3.60	22.65	13.7	47.2
R	—	10.35	3.52	30.42	11.7	44.0
Spray-dried	—	19.4	2.63	1.47	13.4	63.0

[a] It was necessary to wet carrot top SM before extraction, since it was too dry and clogged machinery.

This modification takes advantage of the optical system of the analyzer and does away with the necessity of collecting and analyzing fractions.

RESULTS AND DISCUSSIONS

Table I presents yield data for the plants studied. The reported yields of expressed juice should be looked at in relative terms only. Yields will vary with maturity, moisture content, and the efficiency of the machine used to express the juice. One important consideration is that our equipment was relatively simple and not as efficient as better designed and constructed machinery will be. Thus while our compositional data are representative, absolute yields will be larger with better equipment. The yield data in Table I indicate that a significant amount of both total solids and nitrogen was not heat-precipitated from the expressed juice. Higher yields may be obtained by modifying the precipitation step—for example, Sur (1967) found that he could precipitate up to 96% of the protein from water hyacinth-expressed juice, by lowering the pH of the juice to 3.5 before heating. Rather than precipitating the protein, the juice could be handled somewhat like milk. For example, alfalfa juice was spray-dried by Hartman *et al.* (1967) to form a green powder; but corn juice gave a sticky, hydroscopic product. The spray-dried products will contain amino acids, carbohydrates, lipids, minerals, and other material, including toxic substances that are soluble in water or bound to protein or particulate matter separated from the fiber. This additional material might lower the

Table III. Amino Acid Composition of Starting Material (SM), Residue (R), Expressed Juice (EJ), and Precipitates (PPT) of Waste Plant Material

	Amino Acid Composition, G. Amino Acid per 16 G. N																	
	Essential								Nonessential									
	Lys	Phe	Met	Thre	Leu	Ileu	Val	Tryp	Arg	His	Tyro	CysH	Asp	Ser	Glu	Pro	Gly	Ala
Lakeweeds																		
SM	5.5	5.3	3.3	4.8	8.5	4.9	6.4	1.6	4.5	1.9	4.2	2.1	12.4	5.1	13.7	2.9	6.0	7.2
R	5.7	5.4	2.4	3.9	8.6	4.8	6.5	3.0	4.8	2.0	3.8	1.2	10.3	4.7	12.7	3.4	5.6	6.5
EJ	5.7	5.0	1.9	4.9	8.0	5.0	4.6	1.3	5.2	2.2	2.9	2.2	11.6	4.7	12.0	3.7	5.7	6.7
PPT	6.7	6.2	2.4	5.3	9.9	6.1	7.3	0.9	5.6	2.2	4.5	1.1	12.3	5.2	14.5	5.0	6.7	7.6
Beet tops																		
SM	5.5	5.2	1.7	4.1	7.9	4.4	4.0	0.8	4.5	2.3	4.1	0.3	8.3	3.8	12.5	3.6	5.2	5.3
R	4.4	5.0	1.7	3.9	7.3	4.1	5.1	0.7	3.8	1.8	2.3	1.3	8.2	3.9	11.7	3.7	5.0	4.8
EJ	4.9	4.4	1.7	3.7	7.4	4.1	5.1	0.7	3.8	1.7	1.6	1.0	8.7	3.6	13.4	2.2	4.7	5.0
PPT	5.3	6.3	1.1	5.4	9.9	5.4	6.7	1.1	5.0	1.8	5.2	0.7	10.7	4.9	13.4	4.0	5.9	6.7
Potato vines																		
SM	5.3	3.9	1.1	3.1	6.1	3.7	4.3	1.5	3.9	1.9	1.4	0.9	9.0	2.9	11.5	3.4	4.1	4.5
R	4.8	5.8	1.8	4.8	8.2	5.3	7.1	1.0	3.5	1.3	2.9	1.1	10.5	4.5	12.5	4.5	8.9	11.3
EJ	4.5	5.1	2.2	4.8	7.9	4.8	6.9	0.9	3.2	1.3	0.7	1.2	13.4	4.0	14.6	4.5	5.1	6.4
PPT	4.8	9.4	2.9	7.1	12.5	7.7	10.2	1.8	5.4	2.5	5.8	1.1	13.4	5.6	16.6	5.3	8.1	9.0
Lima bean vines																		
SM	6.3	7.5	1.6	5.4	10.4	6.5	7.2	1.5	4.7	2.9	4.3	1.3	14.5	6.7	17.2	4.3	5.3	5.9
R	6.0	7.2	2.5	7.8	10.2	6.0	6.4	1.2	4.6	2.8	4.0	1.5	17.2	6.9	16.7	3.9	6.0	6.3
EJ	5.7	7.2	1.9	5.0	10.0	6.2	7.0	1.0	4.4	2.8	4.0	1.2	14.8	6.7	17.9	3.8	4.9	5.6
PPT	6.1	6.9	2.0	4.5	10.2	5.9	6.6	0.9	7.1	2.7	4.5	0.8	11.8	5.6	14.9	4.0	4.7	5.7
Carrot tops																		
SM	6.0	12.2	2.8	7.4	12.4	7.6	10.5	2.0	6.1	2.4	8.1	1.4	15.7	6.1	18.1	6.3	9.0	9.8
R	5.8	5.8	2.7	4.7	9.7	5.5	6.6	1.1	4.9	2.2	3.9	1.0	10.8	5.1	12.8	4.6	5.9	7.0
EJ	6.2	5.4	1.3	5.2	8.3	5.0	5.4	1.1	5.3	2.4	5.5	0.9	9.5	3.8	11.3	3.7	7.3	4.8
PPT	4.4	7.5	3.0	7.9	12.7	7.3	6.9	1.5	4.3	1.9	8.5	1.0	13.1	5.4	16.1	5.4	9.1	7.1
Pea vines, spray-dried	5.6	5.2	1.6	6.3	7.1	4.4	5.9	1.5	5.7	2.3	4.3	1.1	14.7	5.7	12.8	4.8	4.6	6.4

Table IV. Individual (A) to Essential (E) Ratio of Selected Materials

	A/E Ratios, Mg. Amino Acid per Gram Total Essential Amino Acids										
Amino Acid	1957 FAO/WHO recom-mended pattern	Hen's egg (whole)	Cow's milk	Lakeweed EJ[a]	Beet top EJ[a]	Potato vine EJ[a]	Lima bean vines EJ[a]	Carrot tops EJ[a]	Alfalfa spray dried	Alfalfa precipi-tate	Pea vine, spray dried
Ileu	134	129	127	125	119	123	126	113	116	103	102
Leu	152	172	196	199	214	202	200	188	207	179	165
Lys	134	125	155	141	141	116	116	141	114	123	130
Total aromatic[b]	178	195	197	196	175	148	230	244	215	248	221
Phe	89	114	97	123	128	130	147	122	126	143	121
Tyro	89	81	100	73	46	18	83	124	89	105	100
Total S-containing[c]	133	107	65	96	78	86	63	50	64	60	63
CysH	62	46	17	53	30	31	24	21	20	22	26
Met	71	61	48	48	48	55	39	29	43	38	37
Thre	89	99	91	121	107	125	103	117	108	125	147
Tyrp	45	31	28	8.5	20	24	20	25	27	38	35
Val	134	141	137	114	147	176	143	122	138	125	137

[a] Expressed juice.
[b] Tyro cannot exceed Phe in obtaining total.
[c] CysH cannot exceed Met in obtaining total.

nutritive value of the protein from some species but improve that of others. Since the value of the protein in the juice may be lowered by the action of enzymes or microorganisms, speed and care are necessary in the processing operations. More study of these operations is needed.

As can be seen from Table I, yields vary. Beet tops, potato vines, and pea vines gave yields similar to alfalfa and good silage was made from the residues (Oelshlegel *et al.*, 1969). On the other hand, the yield from lakeweeds was low and the lakeweed silage was rejected by cows. Highest protein yields per acre can be obtained from forage crops like alfalfa, but green waste plant material like that listed in Table I and the production of milk and meat from the fibrous residues could supplement and increase protein supplies.

Table II gives the composition of the various fractions used in our study. The precipitates are higher in protein than the expressed juices, but both are low enough in fiber to be used by nonruminants. Occasionally, one may run into toxin problems such as the trypsin inhibitor in soybean, saponins in alfalfa, or solanine in potato. Further processing of the precipitate or the dried juice by mild heat treatment or solvent extraction may remove these. Bucanan (1968) has recently found that extraction of leaf protein precipitates with lipid solvents increased the digestibility by 10%. Solvent extrac-

Table V. Essential to Total Amino Acid Ratios (E/T) of Selected Materials

	Grams Essential Amino Acids per Gram N[a]		Grams Essential Amino Acids per Gram N[a]
Gelatin	1.05	Beef muscle	2.79
Wheat gluten	1.99	Alfalfa, spray-dried	2.80
1957 FAO recommended pattern	2.02	Beet top PPT	2.94
Beet top EJ	2.16	Lima bean vine PPT	3.03
Potato vine EJ	2.43	Lima bean vine EJ	3.05
Lakeweed EJ	2.51	Lakeweed	3.15
Soybean flour	2.58	Cow's milk	3.20
Fish	2.66	Hen's egg (whole)	3.22
Pea vine[b]	2.69	Carrot top PPT	3.79
Carrot top EJ	2.75	Potato vine PPT	3.96
Alfalfa PPT	2.76		

[a] Cysteine and tyrosine included, if they do not exceed methionine and phenylalanine. EJ = expressed juice, PPT = precipitate.
[b] Calculated on assumption that this protein preparation contains 16% N.

tion removes some of the objectionable dark green coloring matter present in these materials.

The amino acid composition (Table III) agrees fairly well with the supposition that, since leaf protein preparations represent the many proteins of the photosynthetic process and of leaf metabolism, the average over-all composition is similar among different plant species (Stahmann, 1968).

While the best way to arrive at the nutritive value of a protein is by actual feeding trials, amino acid composition studies provide some indication of the nutritive value, although digestibility differences would not be discerned (Sheffner, 1967). Attempts have been made to evaluate proteins by analysis of enzymatic hydrolyzates (Akeson and Stahmann, 1964; Sheffner *et al.*, 1956), but such analysis were not carried out in this study.

The FAO/WHO report of 1965 (FAO/WHO, 1965) recommends that in looking at the amino acid composition of proteins for possible human use two criteria be used: the A/E ratios, the ratios of the individual amino acids to total essential amino acids; and the E/T ratios, the ratios of the total amount of essential amino acids to the total amount of all amino acids. We show these ratios for our preparations in Tables IV and V. A/E ratios of most of the extracted proteins of this study compare well with the FAO/WHO reference pattern, with the exception of methionine. The leaf protein was similar to milk, which also seems to be low in methionine when compared with the FAO/WHO pattern. This deficiency is well known for leaf proteins and methionine supplementation has been suggested. Methionine is commercially available and supplementation should be no economic problem.

E/T ratios suggest that plant protein preparations have a high essential amino acid content. When the data are expressed in this way, some differences in the plant protein preparations are more easily seen. It may be significant that most precipitates have a higher E/T ratio than the corresponding expressed juices.

Most of the crops used in these studies were actually waste material. Well over 21,000,000 tons of vegetable wastes containing 393,000 tons of protein are lost yearly in the United States alone (Kelley, 1958). The use of this material for leaf protein concentrates should seriously be considered. However, the bulk of leaf protein for future use should most likely come from crops which yield the largest amount of protein per acre. Waste material could supplement the preferred crops. Forage crops promise the greatest protein yield, alfalfa, in particular (Akeson and Stahmann, 1966).

We calculated the land area that would be required to produce enough alfalfa protein to meet the minimum protein requirements of all the people in the world. Assuming that alfalfa hay is 20% protein, and the 1964 average U.S. alfalfa hay yields 2.36 tons per acre (U.S.D.A., 1964), one could obtain 472 pounds (214 kg.) of protein per acre by a 50% protein extraction of alfalfa. Considering the world population of 1964 as 3.2 billion and an average protein need of 35.2 grams per person per day (FAO/WHO, 1965) we calculated that the minimum protein needs of the entire world's population for each year could be supplied from the alfalfa protein produced on 301,000 sq. miles of land, an area somewhat larger than the state of Texas (263,000 sq. miles). An equal amount of protein would remain in the fiber, which could be fed to cows and converted into milk and meat. By adding urea to the residue, this may be increased. Such calculations, of course, give only a rough estimate of the land needed. It may be low because more than 35.2 grams may be needed. On the other hand, much higher alfalfa yields are reported from many experimental plots and so our required land estimate could be high, since alfalfa used for hay is often grown on poorer land. One study with excellent growing conditions gave a forage yield of over 20 tons per acre (Vicente-Chandler *et al.*, 1964).

The development of processing procedures for conversion of leaf protein into practical and desirable foods is many years behind that of soybean protein (United Nations, 1968). Soybean can be used as flour or further processed into the texturized foods which are now starting to come on the market. Similar handling and processing techniques should now be studied with leaf protein preparations.

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