EXTRACTABILITY OF PROTEINS FROM VARIOUS LEAVES

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Extracts from fresh leaves of 25 plants were made by mincing the leaves and squeezing the resultant pulp through double fold muslin cloth. Proteinous and non-proteinous nitrogen present in the juice was estimated. Dry matter and total nitrogen of original pulp and the fibre left after three successive extractions of protein was calculated. The results show that leaves of Leguminous and cruciferous plants are better source of easily extractable protein and most of the protein present could be extracted in the first two extractions. The leaves of some plants were mucilagenous which caused an inadequate extraction of the juice. The extractability of protein from leaves with low pH decreased. However, increase in pH 6.5-8.0 helped in the recovery of the non-extractable protein.

Introduction

Tender green leaves are good source of protein. They cannot be taken in large quantities, particularly when they are mature, because they contain large quantities of fibre which is indigestible. However, when this fibrous material is eliminated the product obtained could form a valuable adjunct to the human diet[1] Many attempts have been made in the past twenty years to prepare protein from leaves and to utilize the product for human consumption.[2]

There are two complementary aspects of this project. The extraction of protein from various leaves in the laboratory and its production on large scale. Guha and his associates[3] have made some protein preparations from leaves. Kuppuswamy and his co-workers [4] have analysed these protein preparations but have not given the details of method of extraction. Crook [5] established the conditions under which maximum extraction of protein can be made from tobacco leaves. Byers[6] studied the extractability of protein from leaves of about 60 species growing in Ghana. Singh [7] has given various methods of precipitating the protein from leaf extract and studied the composition of the product thus obtained. Festenstein [8] reported the effects of alkaline solution, detergents and high speed mecerators on extraction of protein nitrogen.

The lack of information about the extractability of protein from leaves and the deficiency of protein in Pakistan was the reason for which the present studies were undertaken. As a result of this interest some leafy plants that grow wildly or are cultivated abundantly and fed to the animals have been tested for the extractability of the protein. In addition to this, autolytic breakdown of the protein in leaf extract have also been investigated to study the breakdown of the protein at room temperature and the loss due to this factor.

Experimental

Leaves.—Protein was extracted from the cultivated as well as the wild species. The leaves were collected early in the morning from the local farms and processed as soon as possible.

Extraction of Protein.—About 100-150 grams of the leaves were stripped from any tough stem and minced in the mincing machine. Samples for dry matter and nitrogen analysis were taken after mixing the pulp. The remaining pulp was weighed and the juice was extracted by pressing it through double fold muslin cloth. In case the minced material was dry or mucilagenous the juice was extracted after mixing the pulp with suitable quantity of distilled water. The extract was centrifuged at 3000 r.p.m. for five minutes and the volume of the juice was measured. The fibrous residue and the residue left after the centrifugation of the first extract were mixed with distilled water equal to the volume of the juice extracted in the first extraction, minced and the juice was extracted as mentioned in the text. For the third extraction this procedure was repeated.

Dry matter and the total nitrogen determinations were done on the initial pulp and the fibrous material left after the final extraction. The volume and pH of the extract was measured. The desirability of changing the pH for maximum extraction of the protein was realised in the case of the leaves of *Phyllanthus embelica* (Amila). This was achieved by adding sodium carbonate solution.

Autolytic Breakdown of Protein.—The percentage breakdown of the protein into peptides and amino acids due to the proteolytic effect of the enzyme during processing was studied by allowing the juice to stand at room temperature (24-32°C.) for 48 hours. A control was also kept at 0°C. The breakdown of the protein was checked after different intervals.

Analysis of the Samples.—Dry matter of the samples were estimated by keeping it at 100°C. for 48 hours. Proteinous nitrogen (Trichloro acetic acid insoluble) and non-proteinous nitrogen (Trichloro acetic acid soluble) was estimated by the micro Kjeldahl procedure using a mixture of copper sulphate, selenium dioxide and potassium sulphate (K_2SO_4 9: $CuSO_4$ 1: SeO_2 0.2) as catalyst. The pH determinations were done by glass electrode.

Results and Discussion

The extractability of the protein from the leaves of 25 plants of different families was determined and the results are classified according to the families. It was found difficult to compare the relative extractability of protein from different leaves because the age was different and many unrelated species were employed. However, the findings of Byers[6] that more protein could be extracted from young foliage than mature leaves was confirmed.

The extractability of proteinous nitrogen in case of Leguminosae varied from 10-66%. In spite of three successive extractions 10-23% of the protein nitrogen present in the leaves mentioned in Table 1A could be extracted. Whereas those mentioned in Table 1B showed 46-63% extraction. The former being a poor source of extractable protein can be used for feeding the cattles and the later can be exploited for protein extraction.

About 55% of the proteinous nitrogen was extracted from *Cassia fistula* (Amaltas) and *Medicago denticulata* (Maina), of which 43% came out in the first extraction from both the plants and the rest was released by two successive extractions. Similar was the case with *Melilotus parviflora* (Sengi) from which 50% was extracted in the first extraction. The amount of extractable protein present in the *Trifolium resupinatum* (Shatala) was 46%. Although only 26% was released during the first extraction, two successive extractions were considered necessary as they yielded 20% more. These leaves contained comparatively less proteinous nitrogen than all the Leguminous plants (Table 1 B), but the yield of protein per acre is more as the plant continues growing even after seven cuts.

The leaves of family Cruciferae can also be used for easily extractable and good quality protein (Table 1C). The extractability was almost similar to legumes (Table 1 B). 31-52% of the proteinous nitrogen was extracted in the first two extractions and the third extraction was found unnecessary since it yielded only 5-9% of the protein present in the residue. The reason for comparatively better extraction and good quality of protein was the low fibre and high nitrogen content of the leaves. Moreover, the leaves were fleshy and the addition of water was not required.

From *Brassica compestris* (Sarson) about 53% protein was extracted. The plant is cultivated for extraction of oil and the leaves are used as pot herb like Spinach. *Brassica napus* (Shaljam) gave better extraction and the extractability of the protein was more than 60%. However, in the case of *Raphanus sativus* (Mooli) and *Brassica oleracea* (Phulgobhi) the extractability was 45 and 35% respectively. These plants are cultivated for edible purpose, but their leaves go waste.

Spinacea oleracea (Palak), *Lactuca sativa* (Salad), and *Coriandrum sativum* (Dhania), (Tables 1D,1E and 1G) are the leaves readily eaten as vegetables. The amount of extractable proteinous nitrogen in these leaves was 46,42, and 72% respectively. These leaves were free from mucilage and the extraction was easy. The percentage extractability was maximum in case of *Coriandrum sativum* (Dhania). This value not only exceeded all the plants mentioned in this paper, but also exceeded those examined at the Rothamsted Research Station. *Daucus carota* (Gajar) in contrast yielded only 29.6% proteinous nitrogen and thus was poor source of protein.

Beta valgaris (Chakundar) (Table 1D) showed an extractability equal to *Melilotus parviflora* (sengi). More than 52% of the proteinous nitrogen was extracted in the first two extractions. The third extraction was also advantageous as 11% more was extracted. This plant is cultivated for the extraction of sugar from its thick tap roots. The leaves can be used for protein extraction.

The most interesting feature about the leaves of Tables 1E and 1F is that during the first extraction they yielded almost the same percentage of proteinous nitrogen. It was the two successive extractions which changed their extractability from 42-50%. Among all these leaves *Cinchorium intybus* (Kashni) seems to be better source of protein. It had lesser amount of fibre which made the extractability of protein easier. *Avena sativa* (Javi) and *Cynodon dactylon* (Ghas) contained large quantity of fibre.

The percentage extraction of protein from the leaves mentioned in Table 1H was the same as in the case of Tables 1E and 1F but the quality of the protein was poor, and fibre content was high.

TABLE I.

Sr. No.	Latin Name	Common name in urdu	% of dry matter of leaves	% of nitrogen in dry matter	pH of Extracts			% of non-proteinous nitrogen extracted			% of proteinous nitrogen extracted			% of total nitrogen extracted
					1st Ext.	Sec. Ext.	Th. Ext.	1st Ext	Sec. Ext.	Th. Ext.	1st Ext.	Sec. Ext.	Th. Ext.	
					(A) Family *Lequminosae*									
1.	*Mimosa pudica*	Lajwanti	25.5	3.66	6.5	6.5	6.5	2.57	1.43	0.87	4.64	3.18	1.9	14.64
2.	*Bauhinia variegata*	Kachnar	15.6	3.44	6.6	6.6	6.6	7.97	4.09	2.64	9.00	5.98	2.64	32.20
3.	*Susbania aegyptica*	Jantar	18.4	4.57	6.5	6.5	6.5	4.35	3.38	3.30	11.80	7.80	3.37	32.90
					(B) Family *Lequminosae*									
4.	*Trifolium resupinatum*	Shatala	17.5	5.48	6.3	6.3	6.3	4.613	2.395	1.941	25.71	11.490	8.638	54.79
5.	*Madicago denticulata*	Maina	17.9	5.15	6.3	6.4	6.4	7.829	4.041	2.356	31.620	16.160	6.928	68.935
6.	*Cassia fistula*	Amaltas	24.1	2.77	6.1	5.9	5.9	9.974	2.602	2.727	43.370	7.435	3.966	70.08
7.	*Melilotus Parviflora*	Sengi	21.7	4.80	6.9	6.7	6.7	11.00	2.858	1.172	50.09	8.794	3.906	77.82
8.	*Cassia absus*	Chaksoo	28.6	3.63	6.4	6.4	6.5	7.29	4.36	4.48	35.61	13.19	17.58	82.53
9.	*Trigonella foenum graceum*	Methi	15.3	6.58	6.8	6.8	6.8	15.810	2.677	1.932	54.170	5.836	2.876	83.30
					(C) Family *Cruciferae*									
10.	*Brassica pleracea*	Phul-gobhi	9.9	6.24	6.1	6.4	6.4	7.885	4.131	1.732	20.850	10.33	4.319	49.26
11.	*Raphanus sativus*	Mooli	12.07	6.11	6.0	6.1	6.1	7.623	2.796	1.525	31.780	9.528	3.812	56.70
12.	*Brassica compestris*	Sarson	12.04	6.69	6.0	6.3	6.3	9.018	3.139	1.883	37.57	10.54	4.898	67.07
13.	*Brassica napus*	Shaljam	11.5	4.05	5.8	6.1	6.1	7.771	2.731	1.718	45.69	6.282	8.590	72.80
					(D) Family *Chinopodiaceae*									
14.	*Spinacea oleracea*	Palak	10.3	4.90	6.2	6.3	6.4	8.756	5.441	2.418	33.34	15.411	7.254	72.63
15.	*Beta valgaris*	Chakundar	9.2	4.68	6.5	6.5	6.5	10.98	3.163	1.689	43.95	7.909	11.26	78.96
					(E) Family *Compositae*									
16.	*Lactuca sativa*	Salad	9.30	3.28	6.2	6.3	6.3	12.12	3.558	3.624	28.420	7.909	5.438	61.06
17.	*Cinchorium intybus*	Kashni	7.4	4.88	6.3	6.3	6.2	6.539	1.974	2.180	28.56	16.332	5.193	60.78
					(F) Family *Graminae*									
18.	*Cynodon dactylon*	Ghas	13.8	3.06	6.1	6.2	6.2	8.146	3.283	5.852	27.73	10.95	6.23	62.19
19.	*Avena sativa*	Javi	17.8	3.90	6.2	6.3	6.3	14.50	3.907	9.173	29.59	10.64	5.208	73.02
					(G) Family *Umbelliferae*									
20.	*Daucus carota*	Gajar	13.3	3.16	6.4	6.5	6.5	4.241	2.120	1.900	17.230	7.54	4.905	37.94
21.	*Coriandrum sativum*	Dhania	12.2	4.16	6.5	6.5	6.6	6.19	5.732	1.599	49.98	14.66	7.326	85.45
					(H) Families *Rutaceae and Amarantaceae*									
22.	*Murraya exotica*	Kamni	35.1	4.36	6.0	6.0	6.0	32.08	7.15	2.13	39.64	5.33	1.64	63.49
23.	*Celosia cristata*	Kalga	15.3	4.30	7.0	7.0	7.0	8.68	3.09	2.11	30.07	10.66	8.85	87.99
					(I) Families *Rosaceae and Euphorbiaceae*									
24.	*Rosa indica*	Gulab	33.8	2.57	5.9	5.9	5.9	6.34	4.52	5.85	9.4	4.26	7.36	37.8
25.	*Phyllanthus embelica*	Amila	25.2	1.90	3.6	3.7	3.7	5.59	2.73	3.57	2.8	—	—	15.37
	Extraction with Na_2CO_3 *at pH* 7-7.5.	,,	25.2	1.80	7.5	7.5	7.5	7.595	5.369	5.217	7.249	6.828	7.058	39.32

The two plants of Table II gave poor yield as only 21% proteinous nitrogen was extracted from *Rosa indica* (Gulab) and 2.8% was extracted from *Phyllanthus embelica* (Amila). This extremely low rate of extraction in the later case was due to low pH. Addition of water and change in the pH from 3.5-7.5 resulted in the release of 21% proteinous nitrogen.

Fig. 1 shows that 3.5% of the protein present in the juice of *Susbania aegyptica* (Janter) leaves was broken down into peptides and amino acids in the first two hours (Temp. 25±2°C.). The juice when kept at room temperature (25-32°C.) for

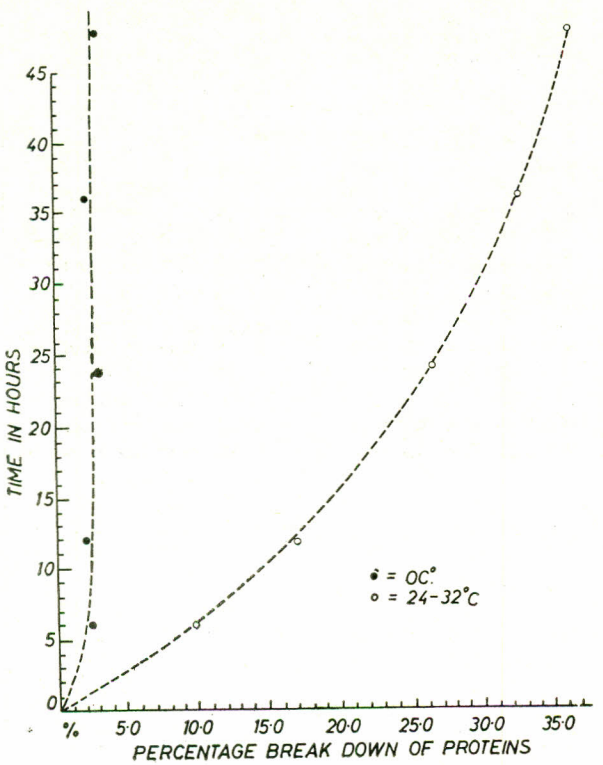

Fig. 1. Autolytic break down of proteins in the leaf extract from *Sesbania aegyptica*.

24 hours showed 26.5% breakdown. Singh [9] reported a similar breakdown (7-40%) when juice of various plant leaves was incubated at 37°C. for two hours. On the basis of these results it is evident that the juice can be kept safely for 1-2 hours without any appreciaable loss in the protein content during winter.

Conclusions

No relationship could be established between the percentage of total nitrogen in the leaves, percentage of dry matter and percentage extractability. Possibly this may be due to the selection of leaves of different ages. Anyhow tender green leaves showed more extractability than the mature leaves. Apart from all the leaves which are specially cultivated for edible purposes, *Trifolium resupinatum* (Shatala), *Medicago denticulata* (Maina), *Cassia fistula* (Amaltas), *Melilotus parviflora* (Sengi) of leguminosae; *Brassica oleracea* (Phulgobhi), *Brassica napus* (Shaljam), *Raphanus sativus* (Mooli) of Cruciferae; *Beta valgaris* (Chakundar) of Chinopodiaceae; *Cinchorium intybus* (Kashni) of Compositae and the leaves of Graminae are good sources for the extraction of protein on a commercial scale. In particular the leaves of Leguminosae and cruciferae are valuable sources of easily extractable and fine quality protein. The protein extracted from these sources can be used to supplement protein deficiency.

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References

1. K. Ereky, B.P., 270, 629.
2. N. W. Pirie, Chem. Ind., **61,** 45 (1942); D. J., Branscombe Nature, Lond., 142,251, (1942); R. E. Slade, D. J. Branscombe and J. C., Mc Gowan, Chem. Ind., **64,** 194 (1945), Anon., Fm. Mechaniz., **4,** 478, 1952. J. M. A., Tilley, M. L. Barnes. and W. F., Raymond, ibid., **6,** 481 (1954).
3. B. C. Guha, Lancet, **i,** 704 (1960).
4. S. Kuppuswamy, M. Srinivasan, and V. Subrahmanyan, *Proteins in Foods*, Spec. Rep. Series No.33 (New Delhi; Indian Council of Medical Research 1958), pp. 221-244.
5. E. M. Crook, Biochem. J., **40,** 197 (1946).
6. M. Byers, J. Sci. Food Agric., **12,** 20 (1961).
7. N. Singh, Biochem. Biophys. Acta., **45,** 422-428 (1960).
8. G. N. Festenstein, J. Sci. Food Agric., **12,** 305 (1961).
9. N. Singh, J. Sci. Food Agric., **13,** 325-332 (1962).