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BIOCHEMICAL ANALYSIS OF LEAF PROTEIN CONCENTRATES PREPARED FROM SELECTED PLANT SPECIES OF TAMIL NADU

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ABSTRACT

Protein is an indispensable constituent in human food. To sustain development of human, production of protein is a must. Attempts are being made to develop a technique to evaluate several precious sources of unconventional protein to diminish proteinCaloric malnutrition problem. These include isolation of oil seed protein, fish protein, single cell protein production of consumable protein from cellulosic waste, and Leaf protein. The leaf protein concentrate can be considered as a readily available and cheap source of protein. The paper envisaged the biochemical analysis of leaf protein concentrates of thirty plants collected from Tamilnadu India. The result indicates that nearly 80% of the plants are suitable for preparation of Leaf Protein Concentrates for human as well as animal nutrition.

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INTRODUCTION

Green cover on the earth indicates the existence of life and presumptuous. Intake of nutritious food is an essential aspect human and animal nutrition. Because of increasing population, people are dying from hunger related causes every day and 75% of the population of the countries in Asia , Africa, central and South America receive only 60 % or even less protein than they need[1]. Thus increasing world population every year will make food more and more inaccessible to the growing population. Advances in science and have paved the way to get an astonishing achievement in the agricultural field. Because of this in India is presently one of the leading countries in the production of food, industrial development science and technology etc. But at the same time India has the misfortune of having about 75 million malnourished children below the age of five years, next to Bangladesh, and Nepal. Most of the people living on or below poverty line and suffering from protein deficiency.i.e Protein caloric malnutrition(PCM). The term “Kwashiorkor’ has been widely adapted to describe the protein malnutrition that is found in every economically underdeveloped country. It has been reported that

low protein diet affect hepatic and muscular glycogen level[2]It has been found that children up to the age of 60 months with severe PCM exhibited increased chromosomal aberrations in peripheral lymphocytes and bone marrow cells[3]These abnormalities persisted even after the had attained the normal height and weight. In addition to this serum leptin levels also lower in patients with anorexia nervosa and protein caloric malnutrition[4].

The minimum protein requirement is estimated to be approximately 0.5g/Kg of body weight in adult Human[5]In growing child the minimum requirement for optimum growth may be more than 3g/Kg of body weight. Pregnancy and lactation would increase the minimum required for nitrogen balance[6]. This increasing demand of food protein can be met by either, substantial increase in overall efficiency of farming or by searching novel or unconventional source of protein. Pirie[6] (1942) explained the real potential of leaf protein (LP) and its use as human food during the II world war. For two decades after the war, several research workers took interest in LP work[7,8,9,10,11,12,13,14]. The Leaf Protein Concentrate (LPC) is protein rich product, containing 40-70% Protein the drymatter (DM) along with appreciable quantities β-Carotene(Pro-vitamin A), Vitamin E, and minerals. The LPC can be used as protein-vitamin –mineral supplement in poultry calf or even in human nutrition[15,16,17]The nutritive value of leaf protein depends on its amino acid composition. A comparison of leaf protein with the provisional pattern of the FAO reference protein (FAO 1965) shows that the LP has enough quantities of essential amino acids, though the amount of methionine is sometimes marginal. Leaf protein is therefore not nutritionally as good as the animal protein such as milk meat and egg, but it is than cereals, legumes and seed protein[18]In a six month feeding trial, LPC was found suitable as a protein supplement in pre school children[19.]

In India attempts were made by late B.C. Guha in 1943 to use leaf protein from water hyacinth and other species[20]The work was then initiated by Subramanyam and his colleagues[21] at CFTRI, Mysore. From 1965 onwards N. Singh and his colleagues at CFTRI explored the possibilities of using green LP in human food and attempts were made to develop an integrated technology for fodder fractionation, suitable for small scale production of LP in Indian villages. Subsequently the work on this aspect was initiated at several other places. The yields of leaf protein from Tithonia , bajra, mustard, wheat and *Sesbania*reached 689,325,609,766 and 1466 kg/ha in 98,93, 71, 70 and 341 days respectively[22]About 50% of protein N can be extracted from this crop[23,24]The protein N extractability of this crop was found to be similar to that of Lucerne and protein concentrate prepared from this crop, containing 51.9 % crude protein, is nutritionally superior with a value of protein efficiency ratio (PER) exceeding 2.0[25]The earlier studies shows that Lucerne is a highly productive crop with consistent performance. The crop yielded over 150 t fresh vegetation , 25 t dry matter 6t crude pretein and 3.2 t extractable protein per hectare when harvested 14 to 16 times in a year[26,27,28].

MATERIALS AND METHODS

Pirie (1942) advocated the exploitation of green leaves as a source of protein in human nutrition. The proteins synthesized in the leaves are nutritionally far superior than the conventional protein sources. However, as the proteins in leaf are associated with indigestible fibrous or cell wall material, the leaves are not suitable in human diet as a source of protein, though leaves of many plants are consumed as vegetable and salad mainly as source of dietary fiber, minerals and vitamins. However, the fibrous material limits consumption of green leaves as a source of protein. In order to make available, the protein in leaf for humandiet, it is necessary to separate them from fibrousmaterial. Pirie suggested a process called as ''Green Crop Fractionation (GCF)" for this purpose.

During GCF ,the fresh green foliage is macerated to rupture the cell . The macerated plant material, called as pulp, is then pressed and the juice released during pressing is then employed for the isolation of protein in it. Either heating or acidification of the juice result s into coagulation of

proteins resulting into a curd referred to as leaf protein concentrate (LPC) ,which is separated from remaining portion of the juice by filtration . The LPC is dark green in colour with 40-70% curd protein in its dry matter (DM) depending on the species from which it is extracted. Inaddition, it contains appreciable amount of minerals, lipids and vitamins. The incorporation of LPC in human nutrition as protein-vitamin-mineral rich concentrate has been proved to be useful to overcome protein deficiency.

Attempts have been made during present investigation to prepare LPC from green foliage of thirty plant species of growing in Tamil Nadu. The LPC prepared using domestic appliances and the yield of LPC dry matter (LPC-DM) per Kg of foliage was recorded. The chemical composition of the LPC sample was then studied to recommend suitable species for optimum production of high quality of leaf protein concentrate (LPC).

Fresh green foliage of *ErythrrinaVariegata* (Fabaceae), *Cassia occidentalis*L (Fabaceae), *Cassia tora*L(Fabaceae), *Sesbaniagrandiflora* (L) Poir. In .Lam(Fabaceae), *Vignamungo* (L) Hepper, Kew Bull. (Fabaceae), *Phaselous Vulgaris.* L(Fabaceae), *Phaseloustrilobus*Ait (Fabaceae), *Trigonellafoenum-gracum L.* (Fabaceae) *Madicagosativa* L. (Fabaceae), Cucurbita maxima duch (Cucurbitaceae), *Cucumissativus* L. (Cucurbitaceae), *Benincasahispida* (Thunb) Cogn. (Cucurbitaceae), *Cocciniagrandis* (L) Voight (Cucurbitaceae), Manihotesculenta. Crantz(Euphorbiaceae,) *Solanumnigrum* L.(Solanaceae), *Solanumtrilobatum* L. (Solanaceae), *Centellaasiatica*(L) Urban. (Apiaceae), *Moringaoleifera.* Lam. (Moringaceae), *Eclipta alba* (L) Hsaak. (Asteraceae), *AdathodaVasica.* Nees (Acanthaceae), TalinumportulaceifoliumForsk.
Asch.(Portulaccaceae), *Souropus androgynous* (L) Merr. (Euphorbiaceae) were collected from the field, early in the morning. The leaves of *Brassica oleraceae. Var. capitata(*Brassicaceae), Brassica olearaceae. Var. botrytis *(Brassiceae),Raphanussativus* L. (Brassicaceae), *Coriandrumsativum* L. (Chenopodiaceae), *spinaciaoleraceae* L. (Chenopoidiaceae), *Amaranthusviridis*L. (Amaranthaceae), *Atriplexhortensis* (Chenopodiaceae), were purchased from the vegetable market.

The samples of foliages collected from either field or market were immediately brought into the laboratory for fractionation, washed with to remove adhering dust and mud particles. The foliage was then minced to a fine pulp using domestic grinder or mortar and pestle. One Kg of pulp was placed on cotton cloth and manually pressed to extract the leaf juice. The amount of leaf juice obtained per Kg of foliage was then measured and a sample was taken for preparation of leaf protein concentrate (LPC).

For the preparation of LPC, about 20 ml water was taken in a stainless steel container and heated to boil. To the boiling water, 100 ml of leaf juice was slowly added with stirring till the temperature reached to 95 degree C. Due to the heating of juice, proteins in it coagulated to a curd referred as leaf protein concentrate (LPC). The LPC was then separated from remaining portion of the juice called as deproteinized juice (DPJ) by filtration through cheese cloth. The LPC thus prepared was stored in vinegar(2% acetic acid) and sealed in specimen bottles. After storage for up to two months, the LPC was suspended in water and filtrated through Whatman filter paper. It was washed several times to it free from vinegar, dried in oven till constant weight and the yield of dry LPC (LPC-DM) was determined taking into consideration weight of the dry LPC and amount of juice extracted per Kg of green foliage.
The dry LPC was ground in to a fine power and stored in plastic containers for further analysis. The nitrogen (N) content was estimated by mircoKjeldahlmethod[29] and crud protein (CP) content was expressed as N×6.25 .The contents of total ash, acid soluble ash (ASA),acid insoluble ash (AIA) and calcium (Ca) were estimated following A.O.A.C (1970)[30]method . A method described by Fiske and Subba Rau (1925)[31]as described by oser[32](1979) was followed for the estimation of phosphorus (P) a sample of dry LPC was boiled in water , filtrated and the amount of water soluble reducing sugar (WSRS)was estimation in the

filtrated using folin-Wu tubes[32] (Oser , 1979) . A method described byCrampton and Maynard (1938) [33]was followed for the estimation of cellulose. The content of β- carotene was determined according to knuckles et al., (1972)[34] .Crude fat content was measured by extracting the sample with chloroform; methanol (2;1) using Soxhlet extractor .

Table 1. Yield and Chemical composition of Leaf Protein Concentrates (LPCs) prepared from various Plant species from Tamil Nadu.

Sr.No	Plant	Yield of LPC-DM g/Kg	%of dry matter (DM)						
			Nitrogen (N)	Crude Protein (CP)	Total ash	AIA	ASA	Ca	P
1	*Cassia occidentalis*	16.5	6.3	39.8	5.6	0.1	5.5	0.63	0.26
2	*Cassia tora*	22.5	8.1	50.0	5.2	2.2	2.9	0.38	0.32
3	*Erythrinavariegata*	8.9	9.2	58.2	8.7	2.7	6.0	0.18	0.31
4	*Madicagosativa*	24.1	7.9	49.4	9.7	1.7	8.0	0.41	0.36
5	*Phaseolustrilobus*	21.9	6.4	40.1	5.0	3.8	1.1	0.16	0.11
6	*Phaseolus vulgaris*	27.0	8.7	54.6	5.5	2.0	3.5	0.10	0.18
7	*Sesbaniagrandiflora*	17.8	6.1	38.2	2.1	0.5	1.6	0.40	0.36
8	*Trigonellafoenium – graecum*	15.1	6.8	43.0	5.9	2.6	3.3	0.31	0.61
9	*Vignamungo*	12.3	4.5	28.7	7.1	2.3	4.8	0.32	0.18
10	*Manihotesculenta*	20.3	7.7	48.6	5.5	2.1	3.3	0.58	0.15
11	*Souropus androgynous*	25.4	5.0	31.7	4.4	1.9	2.5	0.30	0.23
12	*Solanumnigrum*	18.0	8.7	54.8	4.2	1.0	3.2	0.70	0.18
13	*Solanumtrilobatum*	18.1	5.4	33.8	5.2	1.3	3.9	0.89	0.35
14	*Coriandrumsativum*	12.3	3.0	19.2	6.9	2.6	4.3	0.14	0.22
15	*Cucurbita maxima*	13.7	7.7	48.4	9.8	4.9	4.9	0.14	0.17
16	*Benincasahispida*	20.0	4.8	30.2	5.5	2.0	3.5	0.58	0.29
17	*Cucumissativus*	14.7	7.0	43.7	7.1	3.5	3.6	0.14	0.22
18	*Cocciniagrandis*	20.1	5.8	36.3	10.1	3.1	6.9	0.21	0.21
19	*Brassica oleraceav.botrytis*	16.9	5.0	31.7	3.5	1.7	1.8	0.32	0.19
20	*Brassica oleraceav.capitata*	10.5	4.3	27.3	2.7	1.0	1.7	0.30	0.35
21	*Raphanussativus*	13.1	6.1	38.1	9.9	3.1	6.7	0.41	0.51
22	*Centellaasiatica*	7.7	4.3	27.3	6.3	2.0	4.2	0.90	0.34
23	*Moringaolelifera*	14.6	5.7	36.1	4.9	2.1	2.8	0.46	0.47
24	*Eclipta alba*	9.1	5.5	34.3	10.0	3.1	6.9	0.60	0.13
25	*Adhatodavasica*	14.7	7.2	45.3	11.0	2.5	8.5	0.32	0.47
26	*Talinnumportulacifolium*	12.7	4.5	28.2	10.0	3.8	6.1	0.86	0.13
27	*Carthamustinctorius*	10.1	5.8	36.3	4.6	1.6	2.9	0.28	0.98
28	*Spinaciaoleracea*	11.7	6.1	38.1	9.9	3.1	6.7	0.41	0.56
29	*Amaranthusviridis*	10.9	4.3	27.1	9.2	2.0	7.2	0.31	0.13
30	*Atriplexhortenis*	14.2	5.7	35.9	6.9	2.3	4.5	0.37	0.25
	Mean	16.3	6.0	38.7	6.7	2.3	4.5	0.39	0.28

	s.d.	05.0	1.5	09.5	2.5	1.3	2.2	0.22	0.18
	c.v.	30.0	26.1	24.6	37.7	57.9	49.7	56.6	63.8

Table 1b. Yield and Chemical composition of Leaf Protein Concentrates (LPCs) prepared from various Plant species from Tamil Nadu.

Sr.No	Plant	Water soluble Reducing Sugar(WSRS)	% of Drymatter (DM)					
			Crude Fat	Cellulose	Starch	Total Sugar	β Carotene mg/100 g DM	Gross Energy Kcal/g DM
1	*Cassia occidentalis*	0.45	18.4	1.04	2.16	4.0	2.6	3.1
2	*Cassia tora*	0.17	16.0	1.54	1.80	6.4	3.8	3.9
3	*Erythrinavariegata*	0.59	20.4	2.98	4.14	6.60	9.30	3.7
4	*Madicagosativa*	0.12	10.0	7.0	1.35	6.2	3.42	3.4
5	*Phaseolustrilobus*	0.18	14.4	2.62	3.42	3.6	5.88	3.3
6	*Phaseolus vulgaris*	0.55	13.2	2.80	4.10	7.0	1.9	3.4
7	*Sesbaniagrandiflora*	0.74	21.4	2.08	1.98	3.6	1.0	3.2
8	*Trigonellafoenium – graecum*	0.13	10.1	3.10	1.35	6.4	6.2	3.4
9	*Vignamungo*	0.49	13.2	1.10	3.06	7.2	2.4	3.1
10	*Manihotesculenta*	0.80	12.0	3.10	3.96	6.8	6.06	3.1
11	*Souropus androgynous*	0.32	23.4	8.00	2.16	5.0	3.81	3.3
12	*Solanumnigrum*	0.34	18.8	1.76	2.88	6.0	6.0	3.3
13	*Solanumtrilobatum*	0.34	18.1	2.0	2.40	5.8	3.37	3.2
14	*Coriandrumsativum*	0.34	18.8	1.76	2.88	6.0	6.0	3.3
15	*Cucurbita maxima*	0.81	12.0	3.10	3.96	6.8	6.06	3.1
16	*Benincasahispida*	0.60	18.1	1.56	2.16	5.8	1.84	3.1
17	*Cucumissativus*	0.90	18.4	1.10	3.89	5.9	4.14	3.9
18	*Cocciniagrandis*	0.40	10.8	5.6	2.18	6.9	2.72	3.8
19	*Brassica oleraceav.botrytis*	0.13	9.0	2.48	3.60	6.1	1.40	3.7
20	*Brassica oleraceav.capitata*	0.10	8.5	2.98	3.24	6.4	1.04	3.1
21	*Raphanussativus*	0.11	11.8	8.5	2.42	5.0	3.72	3.2
22	*Centellaasiatica*	0.38	21.4	3.42	1.81	5.0	0.10	3.1
23	*Moringaolelifera*	0.11	21.6	5.20	1.85	5.9	4.00	3.6
24	*Eclipta alba*	0.55	21.5	3.28	1.61	4.1	3.80	3.2
25	*Adhatodavasica*	0.80	9.0	1.7	2.52	6.4	8.41	3.2
26	*Talinnumportulacifolium*	0.32	12.0	3.3	3.96	6.5	3.72	3.6
27	*Carthamustinctorius*	0.20	11.8	1.0	2.42	5.0	7.25	3.2
28	*Spinaciaoleracea*	0.72	11.5	2.1	2.70	6.4	3.20	3.1

29	*Amaranthusviridis*	0.83	10.0	9.4	2.43	4.6	1.22	3.6
30	*Atriplexhortenis*	0.22	9.0	6.0	6.30	4.2	4.33	3.2
	Mean	0.40	14.40	3.30	2.44	5.64	4.33	3.2
	s.d.	0.26	5.72	2.32	1.20	1.09	2.92	0.6
	c.v.	60.5	39.74	70.22	49.9	19.36	67.55	20.1

The amount of total sugar and starch were determined following Sadasivam andManickam (1991)[35] . The chromic acid oxidation method described by O' Shea and Maguire (1962)[36] was used for the estimation of gross energy (G.E) in Kcal/g dry LPC . Total phenol content was determined using Folin -Ciocalteau reagent while tannin content was measured using Folin –Denis reagent as described by O' and Shea and Maguire (1962)[36] was used for the estimation of gross energy (G.E) in Kcal/g dry LPC . Total phenol content was determined using Folin –Ciocalteau reagent while tannin content was measured using Folin – Denis reagent as described by Sadasivam and Manickam (1991)[35]. All samples were analysed in duplicate. The date were statistically analysed following Mungikar[37] (1997, 2003).

RESULTS AND DISCUSSION

The Foliages of all plants were taken for the preparation of LPC and so far as possible they were harvested at a preflowering stage. Most of the foliages were green, soft and lush which did not created any problem during fractionation. The freshly prepared protein concentrates obtained were green in colour, however, they became faint when stored in vinegar for long time.

Table 1a gives information on the yield and chemical composition of LPC samples prepared from 30 species under investigation. The yield of LPC fluctuated widely within the range of 7.70 g to 27.07 g /Kg green foliage with *Centellaasiatica* and*Phaselous vulgaris* respectively (fig.1). On an average the plants under investigation yielded 16.39±5.03 of LPC –DM per Kg green foliage. The coefficient of variation (C.V) for the yield of LPC was 30.07 %. On an average leguminous species like *Cassia, Phaselous,* and*Dolichous* gave good yields of LPC followed by those from Cucurbitaceous species.

The nitrogen (N) percent of dry of dry matter (DM) in LPC ranged from 4.37 to 9.28% . On an average the leaf protein concentrates contained 6.04% N with 26.14 % variation in its values in different species. When calculated on the basis of nitrogen content, the LPCs contained from 19.26 to 58.00% Crude protein with an average value of 38.77±9.57%. The wide variation in protein content (C.V=24.6%) may be due to the variation in protein content in the leaf itself, proportion of LPC and recovery extracted nitrogen in the juice. All these factors affect the yield of LPC[38]However on an average almost all LPC samples were with adequate quantities of protein. The plant material should be recommended for the preparation of LPC if the Yield of LPC –DM is more than 10g/Kg fresh weight and if the resulting LPC contains more than 5% N on DM basis[39]Based on this assumption, nearly 25 species were found to be suitable for leaf protein extraction. An international Biological Programme(IBP) technical group suggested maximum ash content of 3%(on DM basis), if the LPC is to be used for human consumption[40]However, almost all species except *sesbania and brassica showed lower val*ues for ash content. The higher values of ash in the LPC samples recorded during present studies can be brought down to the expectation by thoroughly washing the vegetation with water and by giving acid treatment the LPC after its preparation. All LPC samples contained appreciable amounts of crude fat ranging betweentthe8.5 and 23.4% dry matter . Most of the LPC samples were with appreciable amount of β-carotene(Pro-vitamin A).The content of acid soluble ash fluctuated within the wide range of 1.60 and 9.05 % of DM indicating all LPC samples to be rich in mineral elements . All

LPC samples contained marginalamounts of calcium (Ca) while adequate quantities of phosphorus (P).The amounts of these micronutrients varied widely with the value of C.V of 56.6% for calcium (Ca) and 62.8% for phosphorus (p). In comparisons toprotein, all LPC samples contained very little starch ranging from 0.90 to 4.14% of dry matter (table 1 b) . The starch content also varied widely (C.V=49.9%). The total sugar content fluctuated between 3.6 and 7.2% of LPC-DM with moderate variation among the LPCs (C.V=19.36%) The water soluble reducing sugar (WSRS) content in LPC ranged between 0.11 to 0.90% of DM with a wide variation among various samples of LPC (C.V=60.5%).The variability in starch and reducing sugar content indicated breakdown of a part of starch during processing of the plant material for the preparation of LPC.

CONCLUSION

The use of LPC in human and animal nutrition has long been advocated to overcome protein deficiency and malnutrition. As the leaves are abundantly available and as it contain nutritionally superior proteins, their extraction from leaves can produce a cheap source of protein and vitamins. The use of LPC in human nutrition as a source of protein and vitamin A has been advocated by several workers. Choice of suitable plant material for its foliage to prepare LPC in an important and initial step for providing good quality of proteins supplement for human nutrition. During present investigation about 30 plants were screened for leaf protein extraction , 25 were found suitable as they yielded more than 10 g LPC –DM per Kg foliage and as the nitrogen (N) content in the resulting LPC was above 5% g dry matter (DM) as per the opinion of Singh (1969)[42]. Almost all species were found suitable for leaf protein extraction. On the basis of chemical composition the LPC samples were found nutritionally superior, even though they contained higher proportion of ash than desired. All LPC samples contained safer amounts of anti-nutritional compounds like Phenol and tannin. The overall result thus indicated wide scope for fractionation and production of LPC in Tamil Nadu, with the search of suitable green vegetation.

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