



POTENTIAL OF LEAF PROTEIN CONCENTRATE FROM ARID PLANTS FOR HUMAN NUTRITION

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ABSTRACT

In arid and semi arid areas where prevalence of droughts and famines is a recurring feature, forest cover can in general make valuable contributions to food security and provide income to the rural poor. Protein and calorie malnutrition is widespread in these areas leading to high child mortality rate. Plant species can play an important role in overcoming this by being used as a source of LPC, a highly nutritious food. Leaf protein concentrate (LPC) should be seriously considered as additional protein source in the case of non-ruminants and man especially in drought prone areas. The use of LPC in developing countries as alternative protein source to fishmeal in broiler diet holds tremendous promise as it can lower substantially high cost of fishmeal and eventually the acute shortage of animal protein supply. Potential tropical plants for LPC production have been evaluated and selected for further research by USDA. The present study was aimed to determine the potential of arid zone plants for preparation of LPC. Extraction characteristics of the several plant species have been studied and the quality of LPC prepared from them was investigated. Different fractions, chloroplastic and cytoplasmic proteins, were analyzed for their crude protein contents. Analysis of LPC shows considerable differences in their protein contents, which was found to range from 10.9% to 88.9%. Based on this the *Moringa oleifera*, *Azadirachta indica*, *Achyranthes aspera* and *Tephrosia purpurea* were found best suited for LPC preparation.

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INTRODUCTION

Severe protein energy malnutrition is one of the important factors associated with high infant and child mortality rate. According to recent statistics, the rates of childhood malnutrition in India are among the highest in the world (Anon, 1998; Kapil *et al*, 1999). In Rajasthan state problem of undernourishment among women and children is further intensified with adverse effects of drought. Thus, there is a need for providing necessary preventive and supplementary nutritive preparations to the susceptible population. Leaf protein concentrate is an extremely nutritious food made by mechanically separating indigestible fibre and soluble anti-nutrients from much of the protein, vitamins and minerals in certain fresh green plant leaves. Because it is rich in beta-carotene, iron, and high quality protein, leaf concentrate is very effective in combating malnutrition, especially the anemia and vitamin A deficiency which are prevalent among children and pregnant women in most developing countries (Joshi, 1998). The amino acid profile of LPC indicates that it is nutritionally superior to most cereal and legume seed proteins including cottonseed and soybeans; it also compares favourably with most animal proteins except egg and milk (Betschart *et al*, 1974).

LPC extraction from forestry species can play a very important role in overcoming the problems of protein deficiency. In a study conducted in Philippines, LPC from shrubs and tree fodders was found to have higher feeding value than the leaf meals and can be included at high levels as substitute for soyabean oilmeal for nonruminants (Limcango-Lopez & Devendra, 1989). Potential tropical plants for LPC production were been evaluated and selected for further research by USDA (Telek, 1983). A feeding trial carried out in Coimbatore with LPC as a supplement in basic native diet indicated that the LPC supplemented group had 50% higher weight gain and double the height increase than the control group (Nagy *et al*, 1978). Present study was in continuation to our study on potential of TOF as a source of LPC and was undertaken to investigate the potential use of arid zone plants for leaf protein extraction in Rajasthan state of India. The investigations on extraction characteristics & protein content revealed that a great potential exists for preparation of LPC from TOF. As high phenol content has negative effects on the nutritive value, reduction in the phenol content in LPC was also studied.

MATERIALS AND METHODS

LPC preparation

Fresh green leaves (200-300g) from eight plant species (*Achyranthes aspera*, *Tephrosia purpurea*, *Sesbania sesban*, *Pulicaria angustifolia*, *Withania somnifera*, *Solanum nigrum*

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and *Aerva javanica*) present in open fields were harvested. The leaves were washed well in clean water to remove dust and dirt. Large leaves were cut into pieces. Proteins can be expressed from leaves by rupturing the cells by grinding. So leaves were then ground to a pulp in a wet grinder. Different fractions of proteins are obtained depending on the temperature of coagulation. The juice was pressed as much as possible from the pulped leaves and then heated slowly to 60-65°C. Curd formed in the heated juice was then separated in a tightly woven cloth. Liquid is pressed out of this curd as much as possible. The residue left in the cloth is leaf concentrate. The filtrate is further heated at higher temperatures till further coagulation. The coagulated protein is separated as above. The well pressed curd, the LPC, was granulated to get small uniform sized particles. LPC is dried as quickly as possible after it is made. Care was taken to protect the drying LPC from sunlight, blowing dust, insects, and rodents. It is dried to below 10% moisture and then ground as finely as possible. Finally it is stored in air-tight bottles, in a cool dark place. The first fraction obtained is known as the chloroplastic protein and is mainly from chloroplasts. The protein which precipitates from an extract after removal of the chlorophyll containing fraction, is referred to as cytoplasmic. Depending on the temperature of coagulation by heat treatment, five different categories were found to be present (Telek & Martin, 1983). These are as follows:

Categories

1. Only one green fraction coagulated spontaneously at room temperature
2. Only one green fraction coagulated on heating to 60–70 °C
3. One green fraction coagulated on heating to 60–65 °C and another fraction at 80–85 °C
4. One green fraction coagulated at 60-65°C, another fraction at 80-85°C and a third fraction at 95-100°C.
5. No distinct coagulum by heat treatment

Chemical Analysis of Leaves and LPC

Few leaves (5g) collected as above were dried in shade and powdered in a grinder. The powdered samples of leaves and LPC were digested with H₂SO₄ in a Kjeltex digester and then analysed for their nitrogen content in the Kjeltex Auto Analyzer (Tecator). Kjeltabs (CuSO₄ and K₂SO₄) were used as catalyst and a mixture of bromocresol green and methyl red solutions was used as mixed indicator. The value obtained was multiplied by the factor 6.25 for conversion into the protein content. Phenols were extracted in 50% methanol for 20 minutes at 90°C. Total phenols were quantitatively estimated by Folin ciocalteu's reagent (Bray & Thorpe, 1954) using UV spectrophotometer (Make-EC). Tannic acid (Make LOBA) was used as the standard.

RESULTS AND DISCUSSION

Extraction Characteristics of Arid Zone Trees

LPC was prepared from the leaves of the species viz. *Achyranthes aspera*, *Tephrosia purpurea*, *Sesbania sesban*, *Pulicaria angustifolia*, *Withania somnifera*, *Solanum nigrum* and *Aerva javanica* their extraction and characteristics were determined. The yield of LPC per hectare is reported to be

three times more than the grain crops (Joshi, 1998). In the present study the yield of LPC in fresh leaves was found to range from 0.2% in *Aerva javanica* to a maximum 1.0% in *Achyranthes aspera* (Table 1). These values are much lower than those obtained for the trees (Rathore & Meena, 2004). The distribution of chloroplastic (fraction obtained at 60-65°C) and cytoplasmic (fractions obtained above 70°C) proteins in a leaf juice depends upon many factors viz. plant species, physiological state of the leaf, juice pH and the method of heating and fractionating. Lexander *et al* (1970) reported high chloroplastic to cytoplasmic percentage ratios. Nagy *et al* (1978) however, did not obtain this ratio with some plants. We have obtained high ratios in case of *Achyranthes aspera* (89:11), *Tephrosia purpurea* (74:26) and *Withania somnifera* (75:25). Almost equal distribution is seen in *Pulicaria angustifolia*. (43 : 57).

Table 1 Yield of LPC and ratio of fractionated LPC

Name of Species	Local Name	LPC Yield (%)	Chloroplastic: Cytoplasmic ratio
<i>Achyranthes aspera</i> ,	Apamarg	1.0	89 : 11
<i>Aerva javanica</i> ,	Bui	0.2	-
<i>Pulicaria angustifolia</i>	soneli	2.3	43 : 57
<i>Sesbania sesban</i>	Ekad	0.9	-
<i>Solanum nigrum</i>	Makoi	2.5	-
<i>Tephrosia purpurea</i> ,	Biyani	0.9	74 : 26
<i>Withania somnifera</i>	Ashwagandha	0.8	75 : 25

Extracts of *Aerva javanica*, and *Solanum nigrum* yielded only one fraction at 60-70° C. No additional coagulum was separable on further heating the filtered extract. Similarly, *Sesbania sesban* yielded single fraction at 83 °C. This type of plants were categorised as type II plants. This category of plants which yield only one fraction are not reported in literature. In remaining four other species viz. *Achyranthes aspera*, *Pulicaria angustifolia* *Tephrosia purpurea* and *Withania somnifera* the leaves offered a good source of easily extractable protein. In these the extract could be fractionated into two. These were grouped as type III plants. For *Tephrosia purpurea*, *Pulicaria angustifolia* and *Withania somnifera*, the protein fractions were obtained at 60-65°C and 70°C. In case of *Achyranthes aspera* separable precipitates were obtained at 60-65 °C and 83°C. Out of the eight plant species selected for the study, *Trichodesma amplexicaule* belonged to category of type V plants. It was observed that the pressed juice of this plant is very slimy and coagulation could not be obtained. Negative reaction is believed to be due to pectinaceous material which forms a protective colloid (Telek & Martin, 1983). Type I and IV plants were not observed in the selected herb/shrub species.

Thus, it was found that the exact temperature of coagulation of protein depended on the plant species and varied from one plant to another. The chloroplastic protein is reported to coagulate at around 55°C (Telek & Martin, 1983). This coagulation, however, was found to occur at 60-65°C and also at 70 and 83°C (*Achyranthes aspera*). The leaf proteins of those plants which can be fractionated, have lower levels of tannin content and lower protein-phenol interactions (Telek, 1983). These are, thus, the best sources for extraction of proteins. Out of the eight selected tree species, *Trichodesma amplexicaule* was found unsuitable for LPC preparation as it did not yield a

distinct separable protein coagulate. Type II plants are also not very suitable as they yield only one fraction. Type III plants, *Achyranthes aspera*, *Pulicaria angustifolia*, *Tephrosia purpurea* and *Withania somnifera* should be the most convenient sources of LPC as they give two distinct protein fractions.

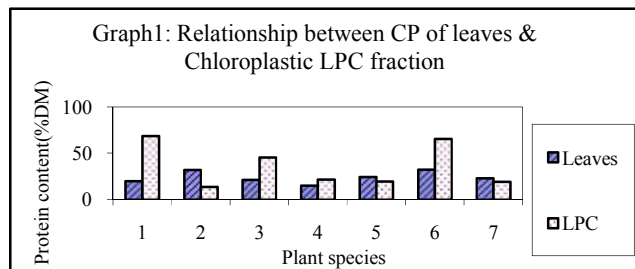
Protein Content in LPC

Chloroplastic protein is reported to be nutritionally superior to cytoplasmic fraction. To study the distribution of protein in the two fractions, their protein contents were determined. The CP contents show considerable variation ranging from 10.9 % to 76.4% and 19.1% to 56.9% in the chloroplastic and cytoplasmic protein fractions respectively (Table 2). The whole leaf protein has been found to contain 50-65 % protein on an average (Telek,1983; Joshi, 1998). Chemical composition and nutritive value of leaf protein concentrates has been studied by Farinu *et al* (1992).

Table 2 Protein content of leaves & LPC fractions

Name of species	Crude Protein content (% DM)		
	Leaf	Chloroplastic	Cytoplasmic
<i>Achyranthes aspera</i>	19.9	68.7	69.6
<i>Aerva javanica</i>	31.9	13.7	-
<i>Pulicaria angustifolia</i>	21.2	45.8	60.4
<i>Sesbania sesban</i>	15.2	21.5	-
<i>Solanum nigrum</i>	24.6	19.6	-
<i>Tephrosia purpurea</i>	32.3	65.6	88.9
<i>Withania somnifera</i>	22.9	19.3	48.1

Protein content of LPC was found to be higher than in the corresponding leaves in *Achyranthes aspera*, *Pulicaria angustifolia*, *Sesbania sesban* and *Tephrosia purpurea*. The protein content of chloroplastic fraction was found to be 3.5 & 2.0 times higher than the CP of leaves of *Achyranthes aspera* and *Tephrosia purpurea*. Similarly, the protein content of cytoplasmic fraction was found to be 3.5 & 2.8 times higher than the CP of leaves of these species (Graph 1). In case of *Aerva javanica*, *Withania somnifera* and *Solanum nigrum* the crude protein content of leaves is higher than the protein content in the LPC. This shows that the protein available is not directly proportional to the CP content. Also all the protein present in the plant leaf is not available and only some can be extracted. The reason for this is attributed to the phenolic content in these plants. Thus, the results show that the plants with high protein content need not be a rich source of available proteins for non-ruminants.



Plant species : 1- *Achyranthes aspera*, 2- *Aerva javanica*, 3- *Pulicaria angustifolia*, 4- *Sesbania sesban* 5- *Solanum nigrum*, 6- *Tephrosia purpurea*, 7- *Withania somnifera*

Achyranthes aspera gives maximum extractable protein, 68.9% and 69.5%, in the chloroplastic and cytoplasmic fractions respectively. This species, thus, appear to be the best

sources for preparation of LPC. *Tephrosia purpurea*, and *Pulicaria angustifolia* can be further investigated for the absence of antinutritional factors and as a potential source of LPC.

Phenol Content in LPC

Presence of phenols may decrease the digestibility and palatability of any feed (Reed, 1995). Before LPC can be added to foods for nutrition, absence of toxic factors should be ensured. LPC prepared from the type III species viz. *Achyranthes aspera*, *Pulicaria angustifolia*, *Sesbania sesban* and *Tephrosia purpurea* was found to have much reduced amount of phenols (Table 3). The phenol content was reduced to amount which is very much safe for injection in case of *Achyranthes aspera* and *Tephrosia purpurea*. In the chloroplastic fractions the reduction in phenol content was found to vary from 48.7% to 95.6%. Maximum reduction was observed in case of *Achyranthes aspera* (95.6%).

Table 3 % Phenol content in leaves and their Chloroplastic LPC fraction

Name of species	Leaves	Chloroplastic	Reduction (%)
<i>Achyranthes aspera</i>	8.5	0.4	95.6
<i>Pulicaria angustifolia</i>	9.9	2.4	76.2
<i>Tephrosia purpurea</i>	2.7	0.8	71.6
<i>Withania somnifera</i>	7.0	3.5	48.7

High phenol content decreases the yield of LPC (Telek & Martin, 1983). In this case no proper trend was observed between phenol content and LPC yield. More suggestive results however, can be obtained by studying the interactions within a species or increasing the number of plants under study.

CONCLUSIONS

LPC offers a major new source of food in the human diet. It is a simple, inexpensive measure to prevent the childhood malnutrition that dooms hundreds of millions of people to a cycle of unnecessary suffering. The study showed that LPC preparation is not very cumbersome process and can be easily extended at village level. Our study indicates that *Achyranthes aspera* due to maximum extractable and fractionable protein can be successfully used for LPC preparation. Also the phenol content in its LPC, an antinutritional factor is also reduced to lower limits. LPC can be incorporated in the midday meal scheme run by the Government. The main component of the food supplied in midday meal in Rajasthan is carbohydrate and the menu is the same every day: *ghoogri*, a gruel made of boiled wheat mixed with gur, with oil and peanuts added in some cases. If this meal is supplemented with LPC species viz. *Achyranthes aspera*, *Moringa oleifera* and *Azadirachta indica* then it will certainly help in improving the nutritional status of the children.

References

Anonymous,1998. United Nations Children's Fund. State of the World's Children. New York, UNICEF.
 Betschart, A.A. and Kinsella, J.E. 1974. Influence of storage on composition, amino acid content and solubility of Ssoybean leaf protein concentrate. *J Agr. Food Chem.*, **22**(1), 116.

- Bray H.G. and Thorpe W.V. 1954. Analysis of phenolic compounds of interest in metabolism. *Meth. Biochem Anal.* **1**, 27-52.
- Farinu G.O., Ajiboye S.O. and Ajao S. 1992. Chemical composition and nutritive value of leaf protein concentrate from *Leucaena leucocephala*. *J. Sc. Food and Agri.* **59**(1), 127-129.
- FSI. (Forest Survey of India). 2000. State of Forest Report 1997, Forest Survey of India.
- Ministry of Environment and Forests. Dehradun.
- GFD, 1984. Gujarat Wood Balance Study. Government of Gujarat, Ahmedabad, India (cited in Tejwani).
- Joshi R.N. 1998. Leaf Concentrate: A good food source to control vitamin A deficiency. *Sight and life.* **4**, 17-21.
- Kapil, U., Monica, T., Pathak, P. and Nayar D. 1999. Nutrient Intake and Consumption of Supplementary Nutrition by Severely Malnourished Children in two ICDS Projects in Rajasthan State, *Indian Paediatrics*, **36**(8), 799-802.
- Kleinn, C. 2000. On large-area inventory and assessment of trees outside the forest. *Unasylva*, **200**(51): 3-10.
- Kotwal, P.C. and Bhattacharya, P. 2000. Extent and status of Trees outside Forests. Concept paper presented in the workshop on "Extent and status of Trees outside Forests" held during June 5-7 2000, at IIFM, Bhopal.
- Lexander, K., Carlsson, R., Schalen, V., Simonsson, A. and Lundborg, T., 1970. Quantities and qualities of leaf protein concentrates from wild species and crop species grown under controlled conditions. *Ann Appl Biol.* **66**, 193.
- Limcangco-Lopez P.D. and Devendra C. 1989. The use of shrubs and trees fodders by nonruminants. Shrubs and tree fodders for farm animals. Proceedings of a workshop in Denspar, Indonesia, 24-29 Jul, 1989. International development research center. No. **276e**, 61-75.
- Nagy, S., Telek, L., Hall, N.T. and Berry R.E., 1978. Potential food uses for protein from tropical and subtropical plant leaves. *J Agric Food Chem.* **26**(5), 1016-1028.
- Prasad R, P.C Kotwal, and D.N Pandey, 2000. Trees outside Forests in India: A National assessment. (Mimeographed), IIFM, Bhopal, India.
- Rathore M. and Meena R. 2004. Trees Outside Forest as a Source of Leaf Protein Concentrate for Improving Nutrition. Paper presented in the IUFRO International Conference on Multipurpose Trees in the Tropics held on 22-25 Nov'04 at AFRI, Jodhpur.
- Reed, J.D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *J. Anim. Sci.* **73**, 1516-1528.
- Telek, L. and Martin, F.W., 1983. Tropical plants for leaf protein concentrates. In *Leaf Protein Concentrates* (eds., Telek L. & H.D. Graham) AVI Publishing Company, Inc, Westport, Connecticut. pp. 81-132.
- Telek, L. 1983. Extracting Protein from Tropical Plants : In OTA Workshop proceedings, "Plants-The Potentials for Extracting Protein, Medicinal, and Other Useful Chemicals." Congress of the United States, OTA, Washington, DC. pp. 78-110.

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