



STUDIES ON PREPARATION OF DIFFERENT TYPES OF LEAF PROTEIN CONCENTRATES FROM SOME WILD AND CULTIVATED PLANTS

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ABSTRACT :

Protein availability in the nutrition of overall population is always constrained due to its mere resources. To fulfil the demand there is always a need of new avenues which is to be considered by the researchers of this field. The present investigation considered the leaf protein concentrates (LPCs) as an alternative source of protein for the demand. 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 leaf protein concentrate (LPC), a highly nutritious food. LPC should be considered seriously as it can serve as an additional protein source in the case of non-ruminants and man, especially in drought prone areas (Rathore, 2010), To prepare leaf proteins the green foliages from 7 plants were selected i.e. Berseem (*Trifolium alexandrinum* L.), Alysicarpus vaginalis L. var. stocksii., Alternanthera paronychioides St. Hil., Cabbage (*Brassica oleracea* L. var. capitata), Radish (*Raphanus sativus* L.), Adulsa (*Adhatodavasica* Nees.), and Bauchi (*Psoraliacorylifolia* L.). The selected plants were fractionated and subjected to prepare various LPCs as suggested by Pirie (1971). Maximum dry weight of unfractionated LPC was found in *Psoraliacorylifolia* L. (80.52 g) and minimum in *Raphanus sativus* L. (20.83 g). Maximum dry weight of chloroplastic LPC was also found in *Psoraliacorylifolia* L. (76.47 g) and minimum in *Brassica oleracea* L. (17.96 g). Maximum dry weight of cytoplasmic LPC was found in *Adhatodavasica* Nees. (4.17 g), and minimum in *Raphanus sativus* L. (1.27 g). The yield of cytoplasmic LPC is always found less than the other two LPCs. However, cytoplasmic LPC is nutritionally superior than the unfractionated and chloroplastic LPC.

Key words: leaf protein concentrates, heat coagulation. *Alternanthera paronychioides* St. Hil. Unfractionated LPC.

INTRODUCTION

Protein calorie malnutrition (PCM) should not be viewed as a mere nutritional deficiency disorder. It is a consequence not only of inadequate food intake, but also of poor living conditions, unsatisfactory environment and lack of primary health care. In other words PCM is a primarily a diseases of socioeconomic inequalities and of misdistributions of food. The dietary protein requirements of man are still a matter of controversy. The minimum protein requirement is estimated to be approximately 0.5 g/Kg of body weight in adult human (Mertz, 1969). In growing child, the minimum requirement for optimum growth may be more than 3g/Kg of body weight. Pregnancy, lactation would increase the protein requirement for nitrogen balance (West *et al.*, 1970).

The leaf protein concentrates (LPC) can be prepared by various methods includes, heat coagulation, acid coagulation, alum precipitation, anaerobic fermentation technique and centrifugation etc. Out of this heat coagulation method was found most suitable for various crops.

The heat coagulation method includes heating of the leaf juice at above 90°C result into leaf protein concentrate (LPC) which is dark green in colour. This type of LPC generally called as unfractionated LPC or whole LPC. Due to its colour and flavour it is not utilized at large extent for human consumption as recipes. In order to prepare colourless or non-green LPC, Pirie (1971) suggested a method of differential heat coagulation wherein the juice is heated to 60°C and subsequently at 95°C. After filtration, due to heating at 60°C proteins associated with chloroplast coagulate along with chlorophyll pigments resulting into dark green LPC referred as "Chloroplastic LPC". When green chloroplastic LPC is isolated from the juice and the filtrate remaining behind is again heated at 95°C, the remaining proteins from cytoplasm precipitate and coagulate, resulting into yellowish to white protein concentrate referred as "Cytoplasmic LPC".

The ratio of chloroplastic and cytoplasmic fractions, made by the controlled heating of leaf extracts, has been measured for several species (Subba Rau *et al.*, 1969; Byers and Davys, 1964 and Byers, 1965) and in vivo (Henery and Ford, 1965; Subba Rau *et al.*, 1969) and in vitro (Byers, 1967) digestibilities of some fractions have been determined. These cytoplasmic fractions are more than 90% digestible, compared with digestibilities of 50%, or less, for chloroplastic fractions and 60-80% for most whole leaf proteins (Byers, 1971). Feeding experiments are in agreement with these analytical results. The biological value (BV) of freeze dried LPC preparations fall in the range of 70 to 80 % while true digestibility (TD) is about 75 to 85% (Akeson and Stahmann, 1965; Buchanan, 1969; Kohler and Bickoff, 1971; Woodham, 1971; Subba Rau *et al.*, 1972).

Meimbanet *al.* (1982) suggested the fortification of biscuits with cassava leaf protein concentrate (LPC). The statistical analysis indicated that up to 2 percent LPC fortification was acceptable. Their finding suggested that fortification of wheat flour with cassava LPC improved the essential amino acid balance of the final baked product. Agbede (2005) works on nutritional significance of leaf meals, protein concentrates and residues from some tropical leguminous plants. His analytical information suggested that the LPCs from these plants could be used as protein supplements in human feeding, the feeding of the LMs (leaf meals) or LPC fibrous residues to the ruminant animals either solely or in combination with other forages appears feasible especially under feedlot.

MATERIALS AND METHODS

Selection of plants: For the present work green foliages from 7 plants were selected to prepare leaf protein concentrate viz., Berseem (*Trifolium alexandrinum*L.), *Alysicarpus vaginalis*L. var. stocksii., *Alternanthera paronychioides*St. Hil., Cabbage (*Brassica oleracea* L. var. capitata), Radish (*Raphanus sativus*L.), Adulsa (*Adhatodavasica*Nees.), and Bauchi (*Psoraliacorylifolia*L.). These plant material were authenticated at Department of Botany, RTM Nagpur University, Nagpur. These plants were collected from different places. Berseem was collected from WaluSangopan Kendra, Nagpur. Bauchi was collected from KrishiVidyapith Campus, Nagpur. Cabbage and Radish were collected from local vegetable market. *Alternanthera*, *Alysicarpus*, and *Adhatoda* were collected from RTM Nagpur University campus, Nagpur.

Heat coagulation method: - This method is based on heating of juice at 95°C. It was utilized to produce unfractionated LPC. For this purpose, a sample of 100 ml juice was slowly added to 20 ml boiling water with continuous stirring, as a result proteins in juice coagulated resulting into green colour curd called as leaf protein concentrate (LPC). During whole process the temperature was maintained at 95°C. This heated juice was then filtered through preweighed Whatmann filter paper No.1. During filtration the yellowish filtrate was obtained which is called as whey or deproteinized leaf juice (DPJ). The green coloured curd (LPC) along with filter paper and DPJ was dried at 55°C in hot air oven. The amount of the dried LPC and DPJ was recorded per Kg of fresh green foliages.

Differential heat coagulation method: - This is based on heating of juice at two different temperatures i.e. at 60°C and 95°C. For this purpose, a sample of 100 ml juice was added slowly to beaker containing 20 ml

distilled water with continuous stirring and heated to 60°C, as a result of which chloroplastic proteins in juice get coagulated resulting into a green curd referred as chloroplastic LPC. The heated juice was then filtered through pre-weighed Whatmann filter paper No.1. The filtrate remaining behind was then heated to 95°C. The remaining proteins in cytoplasm get precipitated and coagulated resulting into yellowish to white protein precipitate referred as cytoplasmic LPC. The heated juice was then filtered through preweighed Whatmann filter paper No.1, to get cytoplasmic LPC. Both the LPCs along with filter paper were then dried at 55°C in hot air oven. The amounts of both dry LPCs were recorded.

RESULT AND DISCUSSION

Table and fig. reveal the dry weight of unfractionated, chloroplastic and cytoplasmic LPC from selected plants. Maximum dry weight of unfractionated LPC was found in green foliage of *Psoraliacorylifolia*L.(80.52 g) and minimum in *Raphanussativus*L.(20.83 g). Maximum dry weight of chloroplastic LPC was found in green foliages of *Psoraliacorylifolia*L.(76.47 g) and minimum in *Brassica oleracea*L. (17.96 g). Maximum dry weight of cytoplasmic LPC was found in green foliages of *Adhatodavasica*Nees.(4.17 g), and minimum in *Raphanussativus*L.(1.27 g).

Details of the yield in table suggested that chloroplastic LPC from all wild plants are relatively higher than that of *Brassica oleracea*L. But in case of cytoplasmic LPC, *Adhatodavasica*Nees. and *Psoraliacorylifolia*L. showed higher yield than that of *Brassica oleracea*L., whereas, it was lower in *Alysicarpus vaginalis*L. and *Alternanthera paronychioides*St. Hil. A comparison between chloroplastic and cytoplasmic LPC content of all plants showed that the content of cytoplasmic LPC was always lower than that of chloroplastic LPC. This observation was earlier reported by Jadhav (1997) and other.

Gogle (2000) reported the chloroplastic and cytoplasmic LPC content of radish and cabbage as 21.60, 2.17 and 37.80, 6.15 g/Kg respectively. Whereas in present investigation the chloroplastic and cytoplasmic LPC content of radish and cabbage are obtained as 19.56, 1.27 and 17.96, 3.55 g/Kg respectively. This variation between reported and obtained value for chloroplastic and cytoplasmic LPC content is might be due to the duration of processing of juice, regional and seasonal difference as well as maturity level of the plants. It is reported that as soon as the juice liberated, enzymes in it; which is responsible for autolysis and reduces the protein content (Singh, 1962). For this, the delay between pulping, pressing and subsequent heating of released juice should be avoided to obtain maximum recovery of leaf protein (Pirie, 1978).

Table: Preparation of unfractionated, chloroplastic and cytoplasmic leaf protein concentrates from different plants.

Sr. No.	Name of the plant's	Dry weight of LPCs, g / Kg fresh green foliages			Cytoplasmic: Chloroplastic LPC ratio.
		Unfractionated	Chloroplastic	Cytoplasmic	
1	<i>Alysicarpus vaginalis</i> L.	71.15	64.35	3.31	1: 19.46
2	<i>Trifolium alexandrium</i> L.	31.64	30.05	1.59	1: 18.89
3	<i>Alternanthera paronychioides</i> St. Hil.	72.41	61.83	2.34	1: 26.42
4	<i>Raphanussativus</i> L.	20.83	19.56	1.27	1: 15.42
5	<i>Brassica oleracea</i> L.	21.51	17.96	3.55	1: 5.05
6	<i>Psoraliacorylifolia</i> L.	80.52	76.47	4.05	1: 18.88
7	<i>Adhatodavasica</i> Nees.	42.42	38.76	4.17	1: 9.29
-	Mean	48.60	44.10	2.90	-
-	Std. Deviation	25.60	23.40	1.17	-
-	Std. Error	9.67	8.84	0.44	-
-	Coefficient of variation	52.58%	53.00%	40.38%	-

CONCLUSION:

It could be concluded from the results of the present investigation that the selected wild plant with cultivated plants can be employed for the preparation of different types of LPCs. However, this LPC should be stands with digestibility and suitability parameters prescribed for the human consumption. It is revealed from the above result that preparation of green chloroplastic LPC and white cytoplasmic LPC can be undertaken by differential heat coagulation; however, the yield of cytoplasmic LPC is low in comparison to that of chloroplastic LPC.

REFERENCES

1. Agbede, J. O. 2005. Nutritional significance of leaf meals, protein concentrates and residues from some tropical leguminous plants. *Journal of Food, Agriculture and Environment*, **3 (1)**: 25-30.
2. Akeson, W. R. and Stahmann, M. A. 1965. Nutritive value of leaf protein concentrate, an in vitro digestion study. *J. Agric. Food Chem.*, **13**: 145.
3. Buchanan, R. A. 1969. Effect of storage and lipid extraction on the properties of protein. *J. Sci. Food. Agric.*, **20**: 359.
4. Byers, M. and Davys, M. N. G. 1964. *Rep. Rothamsted exp. Stn.*, pp. 96.*
5. Byers, M. 1965. *Rep. Rothamsted Exp. Stn.*, pp. 121.*
6. Byers, M. 1967. II. The action of papain on protein concentrates extracted from leaves of different species. *Sci. Fd. Agric.*, **18**: 33.
7. Byers, M. 1971. Amino acid composition and in vitro digestibility of some protein fractions from three species of leaves of various ages. *J. Sci. Fd. Agric.*, **22**: 242-251.
8. Gogle, D. P. 2000. "Studies on various products obtained during green crop fractionation". Ph.D. Thesis, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.
9. Henery, K. M. and Ford, J. E. 1965. The nutritive value of leaf protein concentrate determined in biological tests with rats and by microbiological methods. *J. Sci. Fd. Agric.*, **16**: 425.*
10. Jadhav, R. K. 1997. "Studies on the preparation of protein concentrates from green leaves". Ph.D. Thesis, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.
11. Kohler, G. O. and Bickoff, E. M. 1971. In "Leaf Protein: its agronomy, preparation, quality and use" (Pirie, N. W., Ed.), IBP Handbook No. 20, Blackwell Scientific Publication, Oxford and Edinburgh, pp. 69.*
12. Meimban, E. J., Bautista III, J. G. and Soriano, M. R. 1982. Studies on the fortification of biscuits with cassava leaf protein concentrate (LPC). *Philippine Journal of Nutrition*, **35 (2)**: 82-86.
13. Mertz, E. W. 1969. *Elementary Biochemistry*. Vakile, Fetter and Simmons Pvt. Ltd., Bombay, pp. 224.*
14. Pirie, N. W. 1971. "The machinery for LPC production." in *Leaf Protein: its agronomy, preparation, quality and use*", Pirie, N. W. Ed., IBP Handbook No. 20, (Oxford, United Kingdom: Blackwell's Scientific Publishers), pp. 56-62.
15. Pirie, N. W. 1978. "Leaf protein and other aspects of fodder fractionation". Cambridge University Press, London.
16. Rathore, M. 2010. "Leaf Protein Concentrate as Food Supplement from Arid Zone Plants". *Journal of Dietary Supplements*, **7**, Issue 2.
17. Singh, N. 1962. Proteolytic activity of leaf extracts. *J. Sci. Fd. Agric.*, **13**: 325-332.
18. Subba Rau, B. H., Mahadeviah, S. and Singh, N. 1969. Nutritional studies on whole-extract coagulated leaf protein and fractionated chloroplastic and cytoplasmic proteins from lucerne (*Medicago sativa*). *J. Sci. Fd. Agric.*, **20**: 355.
19. Subba Rau, B. H., Ramana, K. V. R. and Singh, N. 1972. Studies on nutritive value of leaf proteins and some factors affecting their quality. *J. Sci. Fd. Agric.*, **23**: 233-235.
20. West, E. S., Todd, W. R., Mason, H. S. and Bruggen, J. T. V. 1970. "Text Book of Biochemistry" 4th Edn. The Macmillan Company, Collier Macmillan Ltd., London.*

21. Woodham, A. A. 1971. In: "*Leaf Protein: its agronomy, preparation, quality and use*" (Pirie, N. W., Ed.), IBP Handbook No. 20, Blackwell Scientific Publication, Oxford and Edinburgh, pp. 19.