INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
© VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N)
www.ijrbat.in

IN VITRO STUDY OF PROTEIN DIGESTIBILITY OF LEAF EXTRACT (LE) AND LEAF PROTEIN CONCENTRATES (LPC) OF SOME WILD AND CULTIVATED PLANT SPECIES.

V. G. Manwatkar[1], S.B. Wadaskar[2] and D.P. Gogle[3]
[1]Vidya Vikas Arts, Commerce & Science College Samudrapur, Dist-Wardha-442305.
[2]Khare-Dhere College Guhagar, Dist-Ratnagiri-415703.
[3]Dept. of Botany, R.T.M. Nagpur University Campus, Amaravati, road, Nagpur- 440033.
Corresponding author: D. P. Gogle, Email Id :dr.dayanand.gogle@gmail.com

ABSTRACT:
The biological value of leaf protein is depends upon its digestibility. The Concentrated form of leaf protein is called as leaf protein concentrates (LPC). It is less expensive and most abundant source of available protein and equivalent to most of the animal protein source. The use of leaf protein concentrates (LPC) for the human and animal consumption it is necessary to check its digestibility. In the present investigation, *In Vitro* comparative study of protein digestibility of leaf extract (LE) and leaf protein concentrates (LPC) of ten different wild and cultivated plant species has been carried out. The present experimental finding reveals that the protein digestibility of leaf protein concentrates (LPC) were found to be more (67% to 84%) as compared to leaf extracts (60% to 80%).

Keywords: Leaf protein concentrates (LPC), Leaf Extracts (LE), Plant protein, *In vitro* protein digestibility.

INTRODUCTION:

In most of the under developing countries, most of the peoples live under substandard condition as for as nutritional diet is concerned. According to the food security survey of South Asian Countries, India ranked 69th position (Singh *et al.*, 2014). In India, the consumption of green leafy vegetables is very low and is much below the recommended allowances, therefore majority of the Indians do not get sufficient amount of vitamins and minerals present in leafy vegetables. Every year large number of green material is produced on the earth. With the seasonal changes, it gets disappeared without any use. Of course some of the green leafy material is used in grazing by animals but most of this green leafy material goes waste. Leaf proteins (LP) were considered as major unconventional protein source by many researchers to overcome the problem of protein malnutrition. Secondly the available food sources are not sufficient to meet the demand of ever increasing population. Therefore there is a need to search the alternative protein source. In this regard Leaf Protein Concentrates (LPC) is good and cheap source of protein found in the leaves of plants. Leaf Protein Concentrates (LPC) can be predominantly used for human as well as animal nutrition.

By considering the importance of Leaf Protein Concentrates (LPC) for the human and animal consumption, the aspect of significance of digestibility of protein should be taken for consideration (Mohamed et. al, 2009). In vitro enzymatic digestion study suggested that leaf protein have a high biological value (BV), generally less than milk and close to beef (Akeson and Stahmann, 1965). Duckworth and Woodham (1961); Duckworth et al. (1961) and Waterlow (1962) have studied feeding experiment with chicks, rats, pigs and also infants recovering from malnutrition suggested that leaf protein could be a valuable protein supplement. Henry and Ford (1965) studied protein concentrates prepared from 14 different species and found that it has a biological value (BV) over 70%. The biological value of freeze dried LPC preparations have in the range of 70-80% while true digestibility (TD) is about 75-85% (Buchanan, 1969; Kohler and Bickoff, 1971; Woodham, 1971). Therefore, objective of the present studies is to

investigate in-vitro digestibility of Leaf Protein Concentrates (LPC) of different plant species

METHOD AND MATERIAL:

During the present investigation, ten different plants viz. *Brassica juncea* (L.) Czern. & Coss, *Brassica napus* L., *Brassica oleracea* var. Botrytis L. (cultivated) and *Chenopodium album* L, *Goniocaulon indicum*, (Klein ex Willd). C.B. CL, *Celosia argentea* L., *Vigna trilobata* (L.)Verde, *Digera muricata* (L.) Mart, *Tridax procumbens* L. and *Ocimum americanum* L. (wild) were chosen as protein source. Analysis of oven dried leaf juices of experimental plants was also conducted to compare with that of LPC. The LPC were prepared by following the method suggested by N. W. Pirie (1966). The LPC thus prepared was oven dried at 50 – 600C and the dried LE were used for protein digestibility studies.

Protein Digestibility in Vitro: - The total protein digestibility was carried out by pH-stat method given by Hsu *et al.* (1977).

Reagents:

i) Glass distilled water.

ii) Three enzyme solution: - Three-enzyme solution was prepared by dissolving 1.6mg trypsin, 3.1mg chymotrypsin and 1.3mg peptidase of sigma grade in one ml glass distilled water.

iii) Bacterial Protease solution: - Bacterial Protease solution was prepared by dissolving 7.95mg of pure protease (Streptomyces grisus of sigma grade) in one ml glass distilled water.

Procedure: The leaf extract and leaf protein concentrate sample was prepared by passing through 80-mesh screen and the amount of sample taken for analysis so that it contains 6.25mg of protein per ml. The sample was taken in a small beaker containing 10ml glass-distilled water. The sample was allowed to hydrate for one hour at 50C in refrigerator. The pH of sample and three-enzyme solution was equilibrated to 8.0 at 370C on pH meter. 1ml of three-enzyme solution was added to the sample suspension with continuous stirring on magnetic stirrer cum hot plate at 370C.

After exactly 10min. from the time of addition of three-enzyme solution while being stirring, 1ml of bacterial protease solution was added to the sample and immediately transferred the solution to 550C water bath. The solution was transferred back to 370C water bath after exactly nine min. from the addition of bacterial protease solution; it means that in total nineteen min. after the addition of three-enzyme solution. The pH of the hydrolysed sample was measured after exactly 10min. from the addition of bacterial enzyme solution this is called as 20min. pH. The control HNRC (Sodium Caseinate) was run before each set of the test sample and it has 20min. pH of ± 6.42.

Calculation: - In Vitro Protein Digestibility was calculated by using the following equation.

% Digestibility = 234.84- 22.56 X

Where X is the pH after 20min. incubation.

RESULTS AND DISCUSSION

The results of in vitro protein digestibility of leaf extracts and leaf protein concentrates is given in Table No.1. In leaf extract (LE), the highest protein digestibility was found in Digeramuricata (80.30%). The species like *Chenopodium album, Brassica oleracea, Celosia argentea, Vignatrilobata* and *Tridax procumbens* also showed more than 70% protein digestibility. About 69.70% and 68.57% protein digestibility was observed in *Brassica juncea* and *Brassica napus* respectively. The lower digestibility was observed in *Ocimum americanum* and *Gonocaulon indicum* which was 60% and 63.16% respectively.

The higher percentage of protein digestibility in leaf protein concentrates (≥84%) was found in *Chenopodium album, Brassica oleracea, Celosia argentea, Vigna trilobata* and *Digeramuricata*. However, *Brassica juncea, Brassica napus, Goniocaulon indicum* and *Tridax procumbens* have showed more than 75% protein digestibility *in vitro*.

Minimum digestibility was observed in *Ocimum americanum* which was 67.56%.

The results showed that, the protein digestibility was found higher in leaf protein concentrates as compared to leaf extract. The protein digestibility data indicated that, there was significantly higher percent was found in *Chenopodium album, Brassica oleracea, Celosia argentea, Vigna trilobata* and *Digera muricata*. The minimum variation was observed in *Brassica juncea* and *Brassica napus* in leaf extract and leaf protein concentrates. These results on protein digestibility in the present study were comparable with earlier findings reported by various workers even though they adopted different methods for preparation of LPC. The protein digestibility for freezing concentrates, isopropanol extracted freezing concentrates and water washed-isopropanol extracted freezing concentrates was found 91.97%, 81.79%, and 81.41% respectively as reported by Hernandez *et al.* (1997). Similarly, the leaf protein concentrates prepared from Cassava leaf by ultrafiltration and thermocoagulation showed 85% and 80% respectively as reported by Castellanos et al. (1994). It was also showed that the Leaf Protein Concentrates prepared from Cassava leaf were highly digestible in the feeding trial experiment on catfish and it is considered as alternative plant protein source (Fagbenro and Olurole, 2016). Diwanji *et al.* (1997) studied the protein digestibility in vitro from 11 aquatic plants and reported in the range of 69.8% to 42.1%. Similar results were also reported by Maliwal (1983) from 9 different plant species in the range of 86% to 30%. Similarly the seed and leaf flour of *Moringa oleifera* has also shows comparable in-vitro protein digestibility which was 61.12% and 57.22% respectively as reported by Mune Mune *et al.* (2016). These reports are comparable for protein digestibility in vitro in the present investigation. The variations found among the species were characteristics of individual plant species.

CONCLUSION:

The leaves of wild and underutilised plants can be successfully exploited for the preparation of LPC and as a source of protein however, it should have low level of antinutritional factors. The finding in the present investigation as compared wild and underutilised plant species the highest protein digestibility *in vitro* was observed in wild plant species and as compared to leaf extract(LE) and leaf protein concentrate (LPC) the *in vitro* highest protein digestibility was found in LPC .

REFERANCE :

Akeson, W.R. and Stahmann, M.A (1965). Nutritive value of leaf protein concentrates- an in vitro digestion study. *J. Sci. Fd Agric.***13:** 139.

Buchanan, R.A. (1969). Effect of storage and lipid extraction on the properties of leaf protein.*J. Sci. Fd Agric.* **20:** 359-364.

Castellanos, R; Altamirano, S.P. and Moretti, R.H. (1994). Nutritional characteristics of Cassava (*Manihotesculenta*Crantz) leaf protein concentrates obtained by ultra-filtration and acidic thermo-coagulation.*Plant foods for Human Nutrition.***45:** 357-363.

Dewanji, A; Chanda, S; Si, L; Barik, S. and Matai, S. (1997).Extractability and nutritional value of leaf protein from tropical aquatic plants.*Plant Foods for Human Nutrition.***50:** 349-357.

Duckworth, J. and Woodham, A.A. (1961).Effect of sources of raw material and method of drying on protein value for chicks and rats. *J. Sci. Fd Agric.* **12:**15.

Duckworth, J; Hepburn, W.R. and Woodham, A.A. (1961).*J. Sci. Food Agr.***12:** 16.

Fagbenro, O.A. and Olurole, A.O.(2016). Digestibility of nutrients in Cassava leaf protein concentrate of *Oreochromisniloticus*(Cichlidae) and *Clariasgariepinus* (Claridae). *African J. Fisheries and Aquatic Resource Management,* 1 [1]: 13-19.

Hernandez, T; Martinez, C; Hernandez, A. and Urbano, G. (1997). Protein quality of alfalfa protein concentrates obtained by freezing. *J. Agric Food Chem.* **45:** 797-802.

Henery, K.M. and Ford, J.E. (1965). The nutritive value of leaf protein concentrate determined in biological tests with rats and microbiological tests. *J. Sci. Fd Agric.***16:** 425.

Hsu, H.W; Vavak, D.L; Satterlee, L.D. and Miller, G.A.A. (1977).Multienzyme technique for estimating protein digestibility.*J. Sci. Food. Agr.***12:** 16.

Kohler, G.O. and Bickoff, E.M. (1971). In "Leaf Protein: its agronomy, Preparation, Quality and Use." IBP Handbook 20.N.W.Pirie (Editor).Blackwell Scientific Publications, Oxford and Edinburgh.Pp. 69.

Maliwal, B.P. (1983). In vitro methods to assess the nutritive value of leaf protein concentrate. *J. Agric Food Chem.***31(2):** 315-319.

Mune, M.A., Emillenne, C.N., Christian, B.B., and Samual, R.M. (2016). A comparison on the nutritional quality of proteins from *Moringaoleifera*leaves and seeds. Research Article in *Cogent Food & Agriculture,* **2**: 1213618.

Mohamed, T. K., Kexue, Z., Amadou I., Tarawalie, F.andHuiming Z.(2009).Functionality, *in Vitro* Digestibility and physicochemical properties of two varieties of defatted foxtail Millet protein concentrates. *Int. J. Mol. Sci.* **10:** 5224-5238.

Pirie, N.W. (1966). Leaf protein as human food.*Science.***152:** 1701.

Singh, S., Varshney, V.K., Wah, N., and Khan, L.H. (2014). Isolation and biochemical analysis of leaf protein concentrate from the leaves of *Shorearobusta*. *Pakistan J. of Nutrition.***13(9):**546-553.

Waterlow, J.C. (1962). The absorption and retention of nitrogen from leaf protein by infants recovering from malnutrition.*Brit. J. Nutr.***16:** 531-540.

Woodham, A.A. (1971). The use of animal test for the evaluation of leaf protein concentrates. In: Pirie, N.W. (ed), Leaf Protein: Its Agronomy, preparation, quality and Use. London, UK: Blackwell, PP. 115-130.

Table No.1. Protein digestibility (%) of leaf extract (LE) and leaf protein concentrates (LPC) of various plants (Dry wt. basis)

Plant Name	Protein Digestibility (%)	
	Leaf extract (LE)	Leaf protein concentrate (LPC)
Cultivated plant sp.		
Brassica juncea(L.) Czern. &Coss.	69.70	76.69
*Brassica napus*L.	68.57	77.60
Brassica oleracea var. *Botrytis*L.	72.41	83.24
Wild plant sp.		
*Celosia argentea*L.	71.28	84.36
Chenopodium album L.	73.54	83.01
Digera muricata(L.)Mart.	80.30	83.24
Goniocaulon indicum (Klein ex willd). C.B. C.L.	63.16	78.61
Ocimum americanum L.	60.00	67.56
*Tridax procumbens*L.	72.86	74.55
Vigna trilobata(L.)Verde.	73.99	84.82
Mean	70.58	79.37
Critical Difference. C.D. (5%)	-	1.997
Critical Difference. C.D. (1%)	-	2.923
Coefficient of Variation. C.V. (%)	-	2.287