Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 19(3), September-December, 2021]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 19(3) September-December, 2021

CONTENTS

Potential of common leaves of India as a source of Leaf Protein Concentrate

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ABSTRACT: Leaf protein concentrate (LPC) is a cheap and vegetarian source of high-quality protein that can be used for dietary enrichment in various food products. The aim of this study was to compare different plants for preparation of LPC. It was found that LPC yield for curry leaves, drumstick leaves and tobacco leaves were 1.64±0.40, 6.60±0.29 and 4.32±0.17 %, respectively. Crude protein content of drumstick and tobacco leaves were 33.01± 2.48 % and 63.72± 2.99 % respectively. Tobacco leaves were identified as best source of LPC and were taken up for further nutritional evaluation. The moisture, total ash, crude protein, fat, crude fibre, carbohydrate and physiological energy value of fresh tobacco leaves were 75.38%, 3.45%, 10.39%, 0.28%, 4.13%, 6.37% and 69.56 kcal/100g, respectively. The moisture, total ash, crude protein, fat, crude fibre, carbohydrate and physiological energy value of tobacco LPC was 9.4%, 6.16%, 62.34%, 8%, 8.7%, 5.4% and 342.96 kcal/100g respectively.

Key words: Curry leaves, drumstick leaves, Leaf Protein Concentrate, tobacco leaves,

Protein has a very significant role in the human body. It helps to repair and build body tissues and allow metabolic reactions. It also provides a structural framework to the body and maintains proper pH and fluid balance. The protein requirement increases in different stages of life like infancy, adolescence, pregnancy and lactation. In recent years the population of the world has increased and consequently protein requirements have also increased. In this era there are limited sources of plant-based protein. Alternative protein sources should be established to fulfill the protein demand (Gorissen *et al*., 2018).

In the leaves, proteins are produced naturally by the process of photosynthesis. The amino acids synthesized are polymerized into less mobile forms and stored as such in the leaves. The build-up amino acids in the leaves have low bioavailability (Adeyeye and Omolayo, 2011) due to different anti nutritious components such as high fibre (Oke, 1973) and anti-nutritional factors (Huisman and Tolman, 1992; Liener, 1989). Leaf protein can be made more bioavailable by extraction or by reduction of anti nutritional factors. Studies clearly indicate that apart from lower methionine content, the amino acid profile from most leaf species compare favourably with those of soya bean, meat, fish and egg and generally, surpass FAO essential amino acid pattern (Adeyeye and Omolayo, 2011). Leaf protein concentrate is a compound in which the protein of the leaves is separated from other constituents present in the leaf. Various studies have shown that the protein quality of leaf protein concentrate is much higher than most of the pulses and some animal products (Morris, 1977).

Curry leaves (*Murraya koenigii*) are mainly found in South East Asia, predominantly in the countries like India, Bangladesh and Sri-Lanka. In recent years curry leaf cultivation is spreading throughout the world due to Indian immigrants. Curry leaves are mainly used in the South Indian cuisine. These are found all over the Indian sub-continent except in the higher Himalayan range (Singh *et al*., 2014). According to a study by Khatoon *et al*. (2011) curry leaves contain 6% protein along with calcium (830 mg), iron (0.93 mg) and beta-carotene (756 µg). The LPC of curry leaves can provide a good amount of protein in diet.

Drum stickleaves *(Moringa oleifera)* are generally

found in countries like Africa, Central and South America, Srinlanka, India, Mexico, Malayia, Indonesia and Phillipines(FAO, 1992). Every part of *Moringa oleifera* tree is useful. It is also used in the treatment of diseases like ascites, rheumatism, venomous bites, and also used as cardiac and circulatory stimulant (Caceres *et al.,* 1992). According to Ogbe and John (2012) *Moringa oleifera* leaves contains 17.01% protein and LPC of *Moringa oleifera* leaves contains 39.13% protein (Sodamade *et al*., 2013).

In 1492 the plant *Nicotiana tabacum* (tobacco) was discovered by the Columbus in Central America. Today tobacco is cultivated on 4.2 million hectare area all over the world (Chouteau and Fauconnie, 1988). In India, tobacco is grown in 0.4 million hectare area mainly cultivated in total fifteen states (Prasad, 2007). Ninety-nine Per cent of total produced tobacco is used for smoking and chewing purpose (Peto *et al.,* 1996). Tobacco leaves contain a high amount of protein and it is also good in terms of nutritional quality (Kung *et al.,* 1980).A research has showed that LPC of tobacco is free from nicotine and it can provide 25% dietary protein to albino rats (Chakraborty *et al*., 1985).

The aim of this study was to assess the LPC production from these three plant species in order to identify the best source for producing LPC.

MATERIALS AND METHODS

Procurement of leaves

Curry leaves and Drumstick leaves were harvested from trees locally available in G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Tobacco leaves were purchased from Etah (Uttar Pradesh), India. The stems were trimmed and leaves were stored at -20^{0}C for further use.

Preparation of LPC

LPC was extracted using the method of Khan and Varshney (2015) with some modifications. Hundred gram fresh leaves were washed with tap water, drained and blended with 600 ml distilled water in a blender. The prepared juice was then filtered with a muslin cloth and residue was pressed to get the remaining juice. The pH of juice was adjusted to 4.5 with 1N citric acid and then it was heated at 90^{0}C for precipitating the protein. Coagulant was filtered with Whatman no. 40 filter paper. The end products were protein cake and clear liquor. The cake was dried in an oven at 40^{0}C until constant weight.

Nutritional analysis of samples

Curry leaves and drumstick leaves were discarded because the yield of LPC from curry leaves was very low and the protein content of drumstick leaves LPC was much lower than tobacco leaves LPC. The samples of fresh tobacco leaves and LPC were analysed in triplicate for nutritional composition. The Per centages of moisture, crude protein, crude fat, crude fibre and total ash were determined by standard methods of the AOAC, 2012. Carbohydrate was estimated by the difference (subtracting per cent moisture, crude protein, crude fibre, crude fat and total ash from 100). The physiological energy was calculated as:

Physiological energy value (Kcal per 100g) = 4×Crude Protein (%) + 4×Carbohydrate (%) +9 × Crude Fat (%)

Statistical analysis

The experiments were done in triplicate and the values determined were presented as means ± standard deviation. The data were subjected to one-way analysis of variance (ANOVA) using the R software. Differences with $p < 0.05$ and p is equal to 0.05 were considered significant.

RESULTS AND DISCUSSION

This study was conducted to identify the best source for leaf protein concentrate. The LPC yield in different leaves were 1.64±0.40, 6.60±0.29 and 4.32±0.17% for curry leaves, drumstick leaves and tobacco leaves, respectively (Figure 1). The minimum LPC was found in curry leaves (1.64%) while maximum in drumstick leaves (6.60%). Significant difference was observed in the LPC yield from different leaves (p=0.009). According to Jadhao

and Bhuktar (2018) the per cent LPC ranged from 1.6 to 3.4% in different *Curcuma* species.

As the LPC yield from curry leaves was significantly lower from other leaves, it was discarded. The crude protein content of the other two leaves was analysed. It was found that crude protein content of drumstick leaves was 33.01± 2.48 % whereas it was 63.72± 2.99 % for tobacco leaves (Figure 2). Crude protein content of tobacco leaves LPC was significantly higher than drumstick leaves LPC (p=0.009). Also, more amount of drumstick leaves were required as compared to tobacco leaves to obtain one gram of protein (Table 1). The value for drumstick leaves was lower than 39.13% as reported by Sodamade *et al*. (2013) but higher than 17.01 % as reported by Ogbe and John (2012). In tobacco leaves LPC, 50-52% protein content was reported by Fantozzi and Sensidoni (1983) and Parmeswaran *et al*. (1988).

As the crude protein content of tobacco leaves LPC was significantly higher than drumstick leaves, tobacco LPC was taken up for further nutritional evaluation. Nutritional composition of fresh tobacco leaves and tobacco leaves LPC is given in Table 2.

The moisture content of tobacco leaves and its LPC was 75.38±2.31% and 9.4±0.22% respectively. Moisture is a very important constituent for shelf life. Moisture Per centage of tobacco leaves LPC from several studies was from 4-8% (Fantozzi and Sensidoni, 1983; Parmeswaran *et al.,* 1988). Studies showed that the moisture content of LPC from *R. denatus* leaves was 13.5%, *U. diocia* leaves was 17.7% (Sachan *et al.,* 2018) and *P. pinnata* leaves was 6.58% (Khan and Varshney, 2015). Moisture content of LPC of this study is little higher than other tobacco LPC studies.

Ash content of fresh leaves was 3.45% in this study. The ash content of blend Macedonia, Smyrna, and Samsun type tobacco was 14.78%, burley type tobacco was 24.53%, Flue cured type tobacco was 10.81%, Maryland type tobacco was 21.98% and Virginia type tobacco was 9.23% (Leffingwell, 1999). Ash content of tobacco LPC was found to be 6.16% whereas other studies reported 7-8%

Table 2: Nutritional composition of fresh tobacco leaves and tobacco leaves LPC

Nutritional Parameters (%)	Fresh leaves	LPC
Moisture	75.38± 2.31	9.4± 2.07
Crude Protein	10.39± 0.65	62.34± 4.54
Crude Fat	0.28±0.04	8.00± 1.22
Crude Fibre	4.13±1.12	8.7± 2.51
Total Ash	3.45±0.19	6.16± 1.98
Carbohydrate (by difference)	6.37±1.87	5.4± 0.79
Physiological Energy Value	69.56 kcal/ 100g	342.96 kcal/ 100g

*All results are mean± standard deviation of three values

Table 1: Leaves required to extract one gram protein

Leaves	Protein (%)	LPC Yield / 100g leaves	Protein (g)/ g LPC	Raw Leaves required to obtain 1 g LPC	Raw leaves required to obtain 1 g protein
Drumstick	33.012	6.604g	0.33	15.14g	49.9 g
Tobacco	63.726	4.322g	0.63	23.13g	36.55 g

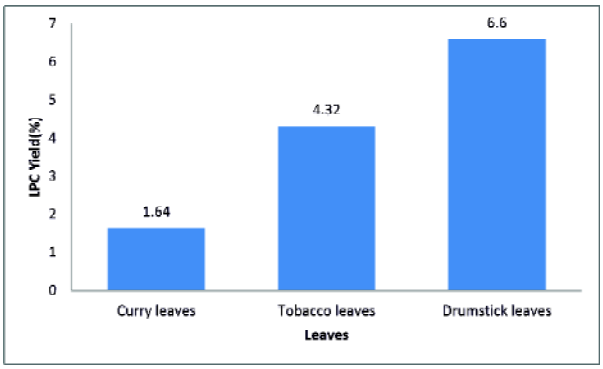

Fig. 1: LPC yield (%) in curry, tobacco and drumstick leaves

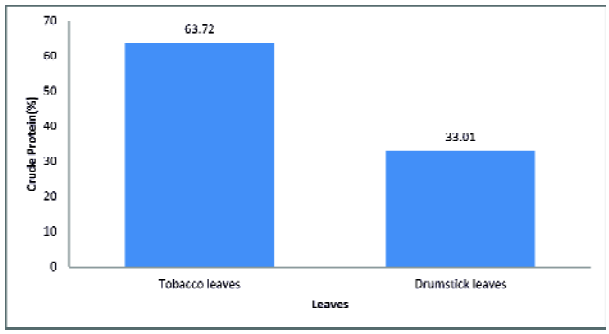

Fig. 2: Crude Protein (%) of LPC from drumstick and tobacco leaves

(Fantozzi and Sensidoni, 1983; Parmeswaran *et al.,* 1988). Ash content of LPC from other leaves such as *R. denatus, U. diocia, P. pinnata, G. heterophylla, E. crassipes* was 16.5%, 19.6%, 3.85%, 2.86%, 4.98% respectively (Khan and Varshney, 2015; Sachan *et al.,* 2018; Tripathi *et al.*, 2014; Virabalin *et al*., 1993). The ash content of tobacco LPC in this study was within reported range.

Protein content of fresh tobacco leaves was found to be 10.39%. According to Leffingwell (1999) 5.61% protein content was reported in air cured tobacco. Protein content of tobacco LPC was found to be 62.34% in this study. Fantozzi and Sensidoni (1983) and Parmeswaran *et al.* (1988) reported 50-52% protein in LPC in their studies. Protein content of tobacco LPC was also higher than LPC from other leaves such as *R. denatus* (9.80%), *U. diocia* (5.05%), *P. pinnata* (43.77%), *G. Heterophylla* (45.75%), *E. crassipe* (61.04%), *G. maculate* (43.6%), *C. callathyrus* (43.4%) but lower than Manihot sp. (68.9%), *A. fatcateria* (66.7%), *S. grandiflora* (70.1%) (Khan and Varshney, 2015; Sachan *et al.,* 2018; Tripathi *et al.*, 2014; Virabalin *et al*., 1993; Tangendja *et al*., 1984).

Fat content of fresh tobacco leaves found in this study was 0.28%. In tobacco LPC fat content was found to be 8% which is similar to the tobacco LPC study of Fantozzi and Sensidoni (1983) but much higher than tobacco LPC study of Parmeswaran *et al.* (1988) who reported 0.5% fat in LPC. Fat content of other leaf LPC such as *R.denatus, U.diocia, P.pinnata, G. heterophylla* was 1.35%, 1.5%, 12.84% and 16.69% respectively (Khan and Varshney, 2015;Sachan *et al.,* 2018;Tripathi *et al.*, 2014).

Crude fibre was 4.13% in fresh tobacco leaves. According to Leffingwell, 1999 different types of tobacco leaves contain crude fibre content in the range of 6.63% to 21.79%. Crude fibre content in tobacco LPC was 8.7%, which was much higher in comparison to another tobacco LPC study that reported 2% crude fibre content (Fantozzi and Sensidoni, 1983). One per cent crude fibre was reported in the study of *U. diocia* leaf LPC (Sachan *et al.,* 2018), 1.84% crude fibre was reported in *G. heterophylla* LPC (Tripathi *et al.*, 2014) and 1.02% crude fibre were reported in *E. crassipe* LPC(Virabalin *et al*., 1993). So, the fibre content found in this study was much higher than others.

Total carbohydrate content in fresh tobacco leaves was 6.37% and in tobacco LPC it was found to be 5.4%. A study on tobacco LPC reported carbohydrate content of 30% (Parmeswaran *et al.*, 1988). LPC *R. denatus* contained 58.85% carbohydrate, LPC *U. diocia* contained 55.15% carbohydrate (Sachan *et al.,* 2018), LPC *P. pinnata* contained 39.83% carbohydrate (Khan and Varshney, 2015) LPC *G. heterophylla* contained 27.40% (Tripathi *et al.*, 2014) and *E. crassipe* contained 32.96% carbohydrate (Virabalin *et al*., 1993). The carbohydrate content of tobacco LPC in this study is much lower than other studies. Physiological Energy Value of fresh tobacco leaves and tobacco LPC was 69.56±1.23 and 342.96 kcal/100g respectively.

CONCLUSION

In this study different plant sources were analysed for preparation of leaf protein concentrate. Curry leaves were discarded as the LPC yield from curry leaves was significantly lower from other leaves. Crude protein content of tobacco leaves LPC was significantly higher than drumstick leaves, hence tobacco LPC was taken up for further nutritional evaluation. LPC of tobacco leaves contained high amount of protein and fair amount of fibre as well as ash. The above experimental results clearly showed that tobacco leaves have good potential for providing leaf protein concentrate.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks ITC Limited for funding the project 'Extraction and estimation of protein by products and nutritional content of tobacco leaves'.

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Received: November 25, 2021
Accepted: December 22, 2021