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Optimization of Production Technique and Nutritional Evaluation of Leaf Protein Concentrate from Tobacco (*Nicotiana tabacum*)

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ABSTRACT

Background: Leaf protein concentrate (LPC) is a cheap and feasible source of high-quality protein. Researchers found the protein quality of LPC is similar to first-class protein food. Tobacco is a protein-rich leaf but contains a high amount of nicotine. According to studies the leaf protein concentrate obtained from tobacco leaves is nicotine-free and also useful to gaining weight in malnourished rats.

Methods: Fresh leaves were blended with distilled water in different leaf water ratio (1:3, 1:6, 1:9 and 1;12). The juice was filtered and coagulated at different pH (acidic, basic and neutral) with different coagulating techniques (conventional heating, Microwave and Ultrasound) to standardize the optimum procedure for leaf protein concentrate extraction. The coagulant was filtered and dried in the oven.

Result: The maximum quantity of crude protein and LPC yield (62.34% and 4.7 g) was extracted with a 1:9 leaf water ratio at acidic pH by using citric acid followed by the conventional heating method.

Key words: Citric acid, LPC, Microwave, pH, Protein, Tobacco, Ultrasound.

Abbreviations: LPC- Leaf protein concentrate, NTLPC- *Nicotiana tabacum* leaf protein concentrate.

INTRODUCTION

Tobacco (*Nicotiana tabacum*) is an economically valuable plant mainly used for smoking and chewing purpose which causes a huge number of death worldwide (Peto *et al*. 1996) In India, tobacco is grown on 0.4 million hectares land. Total fifteen states of India cultivate tobacco (Prasad, 2007), Nowadays India stands third all over the world in terms of tobacco production (800 million kg) (ICAR, 2019). Along with smoking and chewing, it is also used for medicinal purposes, pesticides and food additives (WHO, 2007). Tobacco leaves contain a higher amount of protein and are also good in other nutrients (Kung *et al*. 1980). Research showed that leaf protein concentrate (LPC) of tobacco is free from nicotine and was seen to treat malnutrition by providing 25% dietary protein to albino rats (Chakraborty *et al*. 1985).

Protein concentrate is generally used as a dietary supplement for animals and humans, which is extracted from vegetable or animal sources. Many types of protein concentrates are commercially available in the market such as whey protein concentrate and soy protein concentrate. Generally, whey protein concentrate contains 72.0-76.6% of protein (Kumar *et al*. 2018), soy protein concentrate contains 62.46-63.79% protein (Casas *et al*. 2017). Other than these, some unconventional protein concentrates are rebon shrimp protein concentrate (Suparmi *et al*. 2019) and *P. pinnata* leaf protein concentrate (Khan and Varshney, 2015) Leaf protein Concentrate (LPC) is a concentrated form of protein obtained from leaves along with some amount of carbohydrate, fat and minerals. Studies showed that the protein quality of leaf protein concentrate is similar to soya bean, meat, fish and egg and generally surpasses FAO recommendation of the essential amino acid pattern (Adeyeye *et al*. 2011). Various studies have shown that the protein quality of LPC is much higher than most of pulses and some animal products (Morris, 1977). LPC is also used in animal food as a protein supplement (Abowei and Ekubo, 2011).

Malnutrition is a burning problem in India Recent survey of National Family Health Survey (NFHS) 2019-21 revealed there is no significant improvement in health status, data depict 35.5% of children are stunted and 19.3% children are wasted. (NHFS-5, 2021) According to the UN, every year 2.1 million children in India die before completing the 5th

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year of their life (UN Report, 2019). The World Bank estimates that India is one of the highest-ranking countries in the world with children suffering from malnutrition (World Bank Report, 2013). Good quality protein consumption can help to overcome this situation. Leaf Protein Concentrate (LPC) can be a source of high-quality protein that can relieve kwashiorkor, marasmus and kidney diseases. Along with conventional protein production, unconventional protein production should also be increased. There are many varieties of plants present in the world which contain a good amount and quality of protein such as mulberry leaves thatcontain 28.83% protein on a dry weight basis (Angotra *et al*. 2021), jute mellow (*Corchorus* spp.) contains 25% protein (Ngomuo *et al*. 2017).

This study aims to optimize the extraction procedure of tobacco leaf protein concentrate (NTLPC) in terms of higher LPC yield and protein quantity.

MATERIALS AND METHODS

The research was conducted in 2020-21 at the Department of Foods and Nutrition, College of Home Science, G.B. Pant University of Agriculture and Technology, Pantnagar (U.S. Nagar, Uttarakhand).

Materials

Fresh leaves of tobacco (Azad Kanchan variety) were purchased from Etah (Uttar Pradesh), India. The stems were trimmed and leaves were stored at -20°C for further use.

Extractions of leaf protein concentrate

NTLPC was extracted using the modified method of Khan and Varshney (2015). 100 g fresh leaves were washed with tap water, drained and blended with distilled water with different leaf water ratios in a blender. The prepared juice was then filtered with a muslin cloth and the filtered cake was pressed to get the remaining juice. The juice was coagulated at different pH such as acidic (pH-4), basic(pH-9) and neutral pH (pH-7) with different coagulating techniques. The 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°C until a constant weight was obtained (Fig 1).

The method for extraction of LPC from NTLPC was optimized on the basis of the amount of NTLPC yield (g/100 g) and percentage of protein present in NTLPC obtained using different parameters (Table 1). Each time one parameter was varied and others were kept constant. In every condition, NTLPC yield and protein percentage were recorded which indicated the effect of the variables on the extraction technique.

Table 1: Parameters used for NTLPC extraction.

Parameters	Variables
Leaf: water ratio	1:3, 1:6, 1:9, 1:12
Ph	4 (acidic), 7 (neutral), 9 (basic)
Acid	1N acetic acid, vinegar, 1N citric acid, lemon, 1N HCl
Processing technique	Heating at 90°C, Microwave, Ultrasound

Leaves and water ratio

The fresh leaves of tobacco were blended with distilled water in different ratios. For 100 grams tobacco leaves water amounts were varied as 300 ml, 600 ml, 900 ml and 1200 ml. The NTLPC yield and protein percentage were noted for each of the four-leaf water ratios. In the study of Khan and Varshney (2015) the leaf and water ratios were varied from 1:1 to 1:11 and in the study of Aruna *et al*. (2007), the solid to solvent ratios were varied up to 1:12.

pH adjustment and different acid optimization

After optimizing the leaves and water ratio the pH level was varied into acidic (pH-4), Neutral (pH-7) and basic (pH-9) and NTLPC yield and protein content were recorded. In the study of Khan and Varshney (2015) the pH was also varied into neutral, acidic and basic pH. In the study of Aruna *et.al*. (2007) pH was also varied from pH 4 to pH 13. The natural pH of the tobacco leaf water mixture is slightly acidic (pH-5.7). So, the acidic pH was maintained by using 1N citric acid (32 ml), 1N Acetic acid (200 ml), 1N HCl (7 ml), lemon juice (60 ml) and vinegar (600 ml) which were used in 900 ml of water and leaf mixture in room temperature and basic pH was maintained by using 1N NaOH (8-9 drops) in room temperature and neutral pH was also maintained by using 1N NaOH (2-3 drops). The yield of NTLPC and protein percentage was measured to optimize the most suitable acid. Khan and Varshney (2015) were also used citric acid and acetic acid along with HCl to optimize the extraction method of leaf protein concentrate.

Heating method

After the pH optimization tobacco leaf water mixture was heated at 90°C on a hot plate for 30 minutes. Microwave and ultrasound techniques were also used along with the normal heating method. In the microwave, the leaf water mixture was heated at 120°C for 3 minutes. The water-leaf juice was also treated with ultrasound for 30 min to homogenize the mixture. A combination of ultrasound (30 min) along with the heating (90°C for 15 min) method and Ultrasound (30 min) along with the microwave (120°C for 3 minutes) method was also used. In this experiment total of five methods were used: heating method (90°C), ultrasound method, ultrasound along with heating, ultrasound along with microwave and only microwave heating. NTLPC yield and protein percentage were checked. The study conducted by Cheng *et al*. (2021) also used microwave and ultrasound methods along with the traditional heating method to extract protein from *Moringa oliefera* leaves.

Analysis of crude protein

The samples of NTLPC were analyzed in triplicates for crude protein content. The percentage of crude protein was

determined by the Kjeldahl method (AOAC, 2012). The percent Nitrogen was calculated by the following formula:

$$\% \text{ Nitrogen} = \frac{[(\text{ml of the standard solution-ml of blank}) \times \text{N of acid} \times 1.4007]}{\text{Weight of samples in grams}}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25 \text{ (conversion factor)}$$

Statistical analysis

The experimental study was done in triplicate and the values determined, are presented as means. The data were subjected to a one-way analysis of variance (ANOVA) using the R software. Differences with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Water leaf ratio

The result of different water ratios with the leaves is shown in Fig 2. The NTLPC yield and protein percentage increased from 1.22±0.15% to 4.72±0.19% and 23.62±0.07% to 62.34±0.03% respectively as the water ratio was increased from 1:3 to 1:9 ratio after that protein content increased slightly but LPC yield decreased. The relation of water ratio and LPC yield (p=0.0485) and protein content (p=0.023) was statistically significant. So it may be concluded that the higher levels of water incorporation resulted in a good leaf slurry that helped to increase NTLPC yield and protein content. The study conducted by Virabalin *et al*. (1993) on LPC extraction from water hyacinth used 1:3 leaves water ratio, the study of. Tangendja *et al*. (1984) also used 1:3 leaves water ratio for the LPC extraction process of different tropical plants. Whereas, the study conducted by Khan *et al*. (2015) and Tripathi *et al*. (2014) on *P. pinnata* and *G. heterophylla* leaves showed that the highest LPC yield was obtained by using 1:9 leaves water ratio but the studies of protein extraction from moringa leaves (Soo *et al*. 2021) and tea residue (Qiaoyun *et al*. 2017) concluded 1:30 and 1:70 leaves water ratio gave the best result.

pH

The effect showed the highest NTLPC yield and protein content was found in acidic pH (Fig 3). As the pH was increased the LPC yield (from 4.72±0.19% to 1.7±0.11%) and the protein content (from 62.34±0.03% to 45.21±0.17) were decreased (Fig 3). The effect of NTLPC yield (p=0.033) and the protein content (p=0.006) and the pH level was statistically significant. A study on *P. pinnata* leaves and different tropical leaves also suggested acidic pH for best leaf protein concentrate isolation (Khan *et al*. 2015; Tangendja *et al*. 1984; Merodio *et al*. 1983). A study conducted on purslane leaves varied pH from pH 2 to pH 12 and it was found that the best protein yield was obtained in pH 5.5 (Shanker *et al*. 2018). A study on LPC extraction from water hyacinth leaves also used pH 4 for acid coagulation (Virabalin *et al*. 1993).

Different acids

After optimizing the pH level different acids were used to obtain the best result. (Fig 4) The result of using different acids showed no significant difference in NTLPC yield (p=0.114) but there was a significant difference in the protein content (p=0.009) from 2.9±0.02% to 5.29±0.22% and 33.13±0.06% to 68.19±0.04%.

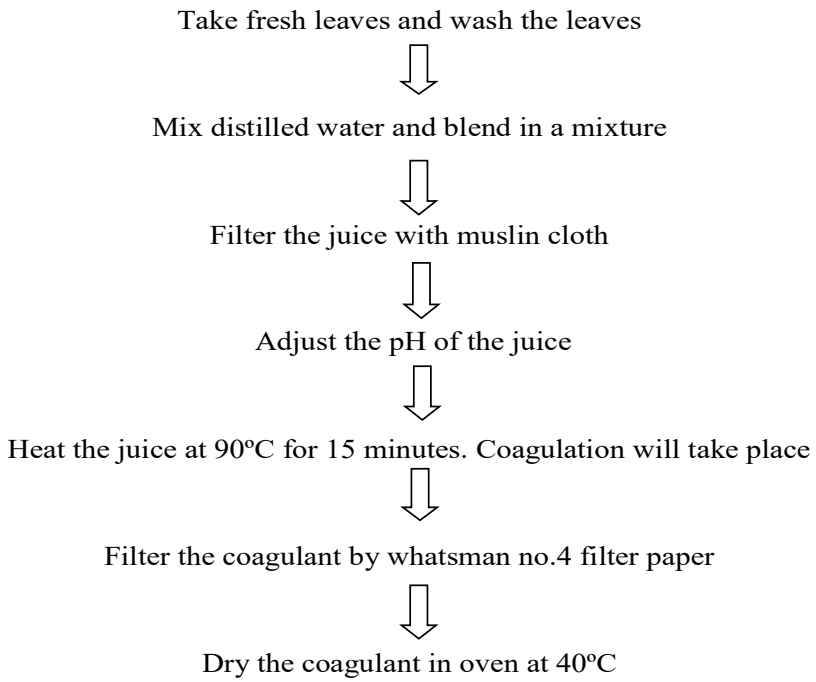

Fig 1: Schematic representation of the procedure of extracting tobacco leaf protein concentrate (NTLPC).

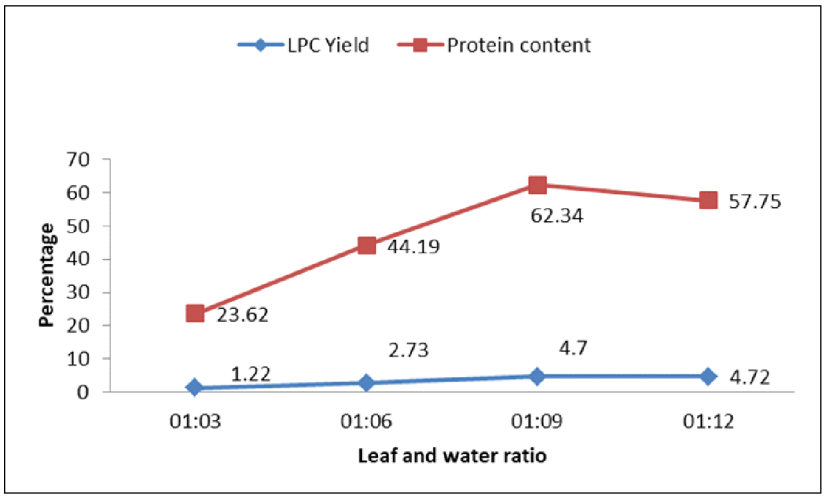

Fig 2: Effect of water concentration on NTLPC yield and protein content.

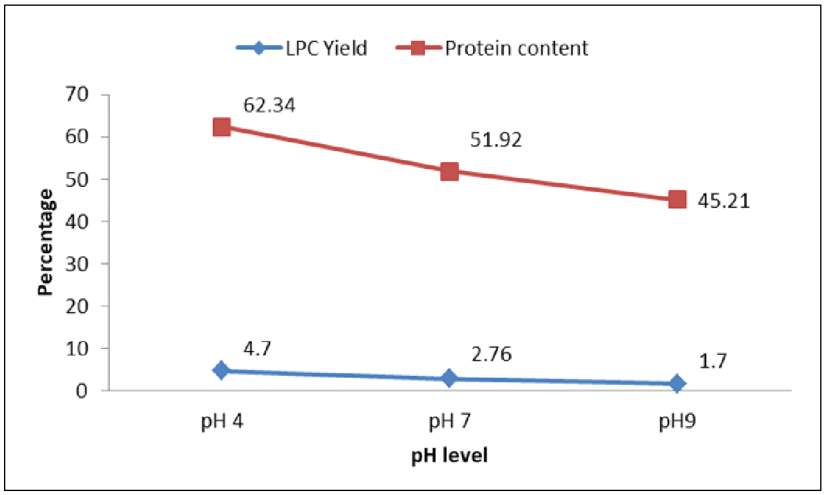

Fig 3: Effect of different pH levels in LPC yield and protein content.

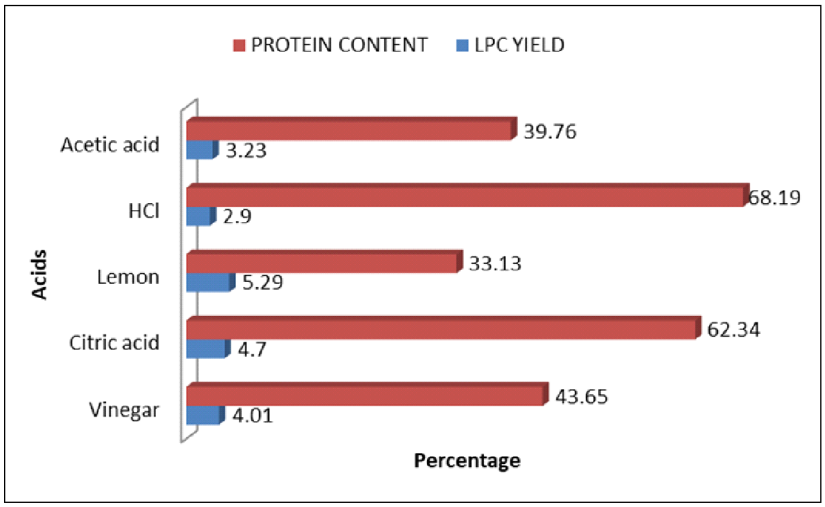

Fig 4: Effect of different acids in LPC yield and protein content.

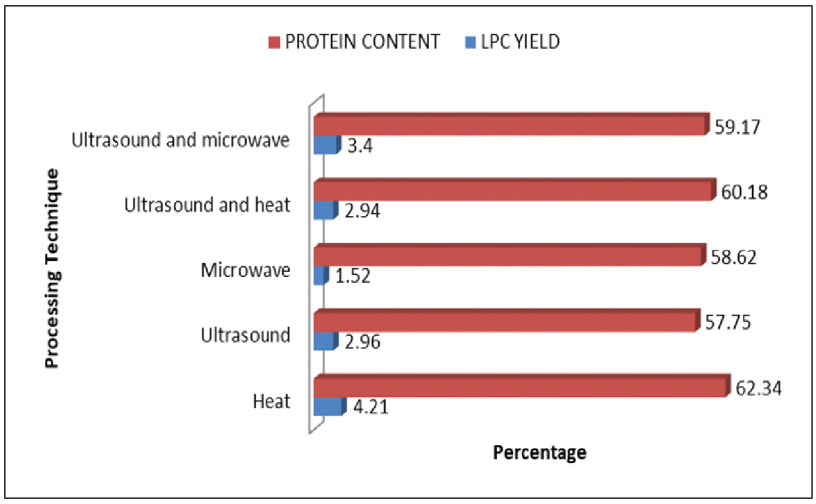

Fig 5: Effect of different techniques in LPC yield and protein content.

The highest NTLPC yield was obtained from lemon and the lowest was from HCl but the highest protein content was obtained from HCl and the lowest was from the lemon. A study conducted on water hyacinth leaves used HCl for acid coagulation (Virabalin *et al*. 1993). Acid coagulation by citric acid is safe for human food consumption. A study on *P. pinnata* leaves LPC also suggests that citric acid is the best option for human consumption (Khan *et al*. 2015).

Heat, microwave and ultrasound techniques

The result of using different techniques (conventional heating, ultrasound, microwave heating, ultrasound along with conventional heating and ultrasound along with microwave heating) showed no significant difference between protein content (p=0.882) but a significant difference was noted in NTLPC yield (p=0.009) range from 1.52±0.02 to 4.72±0.19% to 5.75±0.3 to 62.34±0.03% respectively. The highest NTLPC yield was obtained by heating followed by ultrasound and microwave combined method, ultrasound and heat, ultrasound and microwave. In terms of protein content highest protein content was also obtained by the heating method and the lowest protein yield was obtained by ultrasound (Fig 5). The study conducted on the extraction of LPC from *Diplaziume sculentum* leaves concluded that the yield of protein extraction through an ultrasonic wave is better than the conventional method. (Saha *et al*. 2017).

The four variables were studied to optimize the technique of LPC extraction to derive the maximum protein yield from tobacco leaves. The highest LPC yield was obtained when a leaf water ratio of 1:9 was used, the acid pH-4 gave the best result with citric acid and HCl but citric acid gave the highest amount of yield and protein content as HCl gave a low amount of NTLPC yield. So, citric acid was chosen. Regarding heating technique, conventional heating method gave the best result. Therefore the conventional heating at 90°C gave the best result.

CONCLUSION

As focus on sustainable diet is increasing, leaf protein concentrate has a good potential to be an alternative protein source. *Nicotiana tabacum* is a good source of protein and LPC of tobacco can be utilized as a protein supplement. The study conducted to optimize the extraction procedure for obtaining the maximum yield from tobacco leaves showed that the LPC yield and protein content increased as the leaf water ratio was increased to 1:9 ratio after which protein content decreased, but the LPC yield and protein content decreased as the pH level increased. The highest protein content was observed by the use of HCl but the highest LPC yield was observed by the use of lemon. In the context of heating technique, the best results were found by using the conventional heating technique in terms of both LPC yield and protein content. It may be concluded that the optimized parameters for the producing NTLPC are 1:9 leaves water ratio, acidic pH level 4, use of citric acid followed by simple heating at 90°C. Through this method, 62.34±4.54% protein can be recovered from NTLPC. Heat coagulation involves the simplest equipment and the lowest processing cost. The process standardized for extraction of LPC from tobacco leaves is a safe cost-effective procedure.

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Conflict of interest: None.

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