The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(1): S.I (1), 90-92, 2025 www.thebioscan.com

HPLC ANALYSIS OF LEAF PROTEIN CONCENTRATE OF *BRASSICA OLERACEA* VAR. BOTRYTIS

NEHA SAWANT[1*], MEENAKSHI VAIDYA[2]

[1,2]S.V. K. M's Mithibai College of Arts, Chauhan Institute of Science and Amrutben Jivanlal College of Commerce and Economics (Autonomous) Affiliated to the University of Mumbai, Vile Parle - West Mumbai 400056, India.

[*]Corresponding author Email: nehasawant97@gmail.com

DOI: 10.63001/tbs.2025.v20.i01.S.I(1).pp90-92

KEYWORDS
Brassica oleracea var. botrytis,
Leaf Protein Concentrate (LPC),
HPLC,
amino acids

Received on:

10-02-2025

Accepted on:

07-03-2025

Published on:

09-04-2025

ABSTRACT

Protein is a crucial component of a balanced diet, fundamental to maintaining human health. While protein can be obtained from plant and animal sources, underutilized agricultural by-products provide an alternative sustainable protein source. Brassica oleracea var. botrytis (cauliflower) is a widely consumed vegetable in India, yet its leaves, which are rich in essential nutrients, are often discarded as waste. This study explores the extraction of protein from cauliflower leaves using the Leaf Protein Concentrate (LPC) method, followed by amino acid analysis via High-Performance Liquid Chromatography (HPLC). The HPLC results identified 21 amino acids, including essential amino acids such as lysine (0.28%), threonine (0.23%), and methionine (0.15%), along with significant amounts of glutamic acid (1.54%). These findings highlight the potential of cauliflower leaf protein as a sustainable and nutritious dietary supplement, offering a viable strategy for reducing food waste and addressing nutritional deficiencies.

INTRODUCTION

Protein is an essential ingredient in a healthy, balanced diet. It can be derived from plant and animal sources, with an intake recommendation of 0.83 g protein/kg per day to meet the needs of a healthy adult population, as stated in the 1985 FAO/WHO/UNU report (Schonfeldt et al.,2012). Protein Energy Malnutrition (PEM)is most commonly seen in infants, paediatrics, and children under the age of 5 approximately 24.8% of the population suffer from stunting, 2.21% by overweight, and 6.41% by wasting and severe wasting the survey reports of UNICEF and WHO (Sahare et al.,2023). Proteins play a fundamental role in maintaining the human body function and overall well-being (Chakraborty et al.,2016). The nutritional quality of protein sources is largely determined by their amino acid composition (Liu et al., 2019).

Brassica oleracea var. botrytis (Cauliflower) is one of India's most commercially significant vegetable crops. It is widely consumed across the country and valued for its nutritional benefits (Pareek et al., 2024). However, despite its popularity, a significant portion of the cauliflower plant, particularly its leaves, is discarded as waste. These leaves contain essential nutrients, including high-quality proteins and stable vitamin C, which retain its properties even after cooking (Singh et al., 2019). The protein content in cauliflower is 1.5 to 2.0 times higher than that in white-head cabbage and 2 to 3 times richer in ascorbic acid (Pusik et al., 2018). Effective utilization of cauliflower leaves can significantly reduce food waste while enhancing nutritional intake. Amino acids are essential components for human health, present as free or bound in protein structures. Evaluating the amino acid composition is important to assess the nutritional value of protein sources. Due to their ability to provide separation, chromatographic methods are considered the preferred approach for analyzing amino acids (Lestari et al., 2022).

In this study, protein from *Brassica oleracea* var. botrytis waste leaves are extracted using LPC, an innovative approach aimed at efficiently utilizing agricultural by-products. High-Performance Liquid Chromatography is employed to analyze and quantify the amino acid composition of the extracted protein, ensuring a comprehensive assessment of its nutritional value. This research seeks to explore the potential of cauliflower leaves as a valuable protein source, contributing to sustainable food systems and addressing protein deficiency challenges.

Materials and Methods

Collection and authentication of plant material

Fresh outer leaves of *Brassica oleracea* var. botrytis were collected from the Bhandup vegetable market. All unwanted particles were removed, and the leaves were cleaned to eliminate dust. The Leaf Protein Concentrate (LPC) was then prepared following the method described by Fellows (1987). The authentication of *Brassica oleracea* var. botrytis was conducted at Keshavsrushti Krishi Tantra Vidyalaya in Bhayandar (W), which is

affiliated with Dr. Balasaheb Sawant Konkan Krushi Vidyapeeth Dapoli.

HPLC Analysis (USP-NF) 2021

The aqueous suspension of LPC from *Brassica oleracea* var. botrytis was prepared and filtered through a 0.22-micrometer filter. A 10 µL sample was injected into the C18 column of the HPLC system. The analysis was conducted at room temperature with a detection wavelength of 254 nm, a run time of 10 minutes, and a flow rate of 1 mL/min. The HPLC system used was a Jasco binary setup.

Result

The chromatographic analysis of LPC was conducted to compare its amino acid profile with standard amino acid samples. The resulting chromatogram provides insight into the composition and concentration of individual amino acids present in the LPC extract (Table 1, Figure 1).

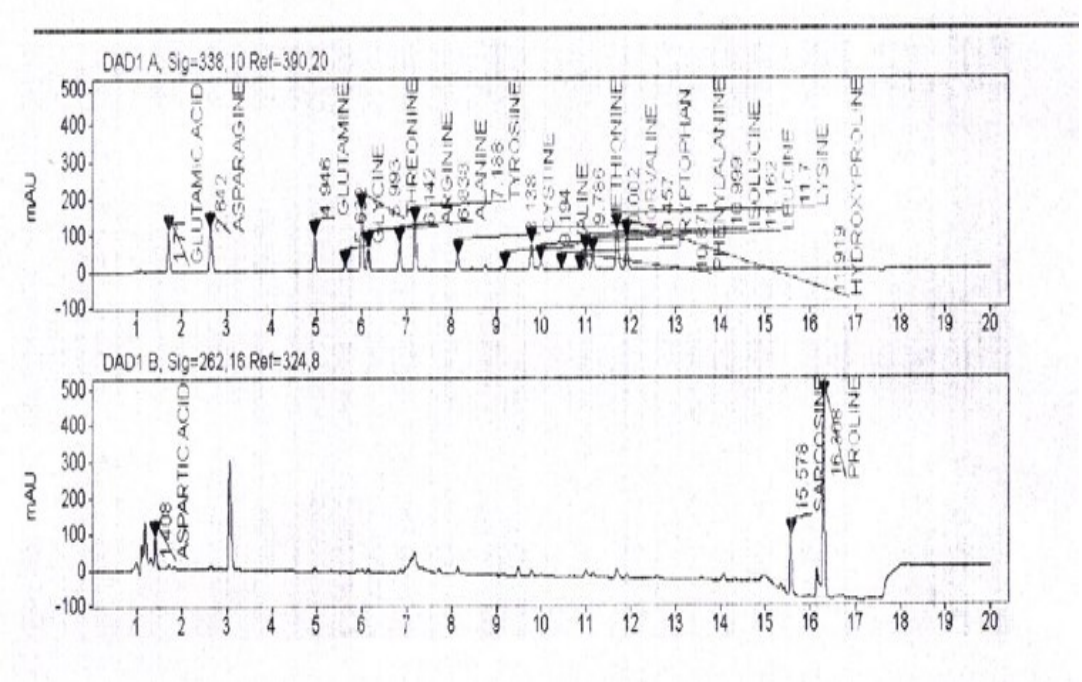

Figure 1: Chromatogram of standards and LPC of *Brassica oleracea* var. botrytis.

Table 1. Amino acid composition of LPC of *Brassica oleracea* var. botrytis.

								Sample Areas	Calculated value of AA	
Peak #	AA Name	AA Abbreviation	Derivative Type	Molecular Wt	Concentration of standard (pmol)	Wt of AA standard (mg/ml)	Standard Area	Sample1 1502-002 (1:2)	Sample (%)	Sample (mg/g)
1	Aspartic Acid	ASP	OPA	133.11	900	0.1198	251.6957	259.688	**0.10**	**98.88**
2	Glutamic Acid	GLU	OPA	147.13	900	0.9853	183.5586	357.905	**1.54**	**1536.92**
3	Aspraragine	ASN	OPA	132.11	900	0.1189	182.4627	495.9964	**0.26**	**258.57**
4	Serine	SER	OPA	105.09	900	0.0946	181.1644	0	**0.00**	**0.00**
5	Glutamine	GLN	OPA	146.14	900	0.1315	242.239	326.312	**0.14**	**141.74**
6	Histidine	HIS	OPA	209.63	900	0.1887	173.2174	0	**0.00**	**0.00**
7	Glycine	GLY	OPA	75.07	900	0.0676	166.8857	46.2914	**0.01**	**14.99**
8	Threonine	THR	OPA	119.12	900	0.1072	235.8648	626.6166	**0.23**	**227.85**
9	Arginine	ARG	OPA	174.2	900	0.1568	170.5647	205.445	**0.15**	**151.07**
10	Alanine	ALA	OPA	89.09	900	0.0802	214.4327	251.561	**0.08**	**75.25**
11	Tyrosine	TYR	OPA	181.19	900	0.1631	236.8674	499.2513	**0.27**	**274.97**
12	Cysteine	CYS	OPA	117.15	900	0.1054	183.1388	140.3269	**0.06**	**64.63**
13	Valine	VAL	OPA	240.3	900	0.2163	263.2231	21.4968	**0.01**	**14.13**
14	Methionine	MET	OPA	149.21	900	0.1343	181.3951	257.1898	**0.15**	**152.32**
15	Norvaline*	NVA	OPA	117.08	900	0.1054	202.2563	83.9814	**0.04**	**35.00**
16	Tryptophan	TRP	OPA	204.23	900	0.1838	62.8643	11.9398	**0.03**	**27.93**
17	PHENYLALANINE	PHE	OPA	165.19	900	0.1487	94.471	5.6641	**0.01**	**7.13**
18	Isoleucine	ILE	OPA	131.17	900	0.1181	174.4489	183.8681	**0.10**	**99.54**
19	Leucine	LEU	OPA	131.17	900	0.1181	178.1079	164.7646	**0.09**	**87.37**
20	Lysine	LYS	OPA	182.65	900	0.1644	212.8808	448.3604	**0.28**	**276.98**
21	Hydroxyproline	HYP	FMOC	115.13	900	0.1036	282.3416	322.2742	**0.09**	**94.62**
22	Sacrosine (IS)	SAR	FMOC	89.09	900	0.0802	364.1397	506.4142	**0.09**	**89.21**
23	Proline	PRO	FMOC	115.13	900	0.1036	331.6421	1541.3092	**0.39**	**385.25**
									4.13	4131.91

The HPLC analysis of LPC from cauliflower leaves identified a total of 21 amino acids, including both essential and non-essential amino acids. The essential amino acids present in the LPC were threonine (0.23%), valine (0.01%), methionine (0.15%), tryptophan (0.03%), phenylalanine (0.01%), isoleucine (0.10%), leucine (0.09%), and lysine (0.28%). The non-essential amino acids detected were aspartic acid (0.10%), glutamic acid (1.54%), asparagine (0.26%), glutamine (0.14%), glycine (0.01%), arginine (0.12%), alanine (0.10%), tyrosine (0.27%), cysteine (0.06%), norvaline (0.04%), hydroxyproline (0.09%), sarcosine (0.09%), and proline (0.39%).

The results indicate that *Brassica oleracea* var. botrytis leaf protein concentrate contains a balanced profile of essential and non-essential amino acids, highlighting its potential as a sustainable and nutritious protein source. The presence of significant amounts of glutamic acid and lysine suggests its potential application in improving dietary protein intake, particularly in regions facing malnutrition challenges.

DISCUSSION

The HPLC analysis of Leaf Protein Concentrate (LPC) from cauliflower leaves identified a total of 21 amino acids, including both essential and non-essential amino acids.

Comparative studies on amino acid profiling in various plant sources further support the significance of these findings. For instance, HPLC analysis of five different Korean cultivars of young radish revealed variations in amino acid composition, emphasizing genetic and environmental influences on amino acid content (Kim et al., 2017). Similarly, the free amino acid profile of 18 samples of tronchuda cabbage (Brassica oleracea L. var. costata DC), harvested over three different months, was determined using HPLC with UV-Vis detection (Oliveira et al., 2008). Furthermore, the amino acid composition of three medicinal plants—*P. hirta*, *E. thymifolia*, and *P. indica*—were analyzed using HPLC, confirming the presence of significant amino acids essential for human health (Prasad, 2017).

In addition to leafy vegetables, wild *Allium* species from the Western Himalayas have also been screened for their amino acid content using High-Performance Liquid Chromatography, reinforcing the importance of plant-based sources in nutritional studies (Pandey et al., 2020). These studies collectively highlight the growing interest in exploring underutilized plant resources for their amino acid composition and potential dietary benefits applications.

CONCLUSION

The study highlights the potential of cauliflower leaves, an often-discarded agricultural by-product, as a valuable source of high-quality protein. The extraction of Leaf Protein Concentrate (LPC) from cauliflower leaves and its subsequent analysis using HPLC revealed the presence of a well-balanced composition of essential and non-essential amino acids. The high content of glutamic acid and lysine further supports its suitability as a nutritional supplement to address protein deficiencies.

REFERENCES

- Chakraborty .S, Santa Datta(2016). Estimation of Macro-Nutrients in Domestically Processed Cauliflower Leaf Powder. International Journal of Science and Research (IJSR), 5(2): 2094–2096.
- Fellows, P. (1987). Village-scale leaf fractionation in Ghana. Tropical Sci., 22, 77-87.
- Kim, J. K., Bong, S. J., & Park, S. U. (2017). Amino Acids Content in Different Cultivars of Young Radish(Raphanus *sativus*). Asian Journal of Chemistry, 29(7): 1427–1430.
- Lestari, L. A., Rohman, A., Riswahyuli, Purwaningsih, S., Kurniawati, F., & Irnawati. (2022). Analysis of amino acids in food using High-Performance Liquid Chromatography with derivatization techniques: A review. Food Research, 6(3): 435–442.
- Liu, J., Klebach, M., Visser, M., & Hofman, Z. (2019). Amino Acid Availability of a Dairy and Vegetable Protein Blend Compared to Single Casein, Whey, Soy, and Pea Proteins: A Double-Blind, Cross-Over Trial. Nutrients, 11(11): 2-13.
- Oliveira, A., Pereira, D., Andrade, P., Valentão, P., Sousa, C., Pereira, J., Bento, A., Rodrigues, M., Seabra, R., & Silva, B. (2008). Free Amino Acids of Tronchuda Cabbage (*Brassica oleracea* L. Var. costata DC): Influence of Leaf Position (Internal or External) and Collection Time. Journal of Agricultural and Food Chemistry, 5: 5216–5221.
- Pandey, H. K., Guglani, A., Kharakwal, G. C., Balakrishna, G., & Bala, M. (2020). Variation in Amino Acids Composition through Pre-column Derivatisation using Phenylisothiocyanate by HPLC in Some Economically Important Less Explored Wild Allium Species of Western Himalayas. Defence Life Science Journal, 5(3):185–191.
- Pareek, J., Girdhar, A., Kumar, M., & Goyal, N. (2024). Constraints Faced by the Farmers in Production and Marketing of Cauliflower in Haryana, India. Asian Journal of Agricultural Extension, Economics & Sociology, 42(5):249–254.
- Prasad (2017). HPLC Analysis of Amino Acid and Antioxidant Composition of Three Medicinal Plants of (Pithoragarh) Uttarakhand Himalayas.Journal of Analytical & Pharmaceutical Research, Volume 6(5):119–122.
- Pusik, L., Pusik, V., Lyubymova, N., Bondarenko, V., Gaevaya, L., Serhiienko, O., Romanov, O., Gryn, L., & Kononenko, L. (2018). Study into formation of nutritional value of cauliflower depending on the agri-biological factors. Eastern-European Journal of Enterprise Technologies, 6: 29–35.
- Sahare, D. S., Rasala, T. M., Kesare, K. P., Gupta, S. S., & Ittadwar, Dr. A. M. (2023). Protein Energy Malnutrition: An Overview for Child Health. International Journal of Pharmaceutical Sciences Review and Research, 80(1):17-22.
- Schönfeldt, H. C., & Hall, N. G. (2012). Dietary protein quality and malnutrition in Africa. British Journal of Nutrition, 108(S2): S69–S76.
- Singh, S., Mishra, M. L., & Singh, P. (2019). An intervention study on utilization of cauliflower leaf powder (CLP) on the nutritional status of selected rural school children of Kumarganj, Ayodhya, Uttar Pradesh, India. Biomedical Research, 30(6),875-881.
- Slobodianiuk, L., Budniak, L., Marchyshyn, S., Skrynchuk, O., & Kudria, V. (2021). HPLC analysis of amino acids content in *Crambe cordifolia* and *Crambe koktebelica* leaves. International Journal of Applied Pharmaceutics,13(4): 111–116.
- United state Pharmacopeia (USP-NF) 2021.