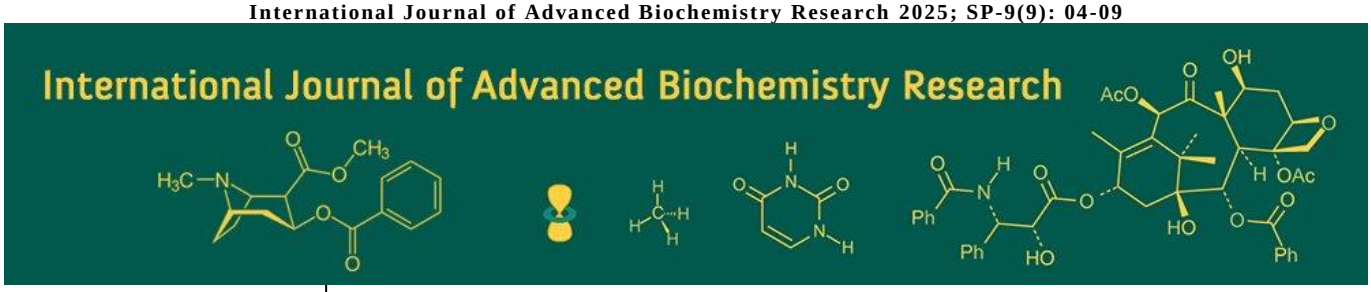

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(9): 04-09
www.biochemjournal.com
Received: 06-06-2025
Accepted: 09-07-2025

Ananya Tiwari
Department of Agricultural Biochemistry, Chandra Shekhar Azad Agriculture University, Kanpur, Uttar Pradesh, India

Pratibha Singh
Department of Biochemistry, ANDUAT University, Kumarganj, Ayodhya, Uttar Pradesh, India

CL Maurya
Department of Seed Science and Technology, Chandra Shekhar Azad Agriculture University, Kanpur, Uttar Pradesh, India

Sarvendra Kumar
Department of Genetics and Plant Breeding, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Prabhas Kumar Shukla
Department of Biochemistry, ANDUAT, University Kumarganj, Ayodhya, Uttar Pradesh, India

Rajeev Kumar
Department of Crop Physiology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

Corresponding Author:
Ananya Tiwari
Department of Agricultural Biochemistry, Chandra Shekhar Azad Agriculture University, Kanpur, Uttar Pradesh, India

Assessment of nutritional attributes in pigeonpea genotypes: Protein quality, carbohydrate fractions, and digestibility patterns

Ananya Tiwari, Pratibha Singh, CL Maurya, Sarvendra Kumar, Prabhas Kumar Shukla and Rajeev Kumar

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i9Sa.5461

Abstract
This study evaluated 20 pigeonpea (*Cajanus cajan*) genotypes across two consecutive cropping seasons (2023-2024) to assess their nutritional potential in terms of protein content, carbohydrate content, and *In vitro* protein digestibility. The pooled data revealed significant genotypic variation. Protein content ranged from 20.39% to 24.67%, with the highest values observed in early maturing genotypes PUSA Arahar-16 (24.65%), ICPL-15 (23.93%), and PAU-881 (22.70%), and in late maturing genotypes AMAR (24.67%), NDA-1 (24.66%), and MAL-6 (23.31%). *In vitro* protein digestibility varied from 42.72% to 82.80%, with ICPL-15 (82.80%), NDA-1 (79.40%), and KA-12-1 (75.34%) demonstrating the highest digestibility. Carbohydrate content ranged from 49.07% to 60.25%, with early genotypes UPAS-120 (60.25%) and ICPL-15 (59.58%), and late genotype AJAD (59.55%) showing the highest levels. Genotypes such as ICPL-15, NDA-1, and PUSA Arahar-16 emerged as promising candidates for biofortification and dietary protein improvement. High carbohydrate genotypes like UPAS-120 and AJAD may be suited for energy-dense food applications, while lower carbohydrate lines like PAU-881 and IPAL-21-1 may be beneficial for low-glycemic diets. The findings provide a valuable basis for selecting nutritionally superior pigeonpea varieties in breeding programs aimed at combating malnutrition and enhancing dietary quality in pulse-based food systems.

Keywords: Carbohydrate, protein, nutrition, genotypes, pigeonpea, digestibility

Introduction
Pigeon pea (*Cajanus cajan* L,) is a important legume and belongs to family Leguminoseae and has a diploid genome comprising 11 pairs of chromosomes (2n = 22) Pulses or legumes are popularly known as "Poor man's meat" and "rich men vegetable" (Singh, 2015)**.** Moreover, because of deeper root system, they are able to utilize the moisture and nutrient available in the lower strata of the soil more efficiently than other cereal crops. But unfortunately, the availability of pulses has been continuously decreasing and consumption of pulses has remained low. Therefore, the question of increasing the production of pulses has been on priority in the "Prime Ministers Twenty Points Programme" launched in the country since 1968. It is a great source of B-complex vitamins, carbohydrates, and minerals. Pigeon pea when supplemented with other cereals provides a well-balanced diet with all essential amino acids and is equivalent to other protein-rich sources such as soybean and whey (Talari and shakappa 2018) [16]. It is a good source of crude protein, fiber, vitamins especially thiamine, riboflavin, niacin, choline and antioxidants (Olagunju *et al.*, 2018) [13]. Legume seeds occupy an important place in human diet all over the world as they are rich sources of proteins. In addition to being used as human food, legumes are also used as animal feed. This is advantageous for farmers because it decreases the need for costly nitrogenous fertilizers and legumes have the ability to fix atmospheric nitrogen, increasing the overall fertility of the soil. (Blazos and Belski, 2016) [8]. Pigeon pea seed is a cheap, nutritious and healthy legume of various uses with healing and medicinal value. It is a rich source of protein, fibre, minerals and vitamins (Fasoyiro *et al.*, 2016) [5]. It also contains anti-nutrients such as tannin, cyanogenic glycosides, hemagglutinin and alkaloids which inhibit the bio-availability of nutrients like proteins (Aruna and Devindra, 2016) [2]. Consuming legumes is crucial to preventing chronic diseases as a result of the phytochemicals they

contain, diseases like cancer and heart disease that protect the organism from oxidative damage and maintain homeostasis in opposition to antioxidants (Khyade and Jagtap, 2016) [7]. Studies on quick dhal processing of Pigeon Pea (*Cajanus cajan*) contains nutritive values of 20-22 percent protein, 1.2 percent fat, 65 percent carbohydrate and 3.8 percent ash. Pigeon peas have anti-nutritional factors such as oligosaccharides, digestive inhibitors, phytates and tannins. The digestive inhibitors and toxicants such as hemagglutinins inhibit the activity of the digestive enzymes such as trypsin, chymotrypsin, and amylase (Onwuka, 2006) [14]. Proteins are the vital components of the human diet and play structural and functional roles in growth and development. The human body needs a constant supply of good quality dietary proteins especially the ones which have a high content of indispensable (previously called, essential) amino acids since the human body is incapable of synthesizing them. From the nutrition point of view, the quality of protein is as much important as is its quantity. The protein quality depends on the content of amino acids especially the dietary indispensable amino acids, the physiological utilization of specific amino acids after digestion (or protein digestibility) as well as on the bioavailability of the amino acids. (Butts *et al.*, 2012) [4]. The nutritional quality of the dietary proteins can be assessed using a variety of different markers and approaches such as amino acid score (AAS), nitrogen balance (NB), protein Efficiency Ratio (PER), net protein ratio, (or retention) (NPR), net protein utilization (NPU), protein digestibility, biological value (BV) and PDCAAS (Boye *et al.*, 2012) [3].

Materials and Methods

Twenty pigeonpea genotypes (10 early and 10 late-maturing) were evaluated over two growing seasons (2023 and 2024) under randomized complete block design with three replications. The lab experiments were conducted at the laboratory of the Department of Agricultural Biochemistry, Chandra Shekhar Azad University of Agriculture & Technology Kanpur and Department of biochemistry & ANDUAT, Kumarganj Ayodhya.

Protein content: The nitrogen content of pigeonpea seed samples were estimated by Micro-Kjeldhal Method (AOAC, 1970) [1]. This method essentially involves digestion of the sample to convert nitrogenous compounds into NH_4 form. Crude protein was determined by multiplying the total Nitrogen content by the factor 6.25. About 200 mg dried defatted powdered samples of each variety of pigeonpea were transferred to Micro-Kjeldhal digestion tube. Then 3 g digestion mixture were added to digestion tubes and finely 5 ml concentrated H_2SO_4 was added and sample was digested in Kel Plus-KES 06 digestion unit at 420 °C for 1 hr or until sample become clear sky blue in colour. After cooling the digested sample was diluted with small quantities of distilled water and transferred to Kel Plus distillation tube and total volume was made up to 25 ml in each tube with distilled water. In each set one blank was run. Now, tube was fitted in distillation unit and run button was pressed, 23 ml of 40% NaOH solution was poured in it and NH_3 liberated by steam distillation, was collected in 250 ml conical flask containing 21 ml of 4% boric acid solution along with mixed indicator was poured. Boric acid containing NH_3 (Ammonium borate) was titrated against N/10 standard HCl until the first appearance of pink colour at the end point. A blank was also titrated against N/10 standard HCl until the first appearance of pink colour at the end point titrated value for blank was recorded. Percent to Nitrogen in the sample was calculated using following formula

$$\text{Nitrogen\%} = \frac{14.01 \text{ x } 0.1\text{N x } (\text{T} - \text{B})}{\text{W} \times 1000} \times 100$$

Protein% = Nitrogen% x 6.25

Where,
N = Normality of HCl
W = Weight of the sample (g)
T = Titrate value
B = Blank value

The estimation was done in triplicate and the mean value was recorded to calculate the crude protein content.

In vitro protein digestibility

In vitro protein digestibility was determined by the modified method of Mertz *et al.*, (1984) [9].

Reagents

- Pepsin reagent: 0.1 M KH_2PO4 p^H-2 containing 0.2% pepsin, 13.6g potassium phosphate was dissolved in 1 litre of distilled water, adjusted pH of the solution to 2 and then dissolved 2.0g pepsin in the buffer.
- TCA: 5%

Procedure: 250 mg of sample was weighed and transferred in a centrifuge tube. 20 ml of pepsin reagent was added. Tube was kept in a shaker incubator at 37 °C, for 3 hours. The centrifuge tube was removed and cooled. 5 ml of 50% TCA was added and centrifuged the contents at 10,000 rpm for 10 min. at room temperature and filtered. 10 ml of aliquot was taken and dried in hot air oven. Dried aliquot was digested for nitrogen determination by Micro kjeldahl method. Digested protein of sample was determined. Protein digestibility was calculated employing the following formula:

Protein digestibility (%) = Digested protein × 100/Total protein

Carbohydrate content

Total carbohydrate was determined by Anthrone method as described by (Hedge and Hofreiter, 1962) [6]. Carbohydrates were first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose was dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm. Accurately weighed 100 mg of moisture free sample was taken into a boiling test tube and hydrolyzed by keeping in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cooled to room temperature. Then neutralized the content with solid sodium carbonate until the effervescence ceased and volume was made up to 100 ml with distilled water. The content was centrifuged and supernatant was collected. 0.5 ml of supernatant was taken in a test tube and volume was made up to 1 ml with distilled water. After cooling the content of

test tube, freshly prepared 4 ml of anthrone reagent (200 mg. of anthrone was dissolved in 100 ml ice cold 95% H_2SO_4) was added to it and the content was heated in a boiling water bath for 8 min., then the content was cooled rapidly and the intensity of green to dark green colour was measured at 630 nm by Spectrophotometer (Systronics 169) against a reagent blank. The carbohydrate content was estimated from a standard curve prepared with known concentration of glucose. The estimation was conducted in triplicate.

Calculation

Amount of carbohydrate present in 100 mg of the sample:

$$\text{Carbohydrate\%} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Results and Discussion

Protein content: This study investigated 20 genotypes across two cropping seasons (2023 and 2024), with pooled data we found protein content in pigeonpea in the range of 14.48 percent to 24.46 percent. offering a clear picture of varietal potential for improving dietary protein intake. Highes tprotein content was found in early varieties/genotypes PUSA Arahar16 (24.65%) ICPL-15 (23.93%), PAU-881 (22.70%) and late varieties/genotypes AMAR (24.67%), NDA-1 (24.66%), MAL-6 (23.31%) and exhibited consistently high protein content across both years. These genotypes may offer significant value in breeding programs aimed at enhancing nutritional quality. ICPL-15 (23.98%), CO-9 (23.96%), and KA-17-1/KA-12-1 (23.15%) provided substantial protein levels, making them strong candidates for dual-purpose use (food and seed).Lowest protein content in early varieties/genotypes was found in IPA-15-6 (20.39%), UPAS-120 (21.12%) late varieties/genotypes lowest protein found in PUSA 211 (21.58%), IPA-15-2 (21.67%) may be less suitable for direct nutritional interventions without enhancement strategies. Singh *et al.*, (2018) [10] reported genotypes such as AMAR, NDA-1, and PUSA Arahar16 are ideal for biofortification strategies and nutritional outreach programs in protein-deficient regions. Moderate protein varieties can be targeted for processing innovations like enrichment or blending with other high-protein legumes. Low-protein cultivars may still contribute to agricultural resilience and could be improved through selective breeding or hybridization. The along data was supported by Wang *et al.*, 2010) [17].

Table 1: Protein Content

S. No	Treatments	Protein Content		
		2023	2024	Pooled
	Early varieties/genotypes			
1	PAU-881	22.66	22.73	22.70
2	PUSA Arahar16	24.61	24.69	24.65
3	VLA-1	22.75	22.79	22.77
4	IPA-15-6	20.38	20.41	20.39
5	UPAS-120	21.35	20.28	20.82
6	Manak	21.09	21.12	21.10
7	Type-21	22.64	22.61	22.62
8	ICPL-15	23.96	23.89	23.93
9	JKM-189	22.31	22.33	22.32
10	CO-9	21.29	21.24	21.27
	Late varieties/genotypess			
11	NDA-1	24.68	24.63	24.66
12	KA-17-1	22.73	22.76	22.74
13	KA-12-1	23.13	23.16	23.15
14	AMAR	24.77	24.57	24.67
15	AJAD	23.21	23.17	23.19
16	K-17-2	23.13	23.09	23.11
17	MAL-6	23.32	23.29	23.31
18	PUSA-211	21.62	21.53	21.58
19	IPA-15-2	21.65	21.69	21.67
20	IPAL-21-1	22.45	22.51	22.48
	SE(m)±	0.498	0.821	0.447
	C.D. at 5%	1.429	2.355	1.283

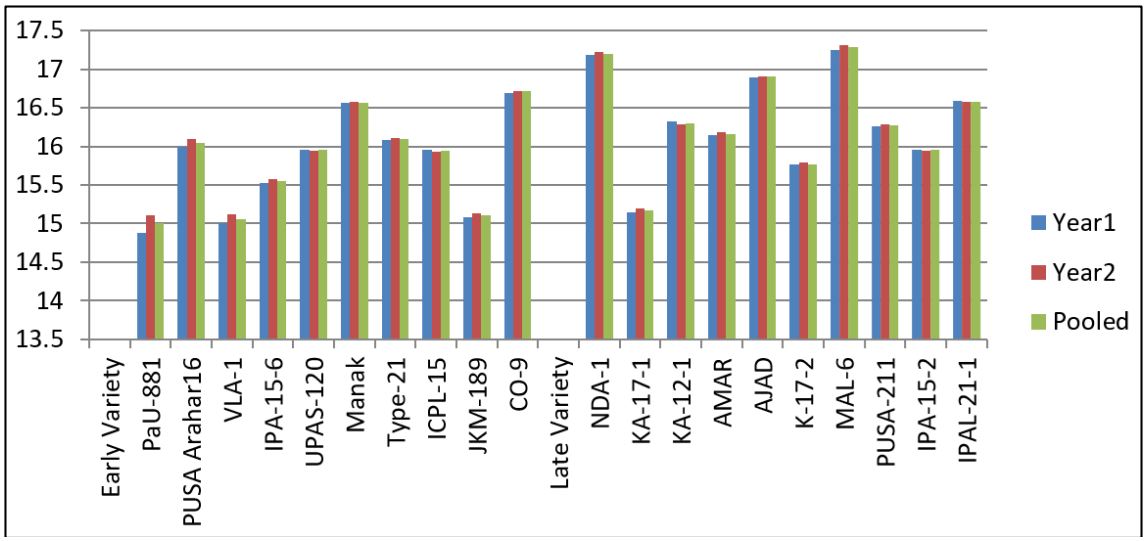

Fig 1: Protein Content

Protein digestibility is a key factor in determining the nutritional quality of legumes, especially in plant-based diets where protein bioavailability is critical. This study assessed 20 pigeon pea genotypes over two cropping seasons (2023-2024), with pooled data providing a reliable basis for early and late varieties/genotype selection based on digestibility performance Results from Pooled Datahighest digestibility recorded in early varieties/genotypes ICPL-15 (82.80%) followed by UPAS-120 (75.20%), PAU-881 (65.55%), CO-9 (62.31%) and late varieties/genotypes highest content found NDA-1 (79.40%) followed by AMAR (70.74%), IPAL-21-1 (68.80%) Reddy & Rao (2017).

Exhibited excellent protein digestibility, indicating superior seed protein structure and enzyme-accessible conformation. Early varieties/genotypes lowest digestibility scores PUSA Arahara16 (42.72%) followed by Type-21 (42.84%), JKM-189 (46.66%) VLA-1 (57.21%) and late varieties/genotypes IPA-15-2 (47.76%) followed by KA-17-1 (51.84%), PUSA-211 (53.56%) recorded the least digestibility, possibly due to stronger anti-nutritional factors or tightly bound protein matrices. High-digestibility genotypes like ICPL-15, NDA-1, and KA-12-1 should be prioritized for nutritional enhancement, especially in populations vulnerable to protein deficiency.

Table 2: *In vitro* protein digestibility

	Treatments	*In vitro* protein digestibility		
		Year 1	Year 2	Pooled
1	PaU-881	65.53	65.58	65.55
2	PUSA Arahar16	42.73	42.71	42.72
3	VLA-1	57.19	57.23	57.21
4	IPA-15-6	59.36	59.32	59.34
5	UPAS-120	75.18	75.21	75.20
6	Manak	61.36	61.39	61.37
7	Type-21	42.85	42.83	42.84
8	ICPL-15	82.81	82.79	82.80
9	JKM-189	46.65	46.67	46.66
10	CO-9	62.29	62.33	62.31
11	NDA-1	79.40	79.41	79.40
12	KA-17-1	51.83	51.85	51.84
13	KA-12-1	75.32	75.37	75.34
14	AMAR	70.75	70.73	70.74
15	AJAD	65.96	65.93	65.95
16	K-17-2	62.93	62.97	62.95
17	MAL-6	65.46	65.48	65.47
18	PUSA-211	53.54	53.57	53.56
19	IPA-15-2	45.74	49.79	47.76
20	IPAL-21-1	68.79	68.81	68.80
	SE(m)±	1.131	1.969	0.912
	C.D. at 5%	3.245	5.648	2.615

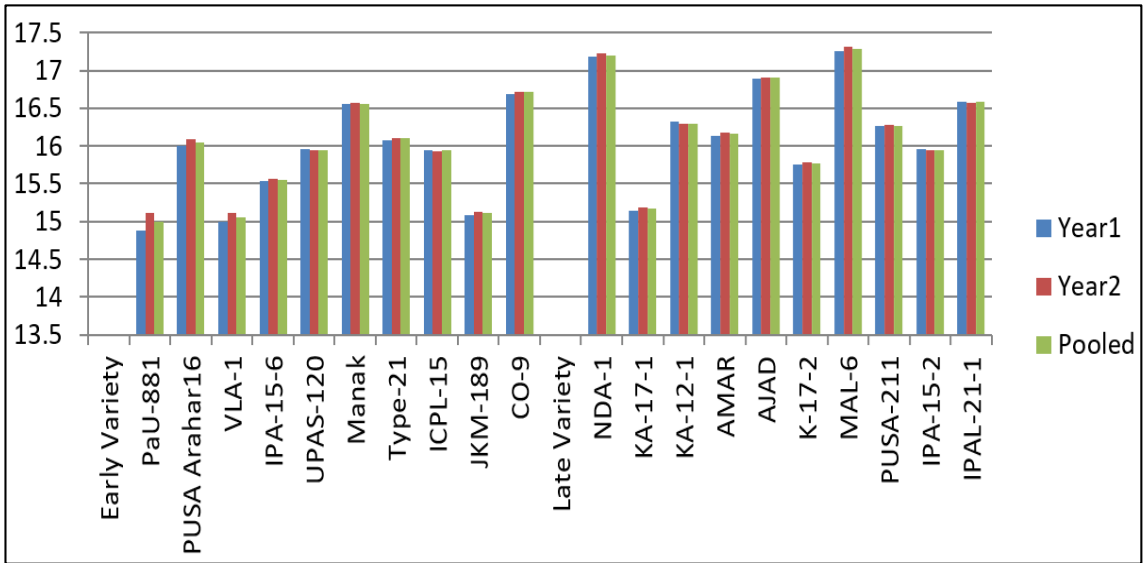

Fig 2: *In vitro* protein digestibility

Carbohydrates

Carbohydrate content is a vital nutritional parameter for pigeon pea, particularly in regions where legumes serve as primary calorie sources. This study compares 20 Early and late maturing varieties/genotypes over two cropping seasons (2023-2024), with pooled data of two years revealing significant varietal differences in early and late varieties/genotypes. The carbohydrate content in the seeds of pigeon pea cultivars ranged from 49.31 to 60.25 percent in early varieties/genotypes and in late maturing varieties/genotypes ranged from 49.07to 59.55 with a mean value of 56.18 percent pooled data analysis. Highest carbohydrate content in early varieties/genotypes UPAS-120 (60.25%), ICPL-15 (59.58%) and late varieties/genotypes AJAD (59.55%), and Type-21 (59.45%) highest varieties/genotypes, highlighting their strong potential for energy-dense food applications. Genotypes such as JKM-189 (58.30%), NDA-1 (58.40%), and K-17-2 (58.28%) maintained consistent values just below the highest bracket. Singh *et al.*, (2018) [10]. Lowest carbohydrate values in early varieties/genotypes IPAL-21-1 (49.08%), PAU-881 (49.31%), and late maturing varieties/genotypes found lowest carbohydrate content PUSA Arahar16 (53.85%) may be preferred for low-glycemic index diets or specialized

nutrition formulation.UPAS-120, ICPL-15, and AJAD may be promoted in nutritional enhancement programs or incorporated into energy-dense food products such as fortified flours or legume-based snacks. Lower carbohydrate genotypes like IPAL-21-1 and PAU-881 are suitable candidates for populations managing metabolic conditions such as diabetes. Breeding programs may prioritize carbohydrate optimization in tandem with other functional traits like protein, digestibility, and micronutrient content.

Table 2: Carbohydrate content

S. No	Treatments	Carbohydrate content		
		Year1	Year2	Pooled
	Early varieties/genotypes			
1	PaU-881	49.30	49.32	49.31
2	PUSA Arahar16	53.86	53.84	53.85
3	VLA-1	55.74	55.77	55.75
4	IPA-15-6	55.72	55.71	55.72
5	UPAS-120	60.10	60.40	60.25
6	MANAK	56.86	56.81	56.84
7	Type-21	59.41	59.49	59.45
8	ICPL-15	59.55	59.61	59.58
9	JKM-189	58.27	58.33	58.30
10	CO-9	56.28	56.27	56.28
	Late varieties/genotypes			
11	NDA-1	58.41	58.38	58.40
12	KA-17-1	54.10	54.30	54.20
13	KA-12-1	53.28	53.25	53.26
14	AMAR	59.29	59.34	59.31
15	AJAD	59.55	59.56	59.55
16	K-17-2	58.29	58.27	58.28
17	MAL-6	56.27	56.32	56.30
18	PUSA-211	54.92	54.95	54.94
19	IPA-15-2	54.97	54.98	54.97
20	IPAL-21-1	49.06	49.09	49.07
	SE(m)±	1.282	1.239	0.900
	C.D. at 5%	3.676	3.554	2.582

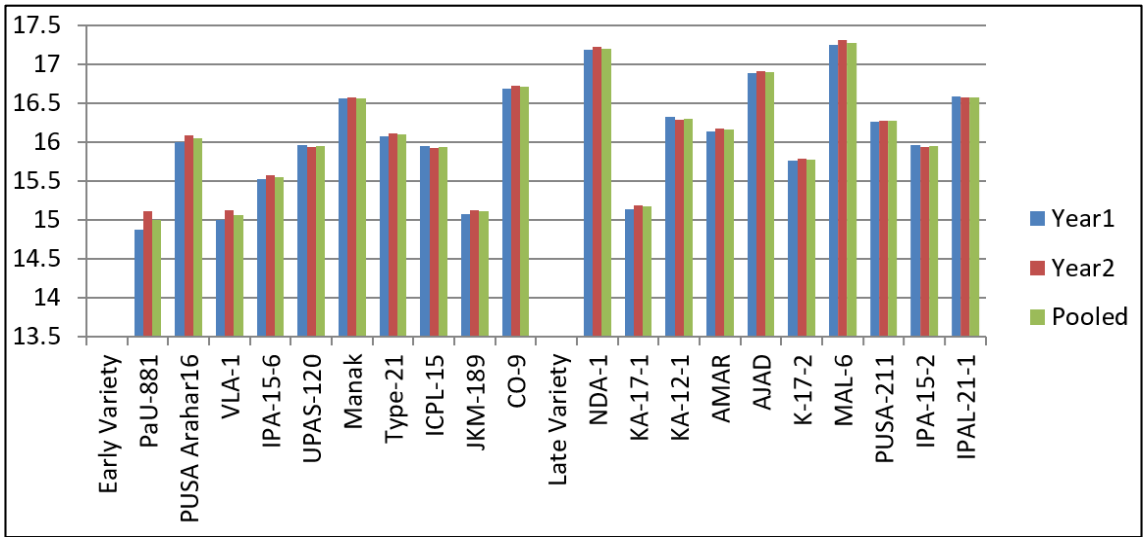

Fig 3: Carbohydrate content

Acknowledgements

The authors wish to extend their heartfelt thanks to the Department of Biochemistry, ANDUAT, Kumarganj and C. S. A. University of Agriculture and Technology, Kanpur, U.P. for their continuous support and valuable guidance. I also gratefully acknowledge the well-equipped laboratory facilities of the Department which provided the necessary instruments and resources essential for conducting the experimental investigations.

Conclusion

Protein Content ranged from 20.39 to 24.67 g/100g, with PUSA Arahar-16, ICPL-15, and AMAR showing high levels, making them ideal for biofortification efforts.Carbohydrate Content peaked in UPAS-120, ICPL-15, and AJAD, positioning them as energy-dense sources suitable for functional foods, protein digestibility were found in varieties/genotypes such as ICPL-15, NDA-1, and KA-12-1 emerge as strong candidates for nutritional

enhancement programs**,** especially in regions facing protein deficiency.

References

1. AOAC. Official Methods of Analysis. 11th ed. Washington, D.C.: Association of Official Analytical Chemists; 1970.
2. Aruna T, Devindra S. Nutritional and anti-nutritional characteristics of two varieties of red gram (*Cajanus cajan* L.) seeds. Int J Sci Res Publ. 2016;6(9):89-95.
3. Boye J, Wijesinha-Bettoni R, Burlingame B. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br J Nutr. 2012;108(S2):S183-S211.
4. Butts CA, Monro JA, Moughan PJ. *In vitro* determination of dietary protein and amino acid digestibility for humans. Br J Nutr. 2012;108(S2):S282-S287.
5. Fasoyiro S, Hovingh R, Gourama H, Cutter C. Change in water activity and fungal counts of maize-pigeon pea flour during storage utilizing various packaging materials. Procedia Eng. 2016;159:72-76.
6. Hedge JE, Hofreiter BT. Carbohydrate chemistry. In: Whistler RL, BeMiller JN, editors. Methods in Carbohydrate Chemistry. Vol. 17. New York: Academic Press; 1962. p. 420-424.
7. Khyade VB, Jagtap SG. Sprouting exert significant influence on the antioxidant activity in selected pulses (black gram, cowpea, desi chickpea and yellow mustard). World Sci News. 2016;35:73-86.
8. Kouris-Blazos A, Belski R. Health benefits of legumes and pulses with a focus on Australian sweet lupins. Asia Pac J Clin Nutr. 2016;25(1):1-17.
9. Mertz ET, Hassen MM, Cairns-Whittern C, Kirleis AW, Tu L, Axtell JD. Pepsin digestibility of proteins in sorghum and other major cereals. Proc Natl Acad Sci USA. 1984;81(1):1-2.
10. Singh J, Kanaujia R, Kumar J, Singh F, Ak S, Singh NP. Genetic variability for antioxidant activity and total phenolic content in four major pulse crops. Novel Tech Nutr Food Sci. 2018;1:1-6.
11. Singh N, Jain N, Kumar R, Jain A, Singh NK, Rai V. A comparative method for protein extraction and 2-D gel electrophoresis from different tissues of *Cajanus cajan*. Front Plant Sci. 2015;6:606-612.
12. Singh SK, Jadhav PV, Nandanwar RS, Patil AN, Wandhare M, Naik RM, *et al.* Assessment of nutritional quality parameters in selected vegetable type pigeon pea genotypes. J Pharmacogn Phytochem. 2018;1:1446-1450.
13. Olagunju AI, Omoba OS, Enujiugha VN, Alashi AM, Aluko RE. Antioxidant properties, ACE/renin inhibitory activities of pigeon pea hydrolysates and effects on systolic blood pressure of spontaneously hypertensive rats. Food Sci Nutr. 2018;6(7):1879-1889.
14. Onwuka GI. Soaking, boiling and anti-nutritional factors in pigeon peas (*Cajanus cajan*) and cowpeas (*Vigna unguiculata*). J Food Process Preserv. 2006;30(5):616-630.
15. Reddy DVC, Sreekanth M, Rani CS, Adinarayana M, Rao GR. Evaluation of pigeonpea genotypes against pod fly, *Melanagromyza obtusa*. [Journal details missing]. 2017.
16. Talari A, Shakappa D. Effect of domestic processing and crude extract of α-galactosidase on oligosaccharide content of red gram (*Cajanus cajan* L.) seeds. Curr Res. 2018;6(3):852-852.
17. Wang X, Gao W, Zhang J, Zhang H, Li J, He X, *et al.* Subunit, amino acid composition and *In vitro* digestibility of protein isolates from Chinese Kabuli and Desi chickpea (*Cicer arietinum* L.) cultivars. Food Res Int. 2010;43(2):567-572.