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The Content Of Protein And Free Amino Acids In The Grain Of Small-Seeded Chickpea Samples

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Abstract: In this study, the total protein and amino acid content of locally grown chickpea (Cicer arietinum L.) genotypes were investigated. The analysis revealed an inverse relationship between yield and protein content. Small-seeded chickpea accessions were observed to have a higher total protein content compared to large-seeded accessions. Specifically, the genotypes k-12104, k-12113, and k-12123 recorded a high protein content, ranging from 25,6% to 26,1%. Furthermore, these genotypes were found to have higher concentrations of many essential (non-essential) and non-essential (essential) amino acids that are important for human health, when compared to the control variety. In particular, elevated levels of amino acids such as methionine, valine, isoleucine, lysine, glutamine, and asparagine were observed. This data is of great importance for improving the nutritional value of chickpeas and for their use as a high-quality protein source. The results of this study serve as a valuable resource for breeders to develop new chickpea varieties that are rich in protein and have high nutritional value.

Keywords: Chickpea, protein, genotype, essential amino acids.

Introduction: Due to global population growth and climate change, the demand for food products is increasing. In developing and emerging countries, a large portion of the population suffers from protein deficiencies, hunger, and various diseases due to food shortages and poor food quality [1, 2]. Animal-based proteins can supplement or even replace animal proteins as an important source of essential amino acids [3, 4].

Chickpea is a significant crop among legumes, distinguished by its high nutritional value. This high nutritional composition is demonstrated by the fact that one-quarter of a chickpea's grain is rich in protein. The protein in chickpea grains easily digestible and provide a nutritious crop for humans and animals [5, 6]. One of the most important goals in agriculture is to create and cultivate ne, high-yielding chickpea varieties that are effective and rich in high-quality proteins [7].

As a rich source of food and protein for millions of people worldwide, chickpeas are considered a key factor in ensuring global food security [8]. The bio-fortification of "dietary protein" in chickpea grains is an economic strategy to mitigate the growing problems associated with malnutrition and the risk of hunger [9]. Due to economic constraints, especially in developing countries, a lack of sufficient protein in the daily diet leads to malnutrition in human population where the daily consumption of animal protein is inadequate. Therefore, increasing the protein content in legumes helps address protein-related malnutrition problems in low-income and underserved countries [10, 11].

Scientific literature indicates that protein content and quantity vary among chickpea types. The protein content of "Kabuli" chickpeas is around 20,55%, which is significantly lower than that of the "Desi" type (29,2%) [12]. Another study conducted by Singh et al. (2021) showed that the protein content ranged from 15,7% to 31,5% [1]. Hall et al. (2017) determined that the average protein content in "Desi" and "Kabuli" was 22,2% and 23,4%, respectively [13].

Globulins and albumins are the main storage proteins in legumes. Globulin proteins in legumes include vicilin (7s), convicilin (15s), and legumin. Other proteins, such as prolamins and glutelins, are present in smaller amounts. Prolamins are rich in glutamine and proline and are soluble in alcohol. Cysteine and methionine concentrations are high in glutelins, and these two amino acids are crucial for human health [14].

Globulins constitute the primary protein in chickpeas, making up 53-60%, while albumins account for 8-12%, prolamins and glutelins for 3-7% and 18-24%, respectively. Another study on the protein content of dried chickpeas found that the amount varied from 20,9% to 25,7% [13]. The determined protein composition showed the following percentages for prolamin, glutelin, albumin, and globulin: 19,38-24,40%, 3,12-6,89%, 8,39-12,31%, and 53,44-60,29%, respectively.

Scientific literature has noted that protein content and quantity vary depending on the chickpea type. The protein digestibility of "Desi" varieties is lower compared to "Kabuli" varieties. Specifically, the digestibility of proteins in "Desi" varieties (63%) was lower than in "Kabuli" varieties (79%). In uncooked chickpeas, protein digestibility ranged from 34% to 76%. Various research sources indicate that the variability in protein digestibility in chickpea grains is between 27% and 89%. Protein digestibility can be enhanced through fermentation of chickpea-based products. Geographical location, environmental variability, the plant's growth period, and the genetic diversity of chickpeas all influence the composition and quantity of chickpea protein [15].

This study aims to analyze the protein and amino acid content in different chickpea genotypes to develop new, high-protein varieties and improve their nutritional value. The findings of this research will serve as a valuable resource for creating high-quality, protein-rich chickpea varieties in the future.

Object And Methods Of Research

A study was conducted at the "Durmon" experimental field of the Institute of Genetics and Plant Experimental Biology of the Academy of Sciences of the Republic of Uzbekistan. The research utilized 10 foreign and local accessions of chickpea (Cicer arietinum L.) from the gene pool of the International Center for Agricultural Research in the Dry Areas (ICARDA).

The total protein content in the grain was determined using the "Keldal" method. This technique involves first quantifying nitrogen and then converting it to protein. The process begins by digesting the organic matter in boiling sulfuric acid until ammonium salts are formed. The ammonium is then converted to ammonia, which is distilled into an acidic solution. Finally, the ammonia is quantified using a titrimetric method to determine the nitrogen content in the tested material.

A homogeneous, finely ground sample of chickpea grain was precisely measured to an accuracy of 0,1% and transferred to a Keldal flask. The experiment was then carried out according to the standard methodological guidelines.

The mass fraction of nitrogen (X) in the analyzed sample, expressed as a percentage, was calculated during the

distillation of ammonia into sulfuric acid using the following formula:

here:

V0 – The volume (in ml) of 0,1 ml/l sodium hydroxide solution used for the titration of 0,05 ml/l sulfuric acid in the control experiment.

V1 –The volume (in ml) of the 0,1 ml/l sodium hydroxide solution consumed to titrate the sulfuric acid in the analyzed solution

K – The correction factor for the titer of the 0,1 ml/l sodium hydroxide solution.

0,0014 – The mass of nitrogen (in grams) equivalent to 1 ml of a 0,05 ml/l sulfuric acid solution

M – The sample mass, in grams g.

The final result of the analysis was the arithmetic mean of five parallel analyses. The results were calculated to three decimal places and then consolidated to two.

The mass fraction of nitrogen relative to the dry matter of the product (X3), in percentage, was calculated from the following formula:

here:

X1 – The mass fraction of nitrogen in the tested sample, in %;

W –The moisture content of the tested sample, in %.

The mass fraction of protein (Y) was determined using the following formula:

Y = KCHX*X1 ,

here:

KCHX – is the conversation factor from nitrogen to protein (6,25).

The content of free amino acids in the samples was determined using the method developed by A. Steven and Cohen Daviel, with a High-Performance Liquid Chromatography (HPLC) instrument. The detection principle of the instrument is based on the derivatization of amino acids with fluorescent labeling reagents (e.g., o-Phthalaldehyde (OPA) or 9-Fluorenylmethyl Chloroformate (FMOC) followed by their detection using a fluorescence detector. This method allows for the accurate and quantitative analysis of various amino acids by separating them from one another.

Results

An analysis of chickpea nursery samples was conducted to identify the highest-yielding samples. These were then selected, and the total protein content in their grains was determined in a laboratory setting using the Kjeldahl method, in comparison to the standard Lalmikor variety.

The analysis revealed that the total protein content in the grains of the chickpea samples ranged from 22,1±0,35% to 26,1±0,44%. Specifically, the small-seeded nursery samples k-12104, k-12113, and k-12123 had protein contents of 25,6%, 25,9%, and 26,1% respectively. The control variety Lalmikor had a protein content of 24,6%.

Furthermore, it was determined that several other samples k-12101 (23.6±0,18%), k-12105 (22,5±0,31%), k-12108 (23,5±0,25%), k-12109 (23,1±0,45%), k-12118 (22,1±0,35%), and k-12135 (23,4±0,32%) - had a lower total protein content compared to the control (Figure 1).

Based on our research, it can be concluded that samples with higher yields tend to have a lower total protein content compared to those with lower yields. It was also found that the protein content in the grains of small-seeded nursery samples was higher than in large-seeded chickpea grains. Other studies have also shown that the protein content in chickpea grains varies depending on the genotype, cultivation conditions, and variety characteristics [15].

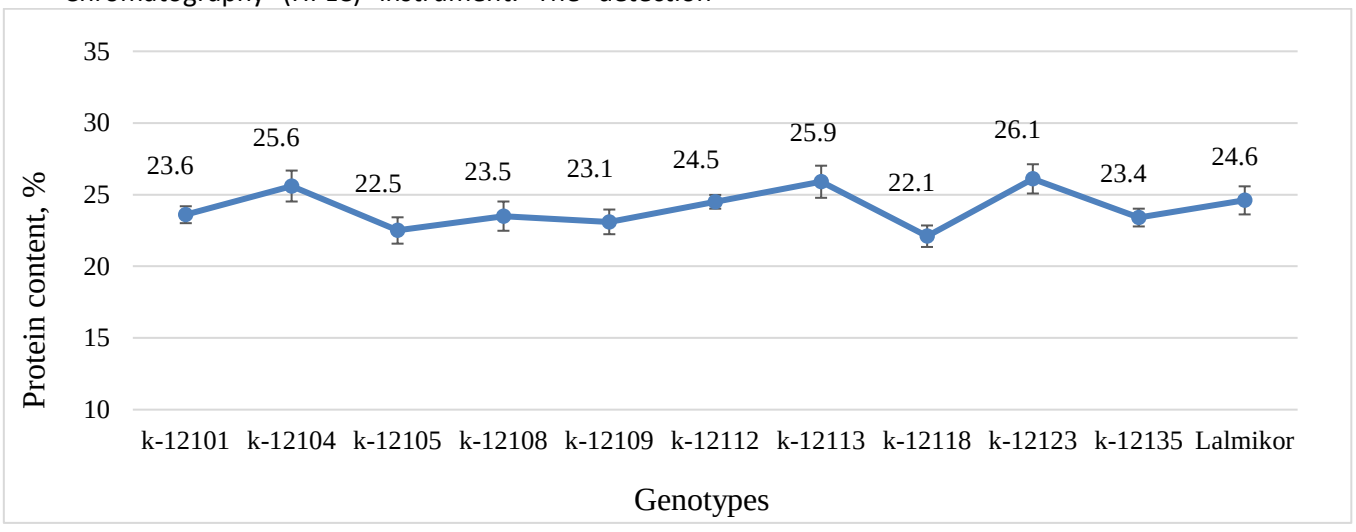

Figure 1. Protein content of samples, %.

In our study, we analyzed the free amino acid content in samples with high total protein levels. The grain composition revealed that the valine amino acid content ranged from 0,30 to 1,21 mg/g. Specifically, samples k-12101 (1,21 mg/g), k-12104 (1,12 mg/g), k-12105 (0,98 mg/g), and k-12135 (0,78 mg/g) had higher valine content compared to the control variety Lalmikor (0,74 mg/g), while the remaining samples had comparatively lower levels.

The isoleucine amino acid content in chickpea grains ranged from 0,12 to 1,95 mg/g. It was found that 70% of the selected samples had higher levels of this amino acid compared to the control Lalmikor (0,65 mg/g).

Lysine, an essential amino acid, was found to be present in chickpea grains at a concentration of 8 to 55 mg per 100 g, depending on the protein quality. The lysine content in samples k-12101 (0,08 mg/g), k-12104 (0,09 mg/g), and k-12112 (0,08 mg/g) was lower than the control Lalmikor (0,11 mg/g), while the remaining samples showed higher levels.

The tryptophan amino acid content in samples k-12101 (0,86 mg/g), k-12104 (0,88 mg/g), k-12105 (0,85 mg/g), and K-12112 (0,82 mg/g) was higher than the control Lalmikor (0,74 mg/g) by 13,94%, 15,9%, 12,9%, and 9,7%, respectively. In contrast, samples k-12109 (0,39 mg/g), k-12113 (0,30 mg/g), k-12118 (0,18 mg/g), k-12123 (0,43 mg/g), and k-12135 (0,45 mg/g) showed a reduction of 39,2-75,6% compared to the control.

Threonine, an amino acid with an indole ring, participates in protein metabolism and serotonin hormone synthesis [16]. The threonine content among the samples ranged from 0,14 to 0,68 mg/g. Samples k-12104, k-12108, k-12112, k-12113, and k-12118 showed higher levels compared to the control Lalmikor (0,33 mg/g).

Methionine a sensitive essential amino acid to oxidation [17], was highest in the chickpea sample k-12118 (0,70 mg/g), which is 30% higher than the control. Other genotypes, including k-12101 and k-12118, also showed higher results compared to the control Lalmikor (0,49 mg/g).

For phenylalanine content, sample k-12112 had the highest result (0,61 mg/g), while k-12104 had the lowest (0,12 mg/g). The difference compared to the control Lalmikor (0,42 mg/g) ranged from 31,1% to 74,4%.

Based on the content of essential amino acids, the highest amounts were found in samples k-12135 for leucine (1,12 mg/g), k-12118 for methionine (0,70 mg/g), isoleucine (1,95 mg/g), for threonine (0,68 mg/g), and k-12109 for leucine (0,97 mg/g), and k-12104 for tryptophan (0,88 mg/g), indicating high grain quality (Table 1).

Table 1

Content of free non-essential amino acids in the grain of chickpea samples, mg/g

№	Catalogue number	Valine	Methionine	Isoleucine	Leucine	Lysine	Threonine	Tryptophan	Phenylalanine
1	K-12101	1,21	0,58	0,43	0,37	0,08	0,14	0,86	0,21
2	K-12104	1,12	0,22	1,22	0,36	0,09	0,39	0,88	0,12
3	K-12105	0,98	0,20	0,95	0,27	0,15	0,33	0,85	0,43
4	K-12108	0,48	0,13	0,64	0,42	0,11	0,35	0,74	0,25
5	K-12109	0,68	0,21	0,66	0,97	0,14	0,16	0,39	0,17
6	K-12112	0,53	0,30	0,95	0,20	0,08	0,53	0,82	0,61
7	K-12113	0,31	0,16	0,12	0,76	0,55	0,46	0,30	0,54
8	K-12118	0,30	0,70	1,95	0,20	0,21	0,68	0,18	0,44
9	K-12123	0,74	0,32	0,72	1,07	0,16	0,18	0,43	0,21
10	K-12135	0,78	0,25	0,76	1,12	0,17	0,19	0,45	0,28
11	Lalmikor	0,74	0,49	0,65	0,65	0,11	0,33	0,74	0,42
mean		0,72	0,32	0,82	0,58	0,17	0,34	0,60	0,33
sd		0,07	0,04	0,11	0,08	0,03	0,04	0,06	0,04
cv		42,2	57,3	57,2	60,2	59,1	50,1	42,5	48,2
range		0,3-1,21	0,13-0,7	0,12-1,95	0,2-1,12	0,08-0,55	0,14-0,68	0,18-0,88	0,12-0,61

Non-essential amino acids, which are produced through plant metabolism, play a crucial role in forming total reserve proteins [18,19]. Aspartic acid is essential for the synthesis of lysine, threonine, methionine, and isoleucine [20]. The aspartic acid content in chickpea grains was found to be 18,4% to 36,2% higher in genotypes k-12109, k-12112, k-12113, k-12118, k-12123, and k-12135 compared to the control Lalmikor (1,11 mg/g).

Table-2

Content of free essential amino acids in the grain of chickpea samples, mg/g

№	Catalogue number	Asparagine	Glutamine	Serine	Glycine	Asparagine	Glutamine	Cysteine	Argenine	Alanine	Proline	Tirosine	Histidine
1	K-12101	0,38	1,12	0,41	0,23	0,80	0,51	0,78	0,46	0,08	0,18	0,85	0,44
2	K-12104	0,34	0,23	0,39	0,23	0,45	0,98	0,64	0,51	0,09	0,30	0,25	0,23
3	K-12105	0,25	0,28	0,37	0,22	0,45	0,44	0,44	0,12	0,08	0,11	0,69	0,74
4	K-12108	0,28	0,31	0,30	0,14	0,28	0,37	0,32	0,11	0,12	0,13	0,24	0,55
5	K-12109	1,51	1,38	0,94	0,87	1,12	0,39	1,02	0,43	0,03	0,19	0,35	0,33
6	K-12112	1,36	0,73	0,70	0,97	1,22	0,28	0,13	0,63	0,05	0,46	0,36	0,42
7	K-12113	1,49	1,43	0,85	0,85	1,70	0,67	0,70	0,24	0,05	0,43	0,82	0,32
8	K-12118	1,36	0,50	0,70	0,96	1,94	0,54	0,92	0,14	0,05	0,46	0,74	0,18
9	K-12123	1,66	1,52	1,04	1,23	1,47	0,42	1,12	0,47	0,03	0,16	0,38	0,16
10	K-12135	1,74	1,59	1,09	1,30	1,15	0,45	1,18	0,50	0,04	0,56	0,40	0,15
11	Lalmikor	1,11	0,98	0,59	0,68	0,79	0,38	1,03	0,25	0,09	0,49	0,66	0,19
mean		1,04	0,92	0,67	0,70	1,03	0,49	0,75	0,35	0,07	0,32	0,52	0,34
sd		0,13	0,12	0,06	0,10	0,12	0,04	0,08	0,04	0,008	0,04	0,05	0,04
cv		57,7	57,9	42,1	61,1	51,9	38,6	45,5	52,5	44,2	53,4	44,4	55,5
range		0,25-1,74	0,23-1,59	0,3-1,09	0,14-1,30	0,28-1,94	0,28-0,98	0,13-1,18	0,11-0,63	0,03-0,12	0,11-0,56	0,24-0,85	0,15-0,74

The content of glutamic acid in genotypes k-12101, k-12109, k-12113, k-12123, and k-12135 was 12,5% to 38,3% higher than in the control variety Lalmikor (0,98 mg/g). Other essential amino acids like tyrosine, histidine, and serine also showed higher values in some samples compared to the control variety.

Alanine is one of the oldest amino acids [21], and despite being present in small amounts in chickpea grains, sample k-12108 recorded a higher value (0,12 mg/g) compared to others. The amino acid proline is considered an important component that increases stress tolerance and photosynthetic efficiency [22]. The average content of proline in chickpea grains is 0,32±0,04 mg/g (Table-2).

The amino acids analyzed above are essential for the body's growth, development, and immune system [23]. The high content of essential amino acids indicates that the selected chickpea samples are rich in high-quality protein.

Conclusion

Analyses revealed an inverse relationship between the yield and protein content of chickpea samples, meaning that samples with higher yields had lower protein content. It was also found that the protein content of small-seeded chickpea genotypes was higher compared to large-seeded varieties. The studied samples showed higher levels of many essential non- essential amino acids- including valine, isoleucine, lysine, and tryptophan- compared to the control variety, indicating their high nutritional value. Specifically, the genotypes k-12104, k-12113, and k-12123 were noted for having a high total protein content. These genotypes also showed high levels of essential amino acids such as glutamine, asparagine, and cysteine, which suggests a high-quality protein composition.

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