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Comparison of Total Protein and Amino Acid Content of Some Legumes Types

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Abstract

In this study, the total protein content of some legumes was determined by spectrophotometer together with amounts of essential and non-essential amino acids were determined by High Performance Liquid Chromatography (HPLC). The total protein amount in legumes varied between 337.75 and 154.15 mg (g dw)-1 , and *Glycine max* (Soybean) was the richest, while *Cicer arietinum* (chickpeas) was the poorest. It was observed that there was no statistical difference between *Pisum sativum* (Pea) and *Cicer arietinum* (Chickpeas), also there is no differences between *Vigna radiate* (L.) (Mung bean) and *Phaseolus vulgaris* (Ispir dry bean) (p>0.05). It was determined that *Glycine max* (Soybean) is the richest in terms of non-essential amino acids except serine, tyrosine and cysteine, while *Pisum sativum* (Pea) is the poorest in terms of glutamic acid, serine and glycine, and *Cicer arietinum* (chickpeas) is the poorest in terms of other amino acids. Among the essential amino acids, while *Glycine max* (Soybean) are richest in terms of histidine and leucine, *Vigna unguiculata* (Cowpea) are richest in arginine, methionine, tryptophan and lysine, and *Vigna radiate* (L.) (Mung bean) are richest in valine, isoleucine and phenylalanine. It was observed *Vigna unguiculata* (Cowpea) is the richest in total essential amino acids, while the poorest was *Cicer arietinum* (Chickpeas). On the other hand, *Glycine max* (Soybean), the richest in terms of total non-essential and total amino acids, while the poorest was *Cicer arietinum* (Chickpeas). The results obtained show that the TEA/TNEA and TEA/TA ratios varies between 65-99 and 39-50 percent respectively, depending on the type of legume type. From these results, it can be seen that all legumes are a good source of protein, but *Glycine max* (Soybean) and *Vigna unguiculata* (Cowpea) are better than the other legume species examined in this study.

Keywords: Legumes, Total protein, Amino acids, HPLC, Spectroscopy

1. Introduction

Legume refers to plants with a shell in their fruit, while pulses refer to only dried seeds [1]. Legumes refer to plants whose fruits are enclosed in a shell, while pulses refer only to dried seeds. Legumes such as beans, lentils, peas and chickpeas are an important source of protein, carbohydrates, vitamins and minerals and are widely consumed worldwide [2]. Grain legumes are environmentally friendly, sustainable protein sources [3]. In addition, grain of legumes has been associated with the prevention of chronic diseases (such as type 2 diabetes mellitus, cardiovascular diseases and colon cancer) [4]. It is reported that legumes have high-quality protein content. Consumption of legumes has health benefits due to components such as proteins, fibers or some minor compounds such as some lipids, polyphenols or bioactive peptides [5]. When protein needs are met from animal protein, the levels of cholesterol and saturated fatty acids, which cause diseases such as obesity, cardiovascular diseases, and cancer, and animal-based diseases, increase [6], [7]. Seed of legumes are environmentally friendly, sustainable protein sources due to their positive effects on soil quality through symbiotic nitrogen fixation, carbon sequestration, nutrients and water retention [3], [8].

Amino acids are generally the building blocks of proteins, which form an important part of the cell structure, and also have different functions such as hormone production, signal transmission and homeostasis of biochemical pathways [9]. In addition, amino acids are important regulators of basic metabolic pathways required for repair, growth, reproduction and immune system in the organism and increase protein accumulation. [10], [11].

As a result of the deficiency or insufficient level of amino acids in the body, protein synthesis cannot occur properly and causes various metabolic disorders [12]. In this study, it was aimed to determine the total protein and essential and non-essential amino acid contents of *Glycine max* (Soybean), *Vigna unguiculata* (Cowpea), *Vigna radiate* (L.) (Mung bean), *Cranberry beans* (Kidney bean), *Phaseolus vulgaris* L. (İspir dry bean), *Phaseolus vulgaris* L. (Gezin dry bean), *Lens culinaris* (Red lentil), *Lens culinaris* (Green lentil), *Pisum sativum* (Pea) and *Cicer arietinum* (Chickpeas).

2. Material and Method

2.1. Material

After the legume samples obtained from the market were washed, kept in an oven at 50° C for 12 hours to ensure their moisture content was constant. 20 gram samples taken from the oven were thoroughly crushed in a blender (Fakir Hausgrate 220 Watt) and turned into powder. 0.2 grams of these powdered samples were transferred into a plastic tube for total protein determination and 1.0 grams were added to glass tubes for amino acid analysis.

2.2. Determination of total protein

Total protein contents were determined according to Lowry [13]. In this method, copper-protein complex is first formed by adding Folin-Ciocalteu solution to the medium, copper-protein complex combines with tyrosine and tryptophan radicals. Then the total protein contents were determined by UV-visible spectrophotometer.

2.3. Determination of Amino Acids

2.3.1. Hydrolysis

1.0 g of the homogenized samples based on dry weight were taken into a glass tube, 5.0 mL of 6.0 N HCl was added and vortexed, and kept at 110°C for 24 hours [14]. Then, the samples were cooled to room temperature, filtered and the filtrate volume was completed to 10 mL with distilled water.

2.3.2. Derivatization

50 µL of standard amino acid solutions or hydrolyzed samples were transferred to 5.0 mL glass tubes and dried at 65 °C under vacuum. Then, 50 µL of reagent 1 solution [(2:2:1 ethanol:water:triethylamine (TEA) (v/v)] was added, vortexed and dried again under vacuum at 65 °C. 50 µL of reagent 2 solution [7: 1: 1: 1 ethanol: water: TEA: phenyl isothiocyanate (PITC) (v/v)] was added to the dried samples and kept in the dark for 30 min at room temperature for complex formation. At the end of this period, the samples were dried again under vacuum at 35 °C [15]. 1.0 mL of mobile phase A and acetonitrile (ACN) mixture (8:2 v/v) was added, vortexed and placed in HPLC vials for analysis.

2.3.3. Amino acid analysis

Amino acid analyses were performed using a Nucleodur 100-5 C18 column (250x4.6 mm, 5µm) on HPLC (SHIMADZU Prominence-I LC- 2030C 3D Model PDA detector) by modifying the methods of Elkin and Wasynczuk [14] and Kwanyuen and Burton [15]. Analyses were performed at 40°C using the gradient program, at a wavelength of 254 nm, with a flow rate of 0.8 mL/min using the mobile phase consisting of solutions A and B. Solution A was 0.07 M CH₃COONa (pH = 6.4 with CH₃COOH) and solution B was a mixture of Acetonitrile (ACN) and water (60:40 v/v)**.**

2.4. Statistical Analysis

All measurements were made in five parallels and the results were given as Mean ± Standard deviation. Statistical evaluation of the results was made using one-way ANOVA test with SPSS version 26. Differences between group means were analysed for significance using Tukey HSD test. Statistical significance level was selected as p<0.05. The same letters shown in the same column in Table 1, 4, and the same row in Tables 2, 3 indicate there is no statistical difference, while different letters indicate a statistical difference.

3. Results and Discussion

To determine the nutritional value of all food products and their compliance with current quality standards, it is necessary to determine the total protein amounts. Therefore, experimentally found the total protein amounts in some legumes are given in Table 1.

Table 1. Total protein contents of legume samples

The level of statistical significance was expressed as $p<0.05$. Different letters in the same column indicate statistical difference, and the same letters indicate no statistical difference

It is seen that the total protein in the investigated legumes is in between 337.75 and 154.15 mg/g dw. Experimental results showed that while *Glycine max* isrich in total protein, *Cicer arietinum* are the poorest in studied samples (Table 1).

James et al. [16] reported the protein content of seven different legume varieties to be in the range of 13.25 and 29.34%. Sarıoğlu and Velioğlu [1] reported that the protein amounts in beans, *Cicer arietinum, lentils* and *Pisum sativum* were 21.75, 18.56, 23 and 19.82 g protein/100g, respectively. Aremu et al. [17] published an article found that the percentage of protein in *Glycine max*, *Vigna radiate* (L.) and lima beans varied between 40-42, 20.24-23.4 and 19.8-23.7, respectively. It has been reported that the percentage of protein in lentils, *Cicer arietinum, Vigna radiate* (L.), mung beans*, Pisum sativum,* cowpeas and Glycine max are 25.1, 17.1, 22.9, 24, 19.7, 24.1 and 43.2, respectively [18]. Our findings for total protein in different legumes are consistent with the literature of the same type of legumes. While the difference between *Cicer arietinum*, Pisum *sativum, Vigna radiate* (L.) and *Phaseolus vulgaris* L. (Ispir dry bean) in terms of total protein is statistically insignificant (p>0.05), the difference between *Phaseolus vulgaris* L. (Gezin dry bean), *Lens culinaris* (Red lentil) and other legumes is statistically significant (p<0.05).

The basic building blocks of proteins are amino acids and grouped as essential and non-essential amino acids. Aspartic and glutamic acids are non-essential amino acids and have important roles in nutritional metabolism, energy requirements, immune responses, oxidative stress and regulation of signalling pathways [19]. Asparagine helps regulate immune function, detoxify ammonia, regulate sugar balance and nitrogen storage [20].

Serine plays an essential role in a broad range of cellular functions including protein synthesis, neurotransmission, folate and methionine cycles and synthesis of sphingolipids, phospholipids [21]. Glutamine serves as a precursor in the biosynthesis of glutathione and plays an important role in antioxidant defense system in the body [22]. Alanine plays an important role in metabolism, such as balancing carbon and nitrogen flow, regulating intracellular pH, and regulating nitrogen-related metabolic events during stress [23]. Pyrroline is an important regulator of basic metabolic pathways required for repair, growth, reproduction and immune system in organisms [24]. Tyrosine is the precursor of epinephrine, norepinephrine, DOPA, and thyroid hormones, and the hydroxyl group of tyrosine also enables the synchronization of metals through oxidative and reductive reactions [25]. Cysteine plays a role in the development of anabolic and catabolic processes in living organisms [26].

It was determined that *Glycine max* is the richest in terms of non-essential amino acids except serine, tyrosine and cysteine, while *Pisum sativum* is the poorest in terms of glutamic acid, serine and glycine, and *Cicer arietinum* is the poorest in terms of other amino acids (Table 1). While there is no difference between *Pisum sativum* and *Cicer arietinum* including *Phaseolus vulgaris* L. (Gezin dry bean), *Phaseolus vulgaris* L. (İspir dry bean) and *Vigna radiate* (L.) in terms of aspartic acid in the legume samples investigated, the others are different from each other (Table 1).

While cysteine in *Glycine max* and cowpeas is statistically indistinguishable, other legumes are different from each other. The amounts of glutamic acid in *Vigna radiate* (L.), *Cranberry beans*,

Phaseolus vulgaris L.(Ispir dry bean) and *Phaseolus vulgaris* L.(Gezin dry bean) are the same with other legumes types. While there is no difference in term of glutamine in *Phaseolus vulgaris* L (Gezin dry bean) and green lentils other legumes types on the other hand, glutamine content of other samples is different (Table 2).

Among the non-essential amino acids in seven different legume varieties, cysteine was reported to be the lowest as 0.56 mg and glycine was reported to be the highest as 13.29 mg per 100 g [16]. The amounts of non-essential amino acids in *Vigna radiate* (L.), white beans, *Cicer arietinum* and green lentils determined by Margier et al. [27] are lower than our findings

Amino Acids	Glycine max	Vigna unguiculata	Vigna radiate $(L.)$	Cranberry beans	Phaseolus <i>vulgaris</i> L. (Ispir dry bean)	Phaseolus <i>vulgaris</i> L. (Gezin dry bean)	Lens culinaris (Red lentil)	Lens culinaris (Green lentil)	Pisum sativum	Cicer arietinum
Aspartic Acid	35.83 \pm 1.24 $\frac{9}{5}$	28.59 ± 0.77 ^t	25.29 ± 0.67 ^d	26.44 ± 0.70 ^{d,e}	25.12 ± 0.66 ^d	24.07 ± 0.63 ^d	21.70 ± 0.65 c	20.14 ± 0.60 b	17.59 ± 0.40 ^a	17.17 ± 0.36 ^a
Glutamic Acid	53.99 \pm 1.63 ^f	39.32 \pm 1.35 \degree	36.38 \pm 1.22 ^d	36.23 ± 1.23 ^d	34.25 ± 1.18 ^d	36.36 ± 1.19 ^d	30.64 \pm 1.14 °	29.99 ± 1.11 ^c	22.75 ± 0.66 ^a	26.86 ± 0.62 ^b
Asparagine	11.61 ± 0.22 s	7.29 ± 0.15 ^f	7.20 ± 0.16 ^f	6.89 ± 0.18 ^f	4.84 ± 0.11 ^e	3.61 ± 0.09 ^d	3.52 ± 0.07 d	2.92 ± 0.06 ^c	2.78 ± 0.04	0.92 ± 0.06 ^a
Serine	17.07 ± 0.35 ^f	16.45 ± 0.36 ^t	12.74 ± 0.23 ^e	17.08 ± 0.36 ^f	16.66 ± 0.35 ^f	11.36 ± 0.21 ^d	11.60 ± 0.23 ^d	10.05 ± 0.21 ^c	7.31 ± 0.14 ^a	$8.89 \pm 0.16^{\mathrm{b}}$
Glycine	19.94 ± 0.50 ^h	12.01 ± 0.22 ^f	15.35 ± 0.33 g	11.75 ± 0.26 ^f	10.89 ± 0.24 ^e	10.66 ± 0.17 ^e	10.16 ± 0.21 ^d	8.51 ± 0.16 c	$4.64 \pm 0.10^{\text{ a}}$	5.71 \pm 0.11 ^b
Glutamine	$14.44 \pm 0.30^{\text{ i}}$	9.50 \pm 0.17 \rm{s}	7.87 ± 0.12 ^e	10.31 ± 0.23 ^h	9.08 \pm 0.17 $^{\rm f}$	4.41 ± 0.11^b	5.18 ± 0.10 c	$4.55 \pm 0.09^{\mathrm{b}}$	5.84 ± 0.12 ^d	$3.49 \pm 0.10^{\text{a}}$
Alanine	22.38 ± 0.46 ^h	14.98 ± 0.32 ^e	19.32 ± 0.42 s	$7.87 \pm 0.14^{\mathrm{b}}$	16.69 ± 0.39 f	11.40 ± 0.22 ^d	10.16 ± 0.21	10.09 ± 0.20 c	$8.18 \pm 0.17^{\text{ b}}$	7.12 ± 0.12 ^a
Pyroline	15.85 ± 0.33 g	12.44 ± 0.22 ^e	12.98 ± 0.23 f	9.65 \pm 0.18 \degree	12.26 ± 0.23 ^e	9.65 ± 0.19 ^c	9.16 \pm 0.16 ^b	$7.43 \pm 0.10^{\text{a}}$	10.25 ± 0.22 ^d	7.62 ± 0.13 ^a
Tyrosine	10.27 ± 0.21 ^f	9.95 ± 0.19 ^f	9.07 ± 0.15 ^e	12.71 ± 0.22 ^h	10.76 ± 0.22 ^g	8.98 ± 0.14 ^e	5.29 ± 0.10 ^d	4.11 \pm 0.08 \degree	$3.84 \pm 0.10^{\mathrm{b}}$	3.32 ± 0.07 ^a
Cysteine	3.57 ± 0.09 g	$4.27 \pm 0.10^{\mathrm{h}}$	3.69 \pm 0.08 g	2.36 ± 0.04 °	$5.67 \pm 0.11^{\text{ i}}$	2.94 ± 0.05 ^f	2.73 ± 0.06 ^e	$2.11 \pm 0.04^{\mathrm{b}}$	2.46 ± 0.04 ^d	1.37 ± 0.04 ^a

Table 2. Amounts of non-essential amino acids in some legumes (mg g⁻¹)

Table 3. Amounts of essential amino acids in some legumes (mg g⁻¹)

The level of statistical significance was expressed as p<0.05. Different letters in the rows indicate statistical difference, while the same letters indicate no statistical difference.

Legumes play a vital role as a significant source of food proteins, containing substantial amounts of essential amino acids such as lysine, leucine, aspartic acid, glutamic acid, and arginine [28]. Essential amino acids are amino acids that cannot be synthesized in the body and must be taken from outside with food. Histidine ensures protein methylation, the protection of haemoglobin structure and function, and the formation of histamine, which has many roles in metabolism [29]. Arginine, which has an important role in nitrogen metabolism, is a nitrogen source for the cell. Arginine, which is needed in physiological and biochemical processes in the cell and in growth and development periods, also serves as a precursor for the biosynthesis of polyamines and nitric oxide [30]. Threonine is a fundamental part of proteins such as collagen and elastin, plays a very important role in fat metabolism and the immune system, and also helps in the synthesis of glycine and serine [31]. Tryptophan plays a role in a number of important biological processes, especially the function of neurons, the immune system, and the homeostasis of the gut [32]. Leucine is an essential branched-chain amino acid that plays a very important role in protein synthesis and muscle recovery. It also facilitates the regulation of blood sugar levels, accelerates wound healing, and increases the production of growth hormone. Isoleucine plays an important role in muscle metabolism and is mostly concentrated in muscle tissue. It is also very important for immunological function, hemoglobin synthesis, and energy management [33]. Alanine ensures intracellular and intercellular reactions and transfers, the safe passage of nitrogen and the regulation of the glucose-alanine cycle. It also plays a role in many events such as the regulation of intracellular pH, the reduction and regulation of nitrogen-related metabolic events during stress [24], [34]. Phenylalanine plays an important role in catabolic events required for defense mechanisms requiring carbon and nitrogen. It is the building block of a wide variety of secondary compounds such as flavonoids, phenylpropanoids, anthocyanins and lignin, which are found in the structural component of cell walls [35]. Methionine plays an important role in metabolic and physiological processes in the development of living cells, cell growth and adaptation to stress [36]. Lysine plays a very important role in protein synthesis, the production of hormones and enzymes, and calcium absorption. Elastin is also very important for energy production, immunological function, and collagen and collagen synthesis [33]. Among the essential amino acids, *Glycine max* is richest in terms of histidine and leucine, *Vigna unguiculata* are richest in arginine, methionine, tryptophan and lysine, and *Cranberry beans* are richest in terms of valine, isoleucine and phenylalanine. It was observed that *Cicer arietinum* were the poorest of all in terms of histidine, tyrosine, methionine, tryptophan and lysine. Valine and isoleucine were least found in *Pisum sativum,* on the other hand leucine and phenyl alanine, were found to be least observed in *Lens culinaris* (Green lentil) and arginine was found to be least in *Phaseolus vulgaris* L. (İspir dry bean).

The amounts of non-essential and essential amino acids, in *Glycine max*, *Pisum sativum*, *Vigna unguiculata*, *Lens culinaris* and *Cicer arietinum* are consistent with findings of Aguilera et al. [37]. It can be said that the amounts of essential amino acids determined by Kamboj and Nanda [38] in *Cicer arietinum*, *Lens culinaris*, *Pisum sativum*, *Vigna unguiculata*, *Phaseolus vulgaris* L and *Glycine max* are compatible with our findings. It can be said that the non-essential and essential amino acid values determined by Khazaei et al. [39] in six legume samples such as *Lens culinaris*, broad beans, *Pisum sativum*, Glycine max, *Cicer arietinum* and beans are compatible with our results.

Audu and Aremu [40] reported that the non-essential amino acids in red *Cranberry beans* vary between 1.7 and 7.2, and the essential amino acids vary between 1.2 and 10.2 mg / g dry protein. It was observed that our findings are compatible with the findings of Audu and Aremu [40].

Total essential amino acid (TEA), total non-essential amino acid (TNEA), total amino acid (TA) and TEA/TNEA, TEA/TA ratios are given in Table 4.

Table 4. TEA, TNEA, TAA (mg g⁻¹) and TEA/TNEA and TE/TA ratios

It was observed that the highest of total essential amino acids was found to be in *Vigna unguiculata*, on the other hand *Cicer arietinum* has the least. It was determined that the richest in terms of total nonessential and total amino acids was *Glycine max,* and the poorest was *Cicer arietinum*.

It is stated that the total amino acid amounts in seven different legume varieties varies in between 47.40 to 122.88 mg/100 g, TEAA contents changes in between 23.29 to 56.21 mg/100g, and TNEAA amounts varies in between 24.11 to 66.67 mg/100g [16].

Since essential amino acids cannot be synthesized in metabolism, they must be obtained from external sources. Amino acid composition is the most important factor in defining food protein quality. According to the Food and Agriculture Organization (FAO/WHO) and the World Health Organization, it is reported that a good protein source should have a TEA/TA ratio of over 40% and a TEA/TNEA acid ratio of over 60% [41]. It is stated that TEAA/TNEAA ratios in different legume varieties varies between 84 to 101percent [16].

The results obtained show that the TEA/TNEA ratio varies between 65-99 percent, depending on the type of legume. The results show that the TEA/TA ratio varies between 39-50 percent, depending on the type of legume.

From these results, it can be said that all legume samples studied are good sources of total protein, TEA and TNEA, on the other hand *Glycine max* and *Vigna unguiculata* are better than other legume species examined in this study.

4. Conclusion and Suggestions

It can be said that the richest total protein in legumes are *Glycine max* and *Vigna unguiculata*, while the poorest are *Cicer arietinum*. In general, it is seen that the best source in terms of essential and nonessential amino acids is *Glycine max* and *Vigna unguiculata*. It can be concluded that legumes are good source of both essential and non-essential amino acids compared to animal protein sources.

Contributions

All the authors have contributed equally.

Conflict of Interest Statement

Authors declare that they have no known conflict of interest.

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