IIOAB JOURNAL

ARTICLE

AMINO ACID PROFILE OF SOME NEW VARIETIES OF LEGUMINOUS SEEDS

Shweta Thakur[1]*, Santosh Kumar Shrivastava[2], Manjul Shrivastava[3]

[1,2]*Department of Applied Chemistry, Jabalpur Engineering College, Jabalpur-482011, INDIA*

[3]*Department of Chemistry, Govt. Autonomous M.H. College of Home Science and Science, Jabalpur-482002, INDIA*

ISSN 0976-3104

ABSTRACT

Background: *Legumes play an important role in the traditional diets of many regions throughout the world. They are low in fat and are excellent sources of protein, dietary fibre, and a variety of micronutrients and phytochemicals. Utilization of legume seeds in daily diet not only will be a good option to overcome problem of protein-caloric-malnutrition among the people of low income group and also it supports the vegetarian community in Indian prospects. Availability of the essential amino acids under legume seeds enhances nutritional benefits. The quality of a seed depends on the total amount of amino acids present in protein. The present investigation is an attempt to determine amino acid composition in selected hybrid varieties of legume seeds viz Glycine max (NRC-37), Vigna radiata (LGG-460), Phaseolus mungo (LBG-20), Cicer arietinum (JG-130) and Lens esculenta (JL-3). The HPLC technique was applied to ascertain nutritional quality for seeds. All the varieties were found rich source of amino acids and presenting high nutritive value.*

THE IIOAB JOURNAL

KEY WORDS
Amino acid; HPLC technique; Leguminous seeds; Protein

Received: 30 Dec 2016
Accepted: 5 June 2017
Published: 5 Aug 2017

***Corresponding Author**
Email: chem.249@gmail.com
Tel.: +91-7208118781

INTRODUCTION

Legumes are cultivated for their seeds from many years. These seeds were used for food consumption and for industrial oil production. Legumes include beans, lentils, lupins, peas, peanuts and other podded plants that are used as food [1]. Legumes are a significant source of protein, lipids, dietary fibres, carbohydrates and dietary minerals. Like other plant based foods, pulses contain no cholesterol and little fat or sodium [2]. Legumes have played an important role in the diets of many regions in the world. It is difficult to imagine the cuisines of Asia, India, South America, the Middle East, and Mexico without picturing soybeans, lentils, black beans, green beans, chickpeas, and pinto beans, respectively [3,4].

It has long been established that protein is an essential nutrient that needs to be obtained from diets. Protein is an indispensable requirement for growth and maintenance of any living creature. However, the ultimate source of protein is its amino acid composition. A protein molecule is a long chain of amino acids linked with peptide bonds (i.e., an amino acid linked to another amino acid). Dietary protein is digested and absorbed into amino acids. These amino acids play central roles both as building blocks of the body's proteins and as intermediates in metabolism, controlling virtually all cellular processes and reactions in living cells [5, 6]. Amino acids are responsible for the production of all the body's enzymes (including digestive enzymes), and also play a key role in normalizing moods, concentration, aggression, attention, and sleep. Amino acids contribute significantly to the health of the nervous system, muscular structure, hormone production, vital organs, and cellular structure. More important, many physiological processes relating to exercise require amino acids for energy, recovery, muscle hypertrophy, and strength gain [7].

Proteins obtained from different food stuffs; supports diet and consider as a main ingredient for healthy life. The rich sources of amino acids are meat, fish, dairy products, and vegetables such as legumes and grains. Legumes have been shown to be rich in dietary protein. However, the utility of protein does not depend on its quantity but is known to be affected by (i) essential amino-acid composition, (ii) amino-acid imbalance, (iii) biological availability of essential amino acid, (iv) digestibility and (v) interference due to anti-nutritional factors [8]. Scientists and nutritionist were concerned about enough quantity of amino acids in diet must be an important factor in order to maintain health. Availability of the essential amino acids under legume seeds enhances nutritional benefits. The present investigation is an attempt to determine amino acid composition in hybrid varieties of *Glycine max* (NRC-37), *Vigna radiata* (LGG-460), *Phaseolus mungo* (LBG-20), *Cicer arietinum* (JG-130) and *Lens esculenta* (JL-3) by HPLC technique.

IIOAB JOURNAL
ISSN: 0976-3104

MATERIALS AND METHODS

Collection of samples

New hybrid varieties of Leguminous seed of *Glycine max* (NRC-37), *Vigna radiata* (LGG-460), *Phaseolus mungo* (LBG-20), *Cicer arietinum* (JG-130) and *Lens esculenta* (JL-3) under investigation were collected from Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (MP). These seed varieties were high yielding, taken short cultivation time and have resistance for diseases. The study was conducted in the Soybean Processing & Utilization Centre, Central Institute of Agricultural Engineering Bhopal, (M.P.) India.

Amino acid determination method

Instrumentation

Amino acids were determined by high performance liquid chromatography LC-10A (Shimadzu Corporation, Kyoto, Japan) according to the method of Cserhati *et.al.* [9] and Kerese *et.al.* [10]. Like an amino acid analyser, this system uses a column packed with the styrene-divinylbenzene copolymer with sulphonic group, i.e. a strongly acidic cation exchange resin (Shim-pack Amino-Na or Amino-Li), for separation. Amino acid injected and separated by means of a binary gradient eluting method using two liquids of standard solution and then, fed to detection unit. When arginine (the final peak) elute, the column was automatically cleaned and equilibrated to be ready for the next analysis. *O*-phthaladehyde (OPA) was used as the derivatizing reagent for detection through a reaction process. In the presence of thiol (SH) compound, OPA react rapidly with compounds with primary amino (–NH2) group into a strongly fluorescent substance. This post column derivatization and fluorescence detection method enables selective detection of each amino acid at high sensitivity.

Sample preparation by hydrolysis

Alkali hydrolysis: Tryptophan destroyed during acidic conditions therefore alkaline hydrolysis is used for determination of tryptophan, which is stable under basic conditions. For alkaline hydrolysis 500 mg finely ground samples were hydrolyzed by adding 4.83g barium hydroxide and 5ml of boiling water. The mixture was evacuated and then heated at 120^{o}C for 8 hours. After hydrolysis, the pH was adjusted to 3 with HCl, and diluted to 25ml with HPLC grade distilled water. 1ml of sample was vacuum dried using flash evaporator and finally dissolved in citrate buffer (0.1M; pH 2.20).

Acid hydrolysis: Acid hydrolysis was carried out with 6N HCl at 110^{o}C to 20-22 hrs in evacuated and sealed tubes. The hydrolysate was filtered and diluted to 250ml. 1ml of sample was vacuum evaporated at 40^{o}C until dryness. The content was dissolved in citrate buffer (0.1M; pH 2.20). 20µl of this derivatized were injected directly into the HPLC.

Chemicals and buffer solutions

i) Buffer Solution (Citrate buffer 0.1M; pH 2.20)
ii) 10% Brij-35™ solution (Sigma USA)
iii) Reaction solution A [Sodium hypochlorite solution (NaClO solution)]
iv) Reaction solution B (OPA solution –Sigma USA)
v) Sample diluents: (0.2N Na^{+} (sodium citrate) pH 2.20)
vi) Standard Amino acid Solution (Sigma USA)
vii) HPLC Grade water (Millipore)
viii) Mobile phase A, B & C

Mobile phase A: 58.8 gm of sodium citrate containing 0.2N sodium (pH 3.20), 210 ml 99.5% ethanol and 50ml (60%) Perchloric acid makes up the volume up to 3 litres with HPLC grad water.

Mobile phase B: 58.8g of sodium citrate containing 0.6N sodium (pH 10), 12.4 g Boric acid and 30 ml 4N NaOH solution make up the volume up to 1 litre with HPLC grad water.

Mobile phase C: For washing only: 4g NaOH diluted up to 500ml with HPLC grad water.

IIOAB JOURNAL ISSN: 0976-3104

Chromatographic conditions

Shim-pack VP-ODS (150mm X 2.0mm) i.d. 5-µm (Equivalent to Shimadzu P/N 228-34937-94) column, protected by C-8 guard, was used for amino acid analysis at column temperature 35°C. Mobile phase was pumped through the column at a flow rate of 0.3ml/minute and the injection volume was 20 µl. Detection was accomplished by Shimadzu Fluoresce detector which was operated using an excitation wavelength of 350 nm and emission wavelength of 450 nm. The chromatographic run time was kept 90 minutes and resolution of amino acid derivatives was routinely accomplished by using binary gradient system.

RESULTS AND DISCUSSION

The nutritional quality of protein depends upon the total amount of amino acids present in it, relative proportion of the constituent amino acid and the degree to which the animal can liberated and utilize the amino acids from the protein i.e. amino acid availability. The amino acids compositions of different varieties of leguminous seeds viz *Glycine max* variety NRC-37, *Vigna radiata* variety LGG-460, *Phaseolus mungo* variety LBG-20, *Cicer arietinum* variety JG-130, *Lens esculenta* variety JL-3 are mentioned in tabular form in the [Table 1] and chromatograms are represented in [Fig. 1] to [Fig. 5]

Table 1: Amino acid composition of new variety of leguminous seeds (g/100g seed sample)

Amino Acids	*Glycine max* NRC-37	*Vigna radiata* LGG-460	*Phaseolus mungo* LBG-20	*Cicer arietinum* JG-130	*Lens esculenta* JL-3
Aspartic acid	0.902	0.821	0.322	0.483	0.063
Threonine*	2.033	0.373	0.901	0.599	1.283
Serine	0.100	0.044	0.167	1.220	1.004
Glutamic acid	-	0.011	-	-	0.073
Proline	0.092	0.016	0.230	0.045	0.317
Glycine	4.002	0.077	0.045	0.554	1.663
Alanine	0.324	0.023	0.200	-	2.201
Cystine	0.691	0.028	0.311	0.232	0.301
Valine*	2.202	1.192	1.188	0.844	1.244
Methionine*	0.561	0.035	0.350	0.088	0.203
Isoleucine*	2.100	1.310	1.298	0.848	1.082
Leucine*	3.332	1.882	1.905	1.600	1.901
Tyrosine	1.435	0.400	0.342	0.493	0.784
Phenylalanine*	2.072	1.401	1.190	0.974	1.072
Histidine*	0.662	0.662	0.662	0.438	0.662
Tryptophan*	0.442	0.659	0.192	1.322	0.066
Lysine*	1.253	1.744	1.522	1.266	1.760
Arginine	0.456	2.001	1.829	1.566	2.110

*Essential Amino Acids

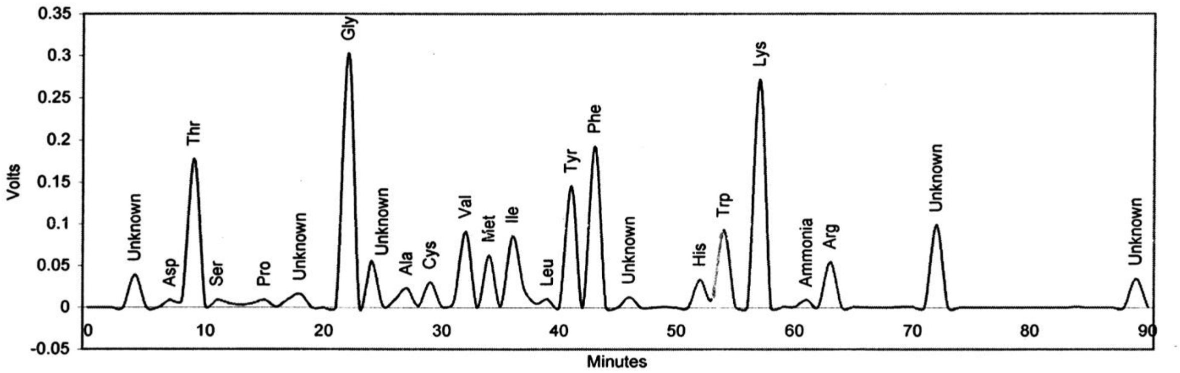

Fig. 1: Chromatogram of amino acid composition of *Glycine max* variety NRC-37.

From the perusal of the data of [Table 1] and [Fig. 1] it appears that in the seed proteins of *Glycine max* variety NRC-37, the percentage of Glycine was found maximum (4.002g/100g). Glycine has several important roles within the body. It is essential for the production of many different acids, including nucleic acids, bile acids, creatine phosphate and porphyrins. This amino acid is closely associated with the central nervous system and the digestive system. Glycine helps with the

IIOAB JOURNAL ISSN: 0976-3104

breakdown of fat by regulating the concentration of bile acids. Glycine is also required for the bio-synthesis of heme. Heme is a key component of haemoglobin. Haemoglobin is essential in the maintenance of red blood cell integrity and optimal oxygen carrying capacity. Glycine is used for treating schizophrenia, stroke, benign prostatic hyperplasia (BPH), and some rare inherited metabolic disorders. It is also used to protect kidneys from the harmful side effects of certain drugs used after organ transplantation as well as the liver from harmful effects of alcohol [11, 12]. However, other amino acids found in increasing order were proline, serine, alanine, tryptophane, arginine, methionine, histidine, cystein, aspartic acid, lysine, tyrosine, threonine, phenylalanine, isoleucine, valine, and leucine. These results also found good agreement with other varieties legume and oil seed [17, 18, 19].

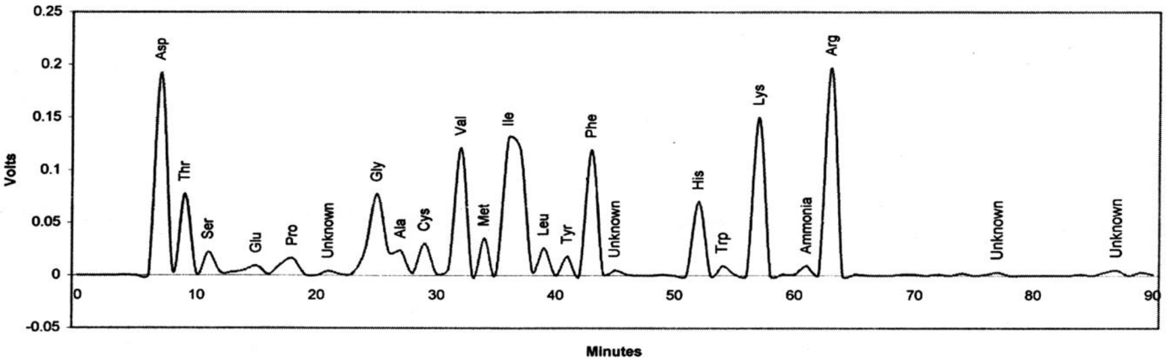

Fig. 2: Chromatogram of amino acid composition of *Vigna radiata variety* LGG-460

Results of [Table 1] and [Fig. 2] shows that the seed proteins of *Vigna radiata* variety LGG-460 was found to contain highest amount of arginine (2.001g/100g). Body uses the amino acid arginine to make nitric oxide. Nitric oxide helps lower blood pressure by relaxing muscles in the blood vessels. It's produced in heart muscles, where it regulates contractions. It may also prevent atherosclerosis by inhibiting the development of plaque in the arteries. Nitric oxide is the active ingredient in nitroglycerin, a medication used to relieve angina, or chest pain caused by coronary heart disease [13, 14]. However, other amino acids also found in the decreasing order were leucine, lysine, phenylalanine, isoleucine, valine, aspartic acid, histidine, tryptophane, tyrosine, threonine, glycine, serine, methionine, cystein, alanine, proline and glutamic acid. These results also found close proximity with other varieties legume seeds [20, 21].

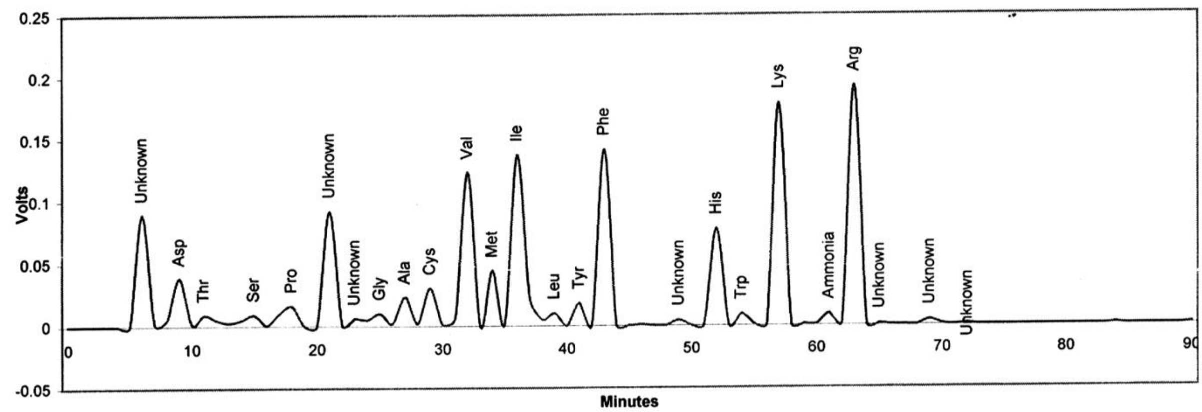

Fig. 3: Chromatogram of amino acid composition of *Phaseolus mungo* variety LBG-20.

Data present in [Table 1] and [Fig. 3], it appears that the seed proteins of *Phaseolus mungo* variety LBG-20 were found to contain highest amount of leucine (1.905g /100g). Leucine is the branched-chain amino acids that enhance energy, increase endurance, and aid in muscle tissue recovery and repair. It also lowers elevated blood sugar levels and increases growth hormone production. It is one of three essential amino acids that increase muscle mass and help muscles recover after exercise. It also regulates blood sugar and supplies the body with energy. These functions make it invaluable when the body is stressed. Leucine is used clinically to help the body heal, and it also affects brain function. It is concluded that the role of leucine *In vivo* is to provide a signal that amino acids are available, which in combination with the signal of energy availability from insulin, stimulates muscle protein synthesis [15]. However other amino acids in the decreasing order were arginine, lysine, leucine, phenylalanine, valine, threonine, histidine, methionine, tyrosine, aspartic acid, cystein, proline, alanine, tryptophan, serine and glycine. These results also found good accordance with the reported values [21, 22, 23].

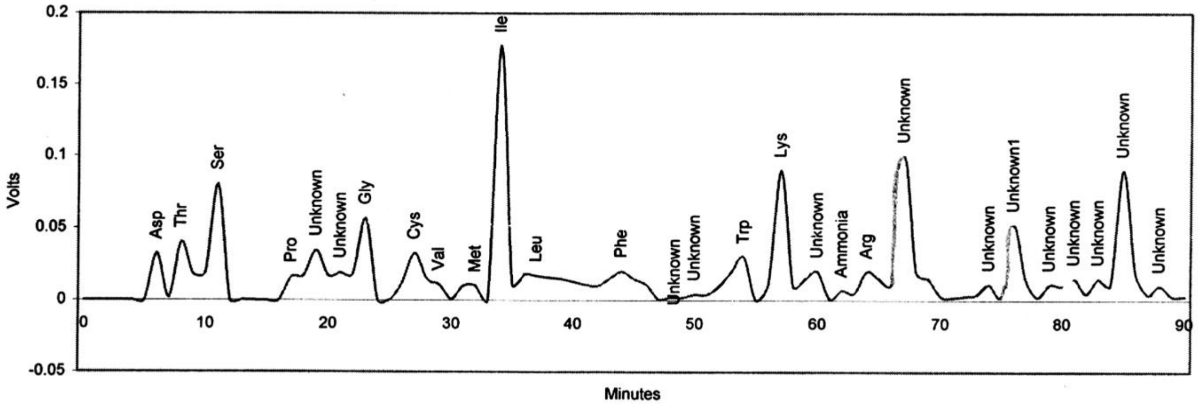

Fig. 4: Chromatogram of amino acid composition of *Cicer arientinum* variety JG-130.

From the data given in [Table 1] and [Fig. 4] it appears that the seed proteins of *Cicer arietinum* (JG-130) was found to contain highest amount of leucine (1.600g/100g) followed by arginine (1.566g/100g). The role of leucine and arginine are discussed in previous varieties of *Phaseolus mungo* and *Vigna radiata* respectively [13, 14, 15]. However, other amino acids in decreasing order were tryptophan, lysine, serine, phenylalanine, isoleucine, valine, threonine, glycine, tyrosine, aspartic acid, histidine, cystein, methionine and proline. These results also found good agreement with other varieties of legumes [20, 24, 25].

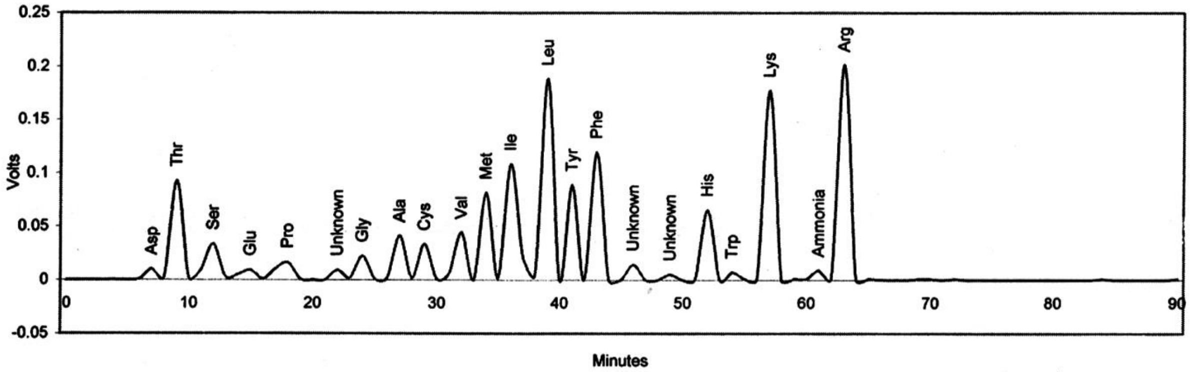

Fig.5: Chromatogram of amino acid composition of *Lens esculenta* variety JL-3.

Results of [Table 1] and [Fig. 5] represent that *Lens esculenta* variety JL-3 was found to contain highest amount of alanine (2.201g/100g). Alanine is one of the simplest amino acids which involved in the energy-producing breakdown of glucose. Alanine plays a key role in glucose–alanine cycle between tissues and liver. In conditions of sudden anaerobic energy need, when muscle proteins are broken down for energy, alanine acts as a carrier molecule to take the nitrogen-containing amino group to the liver to be changed to the less toxic urea, thus preventing build-up of toxic products in the muscle cells when extra energy is needed [16, 17]. However, other amino acids in the decreasing order were arginine, leucine, lysine, glycine, threonine, valine, isoleucine, phenylalanine, serine, tyrosine, histidine, proline, cystein, methionine, tryptophan, glutamic acid, and aspartic acid. Several investigators have also been studied the amino acid composition in some leguminous seeds [22, 23, 24].

CONCLUSION

From the above analysed data it conclude that the varieties of legume seeds taken under this study were found to rich source of some essential (leucine, isoleucine, valine, phenylalanine, lysine & threonine) and non-essential amino acids (glycine, arginine, cystine, tyrosine & aspartic acid). These seeds may support the essential amino acid requirement of human diet except S-containing amino acids. In order to complete this deficiency leguminous seeds were supplemented with other vegetables, cereals and dairy products. Utilization of legume seeds in daily diet not only will be a good option to overcome the problem of protein-caloric-malnutrition among the people of low income group but also it supports the vegetarian community in Indian prospects.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

Authors are thankful to Dr S.D.Kulkarni, Project Director Soya bean processing and utilization centre, Central Institute of Agricultural Engineering CIAE–BHOPAL, MP, India for giving a helping hand for the analysis of the samples. Prof. Dr. R.S. Sharma (HOD, Agronomy) Jawaharlal Nehru Krishi Vishwavidyalaya was gratefully acknowledge for providing seed samples.

FINANCIAL DISCLOSURE

None

REFERENCES

[1] Kurlovich BS, Rep'ev SI, Shchelko LG, et al. [1995] Theoretical basis of plant breeding. The gene bank and breeding of grain legumes (lupine, vetch, soya and bean), Institute of Plant Industry St. Petersburg, VIR. 111:438

[2] Hellendoorn EW. [1976] Beneficial physiologic action of beans. J Am Diet Assoc. 69:248–253.

[3] Messina MJ. [1999] Legumes and soybeans: overview of their nutritional profiles and health effects. American Journal of Clinical Nutrition. 70:439S–450S.

[4] Siddhuraju P, Becker K. [2001] Species/variety differences in biochemical composition and nutritional value of Indian tribal legumes of the genus Canavalia. Nahrung, Molecular Nutrition Food Research. 45(4):224–233.

[5] Burr HK. [1975] Pulse protein: Protein nutritional quality of food and feed. Part II. Fridman, M. (Ed.). p. 119-134.

[6] Attie AD, Scherer PE. [2009] Adipocyte metabolism and obesity. J Lipid Res. 50 Suppl: S395-S399.

[7] Rao N, Shrivastava SK. [2011] Amino Acid Composition in New Variety Seeds of Pea. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2(2):636-644.

[8] Gupta YP. [1988] Nutritive value of pulses, Pulses Crop (Grain Legume). Baldev B, Ramanujam and Jain HK. (Eds.), New Delhi, p.567-574.

[9] Cserhati T, Forgacs E. [1999] Chromatography in Food Science and Technology. Technomic publishing Co. Lancaster, USA, p.158-298.

[10] Kerese I. [1984] Method of Protein Analysis, Ellis Horwood Ltd. Publishers. 336-365.

[11] Gusev EI, Skvortsova VI, Dambinova SA, [2000] Neuroprotective effects of glycine for therapy of acute ischaemic stroke. Cerebrovasc Dis. 10:49-60.

[12] Javitt DC, Zylberman I, Zukin SR, [1994] Amelioration of negative symptoms in schizophrenia by glycine. Am J Psychiatry. 151:1234-1236.

[13] Williams JZ, Abumrad N, Barbul A. [2002] Effect of a Specialized Amino Acid Mixture on Human Collagen Deposition, Annals of Surgery., 236(3):369-375.

[14] Lavie L, Hafetz A, Luboshitzky R, Lavie P. [2003] Plasma levels of nitric oxide and L-arginine in sleep apnea patients, Journal of Molecular Neuroscience, 21(1):57-63.

[15] Garlick PJ, [2005] The role of leucine in the regulation of protein metabolism. J Nutr. 135(6):1553S-1556S.

[16] Nelson, David L.; Cox, Michael M. [2005] Principles of Biochemistry (4th ed.), New York: W. H. Freeman, p.684–85.

[17] Sarjekar P, Shrivastava SK. [2002] Amino acid composition of some conventional and non-conventional leguminous seeds. Asian Journal of Chemistry. 14(2):1071-1073.

[18] Ingale S, Shrivastava SK. [2011] Nutritional study of new variety of groundnut (*Arachis hypogaea L.*) JL-24 seeds. African Journal of Food Science. 5(8):490-498.

[19] Karr-Lilienthal KK, Grieshop CM, Spears JK, Eahey GC. [2005] Amino acid, carbohydrate and fat composition of soybean meal prepared at 55 commercial U.S. processing soybean plant. Journal of Agriculture Food Chemistry. 53(6):2146-2150.

[20] Malleshi NG, Klopfenstein CF. [1996] Proximate composition, amino acid and vitamin contents of malted chickpea, mung bean and their seed coats. Journal of Food Science Technology. 33(6):479-482.

[21] Shrivastava SK, Bajpai RK. [1992] Amino acid composition of five new varieties of Pigeon pea (*Cajanus cajan* (L) Mill.Sp.). Journal of Physical Science. 4(1):82-84.

[22] Iqbal A, Khalil IA, Ateeq N, Khan MS. [2006] Nutritional quality of important food legumes. Food Chemistry. 97(2):331-335.

[23] Ali N. [2003] Processing and Utilization of Legumes. In Takashi Tajima (Ed.). 117-143.

[24] Saxena P, Saxena PL, Shrivastava SK. [2002] Amino acid composition of some new varieties of leguminous seeds. Asian Journal of Chemistry. 14(2):643-646.

[25] Singhai B, Shrivastava SK. [2006] Nutritive value of new chickpea (*Cicer arietinum*) varieties. Journal of Food Agriculture and Environment. 4(1):48-53.