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NUTRITIVE ANALYSIS OF MUNG BEAN VARIETIES OF KHYBER PUKHTOON KHWA REGIONS THROUGH HPLC

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Abstract

Three cultivars of Mung bean viz NM-92, NM-98 and NM-06 were analyzed for their proximate composition. The samples were also tested by HPLC for amino acid content. The data showed that all the varieties had same moisture level. The maximum ash content (4.29%) was present in NM-92, crude fat (2.26%) was highest in NM-98 while NM-06 contained maximum amount of crude protein. About eighteen types of amino acids were detected in each of three varities. Acidic amino acids i.e., aspartic and glutamic acids were in considerable amount ranged from 13 to 23% followed by leucine, isoleucine, alanine, valine, lysine, phenyl alanine,serine and argnine fell in range of 3-8% of total protein. Maximum amount (13.00 and 22.80%) of aspartic and glutamic acids were present in NM-98. Similarly argnine (6.83%) and serine (5.45%) were also in highest amount in this variety. Leucine (7.46%) was maximum in NM-92 variety.NM-06 contained almost all the amino acids in lesser quantity except few like threonine, proline, glycine and alanine. It was concluded from the present study that varieties were of different nutritional value and HPLC was a sensitive method for amino acids determination.

Introduction

Legumes are those species of family leguminosae (fabaceae) which are consumed directly by human beings, most commonly as mature dry seeds, but occasionally as immature seeds enclosed in pods[1]. The leguminous grains, also known as pulses are famous for their high quality protein content. They are mainly composed up of sulphur amino acids and also an indispensible source of the minerals i.e. phosphate, calcium, magnesium and other trace elements. Grain legumes are often considered as meat substitutes for people in less developed countries[2]. In spite of their nutritional importance legumes plants are vital in restoring and building of soil fertility by fixing atmospheric nitrogen in nodule (an outgrowth of roots).One member of legumes known as mung bean (vigna radiate), also called "green gram", mainly grown in tropical regions of Asia is an important summer-growing, annual crop[2,3]. Mung bean has a short growing season and is relatively drought-tolerant. Mung bean is thought to be originated in South and Southeast Asian regions. It is widely grown in India, Pakistan, Bangladesh, Myanmar, Thailand, Philippines, China and Indonesia. It is the second major pulse crop in Pakistan which has covered an area of 200 thousand acre with annual production of about 93 thousand tones equal to 446 kg per ha[4]. In KPK various regions like Malakand, Kohat, Karak, Bannu and D.I.Khan districts are famous for its production however total production in the province is 12.6 tones on 8.1 hectors, so therefore we selected different varieties of Mung bean from different regions of Khyber Pukhtoon Khwa (KPK), Pakistan[3,5].

Mung bean fit well in our cropping system and it is grown as intercrop with other Kharif crops[6]. The green plant is used as fodder in many areas. It is also a good green manure crop. The dried stems and pod wall remaining after threshing are also used as cattle food. It contains isoflavoineds having estrogen and antioxidant activities that used prevention of many diseases such as cancer; it also exhibits antimicrobial and insecticidal activities[7].There are two main varieties golden and green, the name denoting the color of dry seed. The climate and soil requirements of Mung bean are sandy loam soil with moderate moisture content. Mung bean is resistance to most of the diseases and insects pests of legume family.Mung bean is used as supplement for cereal based human diets due to its high lysine content. The grain has about 19.05 to 23.86% protein, 247.67 to 277.3 mg/100g calcium and 5.03 to 12.63 mg/100g iron[8]. The nutritional quality of these grains may be impaired due to the presence of trypsin and other such anti-nutrients. This may cause low protein digestibility and minerals availability in legumes. In rural areas the immature green pods are also used as vegetables. Mung bean stalks, leaves and husks constitutes a significant proportion of livestock feed. The sprouted beans are consumed as cooked vegetable, usually in oriental dishes, or eaten raw in salads[9].

Protein of mung bean is known as complete protein due to the presence of many of essential amino acids. Amino acids are required by man and other animals for their normal growth and health. Every cell of the body contains thousands of different proteins, each digested to carry specific function. And each protein is composed from a basic set of 20 amino acids. The acidic part (carboxylic group) transfers a proton to to the basic part amino group (NH) that results in zwitter ion. Amino acid in the zwitter ion form is electrically neutral because the negative charge is balanced by the positive charge[10].

Amino acids differ from one another with respect to the nature of R (organic group), which may be aliphatic (open chain structure), aromatic (having benzene ring), polar (hydrophilic), non-polar (hydrophobic), acidic (having additional carboxylic group), basic (having additional amino group) or neutral (having equal number of carboxylic and amino group). That's why amino acids are classified on the basis of chemical nature of R group[11]. Nearly all naturally occurring amino acids have L-configuration.

Amino acids are nutritionally classified into two

groups, essential and non-essential amino acids. Those which cannot be synthesized inside human body and must be taken from an external dietary source are called nutritionally essential amino acids (indispensible). They are ten in number and include Threonine, Tryptophan. Histidine, Arginine, Lysine, Leucine, Isoleucine, Methonine, Valine and Phenylalanine and those which can be synthesized by human body are nutritionally non-essential amino acids (dispensable). Examples are Serine, Glycine, Alanine, Proline, Tyrosine, Cysteine, glutamine, Aspartic and Glutamic acid. They participate in many diverse metabolic reactions [7, 12].

Amino acids are the source of soil nitrogen which plays a significant role in the nitrogen cycle. Some play a vital role in the nitrogen transport and metabolism of plants like aspartate and glutamate. They play a key role in the cell metabolism. Methionine is the donor of methyl group, and glycine is the starting material for the synthesis of hemoglobin. They also serve as co-enzymes. Iodine substituted amino acid of non-protein origin are the thyroid harmones. Some amino acids are of therapeutic value while some are employed in the healing of wounds and burns. Amino acids, as zwitter ion act as buffer in the living organisms and serves as carrier of other nutrients as well as carrier of heredity from one generation to the next[13].

In the developing countries, like Pakistan, India and Bangladesh, where mostly people are vegetarian for economic and religious reasons, they usually suffer from protein malnutrition problems as their diet is lacking in lysine, so they are requested to eat food legumes because they are rich in lysine[14].

Keeping in view the importance of legumes this work was an endeavor to highlight the nutritional importance by analyzing mung bean (green gram) for their certain chemical constituent's i.e. proximate composition and amino acid profile.

Material and Methods

Sample collection: Three varieties of Mung bean i.e. NM-92, NM-98 and NM-06 were collected from National Institute for Food and Agriculture (NIFA) Research Centre TARNAB, Peshawar. All the samples were collected in one kilogram plastic bags and were stored at room temperature.

Sample preparation: Seeds of each variety were shifted from dirt and malformed kernels and then samples were stored at room temperature and one third of the seeds were ground in the grinder and then further ground to fine particles by chopper in the laboratory for chemical analysis.

Proximate composition: Samples of Mung bean varieties were analyzed for crude protein, crude fat, ash and moisture by standard methods of AOAC (1990).

Moisture contents: Moisture was determined by oven drying method. 1g of each sample was accurately weighed in Petri dish. The partially covered Petri dish was placed in oven at 105 °C for 6 hours. After cooling the Petri dish in the desiccators for 30 mint was reweighed. The percent moisture was calculated as**:**

Moisture content (%) = Weight loss on drying x 100/weight of sample

Crude fats: Crude fat was determined by ether extract method using Soxhlet apparatus. 1 g of each moisture sample was wrapped in filter paper, placed in thimble and then introduced into extraction tube. Receiving flasks were filled up to 1/3 with hexane and fitted into apparatus. After complete extraction the hexane from the receiving flasks was evaporated on water bath, the ether extract was dried in an electric oven and the flasks were reweighted. The percent crude fat was determined by using the following formula**:**

% Crude Fat = Weight of ether extract x 100/weight of sample

Crude proteins: One gram of each sample was taken in digestion flasks. 8g of digestion mixture i-e $K_2So_4.CuSo_4$ (8:1) and 12 ml of conc. H_2So_4 were

added. Some pieces of fumic stones were also added to avoid bumping of the solution. Digestion was carried out by heating the mixture becomes clear.

After cooling the digest was transferred to 100 ml volumetric flask and volume was made up to the mark with distilled water. Distillation was carried out in Markam Still Distillation apparatus. 10 ml of digest was introduced in the distillation tube through funnel. Then 10 ml of 40% NaOH was gradually added through the same way. Distillation was carried out for at least 10 minutes and NH3 produced was collected as NH_4OH in a conical flask containing 20 ml of 4% Boric acid solution with few drops of modified methyl red indicator .during distillation yellowish color appears due to Ammonium hydroxide.

The distillate was titrated against standard 0.1 N HCL solutions till the appearance of pink color. A bland (experiment without sample) was also run through all the steps as above. Percent Crude Proteins were calculated by multiplying the percent nitrogen with appropriate factor.

$$\% N = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of sample} \times V}$$

Where S= sample titration reading
B= blank titration reading
N= normality of HCL
D= dilution of sample after digestion.
V= volume taken for titration
0.014 = Milli equivalent Wt. of Nitrogen
Crude proteins (%) = % N x 6.25.

Analysis of Amino Acid by HPLC: Six Test tubes (1.5 x 10 cm) were washed and placed in oven at 100 °C for 20 minutes for drying. Each dried test tube was put 40 mg dried ground Mung bean sample, 10 ml 6 N HCL and small portion of phenol was added. All the tubes were placed in vacuum desiccators and 700 mm Hg vacuum was created in order to stop the breakdown of amino acids by oxidation reaction. Desiccators were then placed in oven at 100 °C for three days. 25 microliter of each hydrolysate was transferred to sample tube (6 x50 mm) and was dried in vacuum 50 microliter of dissolving reagent (Methanol + Distilled water + TEA; 2:2:1) was added to sample tube, mixed and dried twice in vacuum. After this 50 of derivatizing reagent containing Methanol, TEA, Water and PITC (7:1:1:1) was added and incubated at room temperature for 20 minutes followed by drying in vacuum for 10 minutes. Methanol was then added to each sample tube and dried twice in vacuum. The residues were then dissolved in 200 microliter of solution containing mm di-sodium hydrogen phosphate and acetonitrile (95:5) 20 microliter was injected in HPLC and the data was recorded by SW 32 software system.

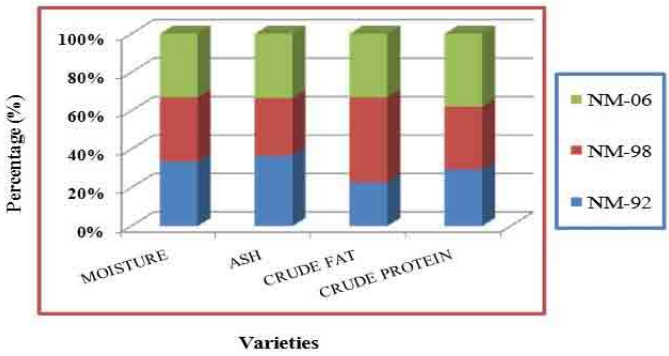
Results and Discussion

Three varieties of Mung beans (NM-98 and NM-06) obtain from NIFA (Nuclear Institute of Food and Agriculture) were analyzed for their important proximate composition and amino acid contact. The data was presented in the form of table and graphs as under.

The data (Table-1) showed that all the varieties had same moisture level. The mineral matter in Mung varieties were in quantity. The maximum ash contact (4.29%) was present in NM-92. Crude fat (2.26%) were highest in NM-06 contained maximum amount of crude protein. The present data was in good line with Agugo and Onimawo (2009) while studying the effect of processing on different pulses containing Mung beans. The reported ash content up to 3.34% crude fat 1.34% and crude protein up to 22.90%. The data was also in good comparison with Adil et al (1980) and Anwar et al (2007) also reported same kind of data while analyzing different Mung bean cultivars.

PARAMETERS %	VARIETIES		
	NM-92	NM-98	NM-06
MOISTURE	4.05	4.04	4.00
ASH	4.29	3.51	3.93
CRUDE FAT	1.15	2.26	1.68
CRUDE PROTEIN	13.20	14.77	17.05

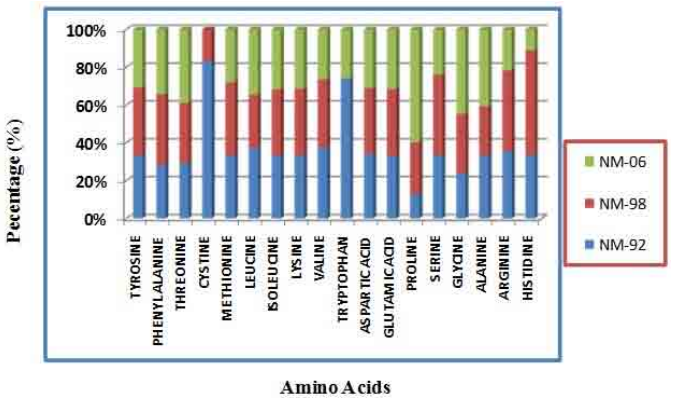
Table 1. Proximate Composition of Mung Bean Varieties.



About eighteen of amino acids were detected (table-2) in each of three varieties. The data showed that acidic amino acids i.e. aspartic and glutamic acids were in considerable amount ranged from 13 to 23% followed by leucine, isoleucine, alanine, valine, lysine, phenyl, alanine, serine and arginine fall in the range of 3-8% of total protein. Maximum amount (13.00 and 22.80 %) of aspartic and glutamic acids were present in NM-98. Similarly Arginine (6.38 %) and serine (5.45 %) were also in highest amount in this variety. Leucine (7.46 %) was in NM-92 variety. NM-06 contained almost all the amino acid in lesser quantity except few like threonine, proline, glycine and alanine. The present data was good in line with Mubarak et al.(2004) who studies the nutritional composition and anti_nutritional factors of mung beans seeds as affected by home traditional processes. The data was also closed to the results of Belton et al. (1984) and Bhatty et al. (1990) who analyzed various Mung bean cultivars for their nutritional.

AMINO ACIDS	VARIETIES		
	NM-92	NM-98	NM-06
TYROSINE	2.57	2.77	2.37
PHENYL ALANINE	3.96	5.16	4.76
THREONINE	2.45	2.65	3.25
CYSTINE	0.25	0.05	0.00
METHIONINE	1.22	1.42	1.02
LEUCINE	7.46	5.66	6.86
ISOLEUCINE	4.04	4.24	3.84
LYSINE	3.49	3.69	3.29
VALINE	4.70	4.50	3.30
TRYPTOPHAN	0.20	0.00	0.07
ASPARTIC ACID	12.80	13.00	11.60
GLUTAMIC ACID	21.00	22.80	20.20
PROLINE	0.73	1.53	3.33
SERINE	4.25	5.45	3.05
GLYCINE	2.56	3.36	4.76
ALANINE	3.65	2.85	4.45
ARGININE	5.63	6.83	3.43
HISTIDINE	1.79	2.99	0.59

Table 2. Amino Acid Profile (g/100g of protein) of Mung bean varieties.



Summary

Mung bean (vigna radiate) belong to family leguminosae (fabaceae) is the second major pulse crop in Pakistan. It is use as supplement for cereal based human diets due to its high lysine content. The grain has about 19.05-23.86 % protein, 247.67-277.3 mg/100gms calcium and 5.30 to 12.63 mg/100gms iron. The nutritional quality of these grains may be impaired due to the presence of trypsine and other such anti-nutritients. In the rural areas the immature green pods are also used as vegetables. Mung bean stalks, leaves and husks constituent a significant proportions of livestock feed. The sprouted beans are consumed as cooked vegetables, usually in oriental dishes, are eaten as raw in salads.

Three cultivars of Mung beans NM-92 NM-98 were analyzed for their proximate composition. The samples were also tested by HPLC for amino acid content. The data showed that all the varieties had same moisture level. The maximum ashes content (4.29%) was present in MN-92. crude fats (2.26) % were highest in MN-98 while MN-06 contain maximum amount of crude proteins.

About eighteen types of amino acids were detected in each of three varieties. Acidic amino acids i.e. aspartic and glutomic acid were in considerable amount range from 13 to 23 % followed by leucine, isoleucine aniline, valine, lysine phenyl alanine, serine and arginine fell in the range of 3-8% of total protein maximum amount (13.00 and 22.80%) of aspartic and glutamic acid is present in MN-98. Similarly arginine (6.83%) and serine (5.45%) were also in highest amount in this variety. Leucine

(7.46%) was maximum in MN-92 variety. MN-06 contained almost all the amino acid in lesser quantity except few like threonine, praline, glycine and alanine

It was concluded from the present study that different verities has different nutritional values and HPLC was a sensitive method for amino acid determination.

Conclusion

It was concluded that Mung bean contained various nutrients in appreciable quantities. All the cultivars contained crude protein in maximum amount which were comparable to those of animals sources. The HPLC method were used for amino acids determination was accurate regularly be utilized for the same purpose. It is recommended the Mung bean due to high nutritional value could be used in daily human diet which would be helpful in fulfilling the protein need of the body. It is also recommended that utmost precautions should be taken while hydrolyzing samples for HPLC recommendation of amino acid, because high risks of oxidation contamination with other protein are present.

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References

1. Adel,a., Y . Shehata, A.M. Thannoun. 1980. Chemical and amino acid composition of Iraqi mung beans. Food Sci. Dept. Faculty of Agri. Univ. of Mosul, Hammam Al-Alil, Mosul, Iraq.171:360-362.
2. Amarteifio, J.O., D. Moholo. 1998. The Chemical Composition of Four Legumes Conmsumed in Botswana. Dept. of Basic Sci, Botswana College of Agri, Gaborone, Botswana. 11:329-332.
3. Anwar,F., S.Latif, R. Przybylski, B.Sultana, M.Ashraf. 2007. Chemical Composition and Antioxidant Activity of Seed of Different Culitivars of Mungbean. Dept. of Chem. & Biochem. Univ. of Lethbridge, Lethobridge,Alberta, Canada. 72:503-510
4. Bhat, R., K. R. Sridhar, S.Seene. 2008. Nutritional quality evaluation of velvet bean seeds (Mucuna Pruriens) exposed to gamma irradiation 59:261-278
5. Bhatty, N., A.H.Gilan, S. A. Nagra. 2000. Nutritional value of mung bean (Vigna Radiation) as effected by cooking & supplementation. Dept. of Rural Home Eco. Univ. of Agric. Faisalabad, Pak. And institute of Chem. Univ. of Punjab, Lahore, Pak: 127:478-481.
6. Cavalieri, A. j.,A. H. C. Hunang. 1980. Carrier Protein-mediated Transport of Neutral Amino Acids into Mung bean Mitochondria. Biology Dept. Univ. of South Carolina, Columbia. 66:588-591.
7. Dzdie, T., J . Hardy . 1996. Physicochemical and Functional Properties of Flours Prepared from Common Bean and Green Mung Beans. J. Agric. Food Chem ., Vandoeuvre Les Nancy, France. 44:3029-3032.
8. Harper, J. E., K. A. Corrigan, A. C. Barbera, M. H.Abd-Alla. 1996. Hypernodulation of Soyabean, Mung bean, and Hyacinth Bean Is Controlled by a Common Shoot Signal. Institute of Agron, Univ. of Study in Catania, Valdsavoia, Cantania, Italy. 5:95-123.
9. Karl, A. j. Wilson, C. Chen.1980. Amino Acid Sequence of Mung bean Trypsin Inhibitor and Its Modified Forms Appearing during Germination. Dept. of Bio. Sci. State Univ.of New York at Binghamton, Binghamton, New York. 66:588-591.
10. King, R.D., P. Puwastien, 1986. Effect of Germination on the Proximate Composition and Nutritional Quality of Winged Bean (Psophocarpustetragonolobus) seed. Institute of Nutr. Ramathibodi Hospital, Mahidol Univ. Rama VI Road, Bangkok Thailand. 58:106-108.
11. Malleshi, N.G., C.F. Klopfenstein. 1996. Proximate Composition, amino Acid and vitamin contects of malted Chickpea, Mungbean and their seed coats. J. of food Sci. and tech. Dept. of Grain Sci. and Tech. Central Food Tech. Res. Institute, Mysore. 33:479-482.
12. Mubarak, A.E. 2004. Nutritional composition and nutritional factor of Mung Bean Seeds (Phaseolus aureus) as affected by some home traditional processes Home Econ. Dept. Faculty of SpecificEdu. Menofiya Univ. Ashmoon City, Egypt. 71:341-349.
13. Nishimura, H., S. Tahara, H. Okuyama, J. Mitzutani. (1972). Mass sectra of sulphur-containing amino acid and peptides Tehrahedron. 28:4503-4513.
14. Thompson, E. W., M. V. Lycock, J.A. Ramshaw, D. Boulter.(1970). The amino acid sequence of Phaseolus aureus L. (mung-bean) cytochrome c. Biochem J.Dept. of Bot. Univ. of Durham, Durham City, U.K. 117:183-192.