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Amino Acid Composition in New Variety Seeds of Pea

Nagabhushana Rao G* and Shrivastava S K †

Department of Applied Chemistry, Govt Engineering College, Jabalpur, Madhya Pradesh- 482001, INDIA.

ABSTRACT

Proteins present in various food stuffs accounts for their nutritive value. In addition, essential amino acids availability adds to improve the nutritional benefits in the seeds. Pulses have been shown to be rich in proteins. It has long been known that proteins are required in the diet of animals and that some proteins are more effective than others in supporting growth. The biological value of a protein may, in general, be considered to depend upon its amino acid composition. Besides building cells and repairing tissue, amino acids form antibodies to combat invading bacteria and viruses; they are part of the enzyme and hormonal system. The nutritional quality of protein depends upon the total amount of amino acids present in the protein , the relative proportion of the constituent amino acid and the degree to which the animal can liberate and utilize the amino acids from the protein i.e. amino acid availability. In the present investigation amino acid composition of Pea (Pisum *sativum)* new variety seeds (Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6) *were* analysed for amino acid composition to ascertain the nutritional quality of the seeds by using FPLC (Fast Pressure Liquid Chromatography).

Key words: Amino acid composition, Fast Pressure Liquid Chromatography

***Corresponding author**

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INTRODUCTION

The growth response in animals depends upon the simultaneous availability of a number of amino acids. The ingested protein is hydrolyzed to its constituent amino acid within the alimentary canal, and that these amino acids are absorbed by the body. Accordingly, it is not the proteins which are required, but rather the amino acids present in the proteins and released by hydrolysis. Over one hundred amino acids have been found in nature. In addition to protein synthesis, amino acids have other biologically important roles of these amino acids twenty are considered "standard amino acids" because they are encoded by the standard genetic code. The twenty "standard amino acids" are separated into two categories i.e. essential and non-essential. Essential amino acids cannot be produced by the body and must be made available through the diet. Non-essential amino acids can be produced by the body within the liver. The correct ratio of essential and non-essential amino acids must be present in sufficient quantities before any muscle can be produced [1]. Besides building cells and repairing tissue, amino acids form antibodies to combat invading bacteria and viruses; they are part of the enzyme and hormonal system; they build nucleoproteins (RNA & DNA), they carry oxygen throughout the body, and are part of all muscular activity. Neurotransmitters are manufactured in the brain from the amino acids we extract from foods, and their supply is entirely dependent on the presence of these precursor amino acids. Without adequate amino-acid conversion, neurotransmitters are no longer produced in sufficient amounts. These deficiencies and nutritional imbalances may provoke ‘emotional’ symptoms, including depression or anxiety [2]. There are twenty amino acids of general occurrence i.e. these are usually found in all proteins. However plants, micro organisms and anti bodies excrete by organisms have continued to provide new amino acids of diverse structure [3].

Ion-exchange chromatography uses post-column derivatization in that the amino acids are separated by means of ion exchange, and derivatives are formed after they have emerged from the column so that they may be quantified. The most common derivatization procedure is that using ninhydrin with subsequent determination of optical density. In contrast, pre-column derivatization, as is used for gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC), uses columns to separate the amino acid derivatives. These derivatives, after emerging from the column, are then quantified by various detection devices. GLC and HPLC procedures are frequently more rapid than ion exchange procedures, but their major limitations often lie in the preparation of the derivatives rather than in the chromatography as such [4].

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MATERIALS AND METHOD

In the present study, six new variety, healthy and matured legume seeds of Pisum sativum, (Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6) under consideration are collected from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur and were studied for their Amino acid composition by FPLC(fast pressure liquid chromatography) Technique.

Principle and Method of Amino Acid Analysis-

Shimazu's High Performance Amino Acid Analysis System (LC-10A) is an HPLC (High Performance Liquid Chromatography) system including a high performance LC-10A mobile phase pump, where a unique post column derivatization detection unit is mounted on an ordinary separation unit. Like an ordinary amino acid analyzer, this system uses a column packed with the packed with a 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 are separated by means of a binary gradient eluting method using two liquids of standard solution and then, fed to detection unit for arginine, the column is automatically cleaned and equilibrated to be ready for the next analysis.

O-phthaladehyde (OPA) is 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 ($–NH_2$) group into a strongly fluorescent substance. This post column derivatization and fluorescence detection method enables selective detection of each amino acid at high sensitivity. Imino acid such as proline and hydroxyproline can also be detected by converting them into compounds with the $–NH_2$ group by adding a sodium hypochlorite solution before the OPA reaction. Preparation of the reagent for reaction becomes easier by using the odorless solid N-acetyl-L-cystein then in the case when foul-smelling β-marcaptoethanol or ethanethiol is used, and sensitivity to proline is increased.

Amino Acid Analysis Using Shimadzu FPLC (Fast Pressure Liquid Chromatography) System

The Shimadzu Fast Pressure Liquid Chromatograph "Amino Acids Analysis system" has a unique reaction detecting unit on Shimadzu FPLC system and enjoys the following features:

1) The system employs reaction detection method using OPA (o-phthalaldehyde) and has very good detecting sensitivity.
2) The system includes FPLC equipment as a main unit and can analyse not only free amino acids but also various hydrolysed amino acids.

Derivatizing Reagent: Derivatization done post- column with o-phthalaldehyde reagent.

Separation: Like ordinary Amino Acids Analyser, this system uses styrene–divinyl benzene copolymer with sulfonic acid, i.e, strongly acidic cation exchange resin, (sodium type) for separation.

Detection: In the presence of SH compound, OPA reacts with a compound having - NH_2 (such as primary Amino Acids) into strongly fluorescent substance. If sodium hypochlorite is added, imino acids such as proline also react with OPA. This system makes use of these reactions in detecting amino acids.

Flow rate - 0.3ml /min

Wavelength - 350-450nm

Injection volume - 20µl.

PREPARATION OF MOBILE PHASE

Chemical composition of mobile phase:

Name of Chemicals	Liquid A	Liquid B	Liquid C
Sodium Normality (N) pH	0.2 3.20	0.6 + 0 (about 0.1) 10.00	0.2 pH not adjusted
Sodium Citrate $2H_2O$ (g) Sodium Hydroxide (g) Boric Acid (g) Ethanol (99.5%) (ml) Perchloric Acid (60%) (ml) 4N NaOH solution (ml)	58.8 - - 210 50 -	58.8 - 12.4 - - 30ml	- 4 - - - -
Final Volume (l)	3	1	0.5

FILTRATION OF MOBILE PHASE:

Filter the prepared solutions A, B and C through 0.45 µm membrane filter care is taken so that the solutions do not mix with each other and that they are not contaminated with ammonia.

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PREPARATION OF REACTION REAGENT

- PREPARATION OF BUFFER

Chemical composition (Quantity in gms molar concentration)

Sodium Carbonate 122.2 g/ 0.384M

Boric Acid 40.7 g/ 0.216M

Potassium Sulphate 56.4 g/ 0.108M

Dissolve the above reagents in water to make 3 liter solution. Use this buffer without pH fine adjustment.

Preparation of 10% Brij-35 solution (Brij-35^{TM} stands for polyoxyethylene lauryl ether.)

Weigh out 10g of Brij to make 100 ml of solution. Put the bottle in warm water to facilitate dissolution of Brij-35.

PREPARATION OF REACTION SOLUTION A

Sodium hypochlorite solution (NaClO solution)

Weigh out 500ml of boric acid-carbonic acid buffer by a measuring cylinder and 0.2 ml of commercially available sodium hypochlorite aqueous (effective chlorine concentration around 7-10%) solution. Mix them thoroughly and filter through glass membrane filter No.3.

Note: Do not add Brij-35 solution to this solution.

PREPARATION OF REACTION SOLUTION B (OPA solution)

When 2-mercaptoethanol is used:

Chemical Composition

Name of Chemicals	Quantity
OPA Ethanol 2-mercaptoethanol 10% Brij-35 solution	(OPA 0.08%) 400 mg 7 ml 1 ml 2 ml Add buffer to make 500 ml of solution.

Chemical Composition

Name of Chemicals	For normal analysis	For measurement of sensitivity increase of proline
OPA	400 mg	800 mg
Ethanol	7 ml	14 ml
N-acetyl cysteine	500 mg	1000 mg
10% Brij-35 solution	2 ml	2 ml
	Add buffer to make 500 ml of solution	Add buffer of make 500 ml of solution

PREPARATION OF SAMPLE DILUENT

Sample diluent (0.2N Na^+ (sodium citrate) pH 2.20)

Weigh out 9.8g of sodium citrate. Add about 400 ml of distilled water to dissolve the sodium citrate. Then, add 8 ml of perchloric acid and 0.05ml of n-caprylic acid. Add distilled water to make 500ml solution. Then, adjust the pH of the solution of 2.20 by adding perchloric acid.

Preparation of standard Amino acid Solution

Rinse the measuring flask (10ml) with sample diluent (Citrate buffer of 2.2 pH). Put 400µl of b-type mixed amino acid solution in the measuring flask and add sample diluent to make 10ml solution. The concentration of the amino acids in the mixed amino acids solution may differ by some degree. Mostly, however, their concentration in undiluted solution is 2.5mol/ml. Since the mixed amino acids solution is diluted 25 times, concentration of each amino acid in the resultant standard solution is 0.1 mmol/ml. Therefore, 10ml of standard sample solution contain 1 n mol (1x 10^{-9}) of each amino acid. The samples were subjected to alkali hydrolysis followed by acid hydrolysis and analysed.

Samples were subjected to alkali hydrolysis by adding 4.83 g Barium hydroxide and 5 ml of boiling water to 500 mg of sample. The mixture was evacuated and then heated at 120° C for 8 hour's. After hydrolysis, the pH was adjusted to 3 with HCl filtered and diluted to 25 ml with HPLC grade distilled water. One ml of sample was vacuum dried using flash evaporator and finally dissolved in citrate buffer (0.1 M; pH 2.2).

Acid hydrolysis is carried out with 6 N HCl at 110° C for 18-22 hours in evacuated and sealed tubes. The hydrolysate was filtered and diluted to 250 ml. 1 ml sample was vacuum evaporated at 40°C until dryness. The content was dissolved in citrate buffer (0.1 M; pH 2.2) [5, 6].

Table: Amino acid composition of new variety seeds of Pisum sativum (Gm /100 gm seed sample)

AMINO ACIDS	SEED VARIETIES					
	Arkel	**Pusa Pragati**	**IPF-99-25**	**JP-885**	**MM-15**	**JM-6**
Aspartic acid (g)	2.6831	1.7566	2.060	2.4933	2.4657	2.410
Threonine (g,e)	0.9171	1.1940	1.1744	0.7042	1.006	0.9050
Serine (g)	0.5574	0.5725	0.7094	0.9889	0.6991	0.866
Glutamic acid (g)	3.6638	4.4491	3.0132	0.3507	3.5741	3.2866
Proline (g)	0.8967	1.1831	0.7183	0.9291	1.0968	0.5937
Glysine (g)	0.9402	0.8902	1.0353	0.6739	0.8101	0.9285
Alanine (g)	0.8399	0.5253	0.5459	0.6925	1.0738	0.9202
Cysteine (g)	0.2609	0.2090	0.8547	0.1668	1.1231	0.1665
Valine (g,e)	1.0078	0.9477	0.1754	0.9901	1.4209	0.9450
Metheonine (g,e)	0.2129	0.3053	0.7362	0.1844	0.2513	0.2351
Isoluecine (g,e)	0.9867	0.7355	1.213	0.9842	0.9036	1.0045
Leucine (g,e)	1.5619	1.4994	0.5194	1.3029	0.7844	1.0942
Tyrosine (g)	0.6121	0.3720	0.7396	0.6411	0.7248	0.6266
Phenylalanine(g,e)	1.0594	4.8719	0.0688	0.9294	0.8650	0.9195
Histidine (g,e)	0.4964	1.5785	0.8390	0.8158	0.2970	0.1670
Lysine (g,e)	1.7337	0.6778	0.1440	0.9631	1.4065	1.3548
Arginine (g,e)	2.2022	1.7491	1.9951	1.4164	2.0324	1.8329
Tryoptophan (g,e)	0.1813	0.1582	0.1496	0.2014	0.1861	0.2118

g: General amino acids

e: Essential amino acids

RESULT AND DISCUSSION

The nutritional quality of protein depends upon the total amount of amino acids present in the protein, the 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[11.] . The amino acids composition of different varieties of pisum sativum are mentioned in tabular form in the table mentioned above and the graphs obtained are also shown in graph I to IV.

Aspartic acid was found max in Arkel with 2.68 gm, Threonine is max in Pusa pragati and IPF-99-25 with 1.1940 gm and 1.1744gm, Serine was found max in JP-885 with 0.9889gm Glutamic acid is found high in pusa pragati with 4.4491 gm, Proline was max in pusa pragati with 1.1831gm , Glysine was max in IPF-99-25 with 1.0353gm, Alanine was max in MM 15 and JM 6 with 1.0738 gm and 0.9285 gm , cystein was found max in MM 15 with 1.123 gm and IPF 99-25 with 0.8547 gm and found least in JP 885 with 0.1668 gm , Valine was found max in MM 15 with 1.4209 gm and found least in IPF 99-25 with 0.1754 gm , Methionine is high in IPF 99-25 with 0.7362 gm and found least in JP 885 with 0.1844 gm, Isoleucine is max in IPF 99-25 with 1.213 gm and JM 6 with 1.0045 gm and found least in pusa pragati with 0.7355 gm , Leucine was found max in Arkel and pusa pragati with 1.5619 gm and 1.499 gm and found least in IPF – 99-25 with 0.5194 gm, Tyrosine is found max in IPF-99-25l and MM 15 with 0.7396 gm and 0.7248 gm and found least in Pusa pragati with 0.3723 gm, Phenyl alanine was found max with 4.8719 gm in Pusa pragati and found least in IPF 99-25 with 0.0685 gm, Histidine was max in pusa pragati with 1.5785 gm and found least in JM 6 with 0.1670 gm , Lysine was max in Arkel with 1.7337 gm and found least in IPF 99-25 with0.144 g, Arginine was max in Arkel with 2.2022 gm and found least in JM-6 with 1.8329 gm, Tryptophan was max in JM 6 with 0.2118 gm. The above values indicated are in gm/100gm of seed sample.

Several investigators have also been studied the amino acid composition in some leguminous seeds [7-20]. From the above analysed data all the varieties of the pea samples contain good quantities of essential amino acids, suitable for good nutritional supplementation. JP-885 contains considerable amounts of amino acids but have lesser quantities of essential amino acids compared with the other varieties of pea samples. Hence the selection and understanding of composition in seeds for the food products gives a good idea for nutritional supplementation.

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