ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Amino Acid Composition of *Phaseolus mungo* (Black Gram) and *Labeo rohita* (Rohu) In the Perspective of Human Dietary Requirements.

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ABSTRACT

The present study is aimed at investigating the amino acid profiles of germinating black gram (*Phaseolus mungo*) seeds and the cultured fresh water carp (*Labeo rohita*) of the local ponds to evaluate their nutritional status in respect of human dietary requirements. The levels of 15 amino acids (free and protein hydrolysate) were estimated in the two experimental materials by HPLC analysis and relative percentages of amino acids and A/E ratios were calculated. The results indicate that the concentrations of essential amino acids are significantly higher in plant source as compared to fish muscle. Total amino acid content of *L. rohita* muscle tissue was found to be 1,248 nmoles/ml while that in *P. mungo* with coat is 1457.9 µmoles/ml and the pulse without coat had 540.6 µmoles/ml. The percentage of the total essential amino acid content in the chosen fish muscle is 28.42 and that in the pulse with coat and without coat are 31.82 and 37.96 respectively. Phenylalanine content is significantly lower in both the food items and hence may be included in the diet of hyperphenylalaninemia patients with dietary restrictions of phenylalanine intake. The significantly ($P < 0.05$) higher total amino acid and essential amino acid contents in the pulse with coat than that in the pulse without coat is suggestive of the fact that the pulse with coat is preferred for dietary consumption.

Keywords: Total amino acids, Black gram (*Phaseolus mungo*), Edible carp (*Labeo rohita*), HPLC analysis, Relative percentage, A/E ratio percentage.

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INTRODUCTION

Proteins are important for growth and development of the body, maintenance and repairing of worn out tissues and for production of enzymes and hormones required for many body processes. Sources of proteins in human diet could be of plant and/or animal origin. Plant proteins provide nearly 65% of the world supply of proteins for humans with 45%-50% and 10%-15% from cereals and legumes or vegetables respectively [1]. Importance of plant proteins in the average diet varies from the least developed regions (where animal proteins are scarce and poverty precludes the consumption of meat) to the highly developed regions (where animal production is particularly abundant). Most common and affordable animal protein source are fish (other than milk) which contain a good quantity of protein (about 18-20%) including eight essential amino acids like the sulphur containing methionine, cysteine and basic amino acid lysine. Nonetheless, there is now an expanding consumption of protein foods of legume and vegetable origin throughout the world. Among the plant species, grain legumes are considered as the major source of dietary proteins. Protein quality in leguminous seeds does not however reach the same level as in animal products. This is due to various factors, among them the well known are the unbalanced amino acid composition, the low true digestibility of protein and the presence of anitinutritional factors in the seeds [2,3]**.** The major proteins in legume seeds are the globulins which usually account for about 70% of the total protein. Glutelins (10 to 20%) and albumins (10 to 20%) make up the remainder. The principal storage globulins in most legumes are legumin and vicilin, the latter predominating in common bean [4]. Amino acids of pulses, particularly soya, are also found to mitigate cataractogenesis and improve insulin receptor tyrosine kinase activity. The present study is aimed at an evaluation of the plant protein source Vs. animal protein source in terms of total amino acid content and find out if any deficiency would result from the consumption of only plant proteins.

MATERIALS AND METHODS

Processing of experimental material

1 gm of black gram seeds with coat and without coat were separately washed in water and were soaked in water for 8 hrs. Then they were kept for germination for 24 hrs. After the period of germination, the black gram seeds were weighed to get the wet weight. The germinated seeds were then mashed in pestle and mortar with 8 ml of phosphate buffer pH 7.0.

1 gm of muscle tissue was taken from the cultured fish, procured from local fish pond and acclimated to the laboratory for one week, and was mashed in a homogenizer with 8 ml of phosphate buffer pH 7.0.

All the samples were then centrifuged at 3000 rpm for 20 minutes at 4^0 C. The supernatant was stored at 4^0 C while the deposit was dissolved in 5 ml of 1N NaOH and centrifuged at 3500 rpm for 20 minutes at 4^0 C. The supernatant was collected and stored at 4^0 C while the deposit was subjected to protein hydrolysis by dissolving in 6 ml of 6N HCl and keeping in incubator shaker at a temperature of 60^0 C for a period of 24 hrs. Then the tubes were centrifuged at 3500 rpm for 15 minutes. The supernatant was filtered and neutralized with 1N NaOH. Finally the filterate was pooled with other supernatants collected during the earlier steps and was analysed for aminoacid content by HPLC.

Pre-column OPA (orthopthalaldehyde) derivatization

Was done for HPLC (High Performance Liquid Chromatography) analysis of amino acids [5]. The Principle is based on the reaction of primary amines with OPA in the presence of mercapto ethanol to form 1-thio substituted 2-alkyl isoindoles. The advantage of the derivatization is that they involve a rapid reaction and high sensitivity and the isoindoles so obtained are well suited for HPLC separation. Mobile phase A is 20mM sodium acetate with 0.018% triethylamine. Mobile phase B consists of 20% of 100 mM sodium acetate, 40% methanol and 40% acetonitrile. pH of both the mobile phases A and B was adjusted to pH 7.2±0.05 with 2% acetic acid. OPA and standard amino acids' mixture of different concentrations (1nm, 250 pm, 100 pm, 25pm and 10 pm) provided by Hewlett Packard were used as standards. The instrument Agilent 1100 HP – HPLC and chemstation software were used in the present study.

First the instrument was calibrated using 1.0 nm, 500 pm and 250 pm standards individually. 10 µl of the standard was mixed with 60 µl borate buffer and 10 µl of OPA reagent in dilution vial and cyclomixed. From this mixture, 50 µl was injected in the HPLC using Hamilton syringes. Each standard is individually run in the gradient program and the chromatogram obtained. The two consecutive runs that had the same retention time were taken and the average of them was used for plotting the calibration curve. Then 50 µl of the sample mixture was injected in HPLC using Hamilton syringes and from the chromatogram obtained, the area of the peaks was recorded, calibrated along with the standards and used for calculation. Reaction temperature was maintained at 40^0 C, the flow rate of the sample was kept at 0.5 ml/min and the detection wavelength (VWD) was 338 nm. From the values obtained, relative percentages of essential amino acids was calculated using the formula, Quantity of essential/Highest quantity of essential X 100. Also, the A/E ratio (the concentration of each essential amino acid as a percentage of the concentration of total essential amino acids) excluding tyrosine was calculated unlike some studies in which tyrosine was also included.

RESULTS

The amino acids estimated by HPLC analysis include both free amino acids and the amino acids obtained by the acid digestion of the pelleted protein. The chromatograms are presented as Figures 1 to 5. The total concentration of the estimated 15 amino acids is 1,248 nmoles/ml, 1457.9 µmoles/ml and 540.6 µmoles/ml in *Labeo* muscle tissue, germinating black gram seeds with coat and without coat respectively. Total essential amino acid concentrations were 254.9 nmoles/ml, 463.9 µmoles/ml and 205.2 µmoles/ml in *Labeo* muscle tissue, germinating black gram seeds with coat and without coat respectively (Table 1). Relative percentages of all the essential amino acids are extremely low in *L. rohita* muscle tissue while the germinating seeds of *P. mungo* (with coat) had the highest amounts relative to the seeds without coat. Further, the results indicate that important amino acids like Threonine, Isoleucine, Leucine and Lysine are less than 50% in seeds without coat (Table 2). The A/E ratio percentages indicate that in Labeo muscle tissue, amino acids Histidine, Arginine, Phenylalanine and Lysine are low. Histidine, Arginine, Valine, Methionine, Phenylalanine and Lysine are relatively high in seeds without coat suggesting that the loss of these amino acids does not occur with the removal of coat protein(Table 3). However, other important essential amino acids viz. Threonine, Isoleucine and Leucine are in significantly good amounts in black gram seeds with coat. The aromatic amino acid phenylalanine content is significantly low in all the samples under study.

Table 1: Concentrations of the estimated fifteen amino acids present in the muscle tissue of the fresh water fish *Labeo rohita* and the germinating seeds of *Phaseolus mungo* (with and without coat) as analysed High Performance Liquid Chromatography (HPLC).

S. No.	Name of the amino acid	Concentration of amino acid		
		Muscle of *L. rohita* (nmoles/ml)	*Germinating seeds of P. mungo* (µmoles/ml)	
			With coat	Without coat
1	Aspartic acid	21.5	141.2	58.8
2	Glutamic acid	97.2	201.2	61.3
3	Serine	160.1	160.3	61.2
4	*Histidine	6.3	42.8	39.0
5	Glycine	361	270.8	100.9
6	*Threonine	42.8	124.6	27.1
7	Alanine	342.2	171.6	35.4
8	*Arginine	26.9	57.1	36.5
9	Tyrosine	11.1	48.9	17.8
10	Valine	67.3	22.1	18.5
11	*Methionine	20.1	18.2	11.3
12	*Phenyl alanine	6.2	33.2	30.3
13	*Isoleucine	16.6	42.2	6.0
14	*Leucine	32.4	49.2	2.8
15	*Lysine	36.3	74.5	33.7

*Essential amino acids

Note: The amino acid levels represented here for the germinating seeds, with and without coat, represent the sum of the free amino acids and the amino acids obtained by mild acid hydrolysis of the proteins.

ISSN: 0975-8585

Table 2: Relative Percentages of Essential Amino acids in the muscle tissue of the fish *L. rohita* and the germinating seeds of *P. mungo* (with coat and without coat). It is calculated as Quantity of essential amino acid/Highest quantity of essential amino acid X 100 after calculating the concentrations in µmoles/ml.

S. No.	Name of the essential amino acid	Relative percentages of the amino acids (%)		
		L. rohita muscle	*P. mungo* Germinating seeds	
			With coat	Without coat
1	Histidine	0.015	100	91.12
2	Threonine	0.034	100	21.75
3	Arginine	0.047	100	63.92
4	Valine	0.304	100	83.71
5	Methionine	0.110	100	62.09
6	Phenyl alanine	0.019	100	91.26
7	Isoleucine	0.039	100	14.22
8	Leucine	0.066	100	5.69
9	Lysine	0.049	100	45.23

Table 3: A/E ratio percentage of the essential amino acids in the muscle tissue of *L. rohita* and germinating seeds of *P. mungo* (with and without coat). It has been calculated as
A/E ratio Percentage = Quantity of essential amino acid/Total quantity of essential amino acids X 100.

S. No.	Name of the essential amino acid	A/E Ratio Percentage of the Amino Acid (%)		
		L. rohita Muscle	*P. mungo* Germinating seeds	
			with coat	without coat
1	Histidine	2.47	9.23	19.0
2	Threonine	16.79	26.86	13.21
3	Arginine	10.55	12.31	17.79
4	Valine	26.4	4.76	9.01
5	Methionine	7.88	3.92	5.51
6	Phenyl alanine	2.43	7.16	14.77
7	Isoleucine	6.51	9.10	2.92
8	Leucine	12.71	10.6	1.36
9	Lysine	14.24	16.06	16.42

Figure 1: Chromatogram of the Free Amino acids of the germinating black gram seeds with coat, analyzed by HPLC.

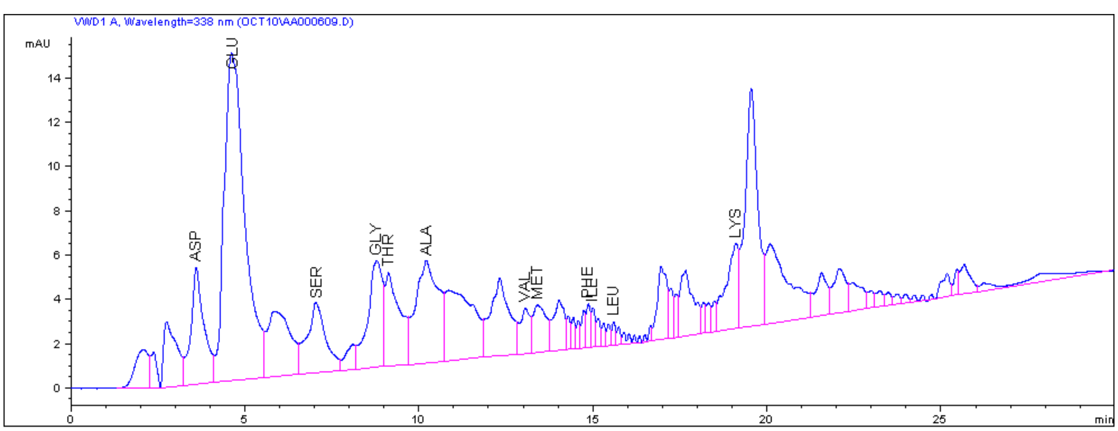

Figure 2: Chromatogram of the proteic amino acids of the germinating black gram seeds with coat, analyzed by HPLC

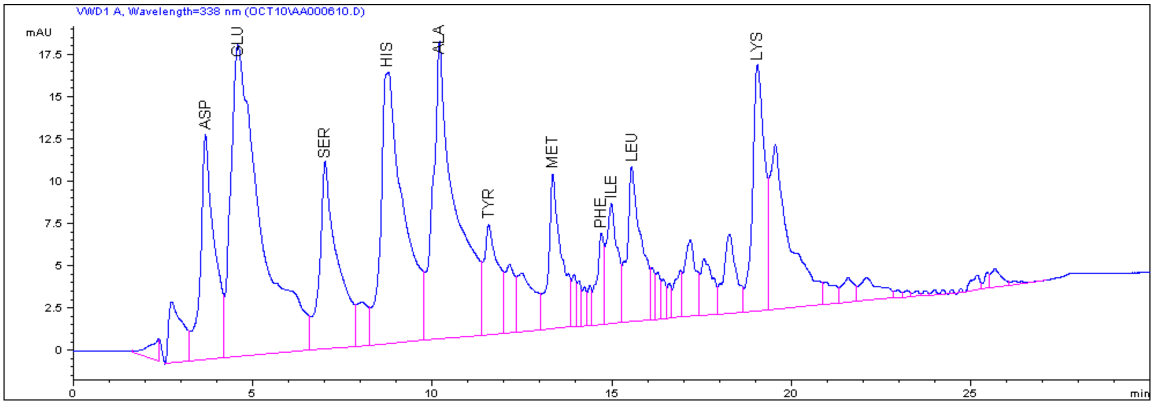

Figure 3: Chromatogram of the Free Amino acids of the germinating black gram seeds without coat, analyzed by HPLC.

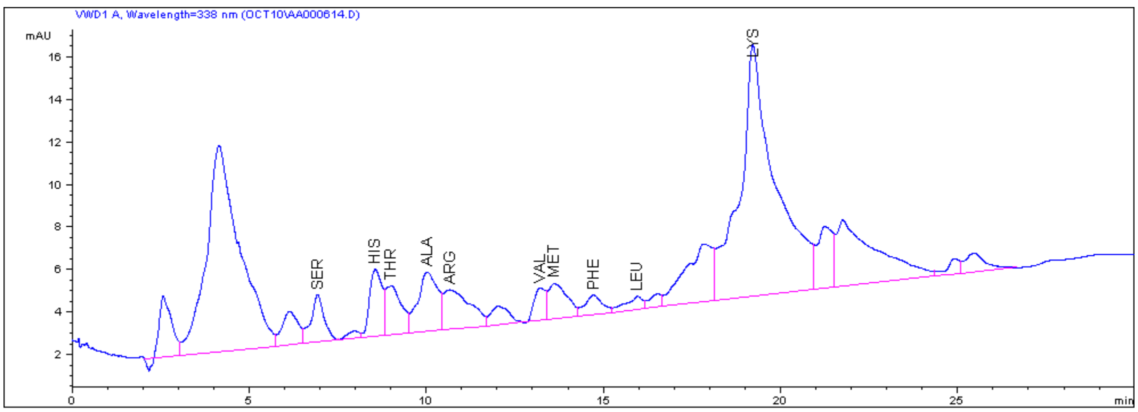

Figure 4: Chromatogram of the proteic amino acids of the germinating black gram seeds without Coat, analyzed by HPLC.

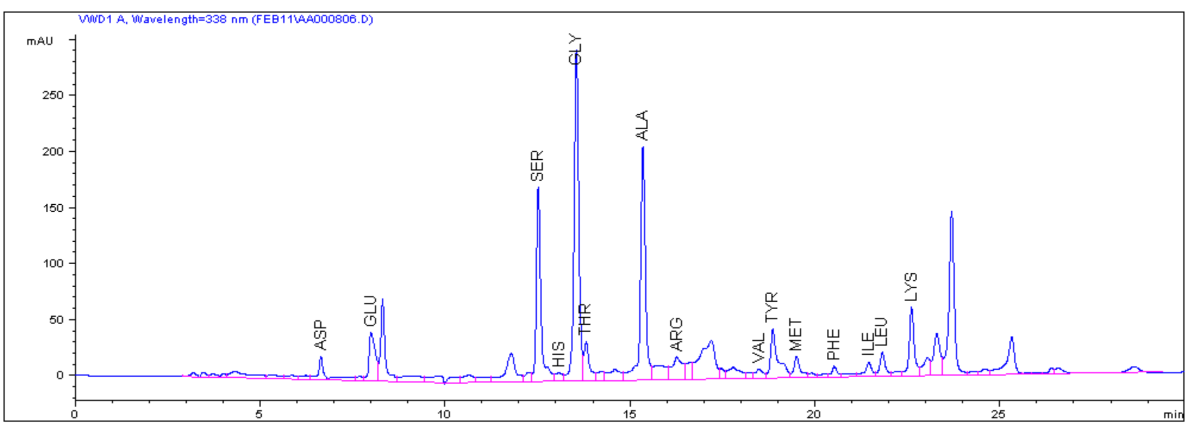

Figure 5: Chromatogram of the total amino acids of the muscle tissue of *Labeo rohita*, analyzed by HPLC

DISCUSSION

Most carbohydrate based diets lack the essential amino acids in either number or quantity and therefore people of poor and developing countries depend on fish consumption. It has been understood that low protein/carbohydrate rich diets like cassava or maize could be complemented by fish. Amino acid profiles of proteins in leguminous seeds are unbalanced when compared to egg protein, the indispensable sulphur-containing amino acids are at a much lower concentration[1]. Sulphur containing amino acids i.e. methionine and cystine are considered as the most critical limiting components of the proteins. In addition some antinutritional factors, mainly proteinase inhibitors have shown a relatively fair content in cystine and methionine. For example, in common beans between 30% and 40% of the total seed cystine has been calculated to be present in these inhibitors[3]. If the nutritional quality of the cultivated legume is poor, then appropriate measures to improve the quality could be taken like proper heat treatment/cooking process/mixture with other dietary foods etc [6].

From this study, relative percentages of methionine are found to be significantly high in germinating seeds of black gram with coat and without coat as well, in comparison with the rohu muscle tissue. But we have not made any attempt to know the sulphur containing amino acid levels of antinutritional factors that could be present in the seeds of black gram.

It has been reported that the various legume protein sources may differ significantly in the amino acid composition. Compared to soya bean protein, the lysine content in pulses tends to be higher and the content of sulphur-containing amino acids (methionine and cystine), and tryptophan tends to be lower [3,4]. Globulins and Albumins, the two protein fractions of the seeds differ in their amino acid composition like sulphur aminoacids and other essential aminoacids like lysine are more in albumins than globulins. The present analysis indicates good quantities of lysine in the germinating seeds with coat, although significantly less in without coat seeds.

Pulses have significant nutritional and health advantages for consumers [7]. They are the most important dietary predictor of survival in older people of different ethnicities [8] and the legume consumption was highly correlated with a reduced mortality from coronary heart disease [9] as well as immunostimulatory activity in rats [10]. It has been concluded from the research studies presented at a 2-day National level conference held by Nutrition Society of India at NIN, Hyderabad, AP, India, that nutritional levels among vegetarians were better as they consume a better mix of nutrients than their non-vegetarian peers. With the result, the performance efficiency would be better, as noted in the families of migrant workers under study, than their non-vegetarian peers. The deliberations made at the conference pointed out the facts like pulses, cereals and milk make a wholesome diet for children; vegetarian foods provide the complete set of nutrients; lysine deficiency in cereals like wheat and rice could be overcome by consuming pulses and milk. These findings corroborate with the results obtained in the present study of a comparison between a legume/pulse and the fish. However, very low quantities of Threonine, Isoleucine, Leucine and Lysine present in plant protein without coat suggest the consumption of the whole grain rather than decoated whole grain.

It is ascertained from the study that regular intake of pulses in the diet would be adequate enough even for growing children and ageing adults with greater protein turnover. Also, the lower market price of pulses make them affordable as compared to the carps like *Labeo rohita* which stand on the higher cost and hence on lesser affordability side by the common man. Other significant finding of the study is that the A/E ratio is low for phenyl alanine in *L .rohita* muscle as well as germinating seeds of *P. mungo*. Generally, PKU patients are put on restrictive protein diet depending upon their blood levels of phenylalanine. But both black gram and the carp rohu could be included in their diet owing to their low phenylalanine levels and good quantities of other amino acids.

CONCLUSIONS

Significantly higher levels of essential and non-essential amino acids in the black gram (P. mungo) seeds suggest that the pulse should be included in the diet regularly to avoid deficiencies and promote good health. Although fish are known to be protein rich, the present study indicates that the levels of amino acids especially essential amino acids are significantly low as compared to pulses. Hence fish consumers too should include adequate quantities of a mixture of legumes in their regular diet for better health.

ACKNOWLEDGEMENTS

The authors sincerely thank the Department of Biochemistry, Sankara Nethralaya, Chennai for their kind technical support in carrying out HPLC analysis. The first author also expresses indebtedness to the management and the Principal of RVR & JC College of Engineering for providing laboratory facilities.

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