



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(1): 1003-1006

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Received: 15-11-2018

Accepted: 18-12-2018

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## A comparative study of the amino acid composition of protein isolates prepared from adzuki bean genotypes [*Vigna angularis* (Willd.) Ohwi & Ohashi]

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**Abstract**

The basic purpose of the study was to compare the amino acid composition, and SDS PAGE characterization of the isolates prepared from three genotypes of rice bean *viz.* EC-340264, Local *Totru* and HPU-51 by using isoelectric precipitation technique. The amino acid analysis indicated that protein isolates were found to have maximum amount of essential amino acids *viz.* histidine in Local *Totru*, methionine in EC-340264, tryptophan in Local *Totru* with values of 1.99, 1.75 and 1.73 g/100g of protein respectively. The non-essential amino acids like serine, arginine and alanine were highest in the protein isolates prepared from genotype EC340264 with values 5.15, 1.76 and 4.75 g/100g of proteins respectively. The isolates were also characterized using the SDS PAGE characterization, revealing the fact that banding pattern of protein isolates showed total 4 bands ranging from 17.0 kDa to 56.23 kD.

**Keywords:** Adzuki bean, amino acid composition

**Introduction**

Legumes have been considered as a cheap and easily available source of high quality protein products such as flour, concentrates and isolates. However, soybean protein is the only one most exploited legume for the industrial purposes and also pea protein to very less extent may be due to the lack of the information regarding functional properties of the other legumes. Traditionally in Japan Adzuki bean is used in preparation of Japanese confections like wagashi, youkan, manju and amanatto. The adzuki bean was also used in the treatment of the diuretic functions and other disease as dropsy and beriberi given in Chinese medicine (Gohara *et al.* 2016) [7]. Adzuki beans is rich source of soluble fibre, also contain folate, potassium, magnesium all of which are good for hearthealth and due to high insoluble fiber content it can be helpful in the problem of constipation and decrease the risk of colon cancer. In east Asian cuisine, adzuki bean is eaten sweetened. Adzuki seeds comprises of 25 per cent protein (on a dry basis), about half of the total protein is globulin (Meng *et al.*, 2002) [2, 3]. Legumin is the second most abundant protein of adzuki bean, which comprises of two types of subunits, acidic and basic with molecular mass of 40 and 20 kDa, respectively (Meng and Ma 2002) [2, 3]. Protein isolates are the most purified form of protein products containing the major concentration of protein but does not contain any dietary fiber like flour and concentrates. Basically isolates were originated from United State around 1950s (Jay and Michael, 2004) [1]. They are easily digestible and incorporated into different food products. Recently, protein isolates have played a major role in the development of new class of formulated foods. Higher concentration of protein with better colour, flavour and functional properties makes it an ideal raw ingredient for use in beverages, infant foods and children milk food, textured protein products and certain types of specialty foods (Olaofe *et al.*, 1998) [4]. The available literature lacks the information on the amino acid composition of different genotypes of adzuki beans. In the given paper efforts were made to study and explore the amino acid composition of protein isolates prepared from three different genotypes of adzuki bean.

**Materials and Methods**

In the present study, protein isolates were prepared from the three genotypes of adzuki beans using isoelectric precipitation.

The adzuki bean seeds were procured from the Department of Organic Agriculture, College of Agriculture, CSK Himachal Pradesh Agricultural University, Palampur. All the isolates were prepared using isoelectric precipitation. The isoelectric precipitated protein fraction (protein isolate) was prepared by using the method of McEwen *et al.* (1974). Adzuki bean flour was stirred for two hours in dilute (0.01N) NaOH solution at a ratio of 1: 10 (w/v). The solubilized protein was separated from the residue by centrifugation for 2 min at 3000 rpm. The residue was washed with double distilled water, again centrifuged and the supernatants were combined. The majority of the protein was then recovered from the supernatant by slowly adjusting the pH to 4.0 with 1 N HCL, after which the protein containing solution was again centrifuged at 10,000 rpm, the precipitates were collected and combined, washed once with the water, stored at -80° C overnight for freezing, then freeze dried in a freeze drier and packed in an air tight container for further analysis. The isolates of the adzuki beans were analysed for the amino acids using High- Speed Amino Acid Analysis (AAA) on 1.8µm Reversed-Phase (RP) columns by the method of Henderson (2007) [10]. Electrophoresis was carried out in the discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (1970) [9] using 12 per cent (w/v) and 5 per cent (w/v) stacking gel. Comparative protein profile of the three genotypes of the adzuki beans was observed by the SDS PAGE. The electrophoresis was carried out using vertical mini biorad SDS PAGE assembly with 12 per cent polyacrylamide gel. The molecular weight of the dissociated proteins was estimated by using pre-stained protein ladder of Mol Bio TM HIMEDIA MBT092 ranging from 11- 245 kDa which was used as a standard. A 12 per cent resolving gel (30% acrylamide stock solution (Merck), 1.5 M Tris-HCl stock solution (pH 8.8), 10per cent SDS, distilled water, TEMED, 10per cent ammonium persulphate), 5per cent stacking gel (30% acrylamide stock solution, 0.5 M Tris-HCl, 10% SDS PAGE, distilled water, TEMED, 10% ammonium persulphate) were prepared and polymerized, chemically running gel solution containing 10 µl N<sup>+</sup>, N<sup>+</sup>, N<sup>-</sup>, N<sup>-</sup> tetramethylenediamine (Merck), and 6 µl to the stacking gel solutions. A marker of known molecular weight pre-stained protein ladder of MolBioTM HIMEDIA MBT092 ranging 11- 245 kDa was also loaded along with the samples. The apparatus was connected with constant current (30 mA) till the bromophenol blue (BPB) reached the bottom of the plate. Later the gel was stained and destained and the molecular weights of the proteins were determined using a standard protein marker.

## Results and Discussion

### Amino acid composition of the adzuki bean protein isolates:

Assessing the quality of the protein is important when considering the nutritional benefit that it can provide. Protein quality is the ability of food to meet the nutritional requirement of the individual species. The quality of protein can be determined by assessing its essential amino acid profile. Essential amino acid composition of the protein isolates given in Table 1 showed that the histidine content was highest in the Local *Totru* (4.39 g/100g) genotype. While, aromatic amino acids (phenylalanine+tyrosine) were recorded highest in the HPU-51 (11.51mg/100g). Lysine content was reported highest in EC-340264 (3.70g/100g). Methionine content was found to be highest in HPU-51 (0.87g/100g). The data revealed that the

tryptophan content was maximum in HPU-51 (11.46g/100g) whereas, isoleucine content was recorded highest in EC-340264 80 (0.32mg/100g). Valine and leucine contents were recorded highest in the Local *Totru* (3.60 and 4.30g/100g respectively). The non-essential amino acids like aspartic acid, glycine, glutamic acid, serine, threonine, arginine, alanine were also analysed and the data is presented in the Table 1. The data revealed that the highest amount of aspartic acid content was present in HPU-51 (9.79g/100g), whereas, glycine content was recorded highest in EC-340264 (4.96g/100g). Similarly, the glutamic acid was highest in EC-340264 (8.22g/100g) and serine content was highest in HPU-51 (2.15g/100g). Threonine content was recorded highest in HPU-51 (5.51g/100g) and arginine in EC-340264 (13.10 g/100g). Alanine content was assessed highest in the EC-340264 (1.36g/100g). The results also revealed that values for essential amino acid i.e. histidine was more in comparison to the required amino acid pattern in the adzuki bean protein isolate except the EC-340264 genotype. Similarly, tryptophan was deficient in Local *Totru* while it was quite high in other two genotypes. ARM was more in all the three genotypes of the adzuki bean in comparison to the reference FAO/WHO amino acid scoring pattern. However, isoleucine, methionine, lysine, valine and leucine were less fulfilling in comparison to the FAO/UNU/WHO reference scoring pattern for the essential amino acid of preschool children. The statistical evaluation revealed that the arginine and tryptophan had the highest variability within the different genotypes with respect to their concentration. Qayyum (2012) [5] while conducting a study on protein isolates from different legumes like chickpea, lentil, broad bean and kidney bean reported histidine in the range of 1.01 to 1.49 g/100g and sulfur containing amino acids in the range 0.99 to 2.06 g/100g. The values of amino acids in various legumes *viz.* chickpea protein isolate, lentil protein isolate, broad bean protein isolates, kidney bean protein isolates, within which essential amino acid like ARM, histidine, isoleucine, leucine, lysine, SAA, threonine, tryptophan, and valine were in the range of 3.58 to 6.64, 1.01 to 1.49, 1.68 to 3.99, 4.65 to 7.52, 3.51 to 4.54, 0.99 to 2.06, 1.61 to 2.17, 0.44 to 0.79, 1.44 to 2.08 g/100g respectively. For non-essential amino acids, the values reported for alanine, aspartic acid, glutamic acid, glycine, serine were 2.75 to 3.78, 10.59 to 17.76, 17.33 to 24.83, 2.40 to 2.74, 4.33 to 5.15 g/100g respectively. The results of the present study are in conformation to those reported in the study.

### Amino acid score for adzuki bean protein isolate

The amino acid scores of adzuki bean protein isolates have been presented in Table 2. The nutritional efficiency of the test protein is evaluated by its potential to meet human amino acid requirements. Amino acid score provides complete picture regarding the presence of individual indispensable amino acids in test protein compared to reference pattern. Protein requirement is at maximum during infancy to satisfy the growing needs for both metabolic activity and protein deposition (Qayyum, 2012) [5]. Amino acid score of adzuki bean isolate was established with the required reference pattern of pre-school children.

The protein isolate from genotype Local *Totru* had highest amino acid score for histidine (286.90) followed by HPU-51 (255.00) and EC-340264 (17.78). Isoleucine content was recorded highest in Local *Totru* (10.33) followed by in EC-340264 (10.32) and the least amount was recorded in HPU-51 (71.43). Amino acid score for lysine was obtained highest in

EC-340264 (71.16) followed by Local *Totru* (57.50) and the least amino acid for lysine was obtained for HPU-51 (53.7). Scoring for the total aromatic amino acids (ARM) revealed that the highest scoring was recorded for Local *Totru* (323.00) followed by EC-340264 (252.39) and lowest scoring was obtained by HPU-51 (210.65). Scoring for threonine content in adzuki bean protein isolate was recorded highest in Local *Totru* followed by HPU-51 and the least in the EC-340264 (181.08) followed by HPU-51 (66.22) and lowest in Local *Totru* (75.38). Valine scoring was highest in the Local *Totru* followed by HPU-51 and atleast in EC-340264 with 90.00,

85.71 and 58.57 values respectively.

The first limiting amino acid of legume i.e. methionine scored highest in HPU-51, followed by EC-340264 and Local *Totru* 33.46, 8.08 and 7.69 respectively. Overall HPU-51 had slightly better amino acid score as compared to the Local *Totru* and EC-340264. Whereas, the results also revealed that sulphur containing amino acid (SAA) methionine is the first limiting amino acid in the Local *Totru* and EC-340264. While isoleucine was the first limiting amino acid in the HPU-51 with protein scores of 8.08, 7.69 and not detected respectively.

**Table 1:** Amino acid profile of adzuki bean protein isolates

S. No.	Amino acid	Fao/Who Amino acid scoring pattern	Genotypes (g/100g protein)			Mean±SD
			EC-340264	HPU-51	Local <i>Totru</i>	
1.	Amino acid		6.28	9.79	9.07	1.85
2.	Histidine	1.8	0.32	3.38	4.59	2.20
3.	Glycine	-	4.96	3.20	3.64	0.92
4.	ARM*	4.6	11.61	14.69	9.69	2.52
5.	Lysine	5.2	3.70	3.40	2.76	0.48
6.	Methionine	2.6	0.21	0.87	0.20	0.38
7.	Tryptophan	0.74	1.34	11.46	0.49	6.10
8.	Iso-leucine	3.1	0.32	0.17	0.31	0.08
9.	Glutamic acid	-	8.22	8.15	6.38	1.04
10.	Serine	-	1.34	2.15	1.63	0.41
11.	Threonine	-	3.52	5.51	3.87	1.06
12.	Arginine	-	13.10	1.21	1.37	6.82
13.	Alanine	-	1.36	1.12	1.09	0.15
14.	Valine	4.2	2.46	2.42	3.60	0.67
15.	Leucine	6.3	4.53	ND	4.50	2.61

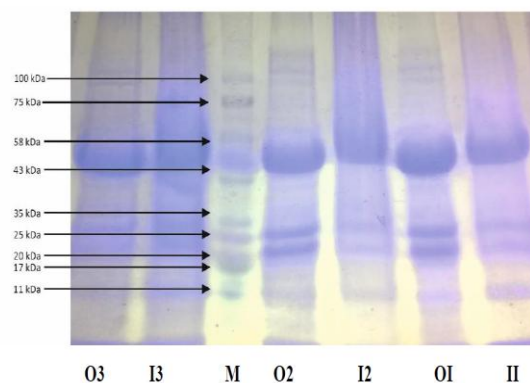
\*ARM(Aromatic amino acids)

**Table 2:** Amino acid score for adzuki bean protein isolate

Amino acid	EC-340264	HPU-51	Local <i>Totru</i>
Histidine	17.78	255.00	286.90
Isoleucine	10.32	10.00	10.33
Leucine	71.90	71.43	75.00
Lysine	71.16	53.07	57.50
*ARM	252.39	210.65	323.00
Threonine	130.37	143.33	154.80
Tryptophan	181.08	66.22	75.38
Valine	58.57	85.71	90.00
Methionine	8.08	33.46	7.69
**LAA	Methionine	Isoleucine	Methionine

\*\*LAA (Limiting amino acids)

\*ARM(Aromatic amino acids)



**Plate -2 SDS PAGE of whole seed protein and protein isolates of three genotypes of adzuki bean**

M = Protein Marker  
 O 1 = EC - 340264 (Whole protein), I 1 = EC - 340264 (Protein Isolate)  
 O 2 = HPU-51 (Whole protein), I 2 = HPU-51 (Protein Isolate)  
 O 3 = Local *totru* (Whole protein), I 3 = Local *totru* (Protein Isolate)

### Molecular weights (kDa) of protein bands in protein isolates of three varieties of adzuki beans by SDS-PAGE

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is one of the most widely used technology for its validity and simplicity for describing protein profiling and genetic structure of crop germplasm (Gept, 1990) [6]. The banding pattern of the protein isolates prepared from three varieties of adzuki bean by SDS-PAGE is depicted in Plate-1. The isolate protein profile showed remarkable variability. A minimum of four protein bands were observed in isolates prepared from different genotypes of adzuki bean. The molecular weight of the proteins were calculated on the basis of relatively mobility of the protein in the marker and it was observed that the molecular weight in the samples ranged in size from 20.90 kDa to 56.23 kDa in protein isolates (Appendix IV). Variability in intensity was observed in some bands that indicated the quantity of proteins cumulating at a particular molecular weight.

Proteins with molecular weight ranging from 48.0 kDa to 100.0 kDa were considered as high molecular weight (HMW) proteins; those in the size ranging from 35.0 kDa to 48.0 kDa were considered as medium molecular weight protein (MMW) and the remaining as low molecular weight (LMW) proteins. The banding pattern of protein isolates revealed total 4 bands ranging from 17.0 kDa to 56.23 kDa which were detected in isolates prepared from all the genotypes. The results were in accordance to results reported by Sai *et al.* (2009) on the SDS-PAGE characterization of the whole protein and the isoelectric protein of red kidney bean, navy bean, and adzuki bean. Chang *et al.* (2012) [8] reported that the SDS-PAGE patterns of isoelectric protein and cryoprecipitate protein were similar to results obtained.

### Conclusion

Legume seeds are among the richest food source of protein and amino acids for human and animal nutrition. In the given study SDS PAGE showed to have proteins with the molecular weight 20.90 to 56.23 kDa in the protein isolates prepared from the adzuki beans. Amino acids composition showed that the HPU-51 had more amount of aromatic acid, methionine and tryptophan content. While lysine and isoleucine was highest in the EC-340264 and valine in Local Totru genotype. The results also revealed that value for essential amino acid i.e. histidine was more in comparison to the required amino acid pattern in the adzuki bean protein isolate except the EC-340264 genotype. Similarly, tryptophan was deficient in Local Totru while it was quite high in other two genotypes. ARM was more in all the three genotypes of the adzuki bean in comparison to the reference FAO/WHO amino acid scoring pattern. However, isoleucine, methionine, lysine, valine and leucine were less fulfilling in comparison to the FAO/UNU/WHO reference scoring pattern for the essential amino acid of preschool children. The amino acid scoring of the protein isolates also revealed that the limiting amino acid i.e. LAA in EC-340264 and Local Totru is Methionine and Isoleucine in HPU-51.

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