Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **3 (2):** 156-161 (2015)

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Research Article

OPEN ACCESS

Electrophoretic Protein profile of few Pulses consumed locally in Mumbai region

Vidya Gajendra Raut, Sopan Namdeo Kharat* and Vijay Damodhar Mendhulkar

Department of Botany, The Institute of Science, 15, Madam Cama Road, Mumbai- 4000 32

*Corresponding Author E-mail: sopankharat@gmail.com

ABSTRACT

In the present analysis marker assisted electrophoretic protein banding profiling was analyzed for five pulses which are sold more in the market and consumed by the people at large scale. The electrophoretic protein profiling was done in the samples of Chana dal (Cicer arietinum, 2n=16), Moong dal (Vigna radiata, 2n=14), Masoor dal (Lens culinaris, 2n=14), Tuvar dal (Cajanus indicus, 2n=22), and Udid dal (Vigna angularis, 2n=22). The protein extraction was done from selected seed samples and the crude protein content was estimated by Lowry's method. SDS-PAGE for the proteins was carried out. The Standard protein marker showed the existence of total 4 bands, with R_F range from 0.16 to 0.74 and molecular weight in the range of 15 to 66 kDa. Cicer arietinum showed 5 bands with R_F range, 0.094 to 0.64 and molecular weight in the range of 09 to 63 kDa, Vigna radiata reveals 8 bands with R_F value ranging from 0.072 to 0.78 and molecular weight in the range of 07 to 75 kDa, Lens culinaris showed 7 bands with R_F, 0.10 to 0.78 and molecular weight in the range of 10 to75 kDa, Cajanus indicus showed 5 bands with R_F 0.33 to 0.76 kDa, and molecular weight in the range 32 to 73 kDa and Vigna angularis showed 6 bands, with R_F 0.14 to 0.79 and molecular weight in the range 14 to76 kDa respectively. Present analysis indicated Cicer arietinum has highest amount of proteins among studied samples. The studies on banding pattern reveals the insight regarding marker assisted identification and location of various protein complexes and the relation between other legume species. In India, leguminous pulses serve as major source for protein intake. The present work was undertaken due to consumption of essential legume protein components and its importance in our daily life.

Keywords: *Electrophoresis, protein profile, SDS-PAGE, Leguminous Pulses.*

INTRODUCTION

Plant materials are the major sources of protein supplements in our dietary system. Legume plants are an important stock of essential protein components. In India, leguminous pulses serve as major source for protein intake because all the people in the society consume pulses. Legume pulses are easily available in most of the part of country. Similarly, economical point of view; it is marketed such as a way that even poor sector of society could use it for dietary purpose. However, marketing status of different legume varieties varies as per the quality and grain size. But it does not affect the protein identity of legume plant at considerable level. Majority of the population in India is economically backward and most of them suffer under malnutrition. This large segment of the population compromises with nutritional status or nutritional value of the food supplements at the cost of economic interest. As a consequence, they receive poor or less nutrients. It is with this sense of realization, a small attempt has been made to study the protein profile of selective and most commonly consume pulses by maximum population. An attempt has been made to collect the samples which are having high consumption or commercial circulation rather than nutritionally good quality sample.

The protein complexity and disfunctioning some time affects the genetical manifestation such as, plasma gene mutation, gene mutation, alteration in gene expression, gene amplification, mitotic crossing over, transposon elements, rearrangements of cytoplasmic genes and previously silent mutated gene. The mutation or alterations in protein quality and quantity may take due to excessive use of fertilizers or residual chemical deposition from fertilizers and its bioaccumulation in the plant.

Electrophoresis is a convenient technique which separate and examine the properties of molecules of high molecular weight such as proteins and enzymes, nucleic acids, complex lipids and carbohydrates. This technique retains the properties of molecules. This separation method usually lean heavily on physical processes and hence chemical properties of the molecules are least affected. This results in the maximum retention of biological activity which the molecules may possess. Proteins can be denatured with sodium dodecylsulphate (SDS). By forming a stable complex that removes native folded structures. The amount of SDS in the complex depends only on the size of the protein, not on charge or sequence. The resulting protein/SDS complex is a random coil that has a negative charge dependent on the size of the protein and not on its sequence. Electrophoretic techniques have proven invaluable significance in study of storage protein of many important plants. Differences in cultivars had been shown using electrophoresis technique in storage proteins of peanut[1,2,3] pea and bean[4]. Protein constituents are one of the most important factors affecting the protein functionality and have been the subject of many investigations. The 7S and 11S subunit of globulin protein together covered 56% of the total chickpea proteins as reported by Chavan et al. (1989)[5]. The composition of subunits of 7S and 11S globulins varies among legume species. The major 7S globulin exists as 6 isomer molecular species, each of which is composed of 3 discrete protein subunit, α and β-subunit[6], with a molecular weight of 80, 76 and 50 kDa[7], respectively. 11S globulin contains both acidic and basic subunits with molecular weight in the range of 27-73 kDa and 20-24 kDa, respectively. Identification of these major protein complexes and their comparative correlation ship within five pulses has been studied and discussed in present work.

MATERIAL AND METHODS

This study was carried out with four leguminous plant species, *Cicer arietinum*, *Vigna radiata*, *Lens culinaris* , *Cajanus indicus*, and *Vigna angularis*. The samples of pulses were collected from the grain market in Mumbai. These samples was decoated , cleaned and grain of uniform physiologically shape and size were selected, for the present work.

Extraction and estimation of protein from seed sample

Decoated grains of samples material of known weight has been taken for the extraction of proteins. The material was grinded well in mortar and pestle to make fine powder with potassium-phosphate buffer (pH 7.4) to fine slurry and then centrifuged at 5000 rpm for 15 minutes. Supernatant was used for protein quantification. Protein content was estimated according to the method of Lowry et al. (1951)[8], as a preliminary analysis to determine the protein concentration required to be used to run gel electrophoresis. The aliquot of 0.2 to 1.0 ml standard bovine serum albumin (200 µg/mL) was pipetted into a series of test tubes and volume was made up to 1.0 mL in each case. 5 mL of alkaline copper reagent was added to all the test tube. The test tubes were allowed to stand at room temperature for 10 min followed by the addition of 0.5 mL of FC reagent. The absorbance was recorded at 660 nm after 30 min against reagent blank[9](Kaempholi et.al.).

Protein profiling using SDS PAGE

Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970)[10]. Equal quantities of samples along with protein molecular weight marker (66, 45, 31, and 15 kDa bands) were loaded into 10% gels. Electrophoresis was performed at constant voltage (100 volts). In SDS PAGE polypeptide chain was separated according to their molecular weight. Sodium dodecyl sulphate (SDS) is an ionic detergent. That denatures proteins by wrapping around polypeptide backbone. The number of SDS molecules bound to a polypeptide chain is approximately half the number of amino acid residues in that chain.

The protein SDS-complex does not carries negative charges hence move towards the anode and the separation is based on the size of the protein. An anionic disc gel electrophoresis (SDS-PAGE) was carried out essentially according to the method of Laemmli (1970). Seeds were grinded to fine powder with the help of mortar and pestle. The sample buffer (400 µl) added in fine seed flour as extraction liquid and bromophenol blue (BPB) follow the movement of protein in the gel. The chemicals used for the extraction of protein buffer contained 0.5 M Tris-HCl (pH 8.8), 0.2% SDS, 1% 2-mercaptoethanol, 30% polyacrylamide. APS 5%, TEMED 10µl for 12% gel. The samples were centrifuged at 10,000 rpm for 10 min at room temperature. After centrifuging samples, the crude proteins were recovered as clear supernatant on the top of the tube. Then the supernatant were transferred into eppendorf tubes and were stored at 4^0C until gel electrophoresis. The molecular weight of the dissociated polypeptides was determined using molecular weight markers Albumin (66 kDa), Ovalbumin(45 kDa), Carbonic anhydrase (31 kDa), and Lysozyme (15 kDa). After the electrophoresis, the gel was stained with coomassie brilliant blue R-250 for 1 hour and distained with 10% acetic acid solution. Depending upon the presence or absence of polypeptide bands, similarity index was calculated for all possible pairs of protein types.

RESULTS AND DISCUSSIONS

It was observed that the legume samples although serve as major protein compliments in diet but they vary in proteomics as an individual varieties. These variations in spite of common similarities are exclusively recorded in their protein band profiling, R_F value and composition. The results obtained in this regards are discussed below.

Protein content

Storage proteins are important components in the seeds. These proteins, on hydrolytic breakdown, provide nitrogen and carbon skeleton for development seedling. A large amount of storage proteins are accumulated in germinating seeds. Seed proteins are classified as, albumins, globulins, prolamins and glutelins (Osborne, 1876). Albumins are water soluble proteins. Globulins are abundant in almost all pulses. Globulin from legume called legumin. The total protein content observed in mg/gm was converted and presented in percentage. The results obtained indicated 26.9% protein content in *Cicer arietinum*, 13.6% in *Vigna radiata*, 18% in *Lens culinaris*, 13 % in *Cajanus indicus* and 12% in *Vigna angularis* (Table-1). *Cicer arietinum* showed the highest protein content compare to other studied samples. Chickpea (*Cicer arietinum*) provides a protein-rich supplements to cereal-based diets. The electrophoretic pattern of the protein are directly associated with the genetic background of the protein and be harnessed to certify the genetic makeup. The higher protein content in *Cicer* also has been reported by Cai et.al, (2002)[11]. Legume seed proteins are composed of water-soluble albumin and salt soluble globulins and their ratio can be altered under the influence of mutated genes and such alteration are responsible to improve nutritional value of the legumes. Inter and intra specific variation in seed protein has been reported in wheat, barley and their wild relatives. SDS-PAGE is a valid technique used for species identification. Each variety of a group exhibit characteristics banding pattern of protein, accordingly, they could be identified.

Determination of Protein Molecular Weight and R_F value

Proteins are a highly diversified class of biomolecules. Differences in their chemical properties, such as charge, functional groups, shape, size and solubility enable them to perform many biological functions. These functions include enzyme catalysis, metabolic regulation, binding and transport of small molecules, gene regulation, immunological defense and cell structure. Determination of the molecular weight of a protein is of fundamental importance to its biochemical characterization. If the amino acid composition or sequence is known, the exact molecular weight of a polypeptide can be calculated.

The range of the molecular weight of the protein in studied samples of pulses revealed variations from 07 kDa to 76 kDa. The greater diversity of protein molecular weight is the identity of legumes as they are protein rich components. The last band travelling maximum distance in the gel indicated molecular weight as 66 kDa for standard, 63 kDa for *Cicer arietinum*. 75kDa for *Vigna radiata* 78 kDa for *Lens culinaris*, 73 kDa for *Cajanus indicus* and 76 kDa for *Vigna angularis* (Figure-1).

This protein band in particular, except in *Cicer arietinum* (molecular weight band 66 kDa) provides a clear view regarding molecular homology of common protein groups as supportively evident by its R_F value also (Table-2).

The molecular weight of an unknown polypeptide is obtained by comparing its position to the standard SDS denatured proteins after electrophoresis. The molecular weights of the standard proteins have been previously determined. After proteins are visualized by staining and distaining, their migration distance is measured. The $\log_{10}$ of the molecular weights of the standard proteins are plotted versus their migration distance. Taking the logarithm R_F allows the data to be plotted as a straight line. The molecular weight of unknowns is then easily calculated from the standard curve. The molecular weight of each band is identified by the standard curve obtained from the standard proteins following Mehdi et al. (2012)[12,13,14].

The R_F value is the distance travelled by a particular molecule from its loading point. The distance measured indicates the size of the particular protein with respect to its molecular weight. Thus, the R_F value determines the molecular weight of a particular unknown band. The different protein bands in studied legume groups showed nearly similar distance from starting point. It indicates the location of protein molecules of similar group. In present analysis we compared R_F value of each sample with the standard protein markers.

In all the studied samples, *Cicer arietinum* exhibit highest R_F 0.66 for band no.5. *Vigna radiata indicated* maximum band number i.e. 08 and the maximum R_F was also recorded as 0.78 compare to other samples of pulses. It was noticed that the last band of all the samples showed R_F value 0.76 to 0.79. That provides a clue about the existence of similar protein structural component except in *Cicer arietinum*. The molecular weight of each band is identified by the standard curve obtained from the standard proteins.

Fig.1. SDS-PAGE protein Electrophoresis with protein markers in collected samples of legume pulses

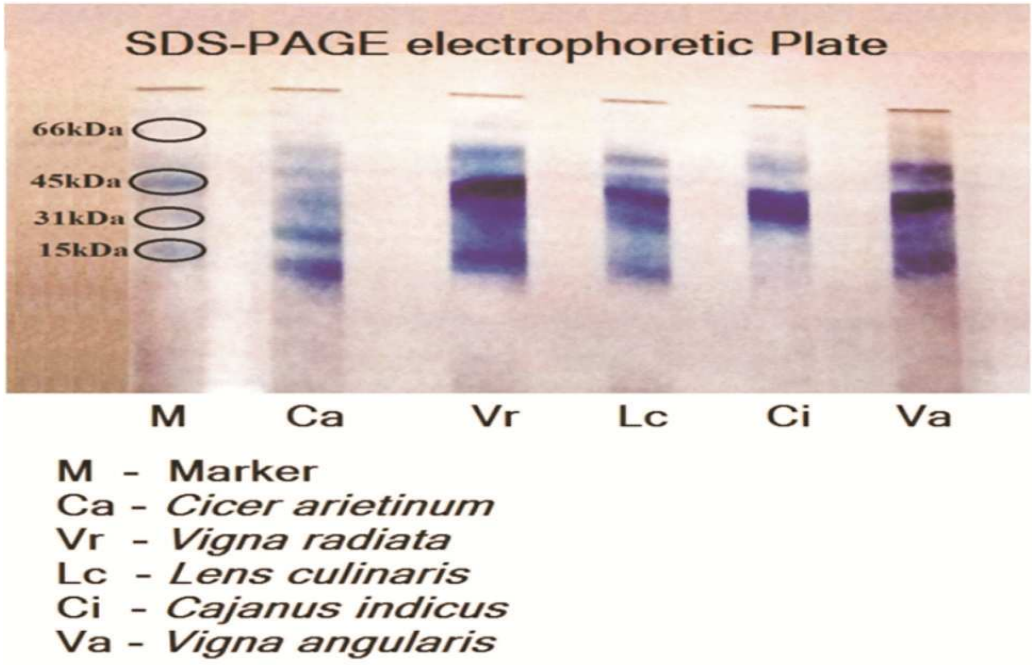

Table - 1. UV-optical density and Percent Protein content in studied samples of collected legume pulses

S.No.	Names of pulses	O.D.	Proteins (In %)
1.	*Cicer arietinum*	0.590	26.9
2.	*Vigna radiata*	0.298	13.6
3.	*Lens culinaris*	0.398	18.0
4.	*Cajanus indicus*	0.288	13.0
5.	*Vigna angularis*	0.266	12.0

Table -2. R_F value Profile and molecular weight for observed protein bands in collected samples of legume pulses

Name of Pulses	Total no. of Bands	Distance travelled by each band (in-mm)	R_F value	Molecular weight in kDa
Standard Protein marker	4	Band no. 1 - 8.0 mm	0.16	15
		Band no. 2 - 16.0 mm	0.33	31
		Band no. 3 - 23.0 mm	0.41	45
		Band no. 4 - 35.5 mm	0.74	66
Cicer arietinum	5	Band no. 1 - 4.5 mm	0.094	9
		Band no. 2 - 12.5 mm	0.26	25
		Band no. 3 - 20.0 mm	0.41	40
		Band no. 4 - 26.5 mm	0.55	53
		Band no. 5 - 31.0 mm	0.64	63
Vigna radiata	8	Band no. 1 – 3.5 mm	0.072	7
		Band no. 2 – 7.5 mm	0.15	15
		Band no. 3 – 16.0 mm	0.33	32
		Band no. 4 – 19.0 mm	0.39	38
		Band no. 5 – 23.0 mm	0.47	46
		Band no. 6 – 26.5 mm	0.55	53
		Band no. 7 – 33.5 mm	0.69	67
		Band no. 8 – 37.5 mm	0.78	75
Lens culinaris	7	Band no. 1 – 5.0 mm	0.10	10
		Band no. 2 – 15.5 mm	0.32	31
		Band no. 3 – 20.0 mm	0.41	40
		Band no. 4 – 23.0 mm	0.47	46
		Band no. 5 – 26.0 mm	0.55	52
		Band no. 6 – 31.0 mm	0.64	62
		Band no. 7 – 37.5 mm	0.78	75
Cajanus indicus	5	Band no. 1 - 16.0 mm	0.33	32
		Band no. 2 - 19.5 mm	0.40	39
		Band no. 3 - 22.5 mm	0.46	45
		Band no. 4 – 32.5 mm	0.67	65
		Band no. 5 – 36.5 mm	0.76	73
Vigna angularis	6	Band no. 1 – 7.0 mm	0.14	14
		Band no. 2 – 23.5 mm	0.48	47
		Band no. 3 – 27.5 mm	0.57	55
		Band no. 4 – 30.0 mm	0.63	60
		Band no. 5 – 35.5 mm	0.74	71
		Band no. 6 – 38.0 mm	0.79	76

CONCLUSION

Protein molecules of plant origin have been studied in the present investigation. Pulses are the main source of dietary protein and thus legumes are largely studied for its protein composition, structure and content. The consumers are cautious about selection of dietary sources specially legume pulses as per their affordability. However, being the common and usual protein source, the pulses are used by poor sector of society, compromising to its quality. Therefore commonly sold pulses were used as a study samples. On the basis of result obtained, it can be concluded that the legume, *Cicer arietinum* represents as a good source of protein with highest amount among studied samples. The highest protein content in the present analysis of pulses observed in *Cicer arietinum* (26.9%). While the lowest content was observed in *Vigna angularis* (12.0%). The studies on the banding pattern provide an insight to identify and locate the presence of similar or variable protein groups in the studied samples.

The R_F value analysis suggests precisely the distance travelled by each band in the studied legume samples. The similarity or dissimilarity in banding patterns thus, can be numerically expressed and correlated. Some bands with similar R_F value have been observed in the studied samples thereby indicating the identical similarity in major legume proteins in the studied pulses. In the cases of variation, the R_F value indicates the species affinity and relationship between *Vigna radiata* and *Vigna angularis*.

The studies on SDS-PAGE electrophoretic banding pattern with known standard protein markers illustrate mainly the presence of an important protein component globulin. The 11S globulin unit was evident in reduced subunit fractions with the varying molecular weights. The globulin sub units with molecular weights of 40 kDa, 43 kDa and 40 kDa was observed in *Cicer arietinum*, *Vigna angularis* and *Lens culinaris* respectively. The occurrence of the bands of similar molecular weight in studied samples also indicates the presence of protein affinity in the legume group, which is suggestive to the biochemical identity and homogeneity with respect to protein components in the pulses.

Acknowledgement

The authors are thankful to the Director and Head, Department of Botany, The Institute of Science, Mumbai for providing Analytical facilities.

REFERENCES

1. Tombs M. P. Variant forms of *Arachin.* Nature. **200**: 1321-22 (1964)
2. Dawson R., McIntosh A.D., Varietal and environmental differences in the proteins of the ground nut (*Arachis hypgaea*). Journal of Science, Food Agriculture. **24**: 597-609 (1973)
3. Hynes M.J. Genetically controlled electrophoretic variants of storage proteins in *Pisum sativum*. *Austrian Journal of Biological Science*. **21**: 827-829 (1968)
4. Adrianse A., Klop W. and Robbers J.E. Characterization of *phaseolus vulgaris* cultivars by their electrophoretic pattern. *Journal of Science food Agriculture*. **20**: 647-650 (1969)
5. Chavan J.K., Kadam S.S. and Salunkhe D.KBiochemistry and technology of chickpea (*Cicer Arientinum* L.) seeds. CRC Crit. Rev. in Food Science and Nutrition. **25**: 107-122 . (1989)
6. Thanh V.H. and Shibaski K. Major proteins of soybean seeds. A straight forward fractionation and their characterization. *Journal of Agriculture and Food Chem*. **24**: 1117-1121 (1976)
7. Qi M., Hettiarachchy N.S. and Kalapathy U. Solubility and emulsifying properties of soya protein isolates modified by pancreatin. *Journal of Food Science*. **62**: 1110-1115 (1997)
8. Lowry et al. (ed) (1951). Biochemical methods IInd edition by Manikam and Sadashivam. New age International pvt. Ltd. Publishers, Tamilnadu Agriculture University, Coimbatore. 8-58 (1996)
9. Kempohalli S.C., Storage Proteins and Trypsin Inhibitors of an Underutilized Legume, *Mucuna* : Variability and Their Stability during Germination. *American Journal of Plant Sciences*. **4**: 910-916 (2013)
10. Laemmli U.K. (1970). Manual on techniques in molecular Biology (proteins)., Nature. 227-680 (1986)
11. Cai R., McCurdy A., and Baik B. K. Textural property of six legume curds in relation to their protein constituents. Washington State University , Pullman, WA, Dept. of food science, Human nutrition and impact. 99164-6376 (2002)
12. Mehdi K., Mohsen F., Farzad M., Rahele M. Study of Leaves and Seed Protein profiles of Chickpea (*Cicer arientinum* L.) under Drought Stress and non Stress conditions, *International Journal of Agriculture and Crop Sciences. IJACS.* **4-6**: 280-283 (2012)
13. Patil., Comparative protein profiling study in wild chickpea and its induced mutants. The experiment. *International journal of Science and Technology.* **18(4)**: 1297-1303 (2014)
14. Sanjeev R., Rajesh M., Madhuri B. and Narendra B. P. V. R. Comparative evaluation of protein extraction methods from few leguminous seeds. *International Journal of Advanced Biotechnology and Research.* **3(2)**: 558-563 (2012)