International Journal of Biomedical Investigation

journal homepage: https://ijbi.edwiserinternational.com

**Original Article**

# Protein analysis and digestibility of few pulses consumed in Indore region

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ARTICLE INFO

Received 19 June 2024
Revised 27 July 2024
Available Online 10 August 2024

*Keywords:*
Pulses
Protein
Digestibility
Analysis
Health
SDS-PAGE

ABSTRACT

**Objective:** Pulses are known as a good source of protein in daily diet. These are cheap and easily available protein options ranging from Rs. 71-156 per kg. The three pulses or dal viz., split Mung (Vigna radiata, 2n=14), Tuvar (Cajanus indicus, 2n=22), and split Urad (Vigna mungo, 2n=22) were tested for comparative protein analysis and their digestibility in this work.
**Methods:** Aqueous extracts of all three pulse samples were prepared by homogenization. The Folin-Lowry method was employed for protein quantification in all the samples. SDS-PAGE was carried out for pulse protein profiling. The in-vitro digestibility of pulses was evaluated by the Biuret method and agar well diffusion assay.
**Results:** The results confirmed that the protein content (18.46mg/100ml) and digestibility of split Mung were the highest as compared to the other two pulses. SDS-PAGE analysis confirmed that split Mung dal showed maximum and 5 different types of protein bands ranging from 20 to 63 kDa revealing its richness in protein quality. The recommended daily intake of protein is 15 gm from 100 gm of pulses and our results showed the highest protein content in split Mung (14%). The differences in in-vitro protein digestibility of the three pulses after being incubated with trypsin were observed in the Biuret assay. The split Mung sample exhibited the maximum level of light purple color development, which was confirmed through comparison with the control. The protein in split Mung was easily broken down by trypsin, as indicated by the appearance of a well defined zone on the agar plate.
**Conclusion:** Mung dal is a suitable protein source for the general population, individuals with health conditions, and those who prioritize their health. This is because it contains a significant amount of protein that is of excellent quality and easy to digest.



## *Introduction*

Pulses are a significant source of dietary protein for the Indian population since they are inexpensive, readily available, and nutritionally valuable. Pulses are regarded as the second most significant staple crops for human consumption, following cereals. They are accessible to all segments of the population [1]. In 2022, the Indian pulses market reached a size of 32.3 million tons and is projected to reach 68 million tons by 2028 [2]. Madhya Pradesh contributes roughly 33% of the overall production of pulses in the country [3]. In 2023,

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https://doi.org/10.31531/2581-4745.1000154

the Global Pulse Protein Market is valued at US$ 1,503.60 million, showing a remarkable growth rate of 4.2% per year [4]. Pulses can be consumed in their whole, in fragmented form, or with the outer covering removed [5]. Arhar (Cajanus indicus), Moong (Vigna radiate), Chana, Urad, and Masoor are among the pulses commonly consumed in India.

Contemporary individuals are increasingly prioritizing their well-being and so, opt for a well-rounded diet consisting of nourishing meals. Nutrition entails the ingestion of a substantial quantity of protein, and individuals predominantly favor plant-based goods due to their elevated nutritional value. Pulse protein is commonly regarded as being devoid of chemicals, hormones, antibiotics, and non-genetically modified organisms (non-GMO). Pulses, with protein content ranging from 17-30%, are an ideal protein source for individuals that prioritize their health [6]. Pulses have a revitalizing effect on the body. Pulses have a slow digestion rate and provide a sense of fullness due to their low calorie content (260-360 kcal/100gm dried pulses) and high levels of carbs and fiber [7]. The iron concentration of this substance, ranging from 3.85 to 6.46mg per 100gm, facilitates the transportation of oxygen throughout the body. This, in turn, enhances energy generation and metabolism. Pulses include dietary fiber that effectively binds toxins and cholesterol in the gastrointestinal tract, facilitating their elimination from the body. This mechanism contributes to enhanced cardiovascular health and reduced levels of blood cholesterol. In addition, pulses serve as a valuable reservoir of vital amino acids, particularly lysine (64mg/gm of protein) and threonine (38mg/gm of protein)), which enhance the protein's nutritional value in the diet [8].

Protein digestibility can be defined as the fraction of protein available for absorption after being ingested in the human body. Digestible protein supplies required amino acids for growth and maintenance of cells and tissues. Higher digestibility is dependent on the hydrolysis of proteins by the protease enzyme. The digestibility of plant protein is between 75- 80% [9]. Pulse protein digestibility can be affected due to the presence of anti-nutrients like phytate, enzyme inhibitors, lectins, phytate, tannins, and saponins which inhibit enzymes involved in digestion. However, processing, cooking, and germination can significantly improve the digestibility of pulse proteins [10]. The digestibility of pulse proteins depends on the type of protein they contain, their structure, and functionality [11].

Pulses are rich sources of carbohydrates (44.3gm/100gm) proteins (25gm/100gm), fats (6gm/100gm), dietary fibers (17gm/100gm), minerals (3gm/100gm) and vitamins like niacin (2.6mg/100gm) thiamine (0.87mg/100gm), riboflavin (0.211mg / 100gm), vitamin C (4.4mg/100gm) [12]. Mung bean is known as poor man's meat [13]. It is a good option for individuals dealing with obesity and diabetes, because of low calories (347gm/100gm). Its consumption can control hyperglycemia, hyperlipemia, and hypertension. Its regular use can prevent cancer and melanogenesis due to the presence of bioactive polyphenols and peptides with hydrophobic amino acids [14].

Minerals like potassium (21%), calcium (14%), and iron (95%) are prominent in Urad [15]. Urad is low in cholesterol, regulates blood sugar levels, and reduces oxidative stress. It also boosts immunity and can effectively manage pain and inflammation [16]. Tuvar dal is known as the "Queen of grains" or Congo pea. It is known to maintain good digestive health, immunity, strong bones and manage diabetes. The high levels of methionine (22.7mg/gm protein), lysine (70.09mg/gm protein), and tryptophan (9.76mg/gm protein) make Tuvar dal, a great source of essential amino acids [17]. Its protein profiling studies have revealed the presence of two globulins i.e. cajanin, concajanin [18].

The majority of the population in India is undernourished which is roughly 15% of the total population [19]. This has left a large segment of the population (74.1%) buying cheaper food with low nutritional value. It is with this sense of realization that a small attempt has been made to study the protein profile of selective and most commonly consumed pulses in the Indore region available at affordable price. These three pulses viz., split Mung, Tuvar, and split Urad were selected, because of their cultivation and consumption in Indore (Madhya Pradesh) region at a larger scale with total production ranging between 286-1181 thousand metric tons. Moreover, the global trend towards plant-based protein consumption has led us to investigate its protein profiling and digestibility. This study aimed to collect pulse samples that are high in consumption with more commercial circulation. It was then followed by quantification of total protein, protein profiling analysis, and protein digestibility assays of the pulse samples.

## *Materials and Methods*

### Sample collection

The sample of pulses were collected from the grain market in Indore. The seeds of Tuvar, split Mung, and Urad (Figure 1) were cleaned, and grains of uniform physiological size and shape were selected for the present study.

**Figure 1:** Seeds of split Urad, Tuvar and split Mung.

Selected seed samples were weighed 2 gm and soaked in water for 15 minutes. Seeds were ground to a fine paste with 10 ml of 0.1 M potassium phosphate buffer (pH 7.4) in a pestle mortar. This fine paste was subjected to centrifugation at 5000 rpm for 10 minutes. After which supernatant was collected for further tests and labeled as aqueous pulse extract.

### Protein estimation

The protein content was determined using the Lowry technique [20]. Quantities ranging from 25 to 400 microliters of standard bovine serum albumin were measured and transferred into a set of test tubes using a micropipette. The volume in each test tube was then adjusted to 1.0 ml. Three test tubes were each filled with 50 microliters of an aqueous extract of a pulse sample. A fixed volume of 2.5ml of the alkaline copper reagent was added to all the test tubes. The test tubes were placed in an incubator and kept at the temperature of the room for a duration of 10 minutes. The measurement of absorbance at a wavelength of 660nm was conducted after a duration of 30 minutes using a colorimeter (HC Memorial Scientific Corporation, Ambala Cantt, India), with a blank serving as the reference. A calibration graph was constructed by plotting the relationship between the absorbance and concentration of a standard protein (BSA). The protein concentration in the sample was determined based on the information provided by the graph. The measurements were conducted in duplicate.

### Protein profiling by SDS-PAGE

The protein from the aqueous pulse extract was examined using discontinuous SDS-PAGE under reduced conditions [21]. The electrophoresis was performed using a 12% separating gel and a 4% stacking gel. Each pulse protein was loaded separately in wells with a 1:1 dilution with sample buffer (containing 50 mM Tris pH 6.8, 2% SDS, 20% glycerol, 2% 2-mercaptoethanol, and 0.04% bromophenol blue) after boiling. Approximately 10ug of each protein was added. The protein bands were separated using a constant voltage of 100 V. After electrophoresis, the gel was treated with a solution of 0.2% (w/v) Coomassie brilliant blue R-250 in a mixture of 50% methanol and 10% acetic acid. A solution containing 30% methanol, and 10% acetic acid was utilized to destain the SDS-PAGE gel. The molecular weight of the pulse protein was evaluated using the Prestained Protein Ladder from Himedia Laboratories Private Limited, Mumbai, India. This ladder has a mixture of 10 proteins that are dyed red, green, and blue, with molecular weights ranging from 11 to 135 kilodaltons (kDa).

### Protein digestibility assays

The digestibility of all three aqueous extracts of pulses was assessed using the Biuret method and agar well diffusion assay, through the catalytic action of trypsin.

### Biuret test

In the Biuret method [22], protein test samples (50ul) were mixed with an equal amount of trypsin (2mg/ml) and incubated at 37° C for 30 minutes. Following incubation, the presence of color was identified by introducing 5 - 7 drops of 0.5% $CuSO_4$ and 1 ml of 10% NaOH (Biuret reagent). The presence of a light purple-violet color signifies a higher degree of protein digestion by trypsin in comparison to the control. The control group consisted of the aqueous extract of pulse protein.

### Agar well diffusion

The agar well diffusion experiment was conducted with minor alterations [23]. The protein digestibility of Moong dal was assessed using the Biuret test, which demonstrated the highest level of digestion. A volume of 50 microliters of trypsin was added to a cooled agar solution with a concentration of 1.5%. The mixture was then placed into a petri dish. Wells were created in this solidified agar plate that had been treated with trypsin. Various amounts of moong dal extract, specifically 25ul, 50ul, 75ul, and 100 ul, were added to the punched wells. A control was prepared using a 50ul phosphate buffer with a pH of 7.4. Following a 12-hour incubation period at ambient temperature, the plate was immersed in a 10% TCA indicator solution for 5 minutes to enhance visibility. The presence of an unobstructed area surrounding the well serves as a reliable predictor of the digestion of pulse protein.

### Statistical analysis

The studies were conducted three times each, and the results were reported as the average value plus or minus the standard deviation (SD). The data was subjected to statistical analysis using the Student's t-test, where p-values less than 0.05 were considered statistically significant.

**Citation:** *Tiwari R, Dave M, Dawar D, et al. Protein analysis and digestibility of few pulses consumed in Indore region. Int J Biomed Investig 2024; 7(1): 154. doi: 10.31531/2581-4745.1000154*

## *Results and Discussion*

Legumes are commonly consumed sources of protein for vegetarians because of their affordability and availability. Pulses are incredibly rich in their nutritional value with proteins (17-30%) present double than in wheat and three times that of rice and being equal to that of meat (18-25%) [24]. These are low in calories (300-340 calories per 100gm) and most suitable for the health of targeted population opting for high fiber, high protein, low glycemic index and gluten free diet with high content of antioxidants.

### Yield of pulse extracts

The extractive yield and color of pulse samples are shown below (Table 1 and Figure 2).

**Table 1:** Extraction yield of extracts of three pulses.

| Sample | Final volume (ml) | Yield | Color of extract |
|---|---|---|---|
| Split Mung | 5.0 | 50% | Light yellow |
| Tuvar | 3.8 | 38% | Dark yellow |
| Split Urad | 4.0 | 40% | Muddy brown |


**Figure 2:** Extracts of Tuvar, Mung and Urad dal.

### Protein content in pulses

Our findings (Table 2) confirmed that the quantity of protein in split Mung dal was found to be the highest (18.46mg/100ml), while it was the lowest in Urad dal (13.12mg/100ml). The protein content observed in mg/100ml was converted and presented in percentage. The results obtained indicated 14% protein content in split Mung, 11% in Tuvar, and 9% in Urad. The standard graph for protein estimation using BSA is presented in Figure 3.

### Protein profiling of pulses

The identification or determination of comparable or varying proteins in the samples under study can be readily accomplished by analysing their banding pattern on SDS-PAGE, taking into account the quantity, location, and molecular weight of the bands. The electrophoretic banding pattern, using recognized standard protein markers, indicates the presence of a significant protein component called globulin (48kDa) in all of the pulse samples. The higher abundance of bands spanning from 20-63kDa in Mung indicates its superior protein quality (Figure 4). Pulses include storage proteins that may be categorized into four categories: albumins, globulins, prolamins, and glutelins. These proteins make up 50% of the total protein found in mature seeds [25]. Globulins are highly abundant storage proteins found in all legumes [10]. Table 3 displays the cumulative count of bands with their corresponding relative molecular weight for all three samples.

**Table 2:** Protein content in studied samples of collected pulses.

| Sample | Optical density (OD) | Protein content (mg/100ml) | Protein (%) |
|---|---|---|---|
| Split Mung | 0.45 | 18.46 | 14 |
| Tuvar | 0.40 | 15.13 | 11 |
| Urad | 0.37 | 13.12 | 9 |

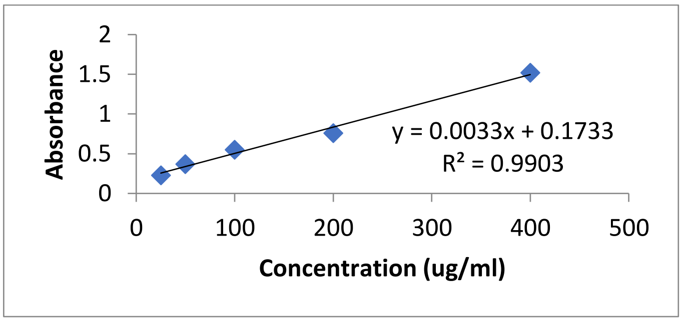


**Figure 3:** Standard graph of bovine serum albumin.

**Table 3:** Number and molecular weight for observed protein bands in collected samples of pulses.

| Sample | Number of bands | Molecular weight |
|---|---|---|
| Standard Protein Ladder | 9 | 11-100 kDa |
| Split Mung | 5 | 20-63 kDa |
| Tuvar | 5 | 20-63kDa |
| Split Urad | 3 | 15-48kDa |

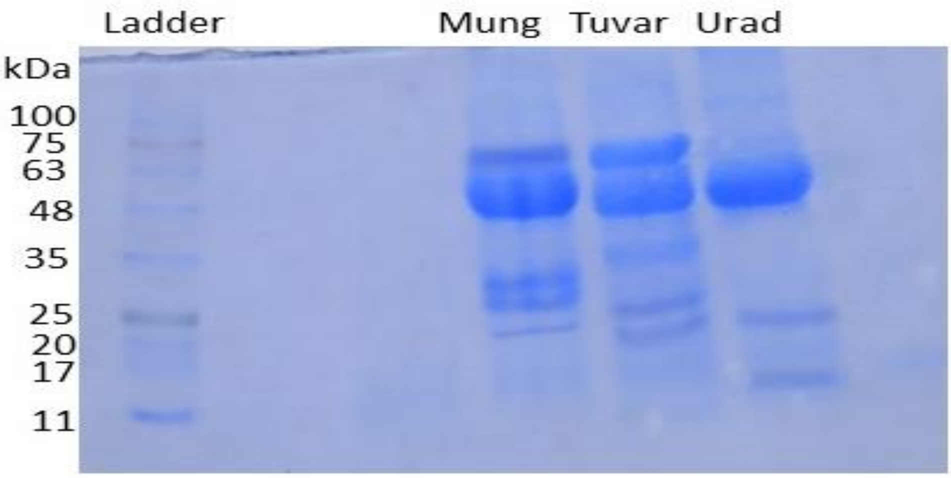


**Figure 4:** SDS-PAGE protein profile with protein ladder in collected samples of pulses.

**Protein digestibility of pulses**

Biuret assay of pulse samples after being incubated with trypsin showed differences in protein digestibility. The Mung extract showed higher protein digestibility as compared to Tuvar and Urad due to appearance of light purple color (Figure 5a) when compared to control (Figure 5b) which demonstrates trypsin action on pulse protein

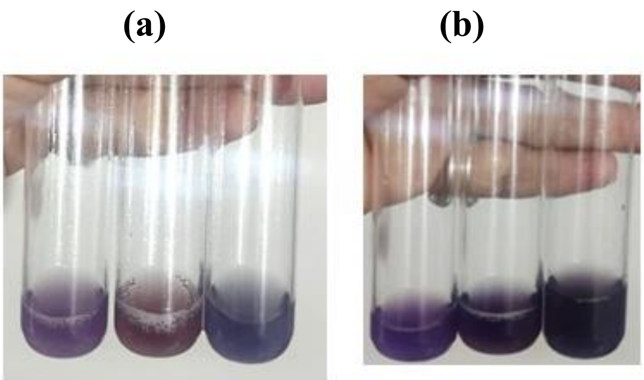


**Figure 5:** (a) Pulses with trypsin action; (b) Control.

Among the three pulse samples tested for protein digestibility by Biuret assay, only split Mung was selected for trypsin action on water agar medium due to its maximum digestibility. The digestibility results were observed in the form of clear zones around wells (Figure 6) for different volumes of pulse extract with zone diameters ranging from 0.5-1.5 cm (Table 4).

The greater digestibility of split Mung can be accounted for due to its high protein content (14%) than Urad and Tuvar. The protein digestibility of split Mung by trypsin completely suggests that its consumption can provide more of its constituent amino acids for absorption and protein synthesis in the body [26]


**Figure 6:** Protein digestibility of Mung by well diffusion.

**Table 4:** Zone diameter for Mung digestibility by trypsin.

| S. No. | The volume of Mung extract | Zone Diameter |
|---|---|---|
| 1 | 25ul | 0.5 cm |
| 2 | 50ul | 0.8 cm |
| 3 | 75ul | 1.1 cm |
| 4 | 100ul | 1.3 cm |
| 5 | 125ul | 1.5 cm |

## Conclusion

Pulses are crucial for ensuring global food security and meeting future nutritional requirements. In 2022, the global population reached 8 billion and is projected to reach 9.7 billion by 2050. There is a growing need for pulse-based products that are rich in protein and fiber, have a low glycemic index, are gluten-free, and include higher levels of antioxidants. A 25% increase in pulse output is necessary to meet the global population's protein requirements. The nutritional composition of pulses in the human diet is influenced by the quality of its protein and its ability to be digested. Pulse ingestion satisfies the body's critical amino acid needs and can be readily digested by cooking, soaking, and processing.
The present investigation suggests that split Mung represents a good source of protein with 18.46mg/100ml protein content. The recommended percentage of daily intake of proteins is typically between 10-35% and our results indicated 14% protein content in split Mung. The protein bands in split Mung dal were more intense and greater in number (5 bands) which indicates that it has good amount of different types of proteins (25gm/100 gm). Trypsin digestibility of protein in split Mung was evident by presence of clear distinct zone on agar plate. On the basis of all these observations, it is clear that split Mung is highly nutritive and valued for its high protein content, easy digestibility which is essential for physiological processes and body growth. Therefore, it can be considered as the most suitable protein option for common and health conscious people and person with health problems as far as demand of nutritious and cheap protein source is concerned.

## Acknowledgements

We would like to thank all the anonymous individuals who helped with this study.

## Conflict of Interest

The authors declare no conflict of interest.

## Funding

The authors did not receive any fundings from any private or government sources.

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