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# Fractionation of rice seed storage proteins in two rice genotypes

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**Abstract**
This study investigated the fractionation of seed storage proteins in two rice genotypes, BPT-5204 and HPR-14, focusing on the estimation and characterization of albumin, globulin, glutelin, and prolamin fractions. Fractionation was based on solubility properties, with subsequent estimation of protein concentrations between the genotypes. The SDS-PAGE analysis further elucidated the various molecular weights for each protein fraction. Our results indicated that glutelin is the most abundant fraction in rice grains, followed by albumin, globulin, and prolamin. Despite employing different rice varieties, minimal differences were observed in protein banding patterns, highlighting the consistency in protein composition across genotypes. This comprehensive analysis enhanced our understanding of rice protein fractionation, putting the groundwork for future research on rice protein functionality and nutritional significance. The findings of this study contribute to the ongoing efforts to optimize rice protein utilization in various food and feed applications, potentially addressing global nutritional challenges.

**Keywords:** Rice, protein fractions, SDS-PAGE

## Introduction

Rice (*Oryza sativa* L.), a monocotyledon plant in the Poaceae family, is a vital staple food for half of the global population. It is widely consumed across the world, especially in South and East Asia, the West Indies, the Middle East, and Latin America, meeting essential calorie requirements (Champagne *et al*., 2004; Sharif *et al*., 2014) [8, 29]. Proteins are a crucial part of the human diet, and each person has to intake the necessary quantity of protein for their age and body weight. Majorly, milled rice comprises 78% of starch, protein stands at second major part (6–7%).

Rice proteins are classified based on their solubility, following the Osborne classification introduced by Osborne in (1924) [25]. Albumin (water-soluble), which readily dissolves in water; Globulin (salt-soluble), showing solubility in saline (salt) solutions; Glutelin (alkali/acid-soluble), capable of dissolving in both alkaline and acidic environments; and Prolamin (alcohol-soluble), which can be dissolved in alcohol. These distinct protein fractions play crucial roles in the composition and functionality of rice proteins. Rice seed storage proteins are synthesized on the rough endoplasmic reticulum (ER) and subsequently are translocated into the ER lumen (Fukuda *et al.*, 2011, Fukuda *et al*., 2013, Tian *et al*., 2013) [10-11, 34].

In rice endosperm, two distinct types of protein bodies have been characterized: PB-I (Type I), which exhibits a lamellar structure and a spherical shape, is particularly rich in prolamin, a class of storage proteins essential for seed development and nutrient provision during germination. In contrast, PB-II (Type II) displays a crystalline structure and an irregular shape, predominantly containing glutelin, another major rice storage protein (Bechtel and Juliano, 1980, Bechtel and Pomeranz, 1978, Ogawa *et al*., 1989, Tanaka *et al*., 1980) [4-5, 33]. Glutelins significantly contribute to the overall protein content in rice seeds. Interestingly, the composition of endosperm storage proteins varies: approximately 60–65% of the proteins reside in PB-II, 20–25% in PB-I, and the remaining 10–15% are composed of albumin and globulin in the cytoplasm. (Ogawa *et al*., 1987) [24].

The protein composition of rice grain milling fractions exhibits significant variation. Research literature reports varied protein solubility fractions influenced by rice variety and extraction methods. Specifically, in brown rice, albumin accounts for 5–10%, globulin 7–17%, glutelin 75–81%, and prolamin 3–6%. In milled rice, these fractions are 4–6%, 6–13%, 79–83%, and 2–7%, respectively. Meanwhile, in rice bran, the proportions changes to 24–43% for albumin, 13–36% for globulin, 22–45% for glutelin, and 1–5% for prolamin (Adebiyi *et al*., 2009, Agboola *et al*., 2005, Cao *et al*., 2009, Ju *et al*., 2001, Juliano, 1985a, Zhao *et al*., 2012) [1, 2, 7, 16, 17, 40]. Notably, glutelin dominates both brown and milled rice, while prolamin—typically a major endosperm storage protein in cereals—remains minor fraction in all rice grain milling, except for oats (Shewry and Halford, 2002) [31]. Rice albumin fraction is only a small percentage of the total protein composition of seeds, roughly 2–6%. It is mostly made up of glycoproteins that are between 16 and 60 kDa, which are especially present in bran (Kawakatsu and Takaiwa, 2019) [18]. Water can be used to extract it, however preventing globulin contamination is a challenge. Although its stated characteristics vary, albumin has a range of molecular weights and isoelectric points. Interestingly, its hydrolysates show enhanced inhibitory activity against the angiotensin-converting enzyme (ACE), indicating potential advantages against hypertension (Uraipong and Zhao, 2016, Uraipong, 2016) [36, 35].

Globulin fraction is salt-soluble due to its net electrical charge (Amagliani *et al.*, 2017, Hamada, 1997) [3, 12], the second most abundant rice seed protein, containing proteins rich in cysteine and methionine but lysine constitutes a lesser amount. They are found in crystalline protein bodies that range from 2–3 µm in diameter. Most of these can be seen in the sub-aleurone layer (Tanaka *et al.*, 1980) [33].

Glutelin, the primary storage protein in rice, is extensively aggregated, disulfide-bonded, glycosylated, and difficult to solubilize. It is considered alkali-soluble (pH > 10) and acid-soluble (pH < 3). Glutelins are generally considered the primary seed storage protein present in rice, unlike other cereals where prolamin holds this value. The bran and endosperm are argued to contain 11–27% and 66–78%, respectively (Mitsuda *et al*., 1967, Sawai and Morita, 1968) [23, 28]. It is mainly found in Protein Body II (PB II) and contains a good amount of lysine, just behind rice albumin. Rice glutelins are composed of high-molecular-weight proteins ranging from 45 to 150 kDa. Divided into two subunits, with MW of 30–40 KDa which are acidic (α) and 19–23 KDa are basic (β) in nature (Hoogenkamp *et al*., 2017, Kawakatsu and Takaiwa, 2019) [13, 18].

Prolamins are also called reserve proteins because they are found in the endosperm area of the grain. They are a minor protein fraction in rice seed, made of heterogeneous polypeptides that are more evenly distributed across the rice fractions compared to other proteins and constitute 2.6–3.3% & about 4% of protein in rice endosperm and bran, respectively (Cagampang *et al.*, 1966, Fabian and Ju, 2011) [9] Prolamins are deficient in essential amino acids but have nonessential amino acids (Braspaiboon *et al.*, 2020) [6]. They are the hydrophobic protein fractions localized in protein bodies I (PBI) (Sugimoto *et al.*, 1986a) [32]. Rice prolamins soluble in aqueous ethanol (60–70%) are about 4% of the bran, the minor fraction in rice seed constitutes 2.6–3.3%.

As regards the essential amino acids, albumin shows the highest content of histidine and threonine, whereas prolamin has the highest proportions of isoleucine, leucine and phenylalanine. Furthermore, globulin has the highest content of the sulphur-containing amino acids cysteine and methionine, while prolamin has the lowest, and glutelin contains a good amount of lysine. Based on their amino acid composition, albumin has been estimated to have the highest and prolamin the lowest biological value among the rice protein fractions (Padhye, 1979) [26]. In this context, this study was done to investigate the concentration of protein fractions and number of proteins in each fractions present in two rice varieties *viz*., BPT-5204 and HPR-14.

## Materials and Methods

### Seed materials

Matured rice seeds of BPT-5204 and HPR-14 were collected from Genomics and Gene Editing Laboratory, Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore.

### Preparation of rice flour

Rice grains were powdered by using a grinder to make amorphous powder form and it was sieved in 100 mesh sieve or 150 µm pore size to remove larger particles.

### Defatting of rice flour

One gram of rice flour was defatted with hexane in the 1:6 ratio by shaking in the 15 ml centrifuge tube for 30 min at 30 °C. The hexane-containing fat was separated from the flour by centrifuging at 10,000 rpm for 20 min at 4 °C.

### Rice protein fractionation

Rice protein fractionation was done based on the solubility properties of each fraction by Osborne classification (Osborne, 1924) [25] of rice protein fractions.

### Extraction of albumin fraction

Defatted rice flour was subjected to protein extraction by mixing in a rotary shaker (300 rpm) with 4 mL of deionized water at 30 °C for 4 h and centrifuged at 10,000 rpm for 20 min to obtain albumin.

### Extraction of the globulin fraction

Defatted rice flour was subjected to protein extraction by mixing in a rotary shaker (300 rpm) with 4 mL of 4% NaCl at 30 °C for 4 h and centrifuged at 10,000 rpm for 20 min to obtain globulin.

### Extraction of glutelin fraction

Defatted rice flour was subjected to protein extraction by mixing in a rotary shaker (300 rpm) with 4 mL of 0.02 M NaOH at 30 °C for 4 h and centrifuged at 10,000 rpm for 20 min to obtain glutelin.

### Extraction of prolamin fraction

Defatted rice flour was subjected to protein extraction by mixing in a rotary shaker (300 rpm) with 4 mL of 70% ethanol at 30 °C for 4 h and centrifuged at 10,000 rpm for 20 min to obtain prolamin.

### SDS-PAGE analysis

Rice protein fractions were separated on 12% and 5% Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) by following the method described by Laemmli (1970) [21]. The SDS-PAGE was performed using a Vertical gel electrophoresis. The glass plates were rinsed with water and wiped with 70 percent ethanol before assembly. The gel preparation, polymerization, loading of samples, and electrophoresis were carried out according to standard protocols. After electrophoresis, the gel was stained with 0.25 percent Coomassie Brilliant Blue G250 and documented using white light.

### Protein estimation
Rice proteins extracted from all three methods were estimated by Bradford protein estimation kit (Product Code ML106, HiMedia Laboratories Pvt Ltd. Maharashtra, India) based on the manufacture protocol with the bovine serum albumin (BSA) as a protein standard.

### Statistical analysis
Statistical analysis of the experimental data was carried out by Analysis of Variance in Completely Randomized Design using Duncan's Multiple Range test (DMRT) in the online statistical tool OPSTAT (Sheoran *et al.*, 1998) [30]. Experimental results were represented as means ± standard error from three replications.

## Results and Discussion
### Estimation of rice seed storage proteins fractions
Rice protein has four protein fractions *viz.*, albumin, globulin, glutelin and prolamin. Rice proteins were fractionated based on their solubility properties, where albumin is soluble in water, globulin is soluble in salt solutions, glutelin is soluble in acidic or alkali solution and prolamin is soluble in alcoholic solution.

After extraction, each fraction was estimated for the protein concentration. Where albumin is found to be 22.33 and 23.78% for BPT-5204 and HPR-14, respectively and globulin was found to be 18.76 and 20.79% for BPT-5204 and HPR-14, respectively. Similarly, glutelin was found to be 35.79 and 36.47% for BPT-5204 and HPR-14, respectively and prolamin was found to be 6.80 and 5.37% for BPT-5204 and HPR-14, respectively as shown in the (Fig. 1) and (Table 1).

Drawing from existing literature (Jayaprakash *et al.*, 2022, Kawakatsu and Takaiwa, 2019, Kim and Jeong, 2002) [15, 18, 19], comparisons indicate that rice consists of 2–6% of total seed protein, 24–37% of bran proteins, and 4–8% of the endosperm. In contrast, Kim and Jeong (2002) [19] reported albumin content in brown rice ranged from 10.1% to 14.1%, globulin from 12.4% to 16.4%, glutelin from 68.6% to 72.8%, and prolamin from 3.6% to 5.3%. Variability of the percentage of protein fractions in HPR-14 and BPT-5204 may be attributed to varietal or genotypic differences. These findings collectively suggest that in rice grain, the glutelin fraction is the most abundant, followed by albumin, globulin, and prolamin.

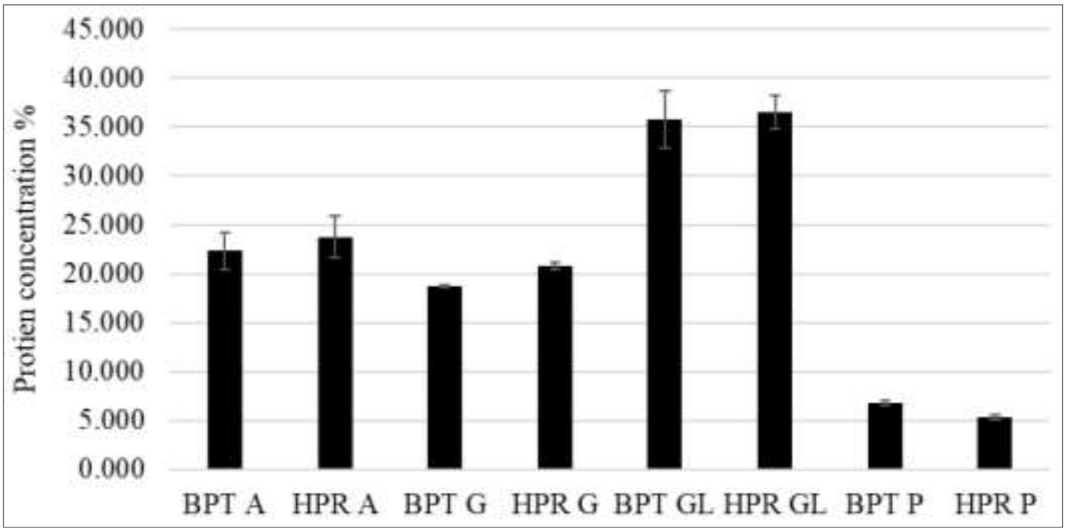


**Fig. 1:** Protein fraction concentration in rice flour, HPR-14 and BPT-5204. HPR-14, BPT-5204. A- Albumin, G- Globulin, GL- Glutelin, P- Prolamin.

**Table 1:** Protein percentage of rice protein fraction in BPT-5204 and HPR-14

| Sl.no | Genotype with protein fraction | Protein percentage mean |
|---|---|---|
| 1 | BPT Albumin | 22.33bc |
| 2 | HPR Albumin | 23.78b |
| 3 | BPT Globulin | 18.76d |
| 4 | HPR G | 20.79cd |
| 5 | BPT GL | 35.79a |
| 6 | HPR GL | 36.47a |
| 7 | BPT P | 6.80e |
| 8 | HPR P | 5.37e |
| | C.D. | 2.70 |
| | SE(m)± | 0.89 |

Values in the same column with different subscript letters (a, b, c, d, & e) differs significantly at p<0.01(DMRT)

**SDS-PAGE analysis of protein fractions**

All the fractions extracted based on solubility were subjected to SDS-PAGE gel electrophoresis to separate proteins according to their molecular weight. The protein electrophoretic profiles of all the four fractions were visualized in the gel, revealing distinct bands for each fraction.

**Albumin and globulin**

In the albumin fraction, bands appeared from molecular weight range of 15 to 75kDa. The bands *viz*.,15kDa, 17 kDa, 23 kDa, 49 kDa, and 75 kDa, with all bands faint except for a prominent band thickness at 17 kDa (Figure 2, Lane 1 and 2). In the globulin fraction, bands were observed at from molecular weight range of 12 to 110 kDa. The bands *viz*., 12 kDa, 14 kDa, 16 kDa, 19 kDa, 23 kDa, 48 kDa, and 110 kDa, with two distinct bands at 16 kDa and 23 kDa (Figure 2, Lane 3 and 4). These results were consistent with the previous studies done on the fractions albumin and globulin by (Iwasaki *et al*., 1982) [14].

**Glutelin and prolamin**

Initially, glutelin is produced as a polypeptide with a molecular weight typically falling within the range of 51–57 kDa (Krishnan *et al*., 1992, Luthe, 1983, Sarker *et al*., 1986, Yamagata *et al*., 1982) [20, 22, 27, 39]. Following this, enzymes catalyse the hydrolysis of the glutelin precursor, generating α and β subunits of the protein. These subunits then undergo polymerization facilitated by disulfide bonds, leading to the formation of macromolecular complexes (Sugimoto *et al*., 1986a) [32]. In this study the glutelin fraction displayed bands from 11 to 110 kDa. The bands *viz*.,11kDa, 14 kDa, 17 kDa, 37 kDa, 48 kDa, 50 kDa, and 110 kDa, showed with two notable bands at 17 kDa and 48 kDa (Figure 2, Lane 5 and 6). Here the bands between 30 to 40 kDa are the α and 17 to 23 kDa are β subunits of the protein after the enzymatic hydrolyzation of the 51 to 57 kDa precursor of glutelin fraction. Although, prolamin fraction have been reported to be showing bands at 12 to 17 kDa (Verma and Srivastav, 2017). In our study the prolamin fraction revealed a single, thick band at 13 kDa (Figure 2, Lane 7 and 8).

These results are consistent with findings from previous studies (Kim and Jeong, 2002, Amagliani *et al*., 2017, Wang *et al*., 2016) [19, 3, 38], albeit with minor variations in molecular weights. Differences in the banding patterns of rice protein on SDS-PAGE observed by earlier research groups could be attributed to variations in protein extraction methods and genetic variation in the protein fractions between the two rice genotypes. Notably, in our study, despite employing two different rice varieties, no discernible differences were observed in the protein bands.

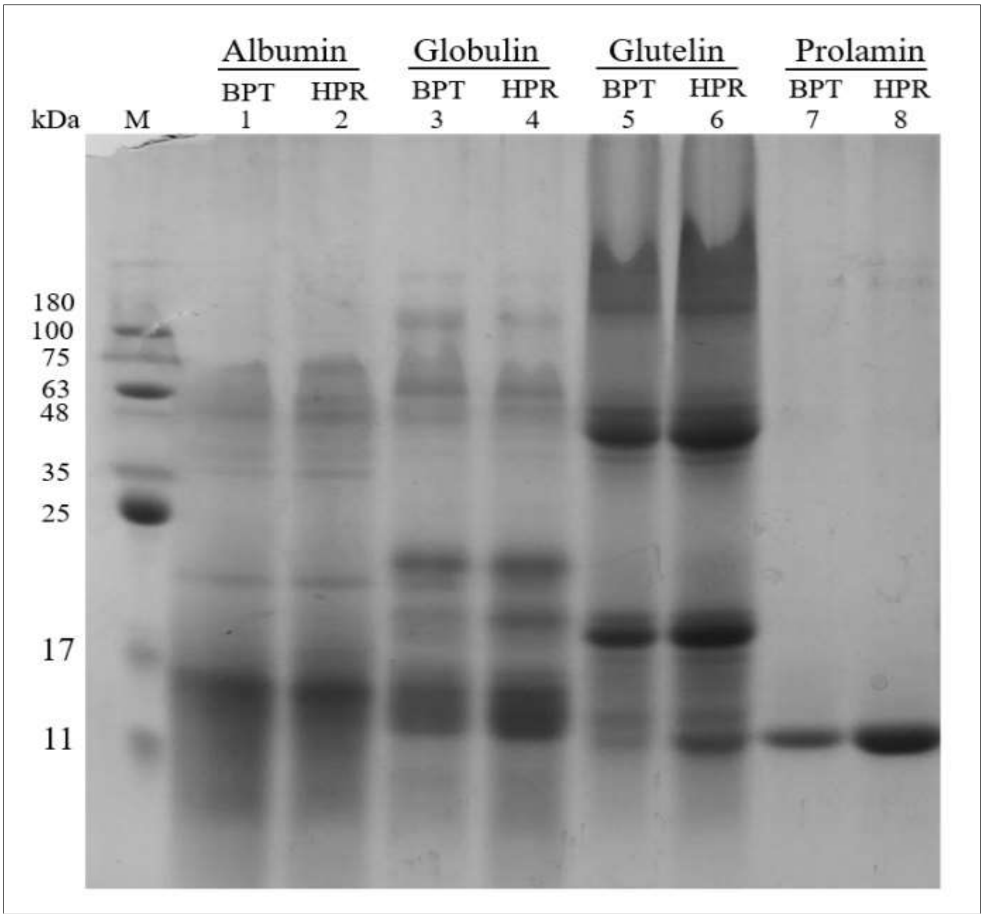


**Fig 2:** SDS-PAGE analysis of protein fractions of rice seed storage proteins of BPT-5204 and HPR-14 genotypes.

**Conclusion**

In conclusion, this study provided a comprehensive analysis of seed storage protein fractionation in two rice genotypes, BPT-5204 and HPR-14, shedding light on the distribution and characterization of albumin, globulin, glutelin, and prolamin fractions. Our findings underscore the significance of glutelin as the most abundant fraction in rice grains, followed by albumin, globulin, and prolamin. Despite employing different rice varieties, minimal variations were observed in protein banding patterns, indicating a consistent protein composition across genotypes. These results deepen our understanding of rice protein fractionation, laying the groundwork for future investigations into rice protein functionality and nutritional implications. Ultimately, this research contributes to the ongoing efforts aimed at optimizing rice protein utilization in diverse food and feed applications, potentially addressing global nutritional challenges, further techniques like LC-MS/MS is required to elucidate more about these proteins and their functions. Our study helps in preliminary understanding of the rice seed storage proteins in BPT-5204 and HPR-14 genotypes for the first time according to our knowledge.

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