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Studies on the Protein Status of White and Brown Rice Grain in Selected Varieties Cultivated in and around Tiruchirappalli District of Tamil Nadu

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

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ABSTRACT

A study was conducted to evaluate the Protein status of White and Brown Rice grain in selected varieties at Anbil Dharmalingam Agricultural College & Research Institute, Tiruchirappalli of Tamil Nadu, India during the period from June 2017 to May 2019. In the present study, sixteen different rice varieties cultivated in and around Tiruchirappalli district of Tamil Nadu as the test rice grains in terms of White and Brown rice in completely randomized design with three replications were tried. Screening and evaluation of protein content in 16 rice varieties were carried out to identify protein rich varieties. Biochemical analysis based on five different traits including contents of albumin(Alb), globulin(Glo), prolamin(Pro), glutelin(Glu) and total or gross grain storage protein (GGSP) were carried out. Results showed that the relative contribution of Albumin as 0.9 to 2.3 g/100 g, globulin as 0.67 to 2.3 g/100 g, prolamin as 0.28 to 2.73 g/100 g and glutelin as 2.0 to 6.18 g/100 g in Brown Rice; Albumin as 0.67 to 2.0 g/100 g, globulin as 0.652 to 2.0 g/100 g, prolamin as 0.20 to 2.3 g/100 g and glutelin as 1.684 to 5.258 g/100 g in White Rice. Results revealed a considerable variation also in gross grain protein contents among Brown and White rice of sixteen cultivars ranged from 5.087 to 9.644 g/100 g and 4.5 to 8.760 g/100 g respectively. Gross grain protein contents were higher in ASD-19, TKM (R) 12 and ADT 37 of Brown rice. Gross grain protein contents were higher in TKM (R) 12, ASD-19 and ADT-38 of White rice. The result on status of

protein in Brown rice showed that ADT-40 had the highest Albumin content. ADT 37 exhibited the highest globulin content. The lowest prolamin content was found in TKM (R) 12, whereas the highest content of glutelin was found in ASD-19. The result of status of protein in White rice showed that TKM (R) 12 had the highest Albumin content. ADT 37 exhibited the highest globulin content. The lowest prolamin content was found in Anna (R) 4, whereas the highest content of glutelin was found in ASD-19. The highest Prolamin to Glutelin ratio was recorded in TKM (R) 12, CR 1009 /Ponmani and Anna (R) 4 for Brown rice. The overall results of this study revealed that ASD-19, TKM (R) 12 and CR 1009 /Ponmani were considered as Top three genotypes suitable for Tiruchirappalli district farmers based on consumer preferences.

Keywords: Albumin; ASD-19; globulin; glutelin; Oryza sativa L.; prolamin; quality traits; rice; rice protein; storage proteins; TKM (R) 12.

1. INTRODUCTION

Rice is grown in more than 100 countries and thousands of varieties are there in rice. Most of the Indian consumers prefer rice as their major dietary source. The harvested form of Rice is called as paddy. Brown rice is considered as whole grain. Brown rice is obtained from paddy by the removal of husk or hull and retaining the bran. Bran is the hard part of cereal grain such as rice.

The bran constitutes nearly 8.5% of the total grain and is highly nutritious [1].

Rice bran is more popular when compare to bran of wheat, maize, oats and millet. In paddy, the vitamins and minerals are majorly distributed in bran portion only where as starch and proteins are distributed in endosperm and embryo portion [2]. Whole grain contains considered amount of fat. Bran contains considered amount of dietary fibers like beta-glucan and pectin which favours the value added products such as bread.

The amount of bran removed is called the degree of milling [3]. The amount of bran obtained by polishing the brown rice at Tiruchirappalli district is approximately 7.5 kg for every quintal. The main product of rice milling is Head rice [4]. Husk, bran and broken rice are the by-products of paddy milling.

Brown rice protein includes both white rice protein and rice bran protein. Milling of paddy yields 70% of rice endosperm as the major product and by-products consisting of 20% rice husk, 8% rice bran and 2% rice germ. Rice bran contains many micronutrients like oryzanol, tocopherol, phytosterol, 20% oil, 15% protein and 50% carbohydrate [5].

Rice bran oil is considered as the healthy food source for biologically significant components. The major fatty acid present in Rice bran oil is Oleic acid, a category of Monounsaturated fatty acid.

Deoiled rice bran contains high level 12%-15% of protein which has yet to be used to its full potential [6].

Pigments are located in the pericarp. Black or purple rice has more phenolic compounds than red rice. Non-pigmented brown rice has less phenolic compounds [7].

Rice polish is derived from the outer layers of the rice caryopsis during milling and consists of pericarp, seed coat, nucleus, aleurone layer, germ and part of sub- aleurone layer of starchy endosperm [8]. Rice polish is a by-product of rice milling industry and is the cheapest source of energy and protein for poultry feeding. It constitutes about 10% of paddy and is available in large quantities in major rice growing areas of the world [9].

Chanput et al. [10] reported that the highest percentage of the total protein in the protein fractions of rice bran is the glutelinfraction (22.7%), followed by the fraction of albumin (21.6%), globulin (17.4), and prolamin (8.1%). This difference may be due to differences in varieties of rice bran. According to Santos et al. [11], differences in rice varieties affect albumin and glutelin fractions.

Protein bodies do not have balanced amino acid composition and are deficient in few essential amino acids. The protein content and its amino acid composition are important traits for consumers to determine the nutritional quality [12].

Immuno blot analysis against sequentially polished rice flour fractions of seven japonica rice varieties revealed that GluA was strongly

localized in the outer region of the endosperm, including the subaleurone layer, whereas GluC was distributed throughout the endosperm [13]. Glutelin is the major rice storage protein, which accounts for 50% of the total seed protein content [13].

Total or Gross protein content is the sum of the Albumin, Globulin, Prolamin and Glutelin contents.

The present study was planned to investigate the rice nutritional quality. No study on status of individual protein content of Brown rice grain of these 16 varieties has been reported. The information available in the literature about fractional composition of storage proteins of rice varieties does not match with these varieties. Therefore, the study was carried out to examine varietal influence on content of composition of storage proteins in rice cultivars cultivated in and around Tiruchirappalli districtof Tamil Nadu. The present study deals with biochemical approaches of the concordant results of individual soluble proteins associated with changes in gross protein content of rice grain and loss of protein due to polishing.

2. MATERIALS AND METHODS

2.1 Materials

This research was conducted at the Anbil Dharmalingam Agricultural College & Research Institute, Tiruchirappalli-620 027, India during the period from June 2017 to May 2019. The Experimental site is geographically located at Altitude 85 MSL, Latitude 10°45' N and Longitude 78°36' E in the Manikandam block of Tiruchirappalli district. The materials used in this research were 16 rice varieties being cultivated in two consecutive seasons in and around Tiruchirappalli districts of TamilNadu as per the recommendations for cultivation practices.

50 g sample of each rice variety were procured from five different sampling stations. Damaged kernels and debris were not considered for observation. Many varieties are unique in their morphological characters of shape, size and colour.

2.2 Methods

2.2.1 Thousand grain weight

The thousand grain weight was determined by means of a digital electronic balance having an accuracy of 0.000 g. One thousand rice kernels were randomly selected from the bulk sample by taking different lot for 3 times and weighed separately.

2.3 Extraction of Soluble Proteins

2.3.1 Osborne extraction method (1907)

The varietal differences in Total protein contents are mostly contributed by quantitative variations of each fractional protein. Whole rice grain contains four types of proteins which can be isolated and characterized, mainly according to their solubility properties, using the Osborne extraction method with minor modifications. Rice seed storage proteins are grouped into four classes based on solubility properties like albumins (water soluble), globulins (salt soluble), prolamins (soluble in aqueous alcohol solutions), and glutelins (soluble in dilute acid or alkali) [14]. The fractional proteins are extracted sequentially.

2.4 Estimation of Protein (Lowry et al. [16])

Protein content of extracted concentrate was determined spectrophotometrically [16]. The amount of protein in sample was calculated from standard curve prepared simultaneously with bovine serum albumin (40-200 µg/ml) as standard.

2.5 Determination of Crude Protein Content (Micro-Kjeldahl Method)

The crude protein content of the fresh weight samples of brown rice was determined as percent total nitrogen by the Micro-Kjeldahl method. Protein percent was calculated by multiplying the percent nitrogen by the factor 6.25 [17].

2.6 Determination of Moisture Content

The percentage of moisture was measured by the methods of Association of Official Analytical Chemists Society [18].

2.7 Statistical Analysis

The experiments, quantification of protein fractions were performed in triplicate, using three independent replicates of each genotype in a completely randomized design. All results were expressed as the mean value. The data obtained were subjected to statistical scrutiny [19]. Wherever, the treat mental differences were significant, critical differences were worked out manually at five percent probability level.

Table 1. The protein and used solvent

SI. no.	Protein name	Solvent used
	Albumin	Double distilled water
	Globulin	1M NaCl
	Prolamin	70% ethanol
	Glutelin	1% Lactic acid containing 1 mmol/L Disodium EDTA. [15]

3. RESULTS AND DISCUSSION

3.1 Results

The results so obtained in both years were analyzed separately and are presented for the period of 2017-2018 since values obtained for the period of 2018-2019 followed a similar pattern. Most of the protein status such as Albumin, Globulin, Prolamin and Glutelin in 2018 followed a trend very similar to that of 2017.

3.2 Discussion

3.2.1 Rice bran

Rice bran is usually not consumed as food because of its high fiber content and possible hull contamination [20,21]. Rice bran is an undervalued by product of rice milling. It is rich in protein, lipids, dietary fibers, vitamins and minerals [22,21]. The composition of rice bran is 15-22% lipids, 34.1-52.3% carbohydrates, 7- 11.4% fiber, 6.6-9.9% ash, 8-12% moisture, and 10-16% highly nutritional protein [21,22,23].

3.2.2 Rice bran protein

The main part of protein source in rice grain is bran fraction. The total protein content in rice bran fraction ranges from 10% to 16% [24,25,26] depending on its cultivars. The predominant protein fraction of Rice bran is glutelin (46.82%) followed by albumins fraction (37.23%), globulin fractions (20.27%) and prolamin fraction (1.18%) [27].

3.2.3 Rice seed storage proteins

Rice seed storage proteins are synthesized on the rough endoplasmic reticulum (ER) and subsequently translocated into the ER lumen. Prolamin is stored in protein body type-I (PB-I), which are ER-derived spherical compartments. Glutelin and α-globulin are transferred to vacuoles via the Golgi apparatus [28,29,30,13] and stored in the irregularly shaped protein body type-II (PB-II) derived from protein storage vacuoles (PSVs) [31,32,13]. Glutelins are stored in the inner region of PB-II as crystalloids with

lattice structures and α-globulin is largely sequestered in the peripheral matrix surrounding crystalloid glutelins [33,34,35]. Glutelin polypeptide is cleaved into an N-terminal half (acidic subunit) and a C-terminal half (basic subunit) in the vacuole by an aspartic protease [36,37,13]. They are conjugated intra-molecularly and inter-molecularly by disulfide bonds to form a higher structural conformation [38,39,40,41,13] and they accumulate with α-globulinin PSVs. Glutelin is encoded by a multigene family. Glutelins consist of 12 full-length gene copies, which are classified into 4 subfamilies (GluA, GluB, GluC, and GluD) based on the similarity of their amino acid sequence [42,43,13]. Some authors have reported that Glutelins are localized in the alueronal and sub alueronal layers and starchy endosperm in developing rice seeds [44,45,46,42,13]. Glutelins are readily digestible by protease enzymes to give the end products of small peptides and individual amino acids [13].

3.2.4 White rice

The milling yield and quality of rice is dependent on the quality of the paddy, the milling equipment used and the skill of the mill operator. White rice grains used for cooking are usually polished by removal of approximately 5 or 8 or 10% of the rice bran. Rice is typically polished to make white rice for reasons of stability and consumer acceptability. The removal of the bran results in the loss of many prebiotic components and beneficial nutrients, including various polyphenols, essential fatty acids, and numerous antioxidants [47,48,49]. The polishing procedure decreases crude protein, fat, mineral and vitamin levels, while increasing the proportion of starch in rice [50]. In general, White rice is comprised of 77% carbohydrate and 7.7% total protein as mean value on Fresh weight basis. The ratio of 10:1 for carbohydrate and total protein is not applicable for all genotypes. Among the cultivars tested, 6.67% of Total protein was recorded as mean value for White rice.

3.2.5 Rice albumin

Wheat albumin (WA), which is a water-soluble protein found in wheat seeds, has hypoglycemic effects attributable to its α-Amylase Inhibition activity [51,52,53,54]. Rice Albumin did not inhibits mammalian α-amylase but has similar function to dietary fibers by suppressing the absorption from the small intestine [54]. Rice Albumin could be useful in diabetes treatment by preventing the absorption of saccharides from the gut, and may effectively suppress hyperglycemia when starch-based and even glucose-based foods are ingested [54].

3.2.6 Rice globulin

Rice globulins consist of α, β, γ and δ-globulins with apparent MWs of 25, 15, 200KDa and higher respectively [55]. Rice Globulin is saltsoluble and contains different polypeptide chains which were stabilized by disulfide linkages [56].

3.2.7 Rice prolamin

Most cereal grains synthesize Prolamin as a major storage protein in their endosperm tissue but in rice it is not so. Prolamin was the most evenly distributed protein of the rice grain. Prolamins are soluble in aqueous alcohol solution. Differences in rice varieties affect prolamin fractions. Prolamin contributes about 3.24-11.6% of Total protein in rice bran [25]. Rice prolamins consist of 60% Cys-rich prolamins and 40% Cys-poor prolamins [57,58].

3.2.8 Rice glutelin

In general, Glutelin was the major or principal protein of whole grain, rice bran and milled rice. Differences in rice varieties affect glutelin fractions.

3.2.9 Grain protein versus plasma protein

Human Serum Albumin (HSA) is a soluble, globular and unglycosylated monomeric protein, it functions primarily as a carrier protein for steroids, fatty acids and thyroid hormones and stabilizes extracellular fluid volume [59]. Some grain proteins are very potent to regenerate plasma proteins. Chronic or acute infection can profoundly influence the production of plasma protein in the body. Myers and keefer [60] have shown low plasma protein levels and inverted albumin-globulin ratio during cirrhosis condition. McKenzie and Elliott [61] reported that surgical infections and stress caused the drain of body proteins and it was controlled by diet and therapy.

Table 2. Biometric observations of rice grain of 16 different varieties [FW]

Where, SEm: Standard Error Mean, SEd: Standard Error deviation, CD=Critical difference. The percentage of husk obtained ranged between 15 and 24

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Table 3. Quantification of storage proteins in brown rice [FW] by Lowry's method

Where, SEm: Standard Error Mean, SEd: Standard Error deviation, CD=Critical difference.

Significant differences were found for protein fractions of selected sixteen genotypes

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Table 4. Quantification of storage proteins in white rice [FW] by Lowry's method

Where, SEm: Standard Error Mean, SEd: Standard Error deviation, CD=Critical difference

Table 5. Protein loss due to polishing

Table 6. Determination of crude protein content in brown rice by Micro-Kjeldahl method

Where, SEm: Standard Error Mean, SEd: Standard Error deviation, CD=Critical difference

3.2.10 Factors affecting contents of soluble proteins of rice grain

Factors influencing on quantitative variation of fractional protein and Gross grain protein content are genotypes, nitrogen uptake ability and grain protein accumulation ability [62]. The wide range of protein content of rice and its fractions is primarily due to such factors as variety, environment, crop season or planting date,

differences in extraction procedures, and nitrogen fertilization [63]. The variations of protein content in different rice accessions might be due to several factors such as water supply, handling, application of fertilizer (soil nitrogen availability), environmental stress (such as salinity and alkalinity, temperatures and diseases), location of growing areas, growing conditions and time which tend to increase the grain protein content [64]. The quantitative

variation of soluble fractional protein of brown rice grain is also influenced by analytical techniques involved in extraction and estimation methods. The differences in quantification of rice proteins between the research groups might be due to the differences in varieties used.

3.2.11 Description

There was a considerable variation in total protein content in brown rice and white rice of the selected 16 cultivars.

Proteins can be classified based on their Total or Gross content as very high (10 to 12%), High (8 to 9.9%), Moderate (6 to 7.9%) and Low (5 to 5.9%) at fresh weight basis for Brown rice [65]. In this study, none of the variety lies under the category of Very high. The Total protein content in brown rice of ten rice genotypes was high. Three genotypes of Brown rice come under the category of moderate and another three genotypes of Brown rice recorded under the low category at fresh weight basis.

3.2.12 In general

In general, the range of Total protein content in Brown rice grain is 5 to 12% on fresh weight basis. Most of the varieties cultivated in Tamil Nadu state of India lies between 7 and 10% of gross grain protein content of Brown rice [65]. The range of Total protein content in White rice is 4 to 10.4% on fresh weight basis. The proportion of each protein fraction will also vary in different rice cultivars. Glutelin is the most abundant protein and makes up between 21 and 66% of Total protein content in Brown rice on fresh weight basis. Globulin is the second most abundant seed storage protein making up 6 to 42% of the Total Protein content of Brown rice on fresh weight basis. The other Protein components Albumin (5 to 32%) and Prolamin (0.77 to 37%) make up the remaining in Brown rice on fresh weight basis. The difference in Protein content between Brown rice and white rice will range from 6 to 30% with respect to Total protein content.

3.2.13 In present study

The protein content of Brown rice ranged from 5 to 9.64% while that of white rice was from 4.65% to 8.76% (Tables 3, 4). Among brown rice varieties, ASD-19 showed the highest content of Protein (9.644%) followed by TKM (R) 12 (9.6%) and ADT-37(9.22%). Among white rice varieties,

TKM (R) 12 (8.76%) was the richest followed by ASD-19 (8.1%) and ADT-38 (7.87%). The protein loss due to polishing ranged from 0.435 g/100 g rice to 1.9 g/100 g rice. i.e. the difference in protein content between Brown rice and white rice ranged from 8.55 to 29.68% with respect to Total protein content. The protein of White rice was decreased about 20 to 29.7% in three varieties after the milling process while that was 10 to 19.9% in ten varieties and 8.5 to 9.9% in another three varieties. This implies that some rice proteins were lost due to polishing and the quantity of protein lost is dependent on the genotype.

The major storage protein of rice grain is the glutelin which recorded from 32.39% to 64.12% of the Total grain protein content of Brown rice at fresh weight basis. In contrast, the aqueous alcohol soluble prolamins (dominant storage protein in other cereals) compose from 2.91% to 31.7% of the Total grain protein content (BR) at fresh weight basis. The salt soluble globulin occupies at the range from 6.93% to 30.65% of the Total grain protein content (BR) at fresh weight basis. The water soluble Albumin contributes at the range from 10.83 to 28.36% of the Total grain protein content (BR) at fresh weight basis of selected genotypes considered for analysis.

In Brown rice varieties, Albumin content (2.3 g/100 g) was highest in cultivar ADT-40, Globulin content (2.3 g/100 g) was highest in cultivar ADT-37, Prolamin content (0.28 g/100 g) recorded as lowest in cultivar TKM (R) 12, Glutelin content (6.184 g/100 g) recorded as highest in cultivar ASD-19. The ratio of Prolamin to Glutelin in studied cultivars vary from 1:1.13 to 1:19.10.

In Brown rice, the contents of Glutelin are similar between ADT-37and TNAU Rice ADT-50; ADT-38 and TRY-3; ASD 20 and IR 50. The contents of Prolamin are similar between ADT-40 and CR 1009 /Ponmani; ADT(R)-46 and IR 50. The contents of Globulin is similar between TKM 10 and ADT(R)-46; ADT-40 and Swarna sub1; TKM (R) 12 and Anna (R) 4. The contents of Albumin is similar between ASD-19 and TNAU Rice ADT-50; ASD 20 and PMK (R) 3; Anna (R) 4 and IR 50. We can find similarity in individual protein content between some varieties inspite of variation in other protein parameters.

In White rice varieties, Albumin content (2.06 g/100 g) was highest in cultivar TKM (R) 12,

Globulin content (2.0 g/100 g) was highest in cultivar ADT-37, Prolamin content (0.20 g/100 g) recorded as lowest in cultivar Anna (R) 4, Glutelin content (5.26 g/100 g) recorded as highest in cultivar ASD-19. The ratio of Prolamin to Glutelin in studied cultivars vary from 1:1.24 to 1:20.46.

In White rice, the contents of Glutelin are similar between ADT-37 and TKM 10; ADT-38 and ADT-44. The contents of Prolamin are similar between ASD-19 and ADT(R)-46; ADT-37 and CR 1009. The contents of Albumin is similar between ASD-19 and ADT-37; ADT-38 and CR 1009; ADT-40, TNAU Rice ADT-50 and Swarna sub1.

Ratio of Prolamin to Glutelin: Among the cultivars tested, Ratio of Prolamin to Glutelin is high in TKM (R) 12 (1:19.1) and CR 1009 /Ponmani (1:6.65).

The variation in Total Protein Content observed between brown and white rice is because of bran portion, which is higher in protein and significantly increase the protein content of brown rice as reported earlier [66,67,68].

Non-Protein Nitrogenous compounds: There was a considerable variation in results obtained between Micro-kjeldahl method and Lowry's method because of the presence of Non-Protein Nitrogenous compounds in Brown rice of sixteen genotypes and ranged between 0.39% and 0.93%.

Compensation mechanism of seed storage protein: Compensation for reduced Seed Storage Proteins by increasing other SSPs is primarily regulated at the transcriptional level. Sulfur-containing amino acids are involved in regulating SSP composition [35].

3.2.14 Loss of SSP is compensated by other SSPs [35]

- Cereal Seed Storage Proteins are encoded by multigenes.
- \triangleright Reduction of any individual Seed Storage Protein content will be compensated by the increase in rest of the Seed Storage Protein content. This was the compensation mechanism behind of SSPs.
- \triangleright There may be a regulatory mechanism behind of maintaining the total nitrogen content or total amino acid content.
- \triangleright SSPs are mainly regulated at the transcriptional or post transcriptional level.

 \triangleright The availability of sulphur may determine which prolamin, sulphur rich or sulphur poor, should be newly synthesised as a storage reserve to maintain the appropriate amino acid balance.

4. CONCLUSION

Brown rice is better than White rice for cooking and human consumption but in general, Rice consumers prefer white rice despite the valuable nutrient content of brown rice which is lost when bran is removed while polishing. Awareness on nutritive value of individual rice genotypes is important to screen for consumption in daily diet. Protein is the one of the Biochemical key factor influencing the eating quality of rice. Rice seed storage protein has important role in human nutrition. The nutritional quality oriented attributes studied in this research will provide vital information for Tiruchirappalli district farmers and consumers of Tamil Nadu. The data's investigated in this study will be useful for breeders to improve the grain nutritional quality in new cultivars. The overall results of this study revealed that ASD-19, TKM (R) 12 and CR 1009 /Ponmani were considered as Top three genotypes suitable for Tiruchirappalli district farmers based on consumer preferences. The studied quality oriented nutritional attributes were found to be competent with rice genotypes available in India and may be upgraded as export quality rice in International market.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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