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Protein status of brown rice grain in different genotypes cultivated in Thoothukudi district of Tamil Nadu, India

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Abstract
A study was conducted to evaluate the Protein status of Brown Rice grain in different genotypes at Agricultural College & Research Institute, Thoothukudi district of Tamil Nadu, India during the period from April 2015 to March 2017. In the present study, twenty different rice varieties inclusive of two traditional cultivars cultivated in and around Thoothukudi district of Tamil Nadu as the test rice grains in the form of Brown rice in completely randomized design with three replications were tried. Screening and evaluation of protein content in 20 rice genotypes were carried out to identify protein rich genotypes. Biochemical research 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. I estimated the relative contribution of Albumin as 0.47 to 1.8 g/100g, globulin as 1.3 to 4.0 g/100g, prolamin as 0.24 to 3.16 g/100g and glutelin as 1.63 to 5.1 g/100g. There was a considerable variation in Gross grain protein contents among Brown rice of twenty cultivars ranged from 6.9 to 9.9 g/100g. Gross grain protein contents were higher in Purple puttu, ASD 16 and ADT 45. The result on status of protein showed that CO (R) 48 had the highest Albumin content. ASD 16 exhibited the highest globulin content. The lowest prolamin content was found in ADT 39, whereas the highest content of glutelin was found in Purple puttu. The overall results of this study revealed that Purple puttu, ADT 39 and ASD 18 were considered as Top three genotypes suitable for Thoothukudi district farmers based on consumer preferences.

Keywords: Albumin, ADT 39, globulin, glutelin, *Oryza sativa* L., prolamin, protein distribution, purple puttu, quality traits, rice cultivars, rice protein, storage proteins

Introduction
Rice is the basic cereal crop for half of the world population. Starch molecule belong to the category of carbohydrates is the major constitute of rice grain. Protein is the second vital component of rice. Rice grain contains considered amount of fat also. Grains may have similar size and shape but differ in amylose or protein content.

Most of the human population depends on rice grain after pulses for the purpose of protein nutrition. Nutritional values of rice vary with different varieties, soil fertility, fertiliser application and other environmental conditions (Deepak kumar verma *et al.*, 2017) [9]. The capability of synthesising and storing the particular protein is based on the genes of the concerned cultivar.

Determining the relative proportion or content of protein is an important trait in determining the nutritional quality of a particular dietary source. However the biological utilisation of a protein is primarily dependent on its digestibility by gastric, pancreatic and intestinal peptidases, the true utilisation can be measured only by *in vivo* digestion (Donald F. Steenson *et al.* 1995) [10]

The classical fraction procedure of Osborne has been used for years to divide cereal proteins into four major groups based on their solubilities. According to Osborne, albumins are soluble in water, globulins are soluble in dilute salt solutions but not water, prolamins are soluble in aqueous alcohols but not water or salt solutions and glutelins are soluble in acid or alkali but not alcohol, water or salt solutions. However, large differences in estimating the relative proportion of proteins or the four major solubility fractions, are found in different cereal species and even within the same species when analysed by different researchers (Meesook Kim *et al.*, 2007) [30].

An effective extraction and estimation procedure is must for accurate assessment of protein content of a particular source. The important quality index has not yet been systematically determined. The relative quantities of each protein fractions are affected by cultivated conditions, genotypes and the analytical methods employed (Heubner *et al.* 1990; Krishnan and white 1995) [14, 25].

Total or Gross protein content is the sum of the Albumin, Globulin, Prolamin and Glutelin contents. Grain protein content is also affected by planting density and weed control (De data *et al.*, 1972).

Rice seed storage protein accumulates in a special organelle called the protein body (PB). Two types of protein bodies exist in rice endosperm (Bechtel and Juliano 1980; Tanaka *et al.* 1980) [2, 42]. Type-I protein body is called as PB-I and Type-II protein body is called as PB-II. PB-I is meant for prolamins and PB-II is meant for globulins and glutelins (Yamaga *et al.* 1982) [48]. The cereal prolamins are broadly classified into (cysteine) cys-poor and cys-rich species (Shewry and Tacham 1999) [37]. Based on amino acid sequence similarity, glutelin are classified into four subfamilies: Glu A, Glu B, Glu C and Glu D (Kawakatsu *et al.*, 2008) [21].

Rice grain contains three portions. Namely, hull or husk, bran and white rice. Hull portion is not suitable for human consumption, bran is meant for protein and antioxidant compounds. White rice is commonly eaten by humans.

The objective of this research was to determine the rice nutritional quality. No study on status of individual protein content of Brown rice grain of these 20 varieties has been reported. The information available in the literature about fractional composition of storage proteins of rice varieties does not match with the preliminary investigation of these varieties in hundred per cent. Therefore, the study was carried out to examine varietal influence on content of composition of storage proteins in rice cultivars of Tamil Nadu. The present study deals with changes in levels of individual soluble proteins associated with changes in gross protein content of rice grain.

Materials and Methods

Materials

This research was conducted at the Agricultural College & Research Institute, Killikulam-628 252, India during the period from April 2015 to March 2017. The materials used in this research were 20 rice cultivars being cultivated in Thoothukudi, Tirunelveli and Kanyakumari districts of Tamil Nadu. 50g 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.

Methods

Extraction

Osborne extraction method (1907)

The varietal differences in 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) (Osborne, 1907) [35]. The fractional proteins are extracted sequentially.

Table 1: The protein and used solvent

Sl. No.	Protein name	Solvent used
1	Albumin	Double distilled water
2	Globulin	1M NaCl
3	Prolamin	70% ethanol
4	Glutelin	1% Lactic acid containing 1mmol/L Disodium EDTA. (Jiang *et al.*, 2014)

Estimation of Protein (Lowry *et al.* 1951) [27].

Concentration of Protein was determined spectrophotometrically at 550nm by Lowry's method using Bovine serum Albumin as a standard.

Statistical Analysis

The experiment was carried out in a completely randomized design. All results were expressed as the mean value. The data obtained were subjected to statistical scrutiny for the parameter under study. The level of significance was considered at $P < 0.05$.

Results and Discussion

Results

The results so obtained in both years were analyzed separately and are presented for the period of April 2015 to March 2017 since values obtained for the period of April 2015 to March 2017 followed a similar pattern. Most of the protein status such as Albumin, Globulin, Prolamin and Glutelin in 2016 followed a trend very similar to that of 2015.

Table 2: Quantification of storage proteins of Brown Rice in selected varieties/hybrid on fresh weight basis.

Sl. No.	Variety/Hybrid	Albumin [g/100g]	Globulin [g/100g]	Prolamin [g/100g]	Glutelin [g/100g]	GGPC [Gross Grain Protein Content] [g/100g]
1	White ponni	0.951	1.500	0.665	3.783	6.899
2	TRY 1	1.100	2.693	0.897	3.938	8.628
3	MDU 5	1.279	1.500	1.050	4.171	8.000
4	ASD 16	0.973	4.025	0.546	4.057	9.601
5	ASD 18	1.585	1.650	0.278	3.935	7.448
6	ADT 36	1.500	2.700	1.056	3.675	8.931
7	ADT 39	1.546	2.533	0.243	4.500	8.822
8	ADT 43	1.227	1.313	2.400	3.583	8.523
9	ADT 45	1.500	2.700	1.200	4.200	9.600
10	ADT 47	0.473	2.066	1.820	4.367	8.726
11	ADT 49	0.800	1.783	2.200	4.075	8.858
12	CO 43	1.250	2.525	2.067	2.850	8.692
13	CO 47	0.700	2.150	2.600	1.633	7.083
14	CO (R) 48	1.800	2.400	1.024	2.495	7.719

15	CO (R) 49	1.417	2.133	1.080	2.858	7.488
16	CO (R) 50	0.700	3.750	1.667	3.017	9.134
17	CO RH3	0.684	3.525	3.162	2.013	9.384
18	CO RH4	0.592	2.712	1.628	4.500	9.432
19	Purple puttu	1.165	3.034	0.600	5.104	9.903
20	Mapillai samba	1.183	2.167	1.876	3.774	9.000
Statistical analysis	SEm	0.032	0.063	0.043	0.073	
	SEd	0.046	0.089	0.061	0.104	
	CD 1%	0.122	0.238	0.163	0.276	
	CD 5%	0.092	0.179	0.123	0.208	

Where: SEm: Standard Error mean, SEd: Standard Error deviation, CD: Critical Difference.

Table 3: Relative contribution of Fractional Proteins in terms of percentage and ratio of Prolamin to Glutelin

Sl. No.	Variety/Hybrid	Albumin [%]	Globulin [%]	Prolamin [%]	Glutelin [%]	Ratio of Prolamin to Glutelin
1	White ponni	13.78	21.74	9.64	54.83	5.68
2	TRY 1	12.74	31.21	10.39	45.64	4.39
3	MDU 5	15.98	18.75	13.12	52.13	3.97
4	ASD 16	10.13	41.92	5.68	42.25	7.43
5	ASD 18	21.28	22.15	3.73	52.83	14.16
6	ADT 36	16.79	30.23	11.82	41.14	3.48
7	ADT 39	17.52	28.71	2.75	51.00	18.54
8	ADT 43	14.39	15.40	28.16	42.04	1.49
9	ADT 45	15.62	28.12	12.50	43.75	3.50
10	ADT 47	5.42	23.67	20.85	50.04	2.40
11	ADT 49	9.03	20.12	24.83	46.00	1.85
12	CO 43	14.38	29.04	23.78	32.78	1.38
13	CO 47	9.90	30.35	36.70	23.05	0.63
14	CO (R) 48	23.31	31.09	13.26	32.32	2.43
15	CO (R) 49	18.92	28.48	14.42	38.16	2.64
16	CO (R) 50	7.66	41.05	18.25	33.03	1.80
17	CO RH3	7.29	37.56	33.69	21.45	0.63
18	CO RH4	6.27	28.75	17.26	47.70	2.76
19	Purple puttu	11.76	30.63	6.05	51.54	8.52
20	Mapillai samba	13.14	24.07	20.84	41.93	2.01

Discussion

Protein

Over 90% of starch and protein are considered to be accumulated simultaneously within two weeks in rice grains (Youichi Ohdaira *et al.* 2011) [34]. Tanaka *et al.* (1995) [43] reported that starch and protein were accumulated rapidly from 5-6 DAF to 18 DAF and from 7 DAF to 20 DAF respectively, based on the temporal component analysis of rice during ripening.

Storage protein itself plays an important role in formation of normal protein body (PB). Both glutelins and globulins accumulate in PB-II, but the influence of the change in their composition on PB-II structure would be different (Kanae Ashida *et al.*, 2011) [20]. PB-I shows the same structure as the indigestible remains of rice in human feces (Tanaka *et al.* 1975) [41].

The protein quality of rice depends on the composition of amino acids (FAO, 1970) [11]. Rice protein is superior because of its unique composition of amino acids and has a special benefit because eight of the essential amino acids are found in delicately balanced proportions (Ahmed *et al.*, 1998) [1]. The amino acid, Lysine is considered to be important for the digestibility of food and for its nutritional quality (Huebner *et al.*, 1990) [14].

Economically poor people depend on rice oriented food. Dietary allowance of protein per day can be recommended as 1g per kilogram of body weight for the young physically working people. Improvement of seed protein content of rice will have positive impact on financially poor people. Resurrection *et al.* (1979) [36] classified protein content more than 10% as high content. Rice with high protein content pre-supposes high gelatinization temperature and tends to be undercooked (Juliano, 1985) [18].

The proportion of each protein differs in different rice cultivars. The protein content of bran fraction of rice grain itself ranges from 10% to 16% depending on its cultivars (Kulp and Ponte, 2000) [26].

Variations in protein content and composition in rice germplasms are valuable resources for the development of rice varieties with preferable protein contents and compositions for specific purposes (Jin-Woong Kim 2013) [17].

Rice Albumin

Albumin from Brown rice grain accounts for about 5.42 to 23.31% of the total grain protein content. Albumins have highest biological value being most readily absorbed and utilized by the body (Mawal *et al.*, 1987) [29]. Mawal *et al.* (1987) [29] identified a 60-K Da glycoprotein as one of the major constituents of rice albumin. According to Hamada (1997) [13], albumins have Mw of 100 KDa or less from rice bran protein. Like other albumins, Rice Albumin are coagulated by heating and are readily soluble in water due to the presence of sufficient net charge and the lack of any extensive disulfide cross-linking or aggregation (Hamada, 1997; Shewry and Casey 1999) [13, 37]. Wei *et al.* (2007) [46] stated that 16-K Da rice albumin had antioxidant activity preventing Cu2+ induced Low-Density Lipoprotein (LDL) oxidation similar to serum albumins. N-terminal aminoacid sequences of rice albumin and serum albumin are homologous (Nakase *et al.*, 1996) [32].

Rice Globulin

Globulin, the second major storage protein fraction in brown rice grain, accounts for 15.4% to 42% of the Total grain protein content. The embryo and outer aleurone layer of the endosperm contain globulin (Wallace and Kriz, 1991) [45]. Alpha-Globulin is localised mostly at the peripheral matrix and surrounds glutelins. Globulins are readily soluble in dilute salt solution. Rice globulin contains different polypeptide chains which were stabilized by disulfide linkages (Hamada, 1997 and Phongthai *et al.*, 2017) [13, 35]. Globulin contains high levels of sulphur-containing amino acids, cysteine and methionine (Krishnan *et al.*, 1992) [24]. Globulin structure is influenced by pH and Ionic strength. Oral administration of rice alpha-Globulin (100mg per day per kg body weight) reduced the atherosclerosis in mice without any alteration of the plasma lipid levels (L. T. Tong *et al.* 2012) [28]. According to Wei *et al.* (2007) [46], globulin fraction from rice grain did not have antioxidant property unless when it is hydrolysed enzymatically by pepsin. Three globulin genes have been reported. The major globulin gene, Glb-1, encodes the endosperm specific 26-K Da α-globulin (Nakase *et al.*, 1966) [32]. The other two globulin genes are REG1 (Rice embryo globulin-1) and REG2 (Rice embryo globulin-2) which encode the embryo-specific 49-K Da globulin, respectively (Sun *et al.*, 1996) [38].

Rice Prolamin

Rice prolamin is relatively low in proline but high in glutamic acid content compared with other cereal prolamins (Waldschmidt-Lietz.E, 1963) [44]. The major amino acid present in prolamins is glutamic acid or glutamine. Nutritional value of prolamin is inferior to glutelin for its low digestibility by human and lower lysine content (Ogawa *et al.*, 1987, Tanaka, 1980) [33, 42]. Prolamin could affect negatively on the quality of cooked rice as prolamin increases the hardness of cooked rice (Furukawa *et al.*, 2003) [12]. Prolamin possesses pharmacological activities such as effective activation of human anti-leukaemia immunity (Chen *et al.*, 2010) [7].

Relative contribution of rice prolamin to the total protein content of rice grain has been underestimated in most of the previous studies. Some scientists have overestimated also. The general factors interferes with the estimation of Prolamins are phenolic compounds and the prolamin itself if it is denatured.

In CO RH3 hybrid, Prolamin occupies one third of the total protein in brown rice grain and In CO 47 variety, Prolamin occupies 36% of the total protein in brown rice grain.

In cereals alcohol soluble Prolamin proteins accumulate during endosperm development and serve as a source of nitrogen, carbon and sulfur for the young developing seedling (Kreis *et al.* 1985) [22].

Rice Glutelin

Glutelin is the major fraction of rice protein and its content ranges about 22.7-40.25% of the total protein in rice bran (Cao *et al.*, 2009; Chanput *et al.*, 2009) [4-5]. Glutelin is soluble below pH 3 and above pH 10 (Juliano, 1985) [18]. Glutelin has the highest molecular weight (MW) among the rice protein fractions (Houston, 1972) [15]. Rice glutelin was composed of acidic subunits (30-39 kDa) and basic subunits (19-25 kDa) which came from a 57 kDa polypeptide precursor. Two subunits were covalently linked to each other by an intermolecular disulfide bond resulting in glutelin molecules with MWs ranging from 64 to 500 kDa (Lasztity, 1996; Cao *et al.*, 2009) [26, 4]. Hamada (1997) [13] found that rice glutelin composed of high MW protein ranging from 45 to 150 kDa; while, Chanput *et al.* (2009) [5] and Xia *et al.* (2012) [47] observed MWs of glutelin at 10-60 kDa. (Phongthai *et al.*, 2017) [35]. Fifteen glutelin genes exist in the rice genome and can be classified into four groups (GluA, GluB, GluC and GluD) (Kawakatsu *et al.*, 2008) [21].

High glutelin containing rice cultivars such as purple puttu, CORH4, ADT 39 and ADT 47 are nutritionally more advantageous for human consumption. Glutelin is the most easily to digest and contains high lysine (Tanaka *et al.*, 1995) [43].

Low ratio of digestible protein, glutelin to total grain protein content and on the other hand, low level of glutelin and high level of prolamin containing rice grains and low glutelin rice cultivars such as CO 47, CORH3 & CO43 can be recommended for patients with abnormal functions of kidney(Mochizuki and Hara 2000) [31].

Relative contribution of rice glutelin to the total protein content of rice grain has been overestimated in most of the previous studies.

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 (Tadashi Tsukaguchi *et al.* 2016) [39]. 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 (Chavan *et al.*, 1978) [6]. 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 (Buresova *et al.*, 2010) [3]. 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.

Previous findings were not satisfactory. These results suggest that Glutelin levels have been overestimated and albumin levels have been underestimated in the endosperm portion of rice grain and these values have been found and followed for long period in the literature for the classes of rice seed storage proteins.

Description

There was a considerable variation in total protein content in brown rice of the selected 20 cultivars.

I can propose a classification method for protein 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. None of the variety/hybrid lies under the category of Very high or low among the cultivars tested in this study. Fifteen genotypes come under the category of high protein and five genotypes come under the category of moderate.

The major storage protein of rice grain is the glutelin which constitute from 21.45% to 54.83% of the gross grain protein content. In contrast, the aqueous alcohol soluble prolamins (dominant storage protein in other cereals) compose from 2.75% to 36.7% of the gross grain protein content. The salt soluble globulins occupies at the range from 15.4% to 41.92% of the gross grain protein content. The water soluble Albumin contributes at the range from 5.42% to 23.3% of the total grain protein content of brown rice of selected genotypes considered for analysis. In general, the range of gross 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. There was a considerable variation in Gross grain protein contents among Brown rice of twenty cultivars ranged from 6.9 to 9.9 g/100g.Among the cultivars, total protein content was highest in cultivar purple puttu (9.9%) followed by ASD 16 & ADT 45(9.6%). The cultivars like White ponni, CO 47 & CO (R) 49 showed the moderate levels of Total protein. Albumin content (1.8 g/100g) was highest in cultivar CO (R) 48, Globulin content (4g/100g) was highest in cultivar ASD 16, Prolamin content (0.243g/100g) recorded as lowest in cultivar ADT 39, Glutelin content (5.104g/100g) recorded as highest in cultivar purple puttu. The ratio of prolamin to Glutelin in studied cultivars vary from 0.63 to 18.54

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 (Taiji kawakatsu *et al.* 2010) [40].

Loss of SSP is compensated by other SSPs (Taiji Kawakatsu *et al.*, 2010) [40]

- Cereal Seed Storage Proteins are encoded by multigenes.
- 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.
- There may be a regulatory mechanism behind of maintaining the total nitrogen content or total amino acid content.
- SSPs are mainly regulated at the transcriptional or post transcriptional level.
- 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.

Conclusion

Consumer interest is increased on health benefits of any food source which they consume. Rice available in thousands of cultivars that vary greatly in their attributes including Total grain protein content and protein fractions. Total grain protein content and each protein fractions are important traits for rice to breed new improved cultivars. Research about storage proteins of cereal like rice continues to expand. The present study suggests that primary importance should be given while selecting a variety to increase the protein content with good nutritional quality. The overall results of this study revealed that Purple puttu, ADT 39 and ASD 18 were considered as Top three genotypes suitable for Thoothukudi district farmers based on consumer preferences. Further, More attention can be given to the genotypes like Purple puttu, ADT 39 and ASD 18 for Rice breeding programs to increase grain nutritional quality. The Rice variety, ASD 16 can be recommended for Atherosclerosis patients. ADT 39 and ASD 18 can be recommended for people having indigestion problems with rice. The provided Biochemical information about protein status of Brown rice of various genotypes may be useful for Rice-recombinant engineers, Thoothukudi district Farmers and Consumers of Tamil Nadu.

References

1. Ahmed SA, Barua L, Das D. Chemical composition of scented rice. Oryza. 1998; 35(2):167-169.
2. Bechtel DB, Juliano BO. Formation of protein bodies in the starchy endosperm of rice (*Oryza sativa* L) – a reinvestigation. Ann. Bot. (Lond). 1980; 45:503.
3. Buresova I, Sedlackova I, Famera O, Lipavsky J. Effect of growing conditions on starch and protein content in triticale grain and amylose content in starch. Plant Soil Environ. 2010; 56:99-104.
4. Cao X, Wen H, Li C, Gu Z. Differences in functional properties and biochemical characteristics of Congenetic rice proteins. Journal of Cereal Science. 2009; 50:184-189.
5. Chanput W, Theerakulkait C, Nakai S. Antioxidative properties of partially purified barley Hordein, rice bran protein fractions and their hydrolysates. Cereal Science. 2009; 49:422-428.
6. Chavan JK, Duggal SK. Studies on the essential amino acid composition, protein fractions and biological value (BV) of some new varieties of rice. J Sci. Food Agric. 1978; 29:225-229.
7. Chen Y, Chen Y, Wu C, Yu C, Liao H. Prolamin, a rice protein, augments anti-leukaemia immune response. J Cereal Sci. 2010; 51:189-197.
8. De Datta, Obcemea SK, Jana WNRK. Protein content of rice grain as affected by nitrogen fertilizer and some triazines and substituted ureas. Agron. J. 1972; 64:785-788.
9. Deepak Kumar Verma, Prem Prakash Srivastav. Proximate composition, Mineral content and fatty acids Analyses of Aromatic and Non-Aromatic Indian rice available online, 2017. at www.sciencedirect.com
10. Donald Steenson F, Shridhar Sathe K. Characterization and Digestibility of Basmati Rice (*Oryza sativa* L. var. Dehraduni) storage proteins. Cereal chem. 1995; 72(3):275-280.
11. FAO. Amino acid content of Foods and Biological data on proteins. Rome Nutrition Division: FAO, 1970, 122.
12. Furukawa S, Mizuma T, Kiyokawa Y, Masumura T. Tanaka K, Wakai Y. Distribution of storage proteins in low-glutelin rice seed determined using a fluorescent antibody. J. Biosci. Bioengin. 2003; 96:467-473.
13. Hamada JS. Characterization of protein fractions of rice bran to devise effective methods of protein solubilization. Cereal Chemistry. 1997; 74:662-668.
14. Huebner F, Bietz J, Webb B, Juliano B. Rice cultivar identification by high-performance liquid chromatography of endosperm proteins. Cereal Chem. 1990; 67:129-135.
15. Houston DF. (Eds). Rice Chemistry and Technology (Volume IV). Minnesota: American Association of Cereal Chemists, Inc, 1972.
16. Jiang *et al.* Proteomic analysis of seed storage proteins in wild rice species of the *Oryza genus*. Proteome Science. 2014; 12:51.
17. Jin-Woong Kim, Byung-Chul Kim, Jae-Heung Lee, Duck-Ryul Lee, Shafiq Rehman, Song Joong Yun. Protein content and Composition of Waxy grains. Pak. J Bot. 2013; 45(1):151-156.

18. Juliano BO. Factors affecting nutritional properties of rice protein. Trans Nat Acad Sci. Technol. 1985; 7:205-216.
19. Juliano BO. Rice: chemistry and technology (2nd Ed) Minnesota: American Association of Cereal Chemists, 1985, 774.
20. Kanae Ashida, Yuhi Saito, Takehiro Masumura, Shuichi Iida. Breeding Science. 2011; 61:201-207.
21. Kawakatsu T, Yamamoto MP, Hirose S, Yano M, Takaiwa F. Characterization of a new rice glutelin gene GluD-1 expressed in the starchy endosperm. J Exp. Bot. 2008; 59:4233-4245.
22. Kreis M, PR Shewry, BG Forde, J Forde, BJ Miflin structure and evolution of seed storage proteins and their genes with particular reference to those of wheat, barley and rye, 1985.
23. In BJ Miflin, ed, oxford surveys of plant Molecular and Cell Biology, Oxford University Press. Oxford, 2:253-317.
24. Krishnan HB, White JA, Pueppke SG. Characterization and localization of rice (*Oryza sativa* L.) seed globulins. Plant Sci. 1992; 81:1-11.
25. Krishnan HB, White JA. Morphometric analysis of rice seed protein bodies. Plant Physiol. 1995; 109:1491-1495.
26. Kulp K, Ponte JG. Handbook of Cereal Science and Technology (2nd Ed, Revised and Expanded). New York: Marcel Dekker, Inc. Lasztity, R. the Chemistry of Cereal Proteins (2nd Ed). Florida: CRC Press, 1996-2000.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagents. Journal of Biological Chemistry. 1951; 193(1):265-275.
28. Tong LT, Fujimoto Y, Shimizu N, Tsukino M, Akasaka T, Kato W *et al.* Sato, Rice alpha-globulin decreases serum cholesterol concentrations in rats fed a hypercholesterolemic diet and ameliorates atherosclerotic lesions in apolipoprotein E-de Cient mice, Food Chem. 2012; 132:194-200.
29. Mawal YR, Mawal MR, Ranjekar PK. Biochemical and immunological characterization of rice albumin. Bioscience Rep. 1987; 7:1-9.
30. Meesook Kim, Yoonhwa Jeong. Extraction and Electrophoretic characterization of Rice Proteins. Nutraceuticals & Food. 2002; 7:437-441.
31. Mochizuki T, Hara S. Usefulness of the low protein rice on the diet therapy in patients with chronic renal failure. Jpn. J. Nephrol. 2000; 42:24-29.
32. Nakase M, Adachi T, Urisu A, Miyashita T, Alvarez AM, Nagasaka S *et al.* Rice (*Oryzae sativa* L.) R-amylase inhibitors of 14-16 kDa are potential allergens and products of a multigene family. J Agric Food Chem. 1996; 44:2624-2628.
33. Ogawa M, Kumamaru T, Satoh H, Iwata N, Omura T, Kasai Z *et al.* Purification of protein body-I of rice seed and its polypeptide composition. Plant Cell Physiol. 1987; 28:1517-1527.
34. Ohdaira Y, Masumura T, Nakatsuka N, Shigemitsu T, Saito Y, Sasaki R. Analysis of storage protein distribution in rice grain of seed-protein mutant cultivars by immunofluorescence microscopy. Plant Prod Sci. 2011; 14:219-228.
35. Osborne TB. The proteins of the wheat kernel. Carnegie Inst.: Washington DC, Phongthai S, Homthawornchoo W, Rawdkuen S. 2017. Preparation, properties and application of rice bran protein: A review. International Food Research Journal. 1907; 24(1):25-34.
36. Resurrection AP, Juliano BO, Tanaka Y. Nutritional content and distribution in milling fractions of rice grains. J Sci. Food Agric. 1979; 30(5):475-481.
37. Shewry PR, Casey R. Seed Proteins. Kluwer Academic Publish-Ers, the Netherlands, 1999.
38. Sun JL, Nakagawa H, Karita S, Ohmiya K, Hattori T. Rice embryo globulins: amino-terminal aminoacid sequences, cDNA cloning and expression. Plant and cell Physiology. 1996; 37:612-620.
39. Tadashi Tsukaguchi, Sumiyo Nitta, Yuri Matsuno. Cultivar differences in the grain protein accumulation ability in rice (*Oryza sativa* L.) Field Crops Research. 2016; 192:110-117.
40. Taiji Kawakatsu, Sakiko Hirose, Hiroshi Yasuda, Fumio Takaiwa. Reducing Rice Seed Storage Protein Accumulation Leads to Changes in Nutrient Quality and Storage Organelle Formation Plant Physiology. 2010; 154:1842-1854.
41. Tanaka Y, Hayashida S, Hongo M. The relationship of the feces protein particles to rice protein bodies. Agr. Biol. Chem. 1975; 39:515-518.
42. Tanaka K, Sugimoto T, Ogawa M, Kasai Z. Isolation and characterization of two types of protein bodies in the rice endosperm. Agri. Biol. Chem. 1980; 44:1633-1639.
43. Tanaka K, Ohnishi S, Kishimoto N, Kawasaki T, Baba T. Structure, organization, and chromosomal location of the gene encoding a form of rice soluble starch synthase. Plant Physiol. 1995; 108:677-683.
44. Waldschmidt-Leitz E. chemical classification of cereal proteins. European Brewery conv., Proc. Congr. 11th Brussels, 1963-1964, 78-84.
45. Wallace NH, Kriz AL. Nucleotide sequence of a cDNA clone corresponding to the maize Globulin-Z gene. Plant physiology. 1991; 95:973-975.
46. Wei C, Nguyen SD, Kim MR, Sok D. Rice albumin N-terminal (Asp-His-His-Gln) prevents against copper ion-Catalyzed oxidations. J Agric Food Chem. 2007; 55:2149-2154.
47. Xia N, Wang JM, Gong Q, Yang XQ, Yin SW, Qi JR. Characterization and *in vitro* digestibility of rice protein prepared by enzyme-assisted micro fluidization: Comparison to alkaline extraction. Journal of Cereal Science. 2012; 56:482-489.
48. Yamagata H, Sugimoto T, Tanaka K, Kasai Z. Biosynthesis of storage proteins in developing rice seeds. Plant Physiol. 1982; 70:1094-1100.