한국농화학회지 Vol. 20, No. 1, March 1977
J. Korean Agricultural Chemical Society

Rice Protein: Its Composition, Structure, Occurence and Biosynthesis

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(Received Dec. 1. 1976)

쌀 蛋白質 : 그의 組成・構造・所在와 生合成

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(1976년 12월 1일)

ABSTRACT

Some of the recent developments and studies in the area of rice protein are reviewed. Protein content and amino acid composition of rice are briefly described. Emphasis is given to characterization of rice protein fractions, effects of protein content on grain properties and lysine content of rice, occurence of protein in rice grain and biosynthesis of protein during grain development.

The important role played by rice protein in formation of its nutritive, technological and culinary qualities has been well established.[1-4] The nutritive value of proteins is to a significant extent determined by their fractional composition, the content of essential amino acids and the ratio of such amino acids.[5] An index such as the ratio of the sum of the essential amino acids(E) to the total protein(or total nitrogen) in the product(T) may give a certain idea on the biological value of the protein.[5] Values of E/T and amino acid scores for rice and certain other food products (Table 1) indicate that rice protein stands appreciably higher than proteins of other plant products.

The main nutritional disadvantage of the rice grain is its low protein content (usually 6 to 8 %). However, content of lysine, the first limiting amino acid,[6] for milled rice ranges 3.2—4.4g/16.8gN,[7] which is higher than that for barley (3.3—3.8g/16.8gN)[8] and for wheat(2.4—4.2g/16.8gN)[9]. Thus, data in Table 1 and lysine content for rice indicate that rice grain, while having low protein content, is characterized by a better quality of protein, that is, by a more fa-

Table 1. Ratio of essential amino acids to total nitorgen (E/T) and amino acid score for several food products[5]

Source of protein	E/T(mg/g)	Amino acid score
FAO scale	2.02	—
Rice	2.61	70
Corn meal	2.78	40
Millet	2.75	70
Soybean meal	2.58	70
Peas	2.59	60
Oats	2.30	80
Rye	2.17	80
Wheat flour	2.02	50

vorable balanced composition of essential amino acids than certain other cereal crops.

The importance of cereal protein in meeting the worldwide problem, so-called protein gap, has been well recognized and efforts have been concentrated on increasing the rice protein and lysine in the grain through breeding[10] or induced mutation.[11] Recently, studies on the characterization of rice protein, effect of protein improvement on protein properties and protein synthesis during grain development have been advanced. This review will discuss some of the more recent developments and studies in the area of rice protein.

Protein content of rice

Next to starch, protein is the most abundant constituent of rice. Rice protein is commonly calculated from Kjeldahl nitrogen by multiplying the factor 5.95, based on the nitrogen content of the major rice protein (glutelin) of 16.8%.[4]

The protein distribution of rice grain may be bran, 14%(germ, 6%); polish, 3%; and milled rice, 83%.[4] However, the distribution varies with degree of milling and the protein content of the rice grain. For example, the protein distribution in fractions of 87% milled rice is bran-polish, 19.4%; embryo, 9.9%; and milled rice, 70.1%.[12]

At 10±1% (w/v) bran-polish removal from brown rice, the difference in protein content between bran-polish and milled rice decreases with an increase in protein content of brown rice, regardless of variety.[13] These results indicate that protein distribution in brown rice becomes more even as protein content increases. Since the protein content of milled rice is highly correlated to that of brown rice,[14,15] Kjeldahl protein of brown rice is a good indicator for milled rice protein.

Although it is generally recognized[7] that brown and milled rices have 8 and 7% protein, respectively, the average value for protein can give only minimal information since protein content of rice in the literature shows wide distribution(Table 2). Moreover, the distibution of mean

Table 2. Protein content of brown and milled rice[a)]

Country	Brown rice(%)	Milled rice(%)
Korea[16]	8.5—9.2	7.6—8.5
Japan[17,18]	7.5—9.7	6.5—9.6
Malaya[19,20]	5.2—11.4	6.6—9.2
Philippines[21]	7.3—15.4	6.5—13.3
Thailand[20]	7.2—12.0	6.5—11.0
Vietnam[20]	6.5—9.1	5.7—8.5

a) Dry weight basis (N×5.95)

protein content of brown rice samples from world collection varies from 5 to 17%.[22]

The wide range of protein content of rice is due mainly to such factors as variety,[5,23] environment,[5,24,25,26] crop season or planting date[26,27] and nitrogen fertilization.[15,26,28,29] The protein content of samples of any variety may vary as much as 6% even when the samples are from the same location.[23] However, it was hypothesized[30] that rice grain having the same specific gravity has the same nitrogen content in the same variety without being affected by the environmental conditions.

Indica cultivars have somewhat higher protein content than japonica cultivars.[21,31] The protein content of rice is higher in waxy type[32] and especially in upland rice[32,33] than in lowland rice.

Amino acid composion of rice

Numerous data on amino acid composition of rice show[1,4,7] large discrepancies due largely to the different hydrolysis conditions among various workers. Several amino acids are slowly destroyed while several others are more slowly released on protein hydrolysis by acid at 110°C. For determining certain amino acids correctly with a 24hr hydrolysis the amino acid correction factors suggested for wheat[34] are also applicable to rice protein.[15,35]

The amino acid composition of nonwaxy and waxy rice is almost identical,[36] indicating that the differences in the grain are mainly physical

Table 3. Amino acid composition of brown and milled rice[a)]

Amino acid	Brown rice		Milled rice	
	Baldi[40]	IRRI[41]	Houston *et al.*[36]	Bressani *et al.*[2]
Alanine	5.3—6.5	5.5—6.5	5.7—6.0	5.7—6.2
Arginine	7.4—9.3	7.6—9.5	8.2—9.1	7.9—8.8
Aspartic acid	8.9—9.5	9.0—10.5	9.2—9.8	9.5—10.4
Cystine	1.6—2.4	1.2—2.1	2.6—2.8	1.4—1.8
Glutamic acid	16.5—19.4	16.9—19.9	17.9—19.3	18.3—21.1
Glycine	4.3—4.7	4.5—5.4	4.6—4.9	4.5—4.8
Histidine	2.0—2.5	2.2—2.9	2.2—2.6	2.5
Isoleucine	3.4—4.0	4.1—4.8	4.7—5.1	4.8—5.3
Leucine	6.5—8.5	7.9—8.9	8.0—8.9	7.8—9.6
Lysine	3.2—3.6	3.5—4.6	3.4—4.0	3.4—4.3
Methionine	1.5—2.1	1.9—2.9	2.7—3.3	1.8—3.5
Phenylalanine	5.1—5.7	5.3—6.0	5.3—5.7	5.6—6.3
Proline	4.1—4.6	4.4—5.5	4.4—4.9	4.7—5.0
Serine	4.6—5.2	4.6—5.9	5.1—7.5	5.7—6.9
Threonine	3.1—3.9	3.6—4.4	3.6—3.8	3.8—4.1
Tryptophan	n.d.	0.9—1.6	n.d.	1.0—1.4
Tyrosine	3.0—4.4	4.4—5.4	4.8—5.6	5.0—5.9
Valine	4.0—6.2	5.9—7.0	6.2—7.2	6.2—7.3

a) g/16.8 g N; By column chromatography

in nature due to starch.[37] Upland and lowland rices also show similar amino acid pattern.[38] Even wild species of rice have similar amino acid composition to cultivated rice.[39]

The amino acid composition of brown and milled rice is shown in Table 3. Although varietal differences exist in the level of some of the amino acids, brown rice tends to have a higher lysine content and a lower glutamic acid content than milled rice.[4] This is due to the presence of the aleurone layer and germ fractions of brown rice, since the milling byproducts(bran, germ and polish) have higher levels of lysine and lower levels of glutamic acid than milled rice.[4] The outer layer of brown rice grains is high in lysine, histidine, aspartic acid, threonine, glycine and alanine and the inner layer is high in glutamic acid, proline and phenylalanine.[42]

Distribution of protein fractions

Although the classical Osborne fractionation method for rice protein by solubility is not mutually conclusive, this classification is still useful in explaining some of the properties of rice protein. Recently the percolation method of Maes[43] has been employed to classify the rice protein.[23, 44-48] Protein extractions can be achieved by successive percolation with water, 5 or 10% sodium chloride and 60 or 70% ethanol, which corresponds to albumin, globulin and prolamin. Residual protein(glutelin) may be calculated by difference or can be extracted with dilute alkali under suitable conditions.[23] The percolation method tends to give more reproducible but lower recoveries of protein fractions than extraction by direct suspension in the solvent(Table 4).

Milled rice has a lower content of albumin and globulin than brown rice (Table 4), since these two protein fractions are more concentrated in the aleurone layers and germ.[23,50]

There is considerable variation in the ratios of albumin-globulin-prolamin-gutelin among reported

Table 4. Protein solubility fractions of brown and milled rice[a)]

	Brown rice			Milled rice		
	Cagampang *et al.*[23]	Lee *et al.*[32]	Linder *et al.*[49]	Cagampang *et al.*[23]	Lee *et al.*[31]	Houston *et al.*[44]
Albumin	4.0—11.6	8.5—13.7	3.0—18.7	2.9—9.9	3.1—4.8	0.3—3.1[b)]
Globulin	7.9—13.4	17.1—20.7	0—12.2	6.6—11.0	6.0—14.0	1.6—7.6[b)]
Prolamin	1.6—4.8	1.7—4.0	2.9—20.6	1.9—4.2	5.8—7.6	1.7—3.4[b)]
Glutelin	74.3—83.3	58.0—70.0	55.1—88.1	76.3—87.0	73.1—84.7	88.2—93.7[c)]

a) Percent of extracted protein
b) By percolation method; Percent of total protein
c) By difference

Table 5. Within-kernel distribution of protein fractions of milled rice[44]

Material[a)]	Weight(%)	Protein (%, db)	Percent of Total Protein			
			Albumin	Globulin	Prolamin	Glutelin[b)]
Flour 1	3.6	12.4	5.9	5.2	0.9	88.0
2	2.5	11.8	4.2	4.0	1.4	90.4
3	1.9	11.4	3.2	2.1	1.4	93.3
4	1.8	10.7	2.9	1.8	1.7	93.6
5	1.6	10.3	2.8	1.9	1.8	93.5
Residual rice	88.6	5.5	2.0	0.9	1.9	95.2
Original rice	100	6.5	2.7	1.6	2.0	93.7

a) Five consecutive layers of the kernel, totaling about 11% by weight, are removed by abrasive milling.
b) By difference

determinations (Table 4). The variability results in part from experimental differences and may reflect environmental differences to some extent.[44] The mean ratio of proten fractions indicates[4,44] that the protein of milled rice is unique among cereal proteins in that it contains about 80% or more glutelin(alkali-soluble protein).

The distribution of protein fractions in milled rice (Table 5) shows that the proportions of albumin and globulin are highest in the protein of outer kernel layers and decrease considerably toward the center; whereas glutelin shows the opposite distribution. The higher contents of albumin and globulin in the outer layers of milled rice may be due to adhering aleurone layer and scutellum.[4]

Prolamin is much more evenly distributed in theproteins throughout the kernel, although there is an indication of higher concentrations in the resi dual kernels than in the portions removed from the outer region (Table 5).

Characterization of protein fractions

Starch gel electrophoresis of the rice protein fractions in the presence of urea shows that globulin has the fastest mobility followed by albumin and prolamin.[46] Most of the glutelin remains in the sample well, but does show some migrating bands.

Amino acid composition of milled rice protein fractions is given in Table 6. Milled rice protein has 10.2-20.2 μeq/g disulfide groups,[52] indicating that two-thirds of cystine content is the disulfide(cystine) form and the rest in the sulfhydryl(cysteine) form.

The amino acid composition of milled rice pr-

Table 6. Amino acid composison of milled rice protein fractions[a)]

Amino acid	Tecson *et al.*[46]			Sawai *et al.*[51]		
				Glutelin		
	Albumin	Globulin	Prolamin	Whole	Subunit I	Subunit III
Alanine	8.7	9.1	6.6	5.6	4.7	6.5
Arginine	8.4	11.0	5.9	11.3	10.9	11.3
Aspartic acid	10.8	7.8	7.9	12.0	10.0	14.7
Cystine	2.9	—	0.3	1.7	2.5	1.1
Glutamic acid	12.5	11.8	21.4	21.7	26.9	13.4
Glycine	6.9	5.9	3.2	5.0	5.6	3.8
Histidine	2.6	1.6	0.9	3.1	2.8	3.3
Isoleucine	4.1	3.0	4.7	5.6	4.6	6.7
Leucine	7.9	6.6	11.3	8.8	8.5	8.7
Lysine	4.9	2.5	0.5	4.1	2.9	5.6
Methionine	2.5	2.3	0.5	1.4	0.7	1.6
Phenylalanine	3.0	3.3	6.3	6.8	7.4	6.2
Proline	6.6	5.5	4.1	5.0	4.8	4.4
Serine	5.2	5.5	5.1	6.5	7.0	6.0
Threonine	4.6	2.9	2.4	4.0	4.0	4.3
Tryptophan	1.9	1.3	0.9	1.4	1.6	0.8
Tyrosine	3.9	5.0	8.7	6.1	5.6	6.8
Valine	8.7	6.2	3.0	7.8	6.7	8.4

a) g/16.8 gN; By column chromatography

Table 7. Number of electrophoretic components of rice protein fractions

Method	pH	Albumin	Globulin	Prolamin	Glutelin
Paper electrophoresis	7.6[31]	1	2	1	—
	10.0[53,54]	3	2	1	3
	13.0[31]	—	—	—	1
Polyacrylamide-gel electrophoresis	8.3[47]	9	6	—	—
	8.65[55]	9—11	3	2	1
Starch-gel electrophoresis	3.1[46-48]	11; 14—17	8; 9	2	—
	8.9[47,48]	9; 15	8; 9	—	—

oteins(Table 6) shows that albumin has the highest amount of lysine among the fractions, followed by glutelin, globulin and prolamin. The amino acid compositions of albumins for normal and waxy rices are similar with exception that the waxy rice has higher lysine content than the nonwaxy one.[48]

Albumin is highly heterogeneous in subunit composition (Table 7). Cellogel electrophoresis at pH 10.5 or 11.5 gives two major and five or six minor albumin bands.[56] Albumins of regular and waxy rices are separated into four and five fractions by gel filtration on G-100 column.[48] Molecular weights for these fractions are 12000, 18500, 26000 and 76000, respectively, for the regular rice and 10000, 18000, 25000, 54000 and

117000, respectively, for the waxy rice.

Globulin is also highly heterogeneous in subunit composition (Table 7). The reduction and subsequent alkylation of globulin results in a preparation with a slower mobility than globulin when subjected to starch-gel electrophoresis in the presence of urea at pH 3.1 Globulins of four japonica and eight indica milled rices have similar amino acid composition.[57] Globulins of waxy and nonwaxy rices also have similar amino acid composition.[48] Both of the globulins of waxy and nonwaxy rices are separated into four fractions with similar molecular weights of 16000, 66000 and 127000 for the three of their fractions.[48]

Globulin of rice kernel consists of three components; alpha-, beta- and gamma-globulin, by Sephadex G-200 chromatography.[50] Alpha-and beta-globulin are more concentrated in endosperm with considerable heterogeneity, while gamma-globulin is a major component in embryo and bran, and accounts for about 20% of the total protein of rice embryo. Gamma-globulin has a sedimentation coefficient of 7S and a molecular weight of 150000.[50]

Gamma-globulin gives three major components, gamma-1, gamma-2 and gamma-3 globulins, on a DEAE-Sephadex A-50 column.[58] These three components are electrophoretically distinct but have almost the same sedimentation coefficient.

Properties of rice embryo gamma-1 and gamma-3 globulins are shown in Table 8. Gamma-2 and gamma-3 globulins have very similar amino termini[58] and they are immunochemically identical.[63] Gamma-1 globulin, a neutral or slightly basic protein, has 4.5% hydroxyproline and small amounts of tryptophan, methionine and cystine.[59] The subunits are associated mainly through hydrophobic bonds and some sulfhydryl groups,[60] and consist of multiple molecular weight species: 36000, 24000, 21300, 14800 and 13000.[61] Gamma-2 globulin, a weakly basic protein, has no hydroxyproline and is composed of three identical major polypeptide chains (molecular weight of 35000) and one minor polypeptide(molecular weight of 13000).[61]

Table 8. Comparison of rice embryo gamma-1 and gamma-3 globulins[50,59,61,62]

	Gamma-1 globulin	Gamma-3 globulin
Shape	Asymmetric (Irregular)	Spheric
Molecular weight	2.0×10^5	1.2×10^5
Sedimentation coefficient	7.26 S	7.1 S
No. of subunits	10	4
Content of α-helix	3%	—
Content of β-structure	38%	—
Composition	18 hexose	15 hexose
	3 pentose	4 pentose
	6 hexosamine	4 hexosamine
	1751 amino acid	1014 amino acid

Rice endosperm globulins are fractionated into three components, G-I, G-II and G-III, by isoelectric precipitation technique.[64] Fraition G-I, a major globulin component, is estimated to be about 40% of the total globulins, and has a sedimentation coefficient of 1.6S. In the presence of urea it dissociates into two portions which recombine when urea is removed. It contains 18.1% nitrogen and has high glutamic acid and arginine, which comprise 43% of the total molecule. The essential absence of histidine and lysine appears unique among cereal proteins. The molecular weight estimated by Sephadex chromatography is 25500 and calculated by amino acid content is 25330. G-III (pH7-soluble protein) shows great complexity of several components by starch-gel electrophoresis and contains a high-sulfur compound.[65]

Prolamin content is the lowest among rice fractions in the rice grain(Table 4), and prolamin has the poorest lysine content than other protein fractions (Table 6). Rice prolamin is relatively low in proline but high in glutamic acid compared with other cereal proteins.[66]

Rice prolamin preparations are characterized by anomalously high ultraviolet absorption at 230nm.[41] Their Lowry protein is higher than Kjeldahl nitrogen,[46] indicating the presence of a phenolic contaminant. This contaminant may be separated from prolamin by gel filtration on Sephadex G-

200 using 0.01N sodium hydroxide as solvent, or by precipitating prolamin from the alcoholic extract with acetone.[46] The resulting preparation has a high nitrogen content of 16.7%. Since the phenolic contaminant can not be dialyzed, it may form a complex with prolamin or have a similar molecular weight as prolamin. Prolamin is also contaminated with glucan.[46] It is unknown whethcr some glycoproteins occur in prolamin as a minor component,

Prolamin is much less heterogeneous than albumin or globulin (Table 7). Starch-gel electrophoresis at pH 3.2 in the presence of 7.5M urea indicates the presence of two prolamin protein bands.[64] Brown rice prolamin is eluted mainly at the void volume on Sephadex G-100 columm,[45] while milled rice prolamin is eluted at V/Vo of 1.45 on Bio-Gel P-300 column,[46] with a molecular weight of 3×10^5.

Glutelin has similar amino acid composition to that of whole milled rice protein.[46] Glutelin is soluble below pH 3 or above pH 10[67] and least soluble at neutral pH region.[67,68] Most glutelin is extracted above pH 11, but the solubility of glutelin is drastically reduced below pH 10. Many of the solvents for the wheat glutelin, such as urea and aluminum lactate, are poor extrants for rice glutelin.[45,46]

Glutelin preparations usually contain sizable amounts of carbohydrate[69] and nucleic acid.[70] Crude glutelin preparations can be purified by simultaneous extraction of impurities and precipitation of glutelin at pH 10 in the presence of sodium chloride.[68] The purified glutelin contains 0.021% phosphorus(nucleic acid), 0.067% pentose and 0.25% hexose,[68] and 17.9% nitrogen which is very high compared with 16.9% of Osborne glutelin.[46] The purification does not change the amino acid composition, but removes two faster migrating bands of the Osborne glutelin on starch-gel electrophoresis in the presence of urea.[46]

The heterogeneity of glutelin is known(Table 7). Rice glutelin gives three fractions by gel filtration on Sepharose 2B with molcular weight ranging from 2 to more than 20 to 30×10^6.[71) Glutelin has a molecular weight of 6×10^5 on Sephares 4B chromatography.[46] Reduction and alkylation of glutelin (S-cyanoethyl glutelin) reduce the molecular weight of glutelin to 6×10^4.[46] The S-cyanoethyl glutelin consists of three subunits, Subunit I, II and III in the ratio of 8:1:1, by carboxymethyl Sephadex G-50 chromatography.[72] Subunit I is a neutral protein and a major constituent of glutelin and Subunit II and III could be a nucleoprotein in native state.[72]

Amino acid data on glutelin and its fractions (Table 6) indicate that glutelin is characterized by high contents of dicarboxylic amino acids, particularly glutamic acid, about 60% of which are in the amide form.[51] Similar amino acid composition is found between glutelins of four japonica and eight indica milled rices.[57] The amino acid composition of glutelin is predominantly determined by that of major Subunit I(Table 6).

The major glutelin subunit exhibits a single sedimentation boundary with 1.4S at a protein concentration of 0.92%, indicating molecular weight of about 20000, which coincides with the molecular weight based on the methionine content.[51] Glutelin and its basic Subunit II and III have glycine as the amino termini.[72] No detectable N-terminal acid is found for Subunit I. The minimum molecular weights of Subunit II and III are 35000 and 43000, respectively, based on the amount of DNP-glycine found. The ratio and molecular weight of glutelin subunits indicate that glutelin is a very large molecule composed of three components polymerized by disulfide linkages.

Effect of protein content on grain properties

Rice samples of the same varieties that differ in protein content indicate that high protein rice seems more resistant to milling, giving lower yields of bran and polish than normal protein rice.[23] Milled samples of high protein rices may be more transluent but darker tan than rices of the same variety with normal protein content.[73]

Flours obtained from the outer layers of milled rice are richer in protein as much as twice than that of original kernel(Table 5). Intensive efforts have been concentrated on separating the high-protein rice flour from the milled rice kernel by peripheral abrasion,[35,44,74-77] and by other methods.[78-80] The amino acid distribution patterns in the protein of flours and residual kernel are remarkably uniform and there is no essential difference in amino acid content as compared with the original kernel.[35,75,77] This uniformity of amino acid composition in the various milling fraitions may reflect the same composition of rice protein bodies with the rice endosperm.[77]

High protein rices tend to have poor cooking and eating qualities.[81-83] Since it is insoluble in water, rice protein influences the rate and extent of water absorption and volume expansion of the starch granules of milled rice during cooking.[81] The amylose to amylopectin ratio is the principal factor affecting the texture of cooked rice,[81,84] but among the samples where the range of amylose content of milled rice is rather narrow (about 6%) protein content may be more important.[81] The decrease in milled rice organoleptic qualities, attributed to a decrease in the cooked rice viscosity and elasticity, is related to increased glutelin content of the grain and especially to its accumulation in the endosperm.[83] However, an increase in protein by 2% points has no adverse effect on the eating quality of boiled rice.[85]

As indicated previously, the protein distribution in brown rice becomes more even as protein content of grain increases.[13] Similarly, the protein distribtion in the endosperm of high protein rice is more even than in low protein counterpart.[73] An 11% protein milled rice has outer layers with 19% protein, while a 5% protein milled rice has outer layers with about 12% protein.[86]

Increased protein in a rice variety is due mainly to an increase in the glutelin content in the grain(Table 9).[23] Milled rice of the high protein samples has about twice as much glutelin as that of the low protein samples. Prolamin content of milled rice also increases accordingly with an increase of protein. As a percentage of total protein, albumin and globulin decrease, while prolamin remains constant as protein content increases. Only glutelin content increases in both quantity and as a percentage of total protein as protein content of milled rice increases. Among rice protein fractions, globulin seems to show least changes as a function of protein content.[32,87]

Effect of protein content on lysine content

Although most of the amino acids of brown and milled rices remain more or less constant with differences in protein content, a few amino acids such as tyrosine, histidine, methionine, phenylalanine, lysine and valine are affected by protein changes.[2,5,15,21-23,26,35,39,41,44] Among these amino acids, lysine is more concerned since it is the first limiting essential amino acid in rice protein.[6] However, there is some evidence[88,89] that lysine is not the first limiting amino acid in rice diets. Apparently, the other proteins in the rice diets are able to compensate for the lysine deficiency of rice protein.

Even though there is an actual net gain in the

Table 9. Contents of protein and pretein fractions in three low-and high-protein milled rices[23]

	Low protein samples			High protein samples		
	Chia-nan 8	T.N.1	BPI-76	Chia-nan 8	T.N.1	BPI-76
Protein(%)	6.8	7.2	8.5	13.1	15.2	16.1
Albumin	0.4 (6.5)	0.6 (9.8)	0.3 (4.5)	0.6 (5.3)	0.5 (3.8)	0.4 (2.9)
Globulin	0.7(11.0)	0.7(10.8)	0.9(12.8)	0.8 (6.5)	1.0 (7.4)	1.1 (8.2)
Prolamin	0.2 (2.7)	0.2 (3.0)	0.3 (3.9)	0.4 (3.2)	0.2 (1.8)	0.6 (4.1)
Glutelin	5.3(78.0)	4.9(76.4)	5.6(78.8)	10.2(85.0)	11.4(87.0)	11.5(84.8)

Parentheses indicate % of total protein

lysine content of rice with an increase in protein content,[26,90] lysine in the protein shows an inverse relation with protein content.[2,5,15,21-23,26,35,39,44] However, the drop in lysine content is only 25% of the corresponding increase in protein.[23] The negative relation between lysine in the prtoein and protein content of the grain becomes negligible in high protein rices.[13,90,91] For example, twenty six samples of high protein rices(8.3-14.5% protein) have lysine content of 2.9-5.1g/16.8gN, showing a correlation coefficient of −0.225.[90] The dye-binding capacity, which has been used to screen high lysine or high protein rices,[91,92] as a function of protein content shows a change of slope at about 12% protein (dry basis).[13,92] Since lysine is the only basic amino acid of rice protein that changes in concentration with changes in protein content,[23] this change in slope reflects the less dependence of lysine content on protein content at high protein levels.

Globulin shows least changes as a function of protein content[32,86] and glutelin, which increases with an increase in protein content (Table 9), has the closest lysine content to milled rice protein.[46] Since only albumin has the highest lysine content than rice protein,[46] the constant lysine content of rice protein above 12% protein is due to the corresponding increase in prolamin and albumin.[13] However, the effect of increased prolamin or lysine content of rice protein would be minimal due to its low lysine content (Table 6).

Although albumin content increases as a percentage of rice as protein content increases,it decreases as a percentage of protein.[13] However, there is no longer a negative correlation between albumin content as a percentage of protein and the protein content in high protein rices.[89] Moreover, rice samples of the same cultivar with more than 10% protein tend to have a constant amino acid composition.[4] Thus, it seems that the constancy of lysine content in high protein rices is due principally to the constant albumin content.

Rat feeding experiments with four milled rice samples(5.7-14.3% protein) show that the protein quality of milled rice at 0—5% dietary protein levels decreases with increasing protein content of rice.[2] However, the decrease in quality is less than proportional to the increase in protein content. The microbiological quality of milled rice samples differing in protein content based on relative growth of *Streptococcus zymogenes* indicates that the drop in microbiological value is only about 10% for an increase in protein content of over 100%.[93] Because of these characteristics of rice protein breeding efforts in improving the nutritional value of the rice grain have been concentrated on increasing the protein content while maintaining the protein quality rather than on improving its protein quality.[14,41]

High-protein and high-lysine mutants from gamma-irradiation of rice seeds have been reported,[94] but they were found to have normal lysine content.[13] It may be probable that high-protein and high-lysine cultivars exist in rice.[5]

Occurence of protein in the rice kernel

A microscopic examination of the inner structure of rice kernel shows that protein granules are contained in the membranes covering the starch granules and in the cell walls.[95] An investigation of saliva-digested cross sections of the rice endosperm indicates that the protein is mainly in the form of membranous network, together with a few inclusions.[96] Protein matrix also occurs in the space between the lossely packed starch granules of rice endosperm.[37] Attempts have been made to fractionate endosperm protein into wedge (or zwickel) and adhering proteins using nonaqueous flotation techniques.[45,97] The amino acid compositions of the two fractions are essentially identical[97] and wedge protein has similar amino acid composition to that of the rice protein.[45]

Most of the protein in rice grain is localized in subcellular storage particles, called protein bodies,[98] which begin to appear at 7 days after flowering in the developing grain.[37] These particles are the main site of localization of glutelin-the reserve protein of rice.[98-100] The protein bodies contain most protein components detected in the starting material(i.e., rice polish) by the techniques of solubility fractionation, electrophoresis on Cellogel membrane and gel filtration on Sephadex G-100 and G-150 column.[101] No significant difference is found in amino acid composition between the protein bodies and rice polish.[101]

The protein bodies are abundant in the periphery of the endosperm, especially in aleurone layer, and their number decreases with decreasing distance from the center of the grain.[13,37,98] This uneven distribution of protein bodies in the endosperm is consistent with the fact that protein content increases with the increase of distance from the center of the grain (Table 5). However, the protein body distribution becomes more even with an increase in protein content of rice,[13] which explains the fact that the protein distribution in the endosperm of high protein rices is more even than that in low protein rices.[73]

The pretein bodies are spherical or oval in shape,[98] 1 to 4μ in diameter,[13,37,98] and stain yellow by iodine.[37,98] Some of them are clumped together forming an assembly of discrete bodies.[101] The ultrastructure of the protein bodies of a high protein rice sample is identical to that of a normal protein milled rice.[102] Small amounts of nucleic acid and phytic acid are found in the protein bodies.[99,101] In addition, ash, phospholipid, nicotinic acid and niacin may occur in the protein bodies.[101]

The rice protein bodies are separated into three fractions by sucrose density gradient centrifugation(45, 55 and 65% sucrose) after enzymatic maceration of rice polish.[98,101] The main components of the fractions are protein, lipid and carbohydrate.[98,99,101] However, they are different not only in their size but also in their protein and lipid contents. The fraction of higher density contains less lipid and more carbohydrate,[98,101] and consists of smaller bodies as compared with other fractions.[98] These results may imply a possible existence of different types of protein bodies in rice endosperm with respect to their composition, structure and biological function.

Electron micrograph of isolated protein bodies reveals that they consist mostly of electron-dense bodies, more than half of which have a limiting membrane and distinct concentric strata structure.[101] The strata structure consists of electron-dense and electron-thin layers arrayed alternatively and electron-dense layers are composed of fine granules. Some of the protein bodies have a uniformly electron-dense structure which might be an unresolved form of the stratified bodies or may represent another type of the protein bodies.

Proteinases, phoshatases, lipases and amylases are found in the protein bodies.[100] The synthesis and accumulation of these acid hydrolases occur during the maturation of the endosperm, indicating that protein bodies perform the functions of lysosomes during germination where mobilization of reserve substances takes place.

Accmulation of protein during grain development

Development of the rice caryopsis is accompanied by morphological changes in the endosperm cells as well as changes in various biochemical constituents. The amounts per kernel of starch and protein increase, while that of water decreases. Starch granules are noted in quantity in the 4-day caryopsis, while protein bodies begin to appear at 7days after flowering.[37] Thus, starch biosynthesis precedes a protein bodies synthesis in the developing rice caryopsis.

The increase in protein content of rice grain results mainly from a corresponding increase in the number of protein bodies in the endosperm.[13,37] However, an increase in protein content is not necessarily accompanied by a change in the size of the protein bodies.[13]

Protein content per grain increases most rapidly from 4 to 16 days after flowering and levels off thereafter.[103] Nonprotein nitrogen remains practically constant in amount per grain.[45] It is a major fraction (about 15%) of the total nitrogen in the 4-day grain,[45] but drops to less than 2-3 % of the total nitrogen in the mature grain.[45,104] Free amino nitrogen progressively increases during the early period of maturing and then decreases in the maturing grain.[103,105,106]

Both albumin and globulin also follow a similar trend to free amino nitrogen.[105,106] Albumin or enzyme protein may act as functional protein in ripening riceseeds.[107] Albumin decreases from 16% of the total protein in the grain at 12days after flowering to 5.6% in the mature grain, while globulin fraction decreases from 17% to 8.5% of total protein.[105] Prolamin, whose change is similar to that of albumin and globulin,[106] shows a sixfold increase per grain during development.[45] The major protein change is the rapid increase of glutelin in the grain between day 4 and day 21.[45] Maximum contents of prolamin and glutelin are attained shortly before maturity.

Gammaglobulins, the major globulin in embryo and bran,[50] are synthesized progressively in rice grain after-flowering.[63] Gamma-1 and gamma-3 globulins, which are localized in scutellum and aleurone cells, increase linearly from 5 to 40days after flowering.[63] The increase in the amounts of gammа-1 and gamma-3 globulins are closely parallel, suggesting th atboth globulins are synthesized at similar sites and by related mechanisms.[63]

Amino acid composition of developing rice grain indicates that the levels of many of the amino acids change significantly during maturation.[45,105] Lysine progressively decreases during grain ripening, however lysine in albumin increases during the early stage of maturation and then decreases toward maturity.[105] Changes in the amino acid composition of globulin follow more closely to that of total protein.[105]

Disc gel electrophoresis shows diffuse and rapidly migrating bands for both albumin and globulin in the grain at 2-4days after flowering.[105] A maximum of 10 protein bands are obtained for albumin, four of which are major bands which are distinct principally at 20 and 30 days after flowering. Globulin shows only one major and three minor bands during grain development. These results are in good agreement with those obtained from mature grain preparation(Table 7).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis gives three major polypeptide subunits for albumin with molecular weights of 8500, 11000 and 16000 from 7days after flowering, and two for globulin with molecular weights of 20000 and 12000.[105]

Protein nitrogen in the rice grain is considered to be derived mainly from the nitrogen already present in the vegetative tissues at flowering.[73] The nitrogen in the plant is mainly in protein form and the ratio of protein nitrogen to free amino nitrogen is constant in the rice plant during the vegetative stage.[108] In the vegetative stage the leaf blades generally have 3 times as much nitrogen as the leaf sheaths plus culms.[109] During grain development, however, leaf blade nitrogen is depleted while the nitrogen of leaf sheats plus culms remains essentially constant,[109] indicating that the leaf blades are the main source of leaf nitrogen translocated to the developing grain.

At the same fertilizer level, rices with high percentage of grain protein translocate more leaf blade nitrogen in the form of amino acids to the developing grains than rices with average protein content and with similar grain yield.[109] This more translocation of leaf blade nitrogen in rices with high protein content may be due to a high concentration of free amino acid in the sap which is being translocated to the developing grains,[110] rather than to differences in translocation rates.

Protein accumulation during the first 2weeks of grain development is affected by the level of free amino acids and the capacity of the intact grain to incorporate amino acids.[103] Developing grains with high protein content tend to have a faster rate of amino acid incorporation than those with normal protein content.[103,109] This indicates that although protein synthesis in high protein and low protein rice grains begins at the same time the rates of synthesis are different between them.

The developing grains with high protein content also have higher concentrations of soluble protein, free amino nitrogen and ribonucleic acid, and higher protease activity than the low protein grains.[103,109] The high protease activity in the developing grains with high protein content may reduce the rate of protein accumulation. However, its effect may not be significant since the protease activity in the developing grain is already low and the enzyme is located mainly in the embryo and aleurone layers and may not be in contact with the protein bodies of the endosperm.[109] Only 9% of the protease activity in the mature rice grain is found in the endosperm.[111]

Addition of nitrogen fertilizer during grain development increases the percentage of grain protein. by simply providing an added source of nitrogen to the developing grain.[108,112] There is some indication that a high percentage of grain protein is not due to a shorter growth duration alone.[109]

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