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SECTION III

CHAPTER 8

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Studies on Protein Quality and Quantity of Rice

Rice (*Oryza sativa* L.) is a principal source of calories and of protein among Asians. Other than starch, protein is the most abundant constituent of the rice grain. Brown (dehulled) rice usually has 8% protein (measured at 12% moisture); milled rice has 7% protein (Juliano 1966). The bran (aleurone layer plus germ) contains more protein than the starchy endosperm. So during milling the proportion of protein lost is higher than the proportion of weight lost.

PROPERTIES OF RICE PROTEIN AND ITS FRACTIONS

The protein of milled rice is unique among cereal proteins in that it contains at least 80% glutelin (alkali-soluble protein). The other Osborne protein fractions are 5% albumin (water-soluble protein), 10% globulin (salt-soluble protein), and less than 5% prolamin (alcohol-soluble protein) (Cagampang *et al.* 1966; Juliano 1965, 1967). Albumin and globulin are concentrated in the aleurone layers and in the germ. Glutelin is insoluble in water because of its high molecular weight which results from crosslinking of the smaller subunits through disulfide bonds. It is heterogeneous and consists of subfractions differing in solubility, electrophoretic mobility, and amino acid composition (Tecson *et al.* 1971). Kjeldahl nitrogen is multiplied by 5.95 to convert it to crude protein based on the 16.8% nitrogen content of glutelin.

Rice protein occurs in the endosperm mainly as discrete particles, 1–4 μ in size, called protein bodies (del Rosario *et al.* 1968; Mitsuda *et al.* 1967). Smaller but more numerous protein bodies are present in the peripheral cells of the endosperm. More protein bodies occur next to the endosperm cell walls. The electron micrograph of the protein bodies shows a limiting membrane and a layered structure inside the protein body (Mitsuda *et al.* 1967, 1969). The protein bodies have the same composition as whole milled-rice protein.

Among the milling fractions, bran, polish, and germ have higher levels of lysine and lower levels of glutamic acid than milled rice (Houston *et al.* 1969; Houston and Kohler 1970; Juliano 1966). This uneven distribution in some of the amino acids may be partly explained by the uneven distribution of the protein fractions that differ in amino acid composition. Albumin, which is concentrated in the bran layers, has the best amino acid balance, followed by glutelin, globulin, and prolamin (Table 8.1) (Tecson *et al.* 1971). Thus, the high lysine content of bran and brown rice compared with milled rice may be explained by the high level of albumin in the aleurone layer and in the germ.

TABLE 8.1

LEVELS OF ESSENTIAL AMINO ACIDS AND CYSTINE OF PROTEIN FRACTIONS AND PROTEIN OF IR8 MILLED RICE (GRAMS PER 16.8 GM N)

Amino Acid	Protein Fraction Albumin	Globulin	Prolamin	Glutelin	Milled Rice Protein (%)
Isoleucine	4.05	3.03	4.68	5.27	4.13
Leucine	7.89	6.56	11.3	8.19	8.24
Lysine	4.92	2.56	0.51	3.47	3.80
Methionine	2.54	2.27	0.50	2.61	3.37
Methionine + cystine	5.40	2.27	0.80	4.09	4.97
Phenylalanine	2.97	3.32	6.26	5.42	6.02
Threonine	4.65	4.55	2.86	3.92	4.34
Tryptophan	1.88	1.34	0.94	1.16	1.21
Valine	8.72	6.18	6.97	7.31	7.21

Source: Tecson *et al.* (1971) except unpublished tryptophan data from International Rice Research Institute.

Among the protein fractions glutelin has the closest amino acid composition to milled rice protein probably because it is the major protein fraction. The high lysine content (3.5–4.0%) of rice protein may be ascribed to its low level of prolamin, the poor-quality fraction. However, lysine is the first limiting amino acid in rice protein as it is in other cereal proteins (Houston and Kohler 1970; Mossé 1966).

Few comparative nutritional studies on brown rice and its milling fractions and on the four protein fractions have been made. The data are difficult to compare because the protein levels, age, sex, and number of rats and feeding periods used differ. Losza and Koller (1954), however, reported the following biological values of milled rice protein and its fractions: whole protein, 81.4%, albumin-globulin, 85.4%; prolamin, 35.0%; and glutelin, 85.4%.

Because it is insoluble in water, rice protein affects the rate and extent of water absorption and the volume expansion of the starch granules of milled rice during cooking (Juliano *et al.* 1965). Milled rice treated with trypsin before cooking becomes very sticky when it is cooked (Barber 1969). The amylose-to-amylopectin ratio is the principal influence on the texture of cooked rice. But among the samples where the range of amylose content of milled rice is rather narrow (about 6%), protein content is more important (Juliano *et al.* 1965).

Many investigators have shown that grain protein content differs among varieties. The protein content of samples of any variety, however, may range up to 6 percentage points even when the samples are from the same location (Cagampang *et al.* 1966; Juliano *et al.* 1964). For example, the protein content of the high-protein variety BPI-76-1 may range from 8 to 14% (at 12% moisture)

and the low-protein variety Intan may have from 5 to 11% protein. Direct comparison of varieties is complicated by differences in growth duration, temperature sensitivity, plant type, and tillering ability among varieties and by differences in soil fertility levels and cultural practices, such as rate and time of application of nitrogenous fertilizers, spacing, etc. (IRRI 1971). Solar radiation available during grain development also affects protein content. Herbicides like Simazine may increase protein content although they may at the same time decrease yield (Vergara *et al.* 1970).

The protein of the other *Oryza* species has the same composition as cultivated rice (Ignacio and Juliano 1968). The protein content (7.4–12.0%) of 17 samples of brown rice representing 11 wild species and 2 interspecific hybrids of genus *Oryza* was negatively correlated with lysine content (3.12–4.48%) and was positively correlated with tyrosine and phenylalanine.

EFFECTS OF HIGH PROTEIN CONTENT ON GRAIN PROPERTIES

A study of samples of the same varieties differing in protein content showed that changes in protein content involved principally the quantity per grain of glutelin and prolamin (Table 8.2) (Cagampang *et al.* 1966). A corresponding increase in the number of protein bodies in the endosperm accompanied the increase in protein content (del Rosario *et al.* 1968), but there was no change in ultrastructure of the protein bodies (IRRI 1970).

TABLE 8.2

MEAN WEIGHT RATIOS AND CONTENTS OF PROTEIN AND PROTEIN FRACTIONS OF MILLING FRACTIONS OF BROWN RICE[1] OF LOW- AND HIGH-PROTEIN SAMPLES OF THREE VARIETIES

Property	Moisture (%)	Low Protein Samples Milled Rice (%)	Polish (%)	Bran (%)	High Protein Samples Milled Rice (%)	Polish (%)	Bran (%)
Weight of brown rice		86.9	2.0	11.1	89.5	1.5	9.0
Protein	12	6.6	11.8	12.4	13.0	16.2	14.7
Albumin	12	0.40	2.81	3.41	0.44	3.65	4.01
Globulin	12	0.69	0.95	3.29	0.84	2.17	3.92
Prolamin	12	0.19	0.40	0.51	0.35	0.69	0.42
Glutelin	12	4.64	4.85	2.10	9.68	6.46	2.39

Source: Cagampang *et al.* (1966).
[1] Mean brown rice protein contents of 7.2 and 13.4%.

The distribution of protein in the endosperm of high protein rices is more even than in low protein rices. An 11% protein milled rice has outer layers with 19% protein, whereas a 5% protein milled rice has outer layers with about 12%

protein (Houston 1967). In addition, high protein rice seems more resistant to milling. High protein rice gives lower yields of bran and polish than normal protein rice (Table 8.2) (Cagampang *et al.* 1966). The polish fraction of high protein rice tends also to contain more protein than the bran from the same sample. Milled samples of high protein rices may be more translucent but darker tan than rice of the same variety with normal protein content.

High protein rices tend to have lower levels of some of the essential amino acids, particularly lysine, than low protein rices of the same variety (Cagampang *et al.* 1966). However, the drop in lysine content is less than proportional to the increase in protein content. These trends are not as consistent among high protein samples (>10%) as they are between low and high protein samples with more than 3 percentage points difference in protein content. For instance, 3 samples of BPI-76 brown rice with 8.0, 10.7, and 14.3% protein had lysine contents of 3.1, 2.9, and 2.9% in their protein.

In a cooperative study with Dr. Ricardo Bressani of the Instituto de Nutricion de Centro America y Panama at Guatemala on the effect of differences in protein content of milled rice on its nutritive quality (Bressani *et al.* 1971) we found that although protein quality tended to decrease as protein content increased, the decrease in quality was less than proportional to the increase in protein content (Table 8.3). Higher growth responses (nitrogen growth indices) were obtained with milled rice diets with 0, 1, 2, 3, 4, and 5% protein than when only the data showing weight gain (diets with at least 3% protein) were considered (Table 8.3). Based on a relative quality value of 75 for casein, the quality

TABLE 8.3

SUMMARY OF PROTEIN QUALITY INDICES FOR FOUR MILLED RICE SAMPLES AND CASEIN BASED ON WEIGHT GAIN IN WHITE RATS

Protein Source	Protein Content[1] at 12% Moisture	0–5% Dietary Protein				Data Showing Net Growth		
		PER[2]	NPR[3]	N Growth Index	Protein Quality[4]	PER[5]	N Growth Index	Relative Quality[4]
Intan	5.68	2.56	3.71	3.49	80	2.04	2.37	47
IR8	7.32	2.20	3.36	3.25	75	2.02	2.30	46
IR8	9.73	1.94	3.07	3.04	71	2.02	2.17	43
BPI-76-1[6]	14.3	1.50	2.57	2.47	57	1.84	2.12	42
Casein	86.2	2.20	3.36	3.23	75	–	3.78	75

Source: Bressani *et al.* (1971).
[1] N X 5.92 for rice protein and N X 6.25 for casein.
[2] Protein efficiency ratio at 5% protein.
[3] Net protein ratio at 5% protein.
[4] Based on a value of 75 for casein.
[5] Protein efficiency ratio of 90% rice diet.
[6] Corrected for differences in casein values between the two feeding experiments.
BPI-76-1 was tested later than the other rice samples.

values for milled rice in the diets with 5% protein or less were about the same as those for milled rice protein which has biological values of 70–75% (Juliano 1966).

In diets containing 90% rice, the protein efficiency ratio (PER) of the 4 milled rice samples showed less difference (1.84–2.04) than at 0–5% in the diets (1.50–2.56) (Table 8.3). Based on a relative quality value of 75 for casein, the quality values for the high rice diets were similar to the relative nutritional value of rice protein of 50% found by Hegsted (1969). In fact, Hegsted (1971) found the relative nutritional value of the BPI-76-1 rice to be 40%, which is very close to our value of 42%.

The apparent utilizable protein (protein content x relative nutritional value) in Intan was 2.9%; in low protein IR8, 3.2%; in high protein IR8, 4.2%; and in BPI-76-1, 6.0%. The corresponding net protein values (net protein ratio x 16.8 x protein content) were Intan, 2.3%; low protein IR8, 4.1%; high protein IR8, 5.0%; and BPI-76-1, 6.2%. These two indices again show that the nutritional value of milled rice increases with protein content.

Carcasses of rats fed rice diets with 5% protein or less generally had lower nitrogen content but higher crude fat content than carcasses of rats fed casein diets (Bressani *et al.* 1971). Hence, protein quality indices of milled rice based on the weight gain of rats are overestimated, since they assume that the nitrogen content of carcasses is the same regardless of protein source. The nitrogen balance index for Intan was 0.49; for low protein IR8, 0.47; for high protein IR8, 0.36; and for casein, 0.53 at protein levels in the diets of 5% and less.

The varietal differences in protein quality may be explained by the decreasing levels of lysine, threonine, sulfur amino acids, and tryptophan in rice protein as protein content increases (Table 8.4) (Bressani *et al.* 1971). The prolamin fraction tended to increase in the protein of IR8 and BPI-76-1 as protein content increased. However, Intan protein had a higher prolamin content than IR8 protein. In addition to the ratio of protein fractions, varietal differences in amino acid composition of prolamin and glutelin from IR8 and BPI-76-1 have been reported (Palmiano *et al.* 1968; Tecson *et al.* 1971). Thus, the amino acid composition of a protein fraction may differ among rice varieties.

The observations on white rats were confirmed by experiments on seven adult human subjects by Dr. Helen Clark at Purdue University. (Clark *et al.* 1971). Clark compared a sample of the BPI-76-1 (14.5% protein) milled rice with Bluebonnet rice (7.9% protein). When equal weights of milled rice of each variety were fed to the human subjects for a week, BPI-76-1 rice caused a highly significant improvement in nitrogen retention (Table 8.5). The mean nitrogen balance was +1.41 gm per day N for BPI-76-1 rice, +0.24 gm per day for Bluebonnet rice, and +0.48 gm per day for Bluebonnet rice plus sufficient nonspecific nitrogen to bring the nitrogen level to that of BPI-76-1. Adding nitrogen to Bluebonnet rice did not influence nitrogen retention significantly. Clark attributed the high nitrogen retention from the BPI-76-1 rice to its high

TABLE 8.4

LEVELS OF ESSENTIAL AMINO ACIDS AND CYSTINE AND RATIO OF FRACTIONS OF FOUR MILLED RICE SAMPLES USED FOR PROTEIN QUALITY STUDIES AT THE INSTITUTO DE NUTRICION DE CENTRO AMERICA Y PANAMA

Property	Intan	Low Protein IR8	Low Protein IR8	BPI-76-1	LSD (5%)
Essential Amino Acids (Gm/16.8 Gm N)					
Arginine	7.90	8.78	8.82	8.58	NS
Histidine	2.45	2.45	2.48	2.48	NS
Isoleucine	4.89	5.10	4.78	5.34	NS
Leucine	7.84	8.32	8.20	9.63	0.92
Lysine	4.27	3.77	3.69	3.35	0.32
Methionine	3.45	3.02	2.44	1.80	0.36
Methionine + cystine	5.26	4.83	3.89	3.32	0.81
Phenylalanine	5.55	5.74	5.66	6.25	0.41
Threonine	4.10	4.10	3.78	3.75	0.20
Tryptophan	1.35	1.26	1.05	0.97	0.08
Valine	6.24	6.55	6.51	7.30	NS
Ratio of Protein Fractions (Parts per Hundred)					
Albumin	3.6	4.5	3.7	2.6	NS
Globulin	9.3	8.6	10.8	8.9	NS
Prolamin	2.4	1.2	1.9	6.2	0.7
Glutelin	84.7	85.7	83.6	82.3	NS
Protein at 12% moisture (%)	5.68	7.32	9.73	14.3	

Source: Bressani *et al.* (1971).

level of all essential amino acids per unit weight as found by amino acid analysis.

The introduction of high yielding semidwarf varieties like IR8 caused some apprehension among nutritionists who thought these varieties might contain less protein than the traditional varieties in farmers' fields. However, IR8 from farmers' fields was found to have the same protein content (8%) as lower yielding traditional varieties (IRRI 1968, 1970, 1971). We found that the protein contents and aminograms of the other IRRI varieties, IR5, IR20, and IR22, are similar to those of IR8.

A study of sources of variation in Kjeldahl protein showed that in unfertilized plots, the variation among panicles from the same hill was as high (6.0% of the mean) as that between hills (IRRI 1971). The variation in protein content of grain from different branches within each panicle was 3%. In the plots fertilized with 120 kg per ha N, the variation in protein content among panicles within a hill was considerably larger than that among hills. Unlike traditional varieties, the percentage of unfilled grains was not always higher in the lower branches of an IR8 panicle so that the grains at the lower branches did not always contain more protein.

TABLE 8.5

NITROGEN AND ESSENTIAL AMINO ACIDS IN EXPERIMENTAL MILLED RICE DIETS AND THE CORRESPONDING MEAN DAILY NITROGEN BALANCE OF SEVEN HUMAN SUBJECTS (GRAMS PER DAY)

Property	Experimental Diets[1]			
	480BPI	480BB	480BB-N	320BB-N
Nitrogen in Diet				
Rice[2]	11.71	6.37	6.37	4.25
Total	12.06	6.72	12.06	6.72
Amino Acids				
Isoleucine	3.60	1.79	1.79	1.19
Leucine	7.77	3.92	3.92	2.61
Lysine	2.26	1.50	1.50	1.00
Methionine	1.32	1.44	1.44	0.96
Methionine + cystine	2.55	2.31	2.31	1.44
Phenylalanine	4.88	2.30	2.30	1.53
Tyrosine	4.92	2.31	2.31	1.54
Threonine	3.07	1.77	1.77	1.18
Tryptophan	0.91	0.55	0.55	0.37
Valine	5.68	2.84	2.84	1.90
Mean Nitrogen Balance	1.41 ± 0.34[3]	0.24 ± 0.11	0.48 ± 0.12	−0.58 ± 0.12

Source: Clark *et al.* (1971).
[1] Diet description states daily intake in grams of each variety of rice, BPI-76-1 or Bluebonnet, and the presence or absence of nonspecific nitrogen source (N: glycine, glutamic acid, and diammonium citrate).
[2] BPI-76-1 contained 2.44% nitrogen and Bluebonnet rice, 1.33%.
[3] Standard error.

A 10-grain sample was found to be optimal for measuring Kjeldahl protein; it showed less variance than a 5-grain sample and was comparable to samples with up to 100 grains. We digested samples manually for 20 min and determined the ammonia in the digested samples with sodium phenate and hypochlorite reagents in an AutoAnalyzer (Juliano *et al.* 1968).

BREEDING FOR HIGH PROTEIN CONTENT

Since the chief nutritional limitation of rice is its low protein content, breeding efforts to improve its nutritive value have concentrated on increasing its protein content without decreasing protein quality (IRRI 1968, 1970, 1971). Screening 7760 entries for high protein content in IRRI's rice collection revealed 126 entries that consistently had a high protein content of at least 13.5% in 2 samples or a mean level of 14% (Juliano *et al.* 1968). The overall mean protein content was 10.6 ± 1.6% at 12% moisture. Many of these entries were *japonica* varieties, which may have high protein content at Los Baños because of their short growth duration and sensitivity to high ambient temperatures.

Six of the high protein entries were crossed by IRRI breeders to IR8. The

lines with good plant type and high protein content were selected through several generations. Some of the F_7 IR1103 lines (IR8 x Chow-sung) may have good yield potential. Some of them gave 2% more protein than IR8 (IRRI 1970). By screening lines in yield trials, several other semidwarf sources of high protein content were identified: IR480-5-9-3-3 (Nahng Mon S-4/2 x Taichung Native I) and some IR1163 lines (IR8/2 x BPI-76-1). IRRI agronomists first identified IR480-5-9-3-3 as a high protein line. Our breeders will make crosses to combine high protein content from these sources. Our goal is to produce 'ines with 2% higher protein content than IR8 while maintaining its high yield.

The most difficult aspect of the breeding program is the variable effect of environment on protein content. The better lines must be tested at several locations, and only those lines that consistently show high protein content should be considered genetically high protein.

Low protein, semidwarf lines of the IR1103 cross and of crosses involving a low protein Philippine variety, Intan, have been identified for genetic and biochemical studies. In a cooperative study with IRRI agronomists and statisticians, we are trying to determine the effect of management practices, such as spacing and nitrogen level, on protein content of these high protein and low protein rices.

EFFECT OF PROTEIN CONTENT ON GRAIN PROPERTIES IN BREEDING LINES

Amino acid analysis of F_4 brown rice of the line with the lowest and the highest protein contents among the six crosses between IR8 and a high protein variety (Table 8.6) showed a decrease in lysine and tryptophan values in the protein as protein content increased (IRRI 1970). These results indicate that genetics and environment have similar effects on the amino acid composition of rice protein. If both parents have normal lysine content in their protein, however, an increase of 2% may not change the lysine content (as reflected by the two IR8 samples in Table 8.4).

A taste panel evaluation of cooked F_7 milled rice of some of these breeding lines conducted by Dr. Luz U. Onate of the University of the Philippines College of Agriculture showed no significant differences in color scores among lines from the same cross that differed by as much as 4.1% protein and had similar amylose content (Table 8.7) (IRRI 1971). Presumably an increase in protein content of 2% has a negligible effect on the color of milled rice. However, the higher color score for BPI-76-1, as compared to IR8, at similar protein and amylose contents, verify the observed darker color of BPI-76-1 (Juliano *et al.* 1965). Crosses involving BPI-76-1 should be screened for this defect.

Milled rice of 5 lines representing 3 of the 6 crosses between IR8 and high protein varieties was analyzed for amino acid composition. The samples, which had 11.4–11.8% protein, contained 3.3–4.0% lysine, 3.4–4.1% threonine, 0.9–1.2% tryptophan, and 3.0–4.6% sulfur amino acids in the protein. The three

TABLE 8.6

CONTENTS OF ESSENTIAL AMINO ACIDS AND CYSTINE OF F_4 BROWN RICE FROM LOW- AND HIGH-PROTEIN PAIRS FROM CROSSES BETWEEN IR8 AND SIX HIGH-PROTEIN VARIETIES (GRAMS PER 16.8 GM N)[1]

Amino Acid	Low Protein		High Protein		r^2
	Range	Mean	Range	Mean	(n = 12)
Isoleucine	4.29–4.81	4.58	4.44–5.06	4.72	0.360
Leucine	7.79–8.02	7.90	7.95–8.82	8.38	0.791
Lysine	4.04–4.57	4.38	3.47–3.93	3.68	–0.838
Methionine	2.16–3.01	2.58	1.89–2.45	2.26	–0.566
Methionine + cystine	3.46–5.05	4.29	3.48–4.34	4.00	–0.351
Phenylalanine	5.17–5.79	5.48	5.34–5.88	5.62	0.310
Threonine	3.91–4.23	4.04	3.55–4.30	3.83	–0.532
Tryptophan	1.14–1.57	1.37	0.92–1.32	1.06	–0.752
Valine	6.44–7.47	6.73	6.22–7.27	6.80	0.151
Protein at 12% moisture (%)	5.4 –7.6	6.7	12.6–15.3	14.4	

Source: IRRI (1970).
[1] Recalculated to 95% nitrogen recovery; mean nitrogen recovery was 93%.
[2] Correlation coefficient with protein content of brown rice.

TABLE 8.7

PANEL SCORES FOR COLOR FOR MILLED RICE OF HIGH-PROTEIN LINES DIFFERING IN PROTEIN CONTENT

Line of Variety Name	Protein at 12% Moisture (%)	Amylose, Dry Basis (%)	Mean Color Scores[1]
IR1100-185-7-4	9.1	26.2	2.2
IR1100-89-2-6	11.1	23.1	2.2
IR1104-64-4-1	8.8	27.1	1.5
IR1103-15-8-5	11.6	25.2	2.2
IR1103-15-9-8	12.9	25.0	1.8
IR8	9.9	26.0	1.5
BPI-76-1	10.0	26.2	2.8
LSD (5%)			1.3

Source: IRRI (1971).
[1] Mean scores of a panel of six. Numerical scores of 1 to 9 were assigned, a score of 1 representing the least expression of color (white); 3 (cream); and 9, the highest expression (brown). Milled rice (100 gm) was cooked in 220 gm of water in electric rice cookers.

IR1103 lines (IR8 x Chow-sung) showed a high level of these essential amino acids. The protein of BPI-76-1 has 0.5% less lysine than the protein of most other rice varieties (including IR8) at the same protein level (Palmiano *et al.* 1968).

Because of such genetic differences in amino acid composition at similar protein levels, brown rice of varieties in IRRI's world collection is being screened for lysine based on dye-binding capacity (DBC). An AutoAnalyzer dilutes the supernatant dye solution and records its color intensity. DBC values are correlated with the lysine content of rice protein (IRRI 1970). Lysine is the only basic amino acid of rice protein that changes in concentration with changes in protein content (Cagampang *et al.* 1966; Juliano 1965, 1967). From the linear regression equation of DBC (absorbance units) as a function of protein content, entries are being selected that have higher DBC than the value derived from the regression equation. The lysine content of the selected entries will be rechecked by direct chemical methods. The slope of regression line (DBC as a function of protein) was lower for entries with 10% and higher protein contents than for the low protein entries. This change in slope reflects the greater dependence of lysine content on protein content at low-protein than at high-protein levels.

BIOCHEMICAL STUDIES ON RICE PROTEIN

Studies on protein accumulation in the developing IR8 rice grain showed that protein nitrogen does not change in concentration but increases in amount per grain (Palmiano *et al.* 1968). Albumin-globulin increases in amount per grain during the first 2 weeks of ripening, reaches an optimum level between 14 and 21 days, and decreases progressively toward maturity. The major protein change is the large increase in glutelin between 4 and 21 days. Prolamin increases six-fold per grain during this period. Aminograms of the developing caryopsis showed that the 4-day grain had a higher level of lysine (6.7%) and a lower level of glutamic acid (17.2%) than the 14-day and mature grains. These changes are consistent with the increase in prolamin after the fourth day from flowering.

Developing grains of lines with high protein content tended to have a higher level of free amino nitrogen and RNA, and a faster rate of amino acid incorporation than those with normal protein content (Cruz *et al.* 1970). The bleeding sap obtained from the culm ten days after flowering also tended to have a higher level of free amino nitrogen in high protein lines (IRRI 1971). Since physiologists find that little net nitrogen absorption occurs during grain development, protein nitrogen in the grain must originate from translocation of nitrogen already in the rice plant (principally the leaf blades) at flowering. Preliminary results indicate that leaf blades of high protein lines have high protease activity (IRRI 1971). We are checking the nature of leaf proteins and their changes during leaf growth and senescence, and, in samples differing in protein content, the levels of the protease and ribulose 1,5-diphosphate carboxylase activities and the rate of amino acid incorporation in the leaf blades during grain development.

SUMMARY

Rice protein is unique among cereal proteins in that it contains at least 80% glutelin. Albumin and globulin are concentrated in the germ and aleurone layer. Endosperm protein occurs in protein bodies 1–4 μ in size. An increase in protein content in a variety results mainly from an increase in the glutelin fraction with a corresponding increase in the number of protein bodies.

The improved high-yielding semidwarf varieties and the traditional varieties have similar protein content and aminograms, even in farmers' fields. The grain protein content of a variety may vary from field to field, between hills, between panicles from the same hill, and between grains from the same panicles.

Rat feeding experiments with 4 milled rice samples (5.7–14.3% protein) showed that the protein quality of milled rice (at $\leqslant$5% dietary protein levels) decreased with increasing protein content of rice. The decrease in quality was less than proportional to the increase in protein content, however. The contents of lysine, threonine, sulfur amino acids, and tryptophan of the protein also decreased slightly with increasing protein content of milled rice. Human subjects fed milled rice containing 14.5% protein retained more nitrogen than subjects fed rice containing 7.9% protein (even when sufficient nonspecific nitrogen was added to make the diets isonitrogenous) because of the higher levels of essential amino acids in the high-protein sample.

IRRI plant breeders are attempting to improve the protein content of an IR8-type rice by at least 2% by crossing IR8 (8% protein) with high-protein varieties. Some promising lines of good plant type and that have consistently higher protein than IR8 in four generations are being tested in yield trials. The changes in aminograms resulting from differences in protein content among these lines were similar to changes observed previously with samples of the same variety differing in protein content.

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Genetic Studies of Wheat Proteins

INTRODUCTION

The average protein content of wheat usually exceeds 12%. This is higher than the protein content of rice, maize, or sorghum, the other widely utilized food cereals. Production environment exerts a strong influence on protein level of wheat. Protein values as high as 18% and as low as 7% are relatively common in the United States.

The cereal grains lack the balance of essential amino acids required for maximum utilization of their protein when they are the main or sole source of daily nutrition. Lysine is in shortest supply in the cereal proteins; thus, it largely fixes the degree of protein utilization.

Increases in the quantity of protein in the grain of wheat and/or improvement in the ratio of essential amino acids in the protein would enhance significantly its nutritional value. The discovery in 1963 (Mertz *et al.* 1964) of the effect of the *opaque-2* gene in maize on the lysine and tryptophan content of its seed protein pointed to the opportunity for significant genetic improvement of protein quality in the cereal species. A gene in barley with effect on lysine similar to that of *opaque-2* was subsequently reported (Hagberg and Karlsson 1969).

This presentation will be concerned with wheat protein research at the University of Nebraska and the outlook for enhancement of nutritional value in wheat by genetic means.

THE NEBRASKA-ARS RESEARCH PROGRAM

Our efforts to improve the nutritional value of wheat by breeding date back to 1954 when Atlas 66, a soft winter wheat from North Carolina was introduced into our wheat breeding program. Atlas 66, selected from a cross Redhart/Noll/2/Frondoso, already had been determined to be higher in grain protein than other soft wheats. Our early research was supported in part by funds from the Nebraska Wheat Commission. It encompassed investigation of the heritability of grain protein, magnitude and stability of the genetic effect, the relationship of grain protein to grain yield, and physiological aspects of the high protein phenomenon.

The Nebraska-ARS protein research was broadened in 1966 to include investigation of the amino acid composition of wheat protein. Funds from the Agency for International Development, U.S. Dept. of State, were utilized to screen the