CEREAL CHEMISTRY

VOL. 43 MARCH, 1966 No. 2

STUDIES ON THE EXTRACTION AND COMPOSITION OF RICE PROTEINS[1]

GLORIA B. CAGAMPANG, LOURDES J. CRUZ, SALVADOR G. ESPIRITU, REMEDIOS G. SANTIAGO, AND BIENVENIDO O. JULIANO[2]

ABSTRACT

Solubility fractionation of the proteins of milled rice, bran, and rice polish of high- and low-protein samples of three varieties indicated that glutelin was the predominant fraction in the whole grain, in the milled product, and in the rice polish. Albumin and globulin were the major proteins of the bran and were concentrated in the bran and polish, whereas prolamin was rather evenly distributed in all three fractions. Differences in the total protein content of the whole grain were due mainly to differences in glutelin content. Increases in the lysine content of milled rice associated with increases in total protein of eight varieties were less than those of other amino acids. The assessment of extraction of protein from milled rice by 18 solvents indicated that alkaline solvents were better extractants than acidic ones.

Rice is a principal source of protein in the diets of Southeast Asia. Environment affects the protein content of cereals (1,2). In rice, environment has caused grain protein content of a variety to vary from 9.0 to 14.7%, a range of about 6% on dry weight basis (3). Such an increase in protein content has been reported to be accompanied by a reduction in the amount of all the essential amino acids of the protein (4,5). However, a recent study of samples of 16 varieties indicated that not all the essential amino acids are affected by protein content (6). The present study deals with changes in levels of various proteins and amino acids which accompanied changes in total protein content of rice.

Materials and Methods

Rough rice samples obtained from the Institute farm were artificially dried with heated air at 40°C. and dehulled with a McGill

[1]Manuscript received July 26, 1965. Contribution from The International Rice Research Institute, Los Baños, Laguna, Philippines. Mailing address: Manila Hotel, Manila, Philippines.

[2]Research Assistant, Research Aides, and Associate Chemist, respectively, The International Rice Research Institute.

sheller. Brown rice samples were milled and polished with a McGill mill No. 3 (7), and the bran and polish fractions and the milled rice were collected for analysis. These milled samples, as well as samples of the original brown rice, were ground in a Wiley intermediate mill with a 40-mesh sieve. All samples were analyzed for moisture content by the vacuum-oven method (8) and for Kjeldahl nitrogen (8). Crude protein was calculated from Kjeldahl nitrogen by multiplying by the factor 5.95.

All samples were placed in low- or high-protein groups in accordance with total protein contents of the brown rice samples, with a protein content of 10% as the dividing point. Samples of bran, polish, and milled rice of varieties Chia-nan 8, Taichung (Native) 1, and BPI-76 were analyzed in duplicate for protein fractions, by a modification of the procedure of Sturgis *et al.* (1). Samples of 10.00 g. of milled rice, or 5.00 g. of bran or polish, were used in these analyses. The defatted samples were shaken four times with 200-ml. portions of each of the four extracting solvents for 2 hr. at 25°C. The solvents used for the various protein fractions were: distilled water to extract the albumin, 5% sodium chloride for globulin, 60% v./v. aqueous ethanol for prolamin, and 0.4% sodium hydroxide for glutelin. Kjeldahl nitrogen was determined on aliquots of the extracts. (The mean coefficient of variability of single extractions of albumin, globulin, or prolamin was 45%.)

The protein of brown rice was extracted by a modification of the percolation method of Maes (9). Brown-rice powder (10 g.) was defatted with cold petroleum ether and mixed with 15.0 g. of Celite analytical-grade filter-aid. A percolation column (25 × 3.5 cm.) was plugged with glass wool and packed with sea sand (E. Merck Ag.), Celite, the sample-Celite mixture, Celite, and sand in that order. All layers except the sample were about 1 cm. thick. The column was covered at the top with glass wool, held in place with a few glass marbles. The column was leached successively at 25°C. with 1-liter portions of the four solvents listed above, and the extracts were then analyzed for Kjeldahl nitrogen. (The mean coefficient of variability of single determinations for albumin, globulin, and prolamin was 6%.) About 90% of each fraction was extracted by the first 250 ml. of solvent. There was no further extraction of protein with 10% sodium chloride, or with 70% v./v. ethanol after extraction with 60% v./v. ethanol.

This same general procedure of extraction was used on a 100-mesh fraction obtained by sieving the 40-mesh powder through a 100-mesh sieve.

The efficiency of extracting protein by a group of 18 solvents was also assessed for the 100-mesh fraction of powdered milled rice of the BPI-76 variety. Samples of rice powder (100-mg.) were shaken for 6 hr. at room temperature with 2 ml. of each solvent, in a Burrell shaker. The samples were then centrifuged at 10,000 r.p.m. The residue was washed with distilled water and total nitrogen determined on the combined extract and washings; or, in the case of nitrogen-containing extractants, the residue was analyzed for total nitrogen. Preliminary experiments with 0.1*N* sodium hydroxide indicated that an extraction of 3 to 6 hr. was optimum and that the extraction efficiency was the same at room temperature as at 4°C. The rice powder clumped with solvents containing Santomerse 1 (sodium dodecyl benzene sulfonate, 40% active ingredient).

Total amino acids were estimated on low- and high-protein samples of milled rice of eight varieties (3) with a Beckman Model 120 amino acid analyzer (6,10). In this "low" and "high" grouping the criterion used was the total protein content of milled rice rather than brown rice, with a protein content of 9% as the dividing point. Nitrogen recoveries ranged from 80 to 95%. Data were readjusted to 95% nitrogen recovery and expressed as g. per 16.8 g. of nitrogen. Tryptophan content was determined colorimetrically (11).

Results and Discussion

The high-protein samples had lower degrees of milling by standard methods (7) than the low-protein samples of the same variety. This was indicated by the lower yields of bran and polish (Table I). Presumably the high-protein samples have harder grains than the low-protein samples, as shown by their greater resistance to milling.

As a percentage of dry weight, the protein content of brown rice, bran, and polish was higher than that of milled rice, particularly in the low-protein samples. Although this may be partly explained by the higher degree of milling noted for the low-protein sample, the protein-rich grain may have a more even protein distribution. Thus, with increases in protein content, the protein of milled rice increases more rapidly than the bran. Consequently, the nutritional benefits of increases in protein of the whole grain are not eliminated in milling as is frequently stated. The loss in protein of brown rice during milling ranged from 11 to 26% at milled rice recoveries of 86 to 90%.

The bran had a lower protein content than the polish in all the high-protein samples and in the low-protein BPI-76 sample. This indicates that the outermost layer of the rice grain may not have the highest protein content. In fact, more-refined milling of successive

TABLE I

WEIGHT RATIO AND CONTENTS OF PROTEIN AND PROTEIN FRACTIONS IN BROWN RICE MILLING FRACTIONS OF LOW- AND HIGH-PROTEIN SAMPLES OF THREE VARIETIES

BROWN RICE FRACTION	LOW-PROTEIN SAMPLE			HIGH-PROTEIN SAMPLE		
	Chia-nan 8	Taichung (Native) 1	BPI-76	Chia-nan 8	Taichung (Native) 1	BPI-76
	%	%	%	%	%	%
Weight[a]						
Milled rice	86.7	88.1	86.0	89.7	88.9	90.0
Rice polish	1.8	2.2	2.0	1.5	1.8	1.2
Rice bran	11.5	9.7	12.0	8.8	9.3	8.8
Protein[b]						
Brown rice	7.75	7.81	9.08	13.6	15.8	16.3
Milled rice	6.78	7.20	8.53	13.1	15.2	16.1
Rice polish	13.8	12.2	14.1	19.0	17.9	18.4
Rice bran	15.5	13.6	13.3	17.4	16.6	16.0
Albumin[b]						
Milled rice	0.43	0.63	0.32	0.63	0.49	0.39
Rice polish	3.60	3.16	2.81	4.52	4.33	3.61
Rice bran	4.31	3.93	3.36	4.21	5.61	3.87
Globulin[b]						
Milled rice	0.73	0.69	0.92	0.78	0.97	1.11
Rice polish	1.26	0.96	1.02	3.53	1.77	2.10
Rice bran	4.17	3.86	3.19	4.65	4.03	4.70
Prolamin[b]						
Milled rice	0.18	0.19	0.28	0.39	0.24	0.56
Rice polish	0.36	0.49	0.54	0.63	0.73	0.99
Rice bran	0.54	0.68	0.52	0.47	0.45	0.53
Glutelin[b]						
Milled rice	5.29	4.89	5.64	10.2	11.4	11.5
Rice polish	5.92	5.10	5.51	6.86	7.41	7.76
Rice bran	2.32	1.80	3.06	2.84	2.81	2.51

[a] Percentage of brown rice, dry weight.
[b] Dry weight basis.

layers of brown rice by Primo *et al.* (12) and Hogan *et al.* (13) demonstrated that the fraction with the highest protein level was not the outermost 5% fraction, but the second-outermost 5% fraction.

Glutelin was the principal protein of the whole grain, of the milled rice, and of the rice polish fraction. The major proteins in the rice bran were albumin and globulin. The mean ratio of albumin:globulin: prolamin:glutelin for the six samples was 37:36:5:22 for bran, 30:14: 5:51 for polish, and 5:9:3:83 for milled rice. This indicates that glutelin was concentrated in the milled fraction and albumin and globulin in the bran and in the rice polish, and that prolamin was the most evenly distributed protein of the rice grain. These protein ratios also indicate that a major portion of the albumin and globulin were removed during milling. Lózsa (14) and Lindner *et al.* (15) had made a similar observation regarding albumin. Some reported ratios of protein fractions of milled rice are 2:8:8:82 (14), 1:12:5:81 (15), and 4:15:3:78 (16). These data on protein distribution agree with the results obtained by Michael *et al.* (17) on barley. Barley endosperm was rich in prolamin and glutelin; the aleurone, in globulin; and the embryo, in albumin and, to a lesser extent, in globulin.

Differences in the total protein content of low- and high-protein samples of the three varieties resulted largely from differences in glutelin content (Table I). Milled rice of the high-protein samples had about twice as much glutelin as that of the low-protein samples. The prolamin content of the milled rice of varieties Chia-nan 8 and BPI-76 also increased twofold with increase in protein content. The corresponding change in prolamin content of Taichung (Native) 1 milled rice was less. In contrast, albumin and globulin contents of milled rice were only slightly affected by protein content.

The above data on the relationship between protein content and the levels of the various proteins of rice were further verified by analysis of five samples of BPI-76 brown rice of different protein levels (Table II). The data indicated that additional glutelin and prolamin accounted for the increase in protein content of brown rice and that protein content did not affect the quantity of albumin and globulin. The mean albumin:globulin:prolamin:glutelin ratio was 5:7:4:84 for the five BPI-76 samples.

TABLE II

SOLUBILITY FRACTIONATION OF PROTEINS OF 40- AND 100-MESH BROWN RICE SAMPLES BY PERCOLATION

Sample	Protein Content	Protein Fraction					Extraction Efficiency
		Albumin	Globulin	Prolamin	Glutelin	Total	
	%	%	%	%	%	%	%
Waxy (40-mesh)							
Niaw Sanpatong	10.60	0.37	0.43	0.19	6.28	7.27	68.6
Gam Pai 41	10.76	0.41	0.43	0.28	6.40	7.52	69.9
Nonwaxy (40-mesh)							
BPI-76	9.08	0.42	0.58	0.27	4.98	6.25	68.8
BPI-76	10.00	0.34	0.45	0.24	6.01	7.04	70.4
BPI-76	12.20	0.38	0.59	0.34	6.70	8.01	65.7
BPI-76	13.91	0.43	0.70	0.34	7.61	9.08	65.3
BPI-76	16.30	0.44	0.61	0.57	8.96	10.58	64.9
Analysis of variance:							
Standard error		0.01	0.04*	0.01**	0.11**		
r[b]		+0.555	+0.522	+0.898**	+0.986**		
Nonwaxy (100-mesh)							
BPI-76	9.74	0.49	0.62	0.14	7.00	8.25	84.7
BPI-76	16.90	0.38	1.02	0.58	12.70	14.68	86.9

[a] Percentage of brown rice, dry weight basis.
[b] Correlation coefficient between the contents of protein and protein fraction in the five samples of BPI-76 brown rice.

These results agree with the findings of Michael *et al.* (17) and Ozaki and Moriyama (18) that increased protein content in a rice variety results from an increase mainly in the amount of glutelin in the grain. In their analyses of protein fractions of brown rice of 29 United States varieties and selections, Sturgis *et al.* (1) found that albumin as a percentage of total protein was negatively correlated to total protein content, whereas prolamin and glutelin contents were positively correlated with protein levels. However, they observed no correlation between globulin level and protein content. Similar changes in the quantity of prolamin and glutelin with a change in protein level have been reported for wheat, barley, and oats (17), and for corn (19). Reported albumin:globulin:prolamin:glutelin ratios for brown rice include 14:20:3:63 (1), 4:8:8:80 (14), 10:4:10:75 (15), and 3.5:10.8:4.4:81.2 (20).

Two samples of waxy (glutinous) rice had protein-fraction levels similar to those of nonwaxy variety BPI-76 (Table II). Presumably, the gross protein composition is not related to the amylose:amylopectin ratio of the starch granules of the rice grain.

The leaching efficiency of the percolation ranged from 64.9 to 70.2% for 40-mesh powders. This is similar to the results obtained by Maes (21), who extracted with water, 40% v./v. isopropyl alcohol, 3.85% v./v. lactic acid, and 0.5% potassium hydroxide.

The particle size of the powdered sample was a critical factor in determining yields by leaching extraction. With a change in mesh size from 40 to 100, the protein extraction yields increased from the 64.9 to 70.4% range to values of 84.7 and 86.9% (Table II). The increase was mainly in the quantity of glutelin. Similar results were implied by data from equilibrium extractions using the method of Sturgis *et al.* (1). The mean protein yield from 20-mesh brown-rice flour reported by Sturgis *et al.* was 62%. Primo *et al.* (16) reported 59% extraction from samples of a similar particle size. The mean recovery in the present study was 89% for 40-mesh milled rice powder.

A series of equilibrium extractions gave higher yields for 40-mesh rice powder than the percolation method adapted from Maes (9). The higher recoveries by the former method may have resulted from further disintegration of the powder during mechanical shaking.

The relative extraction efficiency of various solvents was surveyed on a 100-mesh powder of the variety BPI-76 with 14.6% protein, wet basis. The data obtained (Table III) were from single extractions of 6 hr.; hence, the conditions may not have been optimum for some solvents (22,23). Alkaline solvents were generally better than acidic solvents. However, 4% lactic acid was the best among the acidic sol-

TABLE III
EXTRACTION EFFICIENCIES OF 18 SOLVENTS ON MILLED-RICE PROTEINS[a]

SOLVENT[b]	RESIDUAL PROTEIN	EXTRACTION EFFICIENCY
	mg.	%
0.014*M* Cupric sulfate, 0.004*M* sodium sulfite, 0.05*N* sodium hydroxide	0.4	97
0.1*N* Potassium hydroxide	0.5	97
0.1*N* Sodium hydroxide	0.5	97
3% Santomerse 1, 0.2% sodium bisulfite, 2% sodium carbonate	1.4	90
0.02*M* Cuprammonium hydroxide, 0.05*M* sodium sulfite	3.0	79
0.1*M* Glycine buffer (pH 11.7)	4.0	73
0.5% Sodium lauryl sulfate	4.8	67
4% Lactic acid	7.8	47
3% Santomerse 1	8.4	42
0.1*M* Glycine buffer (pH 1.9)	9.0	38
Ethylene chlorohydrin	9.9	32
2.5*M* Guanidine hydrochloride	10.4	28
0.1*N* Acetic acid	12.7	13
10% Sodium salicylate	12.9	12
0.05*M* Formate buffer (pH 3.5)	13.2	10
0.1*M* Carbonate buffer (pH 10.0)	13.3	9
4*M* *N,N*-dimethylformamide	14.2	3
0.2% Sodium bisulfite	14.2	3
Control (water)	14.2	3

[a] 2.00 ml. solvent and 100 mg. rice powder (14.6 mg. protein) shaken for 6 hr. LSD (5%) = 0.6 mg.
[b] Aqueous solvents, except for ethylene chlorohydrin.

vents tested. Alkaline copper sulfate solutions with sulfite added showed no advantage over dilute alkali alone in dissolving rice proteins. Those containing sulfite have been reported to split disulfide linkages in protein (22). Alkali solutions alone would be expected to extract similar amounts of protein with less modification during extraction. Krishna Murti (24) also found no significant differences between alkali and cuprammonium hydroxide-sulfite reagent as a protein solvent for some plant seeds, including wheat. Optimum extraction periods for cereals of between 4 and 6 hr. have also been reported by Mertz and Bressani (22) for these two reagents and for similar extractants prepared with sodium hydroxide as the added base.

The amount of "residual" protein of rice flour decreased with higher degree of grinding; it was down to only 3% of the total protein with a single extraction with dilute alkali (Table III). Hence, "residual" protein must be essentially an artifact resulting from the incomplete contact between the solvent and the protein of the rice flour. The relatively poorer recoveries of protein from flours of rice as compared with other cereal grains of the same particle size (17,21) are perhaps explained by the smaller size of the starch granules of rice.

TABLE IV

AMINO ACID COMPOSITION OF MILLED RICE[a] OF EIGHT VARIETIES OF LOW- AND HIGH-PROTEIN LEVELS, AND CORRELATION COEFFICIENT BETWEEN AMINO ACID AND PROTEIN CONTENT

AMINO ACID	MILLED RICE[b]				r[c]
	Low-Protein		High-Protein		
	Range	Mean	Range	Mean	
	g.	g.	g.	g.	
Alanine	5.3 – 7.9	6.26	5.4 – 6.5	6.14	−0.126
Arginine	7.6 – 9.4	8.43	7.0 – 9.3	8.19	−0.100
Aspartic acid	9.1 –11.4	10.15	7.4 –13.4	10.40	−0.065
Cystine	0.45– 1.1	0.87	0.31– 1.5	0.95	+0.223
Glutamic acid	17.5 –23.0	20.97	22.1 –25.4	23.22	+0.573
Glycine	3.9 – 5.9	4.84	3.9 – 5.0	4.63	−0.137
Histidine	2.4 – 2.9	2.61	2.1 – 2.8	2.43	−0.338
Isoleucine	4.0 – 5.2	4.47	3.8 – 4.8	4.45	+0.001
Leucine	8.0 – 9.6	8.75	7.0 – 9.8	8.47	+0.048
Lysine	3.4 – 5.0	4.35	3.0 – 4.3	3.66	−0.664**
Methionine	1.6 – 2.6	2.13	0.7 – 2.6	1.82	−0.164
Phenylalanine	4.9 – 6.8	5.68	4.6 – 7.1	5.71	−0.137
Proline	5.3 – 6.9	5.87	4.6 – 6.3	5.42	−0.366
Serine	4.4 – 6.2	5.14	5.2 – 7.8	6.08	+0.401
Threonine	3.2 – 4.6	3.78	3.1 – 4.5	3.74	−0.192
Tryptophan[d]	1.04– 1.51	1.28	1.06– 1.52	1.27	+0.053
Tyrosine	1.9 – 3.0	2.35	2.3 – 4.2	3.34	+0.689**
Valine	3.6 – 6.6	5.63	5.0 – 6.5	5.87	+0.171
Ammonia	2.0 – 2.9	2.45	1.8 – 3.3	2.44	−0.196
Protein content,[e] % of milled rice	6.15– 8.86	7.33	9.27–14.34	11.90	

[a] Calculated to 16.8 g. of nitrogen.
[b] Adjusted to 95% nitrogen recovery of acid hydrolysate by the method of Moore *et al.* (6,10).
[c] Correlation coefficient between protein content and individual amino acids.
[d] By the method of Spies and Chambers (see ref. 13).
[e] Dry weight basis.

The residual protein after successive extractions of the rice powder with water, neutral salt solution, and aqueous alcohol is principally glutelin. In fact, Lózsa (14) defined glutelin in this way rather than as the alkali-soluble extract. Crude rice glutelin extracted with alkali has been noted to be slightly contaminated with albumin, globulin, and prolamin[3] which may have been encased in the glutelin matrix. Although electrophoretic studies (25,26) of the proteins of other cereals indicate that these protein fractions are not entirely group-specific and are highly complex, this solubility fractionation is still a standard method for classifying cereal proteins.

The amino acid data for milled rice indicated a wide range of values among the eight varieties (Table IV). They compare favorably with those reported by other investigators (4–6, 27–29) (Table V). The

[3] S. G. Espiritu, unpublished data, 1964.

TABLE V

AMINO ACID COMPOSITION OF MILLED RICE AS REPORTED BY VARIOUS INVESTIGATORS, AND FAO REFERENCE AMINO ACID PATTERN[a]

AMINO ACID	CHANCEL (27)	TAMURA AND KENMOCHI (28)	TABLE IV	KIK (29)	KYMAL (4)	FAO PATTERN (32)
Alanine	5.33– 6.54	5.75	5.3 – 7.9	4.26		
Arginine	8.42– 9.46	5.97	7.0 – 9.4	8.52		
Aspartic acid	8.66– 9.46	9.00	7.4 –13.4	9.94		
Cystine	2.20– 2.51	2.04	0.3 – 1.5	1.70		
Glutamic acid	17.83–20.55	17.96	17.5 –25.4	17.00		
Glycine	4.38– 4.78	4.40	3.9 – 5.9	5.68		
Histidine	2.18– 2.59	2.01	2.1 – 2.9	2.84		
Isoleucine[b]	4.04– 4.49	3.72	3.8 – 5.2	5.68	3.42– 5.36	4.54
Leucine[b]	8.31– 9.01	7.63	7.0 – 9.8	9.90	7.13–10.5	5.15
Lysine[b]	3.40– 3.55	3.17	3.0 – 5.0	4.82	2.96– 4.45	4.54
Methionine[b]	1.22– 1.74	2.58	0.7 – 2.6	3.12	0.92– 1.52	2.42
Phenylalanine[b]	5.23– 5.67	4.60	4.6 – 7.1	4.54	3.94– 6.66	3.02
Proline	4.33– 4.72	5.33	4.6 – 6.9	4.26		
Serine	5.01– 5.36	4.48	4.4 – 7.8	4.82		
Threonine[b]	3.38– 3.59	3.34	3.1 – 4.6	5.68	2.84– 4.65	3.02
Tryptophan[b]		1.31	1.0 – 1.5	2.41	1.64– 2.99	1.51
Tyrosine	4.84– 5.62	1.46	1.9 – 4.2	5.82		
Valine[b]	5.75– 6.55	5.59	3.6 – 6.6	7.10	6.15– 8.54	4.54
Ammonia	2.18– 2.40	1.96	1.8 – 3.3			
Protein content of milled rice, %[c]	7.60– 9.62	5.20	6.15–14.34	7.02	5.81– 8.91	

[a] Calculated to 16.8 g. of nitrogen.
[b] Essential amino acid.
[c] Dry weight basis.

data of Chancel (27) and of Tamura and Kenmochi (28) were obtained by the same chromatographic technique and differed from data in Table IV mainly in their higher values for cystine. Chancel (27) obtained tyrosine values higher than those reported by the others. The data of Kik (29) and Kymal (4), which were obtained by microbiological procedures, were similar to those in Table IV except for their higher values for valine.

With increases in protein content of milled rice, lysine increased significantly less than the other amino acids (Table IV). In contrast, glutamic acid and tyrosine were positively correlated with protein content. These trends for lysine and tyrosine were previously reported in milled rice of 16 varieties by Juliano *et al.* (6). Kik (5) and Kymal (4) also noted the same trend for lysine, but in addition, they observed a similar trend for the other seven essential amino acids.

This relation between lysine and protein is consistent with the observed increase in the quantity of prolamin as total protein increases, since prolamin has the lowest lysine content of the four rice-protein fractions (15). This finding has also been thought to explain similar negative correlations in corn (19) and wheat (30). The lysine content of rice protein dropped from 4.35 to 3.66% as the total protein content of the samples increased from 7.33 to 11.9%. Actually, there is a net gain in the lysine content of milled rice with an increase in protein content. The drop in the percentage of lysine was relatively less than that reported for wheat and corn, probably because prolamin is only a minor protein fraction in rice. Hence, it is not surprising that Lózsa and Koller (31) found that a larger quantity of protein in rice, from a range of 6.7 to 8.0 to a range of 7.9 to 13.4%, was not associated with a decline in the nutritive quality of the protein.

The nutritional value of rice protein is very important in the rice-eating regions of Asia. Rice protein is deficient in lysine, the sulfur-containing amino acids, and tryptophan as judged by the provisional reference amino acid pattern of the Food and Agricultural Organization of the United Nations (32) (Table IV). These are only tentative standards, however, and a recent appraisal (33) suggested the need for possible changes. The availability of the individual amino acids will also have to be considered in evaluating the quality of rice protein (34).

Acknowledgment

The authors are grateful to Miss Teresita O. Villegas for her assistance in the protein fractionation.

Literature Cited

1. Sturgis, F. E., Miears, R. J., and Walker, R. K. Protein in rice as influenced by variety and fertilizer levels. Louisiana Exp. Sta. Tech. Bull. 466 (1952).
2. Bolhuis, G. G. Some considerations on the influence of external conditions upon the quality of tropical food-crops. Qualitas Plant. Mater. Vegetabiles **10:** 236–247 (1963).
3. International Rice Research Institute. 1963 annual report. The Institute: Los Baños, Laguna, The Philippines (1963).
4. Kymal, P. K. Variation in amino acid content of rice varieties. Ph.D. thesis, Louisiana State Univ. (1955).
5. Kik, M. C., and Hall, V. L. The nutritive value of rice. Arkansas Farm Research **10** (3): 11 (1961).
6. Juliano, B. O., Bautista, G. M., Lugay, J. C., and Reyes, A. C. Studies on the physicochemical properties of rice. J. Agr. Food Chem. **12:** 131–138 (1964).
7. Agricultural Marketing Service, U.S. Department of Agriculture. Rice inspection manual. GR Instruction No. 918–2 (revised). Effective April 1, 1962.
8. Association of Official Agricultural Chemists. Official methods of analysis (9th ed.), Secs. 2.034 and 22.003. The Association: Washington, D.C. (1960).
9. Maes, E. Progressive extraction of proteins. Nature **193:** 880 (1962).
10. Moore, S., Spackman, D. H., and Stein, W. H. Chromatography of amino acids on sulfonated polystyrene resins. Anal. Chem. **30:** 1185–1190 (1958).
11. Spies, J. R., and Chambers, D. C. Chemical determination of tryptophan in proteins. Anal. Chem. **21:** 1249–1266 (1949).
12. Primo, E., Casas, A., Barber, S., and Barber, C. B. Factores de calidad del arroz. IV. Distribución del nitrógeno en el endospermo. Rev. Agroquím Tecnol. Alimentos **3:** 22–26 (1963).
13. Hogan, J. T., Normand, F. L., and Deobald, H. J. Method for removal of successive surface layers from brown and milled rice. Rice J. **67** (4): 27–34 (1964).
14. Lózsa, A. Hazai rizsek fehérje-frakcióinak vizsgalata. Agrokem. Talajtan **2:** 147–160 (1953).
15. Lindner, K., Korpäczy, I., Jaschik, S., and Szöke, K. Der Nährwert der in Urgarn angebauten Reissorten. Qualitas Plant. Mater. Vegetabiles **8:** 25–37 (1961).
16. Primo, E., Casas, A., Barber, S., Barber, C. B., Alberola, J., and Piñaga, F.

Factores de calidad del arroz. IX. Influencia de las fracciones proteicas sobre la calidad del cocción. Rev. Agroquím. Tecnol. Alimentos **2:** 354–359 (1962).
17. MICHAEL, G., BLUME, B., and FAUST, H. Die Eiweissqualität von Körnen verschiedener Getreidearten in Abhängigkeit von Stickstoffversorgung und Entwicklungzustand. Z. Pflanzenernaehr. Dueng. Bodenk. **92:** 106–116 (1961).
18. OZAKI, K., and MORIYAMA, M. The nitrogen metabolism of rice plant. I. Protein content and quality of hulled rice from the plant to which nitrogen was applied at heading. Nippon Dojo-Hiryogaku Zasshi **23:** 146–148 (1953).
19. BRESSANI, R., and MERTZ, E. T. Studies on corn proteins. IV. Protein and amino acid content of different corn varieties. Cereal Chem. **35:** 227–235 (1958).
20. TAIRA, H. Studies on amino acid contents in plant seeds. II. Amino acid pattern of seed protein fractions of Gramineae. Shokuryo Kenkyusho Kenkyu Hokoku **17:** 308–311 (1963).
21. MAES, E. Mehleiweissloslichkeit und Backqualität. VIII. Andere Getreidearten ausser Weizen. Getreide Mehl **14:** 17–18 (1964).
22. MERTZ, E. T., and BRESSANI, R. Studies on corn proteins. I. A new method of extraction. Cereal Chem. **34:** 63–69 (1957).
23. MCINTYRE, R. T., and KYMAL, K. Extraction of rice proteins. Cereal Chem. **33:** 38–44 (1956).
24. KRISHNA MURTI, C. R. Comparative study of alkali and Swan's reagent in the extraction of proteins from plant materials. Proceedings of Symposium on Proteins, Aug. 14–16, 1960, Mysore, p. 50–54. Central Food Technol. Research Inst., Mysore, India (1961).
25. PADMOYO, M., and HÖGL, O. Beitrage zu der Papierelektrophorese des Getreide-Eiweiss. I. Mitt. Gebiete Lebensm. Hyg. **53:** 290–319 (1962).
26. GEHRKE, C. W., OH, Y. H., and FREEARK, C. W. Chemical fractionation and starch gel-urea electrophoretic characterization of albumins, globulins, gliadins, and glutenins in soft wheat. Anal. Biochem. **7:** 439–460 (1964).
27. CHANCEL, J. G. Le riz de Camargue. Etude bromatologique. D. Pharm. Thesis, School Med. Pharm. Marseilles (1962).
28. TAMURA, S., and KENMOCHI, K. Studies on amino acid content of rice. III. Distribution of amino acid in rice grain. Nippon Nogeikagaku Kaishi **37:** 753–756 (1963).
29. KIK, M. C. Nutritional improvement of rice diets and effect of rice on nutritive value of other foodstuffs. Arkansas Univ. (Fayetteville) Agr. Exp. Sta. Bull. 698 (1965).
30. MCDERMOTT, E. E., and PACE, J. Comparison of the amino acid composition of the protein in flour and endosperm from different types of wheat, with particular reference to variation in lysine content. J. Sci. Food Agr. **11:** 109–115 (1960).
31. LÓZSA, A., and KOLLER, K. Estimation of the biological value of rice proteins. Acta Physiol. Acad. Sci. Hung. **5:** 477–487 (1953).
32. FOOD AND AGRICULTURAL ORGANIZATION, UNITED NATIONS. Protein requirements. FAO Nutr. Studies Series 16 (1957).
33. NATIONAL ACADEMY OF SCIENCES-NATIONAL RESEARCH COUNCIL. Evaluation of protein quality. NAS-NRC Publ. 1100 (1963).
34. HARPER, A. E., and DE MUELENAERE, H. J. H. The nutritional value of cereal proteins with special reference to the availability of amino acids. Proc. Fifth Internat. Congr. Biochem. **8:** 82–107 (1963).