Radial Distribution of Protein by Solubility Classes in the Milled Rice Kernel

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Radial distribution of the four solubility classes of protein was determined for eight varieties of milled rice. Five consecutive layers of the kernel, totaling about 10% by weight, were removed by abrasive milling. Original and residual kernels, and all flours, were percolated successively with water, 5% sodium chloride, and 60% ethanol to remove albumins, globulins, and prolamins; glutelins were calculated by difference. Albumin and globulin were concentrated in proteins at the surface of all rices; concentrations decreased toward the kernel center. Globulin generally was somewhat less concentrated than albumin in the outer kernel layers and had less steep gradients toward the center. Globulin and albumin gradients differed independently among varieties. The limiting amino acid, lysine, occurred in 12% greater concentration in the protein of the outer layer of Calrose rice than in the protein of the total kernel.

Ample documentation exists for the presence of a higher protein concentration in the outer portion of the milled rice kernel than in the center (Hogan *et al.*, 1964; Houston, 1967; Houston *et al.*, 1964; Little and Dawson, 1960; Normand *et al.*, 1965; Primo *et al.*, 1962a, 1963; Subrahmanyan *et al.*, 1938). Only scant evidence, however, has been found to show whether or not the composition of the protein varies with its radial location in the kernel.

Williams (1962) reported that the average content of lysine in rice decreased as additional material was removed by milling, while the content of several other amino acids increased. It was implied that the composition and distribution of proteins within the grain were not uniform. Conversely, Normand and coworkers (1966) found very little difference in the amino acid composition of successively removed layers of a sample of Bluebonnet 50 kernels. This would suggest little if any compositional differences in the protein. Neither study, however, examined the protein components directly.

Partial comparisons are possible by use of the Maes procedure (Maes, 1962), developed for comparing proteins of different wheats. This consists essentially of a series of consecutive percolative extractions with different solvents to remove different classes of protein. It has been used on at least one sample of milled rice (Maes, 1964). Cagampang and coworkers (1966) used it to demonstrate the differences in protein composition among milled rice, rice bran, and rice polish.

In the present study, consecutive extractions were made with water, 5% sodium chloride solution, and 60% alcohol to remove, respectively, the classical solubility groups of albumins, globulins, and prolamins. Essentially all the residual protein can be extracted with dilute alkali under suitable conditions (Cagampang *et al.*, 1966), and corresponds to glutelin. Application of this procedure to flours made by consecutively removing layers of the milled rice kernel has made possible the study of the radial distribution of the several protein classes within the kernel.

MATERIALS AND METHODS

Rice Supplies. The nonglutinous milled (white) rices, except Earlirose, were portions of larger supplies of 1966-crop commercially well-milled rices of known single varieties. Caloro, Colusa, and Calrose were grown in the Sacramento Valley, California; Saturn and Belle Patna in Lousiana, and Bluebonnet 50 in Arkansas. Portions were distributed to the Southern Regional Research Laboratory (USDA) and the University of California, Berkeley, to provide common stocks for related experimental programs. Earlirose (1966 California crop) milled rice was prepared according to official government inspection procedures (U.S. Agricultural Marketing Service, 1962). Commercially milled California 1965 Caloro rice and glutinous rice were also examined.

Analytical Samples. A 7-kg. portion of each rice was abrasively milled five consecutive times by passage through a CeCoCo Model D Japanese rice-whitening machine, obtained from Chuo Boeki Goshi Kaisha (Central Commercial Co.), Ibaraki City, Osaka Prefecture, Japan. The flour produced from the surface of the kernels was separately collected after each milling (Houston *et al.*, 1964),

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and sieved through an 18-mesh screen to remove small broken pieces of rice. Flours obtained were defatted by percolation with ethyl ether.

Portions of the original milled rice and the residual kernels were ground to pass 60-mesh and analyzed without defatting, as fat content was very low.

Protein Extraction Procedure. This is a slight modification of the Maes percolative extraction (Maes, 1962). Portions of rice used contained 80 to 150 mg. of nitrogen; this usually corresponded to 5 grams of high-protein flour or 10 grams of ground rice. The sample was thoroughly mixed by mortar and pestle with 10 grams of Celite Analytical Filter Aid and then with 100 grams of sea sand (Baker and Adamson). The percolation column (30 × 3.5 cm., with a 5-mm. outlet) was plugged with glass wool and packed with successive layers of acid-washed coarse river sand, sea sand, sample-Celite-sand mixture, sea sand, and river sand. Each sand layer was about 1 cm. thick, and total packed height of solids was 19 to 20 cm.

Extractions were made by successive percolations with distilled water, 5% sodium chloride solution, and 60% ethanol; eluents were collected in volumes of 350, 300, and 250 ml., respectively. These volumes had been found adequate by preliminary experiments in which the eluate was continuously monitored by an ultraviolet detector at 280 mμ to determine when the eluate no longer contained protein.

Crude protein contents of eluates were calculated by multiplying Kjeldahl nitrogen (determined in aliquots) by 5.95. The glutelin component was calculated by difference. Reported figures are the results of one to six analyses per sample.

Amino acid analyses were made on a Phoenix automatic analyzer with samples hydrolyzed 24 hours at 110° C. with 6*N* HCl in evacuated sealed tubes (Kohler and Palter, 1967).

Starch-gel electrophoresis was performed in 0.017*M* aluminum lactate buffer (pH 3.2) 8*M* in urea for 16 hours at 6 volts per cm.

RESULTS AND DISCUSSION

Evaluation of Data. Comparisons among the reported compositional data are subject to several limitations. The method is empirical, for segregation of proteins into solubility classes is not absolute and depends to some extent on the extractants and conditions used. Solubility separations are not completely clear-cut. Slight overlap of components among the fractions was found in the present work by starch-gel electrophoretic patterns (not shown) of the several classes of extracted protein. Also, values found for the outermost layer may be affected by residual traces of aleurone layer and scutellum.

Protein values are for crude protein based on total nitrogen content. As amino acids are reported to account for 1 to 2% of total nitrogen in milled rice (Kester *et al.*, 1963), they would form a small proportion of material recorded as albumin, and perhaps as globulin.

Reproducibility of data is about ±10% for albumins, prolamins, and the higher values for globulins, and considerably closer (about ±2%) for glutelins. Variability in low globulin values is somewhat more, with an occasional sample differing as much as 50%, because the salt in solution restricts sample sizes to near the lower titration limits. Accordingly, more replications were required for globulin values than for other components.

The analytical separation is not applicable to parboiled rices, because the proteins have been denatured in the parboiling process.

Whole Milled Kernels. The proportions of the several solubility classes making up the total protein varied appreciably in the different samples of rice examined. When the varieties were arranged in order of the proportion of albumin present, as in Table I, there was a regular progression from waxy through short- and medium-grain to long-grain rices. This regularity was not shown for other classes of proteins, nor for total protein; however, the long-grain varieties contained more protein than the others.

There is considerable variation in the ratios of albumin-globulin-prolamin-glutelin, spreading from the 1:4:2:93 of Belle Patna and the 2:3:3:92 of Earlirose to the 2:8:-2:88 of Saturn and the 3:7:2:88 of Mochi Gome.

The mean ratio of albumin-globulin-prolamin-glutelin, 2:4:2:92 for the eight varieties, differs somewhat from the reported values of 4:15:3:78 (Primo *et al.*, 1962b), 1:12:5:81 (Lindner *et al.*, 1961), 2:8:8:82 (Cagampang *et al.*, 1966; Losza, 1953), and 5:9:3:83 (Cagampang *et al.*, 1966). The variability among the several reported determinations undoubtedly results in part from experimental differences, and may reflect environmental differences to some extent. Cagampang and coworkers (1966) found that the ratio varied with protein content in a single variety of milled rice from 5:6:3:84 at 9.1% protein to 3:4:3:90 at 16.3%.

Table I. Protein Distribution in Milled Rices

Variety	Grain Type	Protein, %, Dry Basis	Per Cent of Total Protein			
			Albumin[a]	Globulin	Prolamin	Glutelin[b]
Mochi Gome	Waxy	6.49	3.1	6.6	2.1	88.2
Colusa	Short	6.49	2.7	1.6	2.0	93.7
Caloro	Short	5.77	2.5	3.1	3.2	91.2
Saturn	Medium	6.84	2.1	7.6	1.8	88.5
Calrose	Medium	6.90	1.7	4.3	1.8	92.2
Earlirose	Medium	5.24	1.6	3.0	3.4	92.0
Bluebonnet 50	Long	8.98	1.2	3.2	3.5	92.1
Belle Patna	Long	9.82	0.8	4.4	1.7	93.1

[a] Includes free amino acids.
[b] By difference.

Within-Kernel Distributions. Several regularities occur in the relative proportions of the four protein classes in the outer layers of the eight milled rice varieties. The data in Table II show definitely that the proportions of albumin and globulin are highest in the protein of the outer kernel layers and decrease considerably as some 10 to 15% by weight of the kernel is removed.

The concentration gradient of the albumins is generally steeper, and the proportion in the outer layer tends to be greater, than that of the globulins.

Prolamin is much more evenly distributed in the proteins throughout the kernel, though there is an indication of higher concentrations in the residual kernels than in the portions removed from the outer region.

Glutelin, the major protein, increases in proportion toward the kernel center, in agreement with the decreases in other classes.

This general composition trend confirms and extends the data of Cagampang and coworkers (1966). They pointed out that much albumin and globulin are removed in milling, and that albumin and globulin are more concentrated in proteins of rice polish than in those of the kernel. They did not, however, report further on the distribution in the endosperm.

Gradients. Differences occur for gradients of both albumin and globulin content in the rices examined, and the two gradients vary independently.

In the samples examined, albumin gradients tend to be steepest in the medium-grain rices, followed closely by the long-grain rices. Earlirose, however, has a gradient more like long-grain than the other medium-grain rices. Short-grain and waxy rices have appreciably lower albumin gradients than the other classes. Caloro rice from 1965 and 1966 crops shows very similar gradients, though the values for the 1965 crop (not reported) are somewhat higher over-all.

Globulin gradients show much less variability; no type distinction can be discerned. This lack of interdependence with the albumin contents is emphasized by the low globulin gradient for Saturn, which has one of the steepest albumin gradients. On the other hand, the globulin gradient for waxy rice is steeper than that for albumin.

Table II. Protein

Material	Wt. %	Protein, %, Dry Basis	Per Cent of Total Protein			
			Albumin[a]	Globulin	Prolamin	Glutelin[b]
		Mochi Gome, Waxy, Short-Grain				
Flour						
1	4.8	11.84	5.5	8.5	1.2	84.8
2	3.7	11.48	4.3	5.3	1.3	89.1
3	2.9	10.41	3.7	4.4	1.5	90.4
4	2.2	10.30	2.8	2.4	1.3	93.5
5	1.6	9.88	2.5	1.9	1.3	94.3
Residual rice	84.8	5.77	2.2	2.6	1.8	93.4
Original rice	100.0	6.49	3.1	6.6	2.1	88.2
		Colusa, Short-Grain				
Flour						
1	3.6	12.44	5.9	5.2	0.9	88.0
2	2.5	11.84	4.2	4.0	1.4	90.4
3	1.9	11.36	3.2	2.1	1.4	93.3
4	1.8	10.65	2.9	1.8	1.7	93.6
5	1.6	10.30	2.8	1.9	1.8	93.5
Residual rice	88.6	5.47	2.0	0.9	1.9	95.2
Original rice	100.0	6.49	2.7	1.6	2.0	93.7
		Caloro, Short-Grain				
Flour						
1	3.2	13.57	7.3	8.4	2.1	82.2
2	2.5	12.61	4.2	4.1	2.0	89.7
3	2.1	12.02	3.3	2.8	2.2	91.7
4	1.8	11.25	2.8	1.5	1.7	94.0
5	1.6	10.95	2.9	1.8	1.7	93.6
Residual rice	88.8	5.41	1.8	1.1	2.3	94.8
Original rice	100.0	5.77	2.5	3.1	3.2	91.2
		Saturn, Medium-Grain				
Flour						
1	3.5	15.77	12.5	8.4	1.1	78.0
2	2.8	15.35	7.5	7.1	1.0	84.4
3	2.1	14.52	3.4	7.1	1.0	88.5
4	1.6	13.74	2.7	6.4	0.8	90.1
5	1.3	13.15	2.1	6.1	1.0	91.8
Residual rice	88.7	5.71	0.8	6.1	2.4	90.7
Original rice	100.0	6.84	2.1	7.6	1.8	88.5

[a] Includes free amino acids.
[b] By difference.

Nutritional Factors. The increased concentration of albumin in the outer-layer flours suggests that some could have higher nutritive values on an equivalent protein basis than the entire kernel. This would result from the higher proportion of the limiting amino acid, lysine, in albumin than in other classes of proteins (Juliano, 1966). The resulting lysine increase would, however, be partially offset by the concurrent increase in globulin and free amino acids, both of which apparently have slightly lower lysine contents than the total protein (Juliano, 1966). The effect of the low percentage of free amino acids would probably be very small.

The results of Normand and coworkers (1966) for amino acid contents of 12 successive layers of a single sample of Bluebonnet 50 milled rice show little difference in lysine—or in total aminogram—from layer to layer. However, our results show considerable varietal differences in albumin increase and indicate that our sample of Bluebonnet 50 is among those showing the least difference. Another sample such as Calrose, which has a high albumin concentration in the outer layers, could have appreciable enhancement of lysine in the corresponding protein. That this is so is demonstrated by amino acid data obtained on the present Calrose fractions in connection with another study (Houston *et al.*, 1968):

Material	Lysine, grams per 16.8 grams N
Flour from outer layer (3.2 wt. %)	4.08
Total milled kernel	3.63
Residual kernel (9.3% removed)	3.54

The contrasting results of the two studies on the amino acids of the outer portion of the milled rice kernel emphasize the potential differences among nutritional values of high-protein flours prepared from milled rices and suggest the need for more information on cultural and agronomic effects on rice composition.

From an economic standpoint, a single milling step would probably be used; this would remove about 3% by weight from the milled kernel. The residual kernels would be reduced in protein content only a very few tenths

Distribution in Milled Rices

Material	Wt. %	Protein, %, Dry Basis	Per Cent of Total Protein			
			Albumin^{a}	Globulin	Prolamin	Glutelin^{b}
		Calrose, Medium-Grain				
Flour						
1	3.2	15.11	16.7	8.1	1.7	73.5
2	2.0	14.58	7.8	6.7	1.4	84.1
3	1.6	13.86	4.2	5.0	1.3	89.5
4	1.5	12.97	3.0	3.8	1.3	91.9
5	1.0	12.44	2.2	3.8	1.7	92.3
Residual rice	90.7	6.07	0.7	2.5	1.7	95.1
Original rice	100.0	6.90	1.7	4.3	1.8	92.2
		Earlirose, Medium-Grain				
Flour						
1	2.5	10.59	6.5	4.8	1.8	86.9
2	2.2	10.00	4.0	2.8	2.0	91.2
3	1.8	9.46	2.7	1.5	2.0	93.8
4	1.3	8.92	2.1	1.3	2.1	94.5
5	1.1	8.33	1.6	1.2	2.0	95.2
Residual rice	91.1	4.76	1.0	2.0	3.1	94.1
Original rice	100.0	5.24	1.6	3.0	3.4	92.0
		Bluebonnet 50, Long-Grain				
Flour						
1	3.9	18.74	8.1	7.8	1.7	82.4
2	2.3	20.23	3.9	5.1	1.6	89.4
3	1.6	20.17	2.4	5.1	1.5	91.0
4	1.2	19.81	2.0	3.8	1.7	92.5
5	1.1	19.58	1.4	3.0	1.9	93.7
Residual rice	89.9	8.51	0.6	2.1	3.6	93.7
Original rice	100.0	8.98	1.2	3.2	3.5	92.1
		Belle Patna, Long-Grain				
Flour						
1	2.8	18.21	8.0	7.3	1.1	83.6
2	2.2	19.34	3.6	6.4	0.9	89.1
3	1.7	19.64	2.0	6.4	0.9	90.7
4	1.4	18.80	1.6	5.2	1.0	92.2
5	1.2	18.56	1.4	3.6	1.5	93.5
Residual rice	90.7	8.51	0.4	3.2	1.6	94.8
Original rice	100.0	9.82	0.8	4.4	1.7	93.1

of 1%, and would be almost equivalent to the original ones as a protein source.

One final distinction should be noted between two sources of high-protein rice foods. When the total protein content of rice is raised by cultural means, there is generally a slight relative decrease in the already limiting percentage of the lysine in the total protein (Juliano *et al.*, 1964). However, with the high-protein flours there is either essentially no change or a modest increase in lysine content. Hence, the flours offer foods of potentially increased nutritional value based on both quantity and quality of the contained protein.

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LITERATURE CITED

Cagampang, G. B., Cruz, L. J., Espiritu, S. G., Santiago, R. G., Juliano, B. O., *Cereal Chem.* **43,** 145 (1966).
Hogan, J. T., Normand, F. L., Deobald, H. J., *Rice J.* **67** (4), 27 (1964).
Houston, D. F., *Rice J.* **70** (9), 12 (1967).
Houston, D. F., Allis, Marian E., Kohler, G. O., Meeting of American Association of Cereal Chemists, Washington, D.C., March 31–April 4, 1968; *Cereal Sci. Today* **13,** 105 (1968) (abstract).
Houston, D. F., Mohammad, A., Wasserman, T., Kester, E. B., *Cereal Chem.* **41,** 514 (1964).
Juliano, B. O., International Rice Research Institute, Tech. Bull. **6** (August 1966).
Juliano, B. O., Bautista, G. M., Lugay, J. C., Reyes, A. C., J. AGR. FOOD CHEM. **12,** 131 (1964).
Kester, E. B., Lukens, H. C., Ferrel, R. E., Mohammad, A., Finfrock, D. C., *Cereal Chem.* **40,** 323 (1963).
Kohler, G. O., Palter, R., *Cereal Chem.* **44,** 512 (1967).
Lindner, K., Korpäcsy, I., Jaschik, S., Szöke, K., *Qualitas Plant. Mater. Vegetabiles* **8,** 25 (1961).
Little, R. R., Dawson, E. H., *Food Res.* **25,** 611 (1960).
Losza A., *Agrokem. Talajtan* **2,** 147–60 (1953).
Maes, E., *Nature* **193,** 880 (1962).
Maes, E., *Getreide Mehl* **14** (2), 17–18 (1964).
Normand, F. L., Hogan, J. T., Deobald, H. J., *Cereal Chem.* **42,** 359 (1965).
Normand, F. L., Soignet, D. M., Hogan, J. T., Deobald, H. J., *Rice J.* **69,** (9), 13 (1966).
Primo, E., Casas, A., Barber, S., Benedito de Barber, C., *Rev. Agroquím. Tecnol. Alimentos* **2** (2), 130 (1962a).
Primo, E., Casas, A., Barber, S., Benedito de Barber, C., *Rev. Agroquím. Tecnol. Alimentos* **3** (1), 22 (1963).
Primo, E., Casas A., Barber, S., Benedito de Barber, C., Alberola, J., Pinaga, F., *Rev. Agroquím. Tecnol. Alimentos* **2,** 354 (1962b).
Subrahmaṇyan, V., Sreenivasan, A., Das Gupta, H. P., *Indian J. Agr. Sci.* **8,** 459 (1938).
U.S. Agricultural Marketing Service, "Rice Inspection Manual," G. R. Instruction No. 918-2 (revised) (effective April 1, 1962).
Williams, V. R., *Rice J.* **65** (10), 34 (1962).

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