Amino Acid Analyses of the Proteins of the Major Histological Components of a High-Protein Rice[1]

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ABSTRACT

The major histological components (endosperm, embryo, and aleurone cells plus grain coat) of a high-protein rice (IR 480-5-9) were separated and examined by light and electron microscopy, and the various protein and amino acid analyses were determined. Thus the distribution of protein and of each amino acid in the histological components of the whole grain was obtained, and a satisfactory balance was made with analyses of the whole grain. The histological components that constitute the bran (embryo, aleurone cells, and grain coat) account for less than one-tenth of the weight of the whole grain of IR 480 yet contain about one-seventh of the total amount of protein and one-fifth of the first limiting amino acid, lysine. Nearly 50% more protein and about 20% more lysine were found in high-protein brown rice than in ordinary brown rice and also in high-protein milled (white) rice as compared with ordinary milled rice. The substantial gains in protein and lysine in high-protein milled rice far outweigh the slight reduction in its protein digestibility as compared with ordinary milled rice; consumption of the former variety would be advantageous.

Bradbury et al (1980) described methods for the clean separation of the major histological components (aleurone cells plus grain coat, embryo, and starchy endosperm) of a rice grain (International Rice Research Institute variety IR 32) and reported the protein and amino acid content of each component. For IR 32, the components of the rice bran (embryo, aleurone cells, and grain coat) amount to less than one-tenth of the weight of the grain yet contain one-sixth of the protein and almost one quarter of the lysine.

The twofold purpose of this investigation was to separate and analyze the components of a high-protein rice and to compare it with the previously studied ordinary variety.

MATERIALS AND METHODS

Materials

Brown rice (*Oryza sativa,* L), IR 480-5-9, was obtained from B. O. Juliano of the International Rice Research Institute (IRRI) and

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was selected for its high protein content. Chemicals were reagent grade used without further purification. Constant-boiling-point 6*M* hydrochloric acid was obtained by distillation of BDH analytical reagent grade concentrated hydrochloric acid.

Methods

The morphological integrity of the histological components of IR 480 grains was monitored by light and electron microscopy as described earlier for IR 32 (Bradbury et al 1980). In addition, small pieces of tissue (comprising aleurone cells plus grain coat plus starchy endosperm) were excised from the mid-dorsal regions of IR 480 and IR 32 cut grains that had been softened in water for three days and prepared for microscopy as described by Bradbury et al (1980). Thick (1.5-μm) sections were stained for protein by reaction in 1% aniline blue black (Polysciences) in 7% acetic acid (Fisher 1968) for 1 hr at 70°C to resolve protein bodies.

Amino acid analyses were carried out as described previously (Bradbury et al 1980). As before, about 200 times as much HCl (in milliliters) as sample (in g) was used for hydrolysis, eg, 1 ml of 6*M* HCl per 5 mg of sample. The results, given as residues per 100 amino acid residues, were the mean of three or four analyses made on two separate hydrolysates.

The isolation of the aleurone cells plus grain coat followed the first method described by Bradbury et al (1980), in which cut grains were softened in water for three days and the starchy endosperm was subsequently removed by scraping with a scalpel. This method was also used to obtain preparations of starchy endosperm. The embryo was separated from the whole grain by hand dissection with a needle. Cell wall preparations of the grain coat were obtained by ultrasonication of the rice in decane followed by differential centrifugation to separate the starch granules (Bradbury et al 1980).

RESULTS AND DISCUSSION

Morphological Examination of Histological Components

Aleurone cells and adhering grain coat preparations obtained from IR 480 grains were monitored by microscopy and found to contain very little contaminating starchy endosperm. Cell wall preparations of IR 480 grain coats were essentially devoid of contaminating aleurone cell material. These results, which agree with those reported for IR 32 (Bradbury et al 1980), indicate that the isolation procedures adopted for the ordinary variety of grain were equally applicable to the high-protein variety.

An electron microscopic examination of aleurone cells in aleurone cells plus grain coat preparations revealed no features in IR 480 different from those of IR 32. Protein-carbohydrate body-like inclusions were found in IR 480 aleurone grains.

The only significant difference observed between IR 480 and IR 32 was the incidence of protein bodies in the starchy endosperm. The high-protein variety had a greater number of round bodies that stained blue with aniline blue black (Figs. 1 and 2). This suggests that the starchy endosperm of the high-protein variety has a greater concentration of protein than does the ordinary variety. Similar results have already been reported for high-protein varieties of rice (Bechtel and Juliano 1979). Our morphological observations on the starchy endosperm were supported by analysis.

Amino Acid Analyses of Histological Components

Because of the possible breakdown of amino acids during hydrolysis of the material, due to its high carbohydrate content, the ratio of HCl to sample (v/w) was increased in a series of experiments from 20:1 to 40:1 to 200:1. In this series, the results increased progressively for tyrosine, by a factor of four and for cystine by 60%, serine by 24%, and threonine by 15%. The other amino acids were unchanged. In all recorded analyses the ratio of HCl to sample was 200:1.

The amino acid analyses of the high-protein rice and of its components are given in Table I. Because the starchy endosperm contributes about 91% of the total weight of the whole grain, the amino acid analysis of the two would be expected to be very similar, and this is the case. However, the amino acid analyses of the minor histological components embryo, aleurone cells plus grain coat, and grain coat are very different from that of the whole grain and in particular are much richer in the important amino acid lysine. The protein content (calculated from the area of the amino acid peaks on the chromatogram and the known amount of material loaded on the column) is considerably greater than that for the ordinary rice IR 32 analyzed previously (Bradbury et al 1980). The reverse effect is noted for lysine; the lysine content of the protein of the high-protein rice is less than that of the ordinary rice, which is in agreement with Ignacio and Juliano (1968).

Table II gives the amounts of amino acids and protein in each of the components in 100 g of dry high-protein rice. The sum of the

TABLE I
Amino Acid Analyses[a] of High-Protein Rice(IR 480-5-9) and Its Components

Amino Acid	Whole Grain	Starchy Endosperm	Embryo	Aleurone Cells + Grain Coat	Grain Coat
Alanine	9.6	9.5	10.5	9.7	10.5
Arginine	5.7	6.6	6.7	5.9	4.7
Aspartic acid	9.0	8.6	9.4	8.1	10.5
Cystine	0.4	0.4	0.4	0.7	0.6
Glutamic acid	16.5	15.7	13.5	14.3	6.6
Glycine	8.7	8.3	10.6	8.4	11.6
Histidine	2.6	2.5	3.3	2.6	1.8
Isoleucine	5.2	4.7	4.0	4.9	5.2
Leucine	8.9	9.1	7.9	8.6	9.2
Lysine	3.5	3.2	6.6	4.4	4.7
Methionine	1.4	1.4	1.5	1.5	0.7
Phenylalanine	4.5	4.6	3.7	4.8	5.4
Proline	6.1	6.2	6.4	6.4	7.1
Serine	4.5	5.0	3.1	5.1	6.2
Threonine	3.7	3.6	4.1	3.9	5.0
Tyrosine	2.4	2.6	1.5	2.9	1.4
Valine	7.3	8.0	6.8	7.8	8.8
N recovered, %[b]	97	98	98	101	94
% Protein[c]	10.0	9.1	15.8	14.3	10.2

[a] Residues of amino acid per 100 residues.
[b] Calculated from the areas of the amino acid and ammonia peaks on the chromatogram, the known weight of material loaded on the column, and the known percent nitrogen of the material.
[c] Calculated from the areas of the amino acid peaks on the chromatogram and the known amount of material loaded on the column.

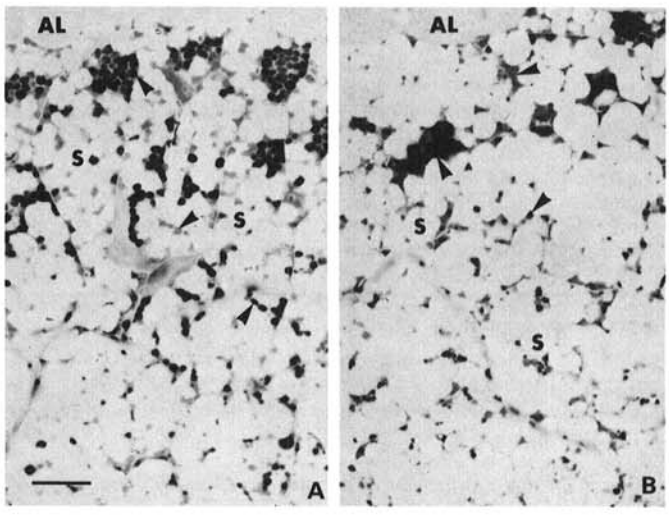

Fig. 1. Light micrographs of 1.5-μm thick epoxy sections of part of the starchy endosperm adjacent to the aleurone layer (**AL**) of the high-protein variety IR 480 (**A**) and ordinary variety IR 32 (**B**). Sections stained with aniline blue black showed more blue-stained protein bodies (arrowed) in IR 480 than in IR 32. Compound starch grains (**S**) are not contrasted. Magnification of both figures is the same (magnification bar = 20 μm).

TABLE II
Amounts[a] of Protein and Amino Acids in the Histological Components of High-Protein Rice

	Embryo (A)	Aleurone Cell + Grain Coat (B)	Starchy Endosperm (C)	Total (D) = (A + B + C)	Total from Whole Dry Grain (E)	Ratio (D/E × 100)
Protein	0.395	0.930	8.281	9.606	10.0	96
	(4.1)[b]	(9.7)	(86.2)			
Alanine	0.0270	0.0568	0.5064	0.5902	0.6026	98
Arginine	0.0375	0.0736	0.7356	0.8467	0.7836	108
Aspartic acid	0.0403	0.0578	0.7262	0.8243	0.9136	90
Cystine	0.0038	0.0099	0.0611	0.0748	0.0803	93
Glutamic acid	0.0627	0.1568	1.4664	1.6859	1.8762	90
Glycine	0.0219	0.0398	0.3612	0.4229	0.4424	96
Histidine	0.0162	0.0307	0.2342	0.2811	0.3051	92
Isoleucine	0.0162	0.0453	0.3956	0.4571	0.5142	89
Leucine	0.0322	0.0806	0.7513	0.8641	0.8923	97
Lysine	0.0300	0.0436	0.2981	0.3717	0.3941	94
	(8.1)	(11.7)	(80.2)			
Methionine	0.0065	0.0163	0.1165	0.1393	0.1408	99
Phenylalanine	0.0196	0.0593	0.4579	0.5368	0.5953	90
Proline	0.0221	0.0440	0.4377	0.5038	0.5227	96
Serine	0.0097	0.0372	0.3030	0.3499	0.3569	98
Threonine	0.0159	0.0326	0.2645	0.3130	0.3378	93
	(5.1)	(10.4)	(84.5)			
Tyrosine	0.0240	0.0392	0.2890	0.3522	0.3663	96
Valine	0.0240	0.0646	0.5447	0.6333	0.6486	98
Percent by weight of each component in dry rice[c]						
	2.5	6.5	91.0	100.0	...	...

[a] Grams of amino acid residues or protein in 100 g of dry rice.
[b] Values in parentheses are the percentages of the particular material (protein or amino acid) in the histological component.
[c] From Juliano (1972).

amounts contributed by each component can be checked against the analysis of the whole grain. The final column in Table II shows a satisfactory check for protein and for each of the amino acids within the experimental accuracy of the method (± 5%).

The percentages of the protein and of the first two limiting amino acids (lysine and threonine) in each of the histological components are given in parentheses in Table II. Although the components of the bran (embryo, aleurone cells, and grain coat) amount to an estimated 9% (Juliano 1972) of the weight of the grain, they contain 13.8% of the protein, 19.8% of the lysine, and 15.5% of the threonine. These are less than the corresponding figures for ordinary rice, in which the components of the bran contain 16.8% of the protein, 23.1% of the lysine, and 18.7% of the threonine (Bradbury et al 1980). This difference is because the additional protein and lysine in high-protein as compared with ordinary rice are located mainly in the starchy endosperm (Table III).

The most useful comparison between the high-protein rice and the ordinary rice is obtained by calculating the total amount of protein and amino acids in a known weight of each of the samples. Table III gives the ratio for the total amount of protein or amino acid in the IR 480 component (whole grain, starchy endosperm, etc.) compared with that in the corresponding IR 32 component. For simplicity, the ratios for all the amino acids have not been included, but the mean ratios are given.

The high-protein brown rice contains 45% more protein than the ordinary brown rice, and the high-protein starchy endosperm contains 49% more protein than the ordinary starchy endosperm, which is in general agreement with Resurreccion et al (1979). Only 9% more protein is in the embryo but 22% more protein is in the aleurone cells plus grain coat of the high-protein than in that of the ordinary rice. Thus the major increase in protein content is concentrated in the starchy endosperm rather than in the bran layers. Similar quantitative results were obtained for the mean ratios for all the amino acids, as shown in Table III. The result for threonine is also similar. The total amount of lysine in the high-protein rice is 18% greater than in normal rice and as with protein, the increase is greater in the endosperm (26%) than in the embryo or the aleurone cells plus grain coat.

The nutritional advantage of eating high-protein milled (white) rice as compared with ordinary milled rice results from the fact that the former contains 49% more protein and 26% more lysine than the latter. However, cooked high-protein milled rice had a lower apparent digestibility (60%) than did cooked ordinary milled rice (66.2%) in Filipino children (Roxas et al 1979). Because substantial protein gains in the high-protein milled rice far outweigh the slight reduction in its protein digestibility, consumption of high-protein rather than of normal rice is clearly advantageous. Because of the importance of digestibility studies, in vitro studies of the proteins of the various histological components of ordinary and high-protein rice are currently in progress.

TABLE III
Ratios of Protein or Amino Acid in the High-Protein Brown Rice[a] Component to That in the Corresponding Ordinary Brown Rice Component[b]

	Whole Grain	Starchy Endosperm	Embryo	Aleurone Cells + Grain Coat	Grain Coat
Protein	1.45	1.49	1.09	1.22	1.31
Lysine[c]	1.18	1.26	1.14	0.97	1.25
Threonine[d]	1.52	1.44	1.04	1.22	1.53
Mean ratio for all 17 amino acids	1.43	1.43	1.09	1.20	1.36

[a] IR 480.
[b] IR 32, from Bradbury et al (1980).
[c] First limiting amino acid in rice.
[d] Second limiting amino acid in rice.

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