

Protein Quality Evaluation of Cooked Rice for Protein Mutants in Growing Rats

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ABSTRACT

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The nutritive value of cooked milled rice of one glutelin mutant *esp-2* and three prolamin mutants *esp-1*, *esp-3*, and *Esp-4* backcrossed twice to Kinmaze and differing in their protein subunit ratios were evaluated by chemical analyses and balance studies in growing rats and compared with the Kinmaze parent. The glutelin mutant had higher lysine content and true digestibility (TD) of protein than did Kinmaze, but all had digestible energy values similar to that of Kinmaze. The *esp-3* prolamin

mutant was confirmed to have lower sulfur amino acids in protein than did the other samples, but lysine was still the limiting amino acid. Biological value of the mutants was similar to that of Kinmaze, but net protein utilization (NPU) of *esp-2* and *Esp-4* was higher than that of Kinmaze. The 10- and 16-kDa prolamin-rich mutant *Esp-4* had even higher TD than that of Kinmaze. The glutelin mutant had best protein quality among the four Kinmaze mutants.

Protein of raw milled rice is completely digestible by rats (Eggum et al 1977). Cooking, however, reduces the digestibility of rice proteins in rats to 85–90% (Eggum et al 1977). Similar values for cooked rice protein were reported in man (Juliano 1985). Fecal protein particles were observed in man on a cooked rice diet (Tanaka et al 1975). They were identified as the core of the large spherical protein bodies (PB I); the crystalline PB (PB II) were readily digested (Tanaka et al 1978). PB I is rich in alcohol-soluble protein or prolamin and constitutes about 20% of milled rice protein (Ogawa et al 1987). Prolamin consists of the major 13a and 13b kDa subunits and two minor 10 and 16 kDa subunits (Ogawa et al 1987). The 10 and 16 kDa subunits had a higher level of sulfur-containing amino acids than did the major 13 kDa subunits (Hibino et al 1989). PB II constitutes 60–65% of milled rice protein, is rich in alkali-soluble protein or glutelin, and has a better quality protein than does PB I. Glutelin consists of 22 and 37 kDa subunits, plus their 57 kDa precursor (Sugimoto et al 1986).

Methods for improving the protein quality of milled rice include increasing the level of PB II at the expense of PB I, and improving the digestibility of PB I by mutation. Using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), Japanese rice scientists screened 3,000 mutant lines (induced by treating the fertilized egg cell of Kinmaze rice variety with *N*-methyl-*N*-nitrosourea) for mutants for prolamin and glutelin subunits (Kumamaru et al 1988, 1991). Four endosperm storage protein (*esp*) mutants were identified. Genetic analysis of these mutants revealed

that all were recessive, except for *Esp-4* (Kumamaru et al 1987, 1990). The designations for the genes were: 13b-L *esp-1*, 57-H *esp-2*, 10/13a-L *esp-3*, and 10/16-H *Esp-4*, where L = low and H = high.

The PB I of the three prolamin mutants were compared with those of Kinmaze (Ogawa et al 1989). CM21 (13b-L) and CM1834 (10/16-H) had a PB structure similar to that of Kinmaze, but they had more PB II. CM1675 (10/13a-L) lacked the typical lamellar structure of PB I. Pepsin digestibility of PB I of CM1675 was similar to that of Kinmaze, but those of CM21 and CM1834 had more. No in vivo digestibility data have been reported because of the sample quantity required.

This study was undertaken to determine the digestibility and quality of protein in cooked Kinmaze rice containing three prolamin and one glutelin *esp* genes, and thus ascertain to what extent these mutations have improved the nutritional properties of rice protein. Cooked rice was used instead of raw rice because cooking reduces the digestibility of PB I, as well as being the form in which rice is consumed. Lack of sample prevented a parallel test on raw rice.

MATERIALS AND METHODS

The rice grains were obtained from F₄ of backcross 2 (B₂F₄) of four CM mutants to Kinmaze. They were from the 1992 crop of these Kinmaze lines grown at Harozaki in Japan. They were dehulled and milled and brought to the International Rice Research Institute, Manila, Philippines (IRRI). They were analyzed for Kjeldahl protein (Eggum et al 1977) and apparent amylose content (Juliano et al 1981). Translucency was measured in duplicate with a Riken Sanno rice meter (brown rice model). Whiteness was measured in duplicate with a Kett model C-3 whiteness meter. Milled rice (600 g) was cooked with water (780 ml) in a Goldstar model RJ-203 SB automatic electric cooker with heater at the lid. The samples were left for an additional 10 min in the cooker after power shut-off before they were cooled, frozen at -20°C,

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and freeze-dried. The freeze-dried samples were air-freighted to Tjele, Denmark for the rat assays.

Nitrogen Balance Studies

Five groups of five male Wistar rats weighing approximately 70 g were used for the N balance experiment, with a preliminary period of four days and a balance period of five days (Eggum 1973, Eggum et al 1989). The rats were housed individually in Plexiglas cages with stainless steel mesh bottoms (allowing separate collection of feces and urine) in a controlled environment of 25°C and 60% rh. Lighting was controlled by alternating 12 hr periods of light and darkness. Each animal received daily 10 g of dry matter throughout the preliminary and balance period. Body weight and diet intake were recorded at the end of the period. During the balance period, urine and feces were collected separately. Metabolic N and endogenous N were determined by adding ether-extracted, freeze-dried egg (100% digestible) equivalent to 4% protein to the N-free (corn starch) diet (Eggum 1973). True digestibility (TD), biological value (BV), net protein utilization (NPU), and digestible energy (DE) were determined from diet, fecal and urine Kjeldahl, and calorimetric analyses (Eggum et al 1989). Rat data were subjected to analysis of variances and Duncan's (1955) multiple range test.

Unreplicated amino acid analysis of diets with internal standards was conducted using the procedure of Mason et al (1980). Tryptophan was determined using the method described by Bech-Andersen (1991). Nitrogen recoveries were 85–89% and were recalculated to 95% recovery. Standard deviation (in grams per 16 g of N) was 0.2 for lysine, 0.1 for cysteine and methionine, and 0.05 for tryptophan. Amino acid score was calculated from the content of lysine, the first limiting essential amino acid, based on 5.5% lysine (WHO 1985) as 100%. Protein quality was calculated by multiplying TD by amino acid score (FAO/WHO 1990).

RESULTS AND DISCUSSION

The milled rice samples were all translucent, except for the *esp-2* line, which had chalky grains mixed with translucent grains and the lowest translucency value (Table I). The *esp-2* line had the highest Kett whiteness value, even higher than that of the Kinmaze parent.

All the lines had low apparent amylose content, like Kinmaze, but *esp-3* and *Esp-4* had higher amylose content (Table I). Crude protein content of the milled rices was also similar, but it was lowest for the chalky *esp-2* and highest for *esp-3*.

Lysine was the first limiting amino acid for all milled rice protein, corresponding to amino acid scores of 50.5–63.5% (Table I) based on 5.5% lysine as 100% (WHO 1985). Lysine values were similar to those of indica milled rices (Eggum et al 1977, 1993c).

The *esp-2* mutant had the highest amino acid score based on lysine, and *Esp-4* had the lowest. The *esp-2* glutelin mutant had higher lysine content than that of Kinmaze and the other rices. Cystine and methionine contents were highest in the *Esp-4* mutant and lowest in the *esp-3* mutant, which is consistent with their respective contents of the high-sulfur 10-kDa and 16-kDa prolamin subunits. The *esp-2* mutant is poor in the 10 kDa subunit, and the *Esp-4* mutant is rich in both 10 and 16 kDa prolamin subunits.

Nitrogen Balance in Rats

Analysis of variance showed that the variance from the milled rice samples was highly significant ($P < 0.01$) for TD and NPU, but it was not significant ($P > 0.05$) for DE and BV (data not shown). Coefficient of variation was 0.47% for DE, 0.69% for TD, and 1.05% for both BV and NPU.

The four cooked and freeze-dried mutant milled rices had DE values that were similar to those of Kinmaze, but *esp-1* had lower DE than did *esp-2* (Table I). However, TD of protein was highest in the glutelin mutant *esp-2*, followed by prolamin mutants *esp-3* and *Esp-4*, Kinmaze, and *esp-1*. Thus, protein of the high-sulfur 10- and 16-kDa prolamin-rich *Esp-4* was even more digestible than was Kinmaze protein. The BV of mutant proteins was similar to that of Kinmaze, but *esp-1* and *esp-2* had higher BV values than did *esp-3*. The NPU was highest in the glutelin mutant *esp-2*, followed by *Esp-4*, *esp-3*, and Kinmaze and *esp-1*.

Protein quality based on amino acid score and TD was highest in *esp-2*, and probably significantly higher than that of the other samples, based on previous data of standard deviation for protein quality of 3.0–3.3% (Eggum et al 1993a). Thus, the three prolamin mutants were probably similar to those of Kinmaze in protein quality. Thus, the reduction of the 13b kDa subunit of prolamin in *esp-1* or of the 10 and 13a kDa prolamin subunits in *esp-3*, as well as the increase in both the 10 and 16 kDa subunits of prolamin in *Esp-4*, had less effect on protein quality than did the higher glutelin precursor 57 kDa subunit *esp-2* of Kinmaze. The results indicate that actual biological tests are required to actually determine the digestibility of proteins in food to confirm the in vitro chemical tests.

The analyses of five cooked indica milled rices (7–9% protein) in growing rats showed: 96–97% DE, 90–97% TD, 73–80% BV, and 70–74% NPU (Eggum et al 1993c). Corresponding values for raw rice were: 97–98% DE, 99–100% TD, 64–68% BV, and 64–68% NPU. Cooked IR36 milled rice (10% protein) had 95% DE, 89% TD, 76% BV, and 68% NPU (Eggum et al 1993b). Thus, Kinmaze (japonica) cooked milled rice had higher TD and NPU values than did indica cooked rices, but Kinmaze (japonica) cooked milled rice DE and BV values (Table I) were similar to those of indica cooked rices. The higher level of waxy gene product

TABLE I
Mean Value and Standard Deviation of Properties of Cooked and Freeze-Dried Milled Rices of B₂F₄ Protein Mutant Lines and Their Kinmaze Parent

Property	Kinmaze	<i>esp-1</i> (CM21)	<i>esp-2</i> (CM1787)	<i>esp-3</i> (CM1675)	<i>Esp-4</i> (CM1834)
Gene	none	13b-L	57-H	10/13 a-L	10/16-H
Translucency (%)	100 ± 0	100 ± 0	41 ± 1	100 ± 0	96 ± 1
Kett whiteness (%)	38 ± 1	38 ± 0	44 ± 1	38 ± 1	40 ± 1
Apparent amylose (% db)	14.9 ± 0.4	14.4 ± 0.1	15.5 ± 0.5	16.3 ± 0.4	16.3 ± 0.3
Crude protein (% wb)	6.75 ± 0.18	7.31 ± 0.0	6.59 ± 0.04	7.53 ± 0.22	7.22 ± 0.04
Lysine (g/16 g N)	3.13	3.47	3.95	3.31	3.27
Cysteine (g/16 g N)	2.20	2.40	2.26	1.69	3.03
Methionine (g/16 g N)	2.56	2.89	2.79	1.64	4.27
Tryptophan (g/16 g N)	1.23	1.30	1.24	1.21	1.28
Amino acid score (%)	56.9	63.1	71.8	60.2	59.5
Nitrogen balance in rats ^a					
Digestible energy (%)	97.1 ± 0.5 ab	96.8 ± 0.6 b	97.6 ± 0.2 a	97.1 ± 0.6 ab	97.1 ± 0.2 ab
True digestibility (%)	95.5 ± 0.8 c	94.6 ± 0.6 d	99.9 ± 0.4 a	97.2 ± 0.9 b	97.1 ± 0.8 b
Biological value (%)	74.6 ± 0.7 ab	75.3 ± 0.8 a	75.2 ± 0.6 a	74.1 ± 0.4 b	74.5 ± 1.0 ab
Net protein utilization (%)	71.2 ± 0.4 c	71.1 ± 0.6 c	75.1 ± 0.4 a	72.0 ± 0.7 bc	72.4 ± 1.4 b
Protein quality (%) ^b	54.3	59.7	71.7	58.5	57.8

^a Means in the same line followed by the same letter are not significantly different at $P < 0.05$ by Duncan's (1955) multiple range test.

^b Amino acid score × true digestibility/100 (FAO/WHO 1990).

in indica rice (Villareal and Juliano 1989) may contribute to the TD value being lower than that in japonica rice, but the amylose extender (high waxy gene product) mutant had a lower TD even in raw rice (Eggum et al 1993b); raw indica and japonica rices have about 100% TD.

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