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FACTORS AFFECTING NUTRITIONAL PROPERTIES OF RICE PROTEIN

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ABSTRACT

Among cereal proteins, rice has one of the highest lysine content, the first limiting essential amino acid. Although parboiling and cooking have no adverse effect on lysine content, they reduce protein digestibility but improve biological value. Some heat processing such as popping, toasting, stack burning, extrusion cooking, and puffing can reduce lysine content and nutritional value of the processed rice. Milling has little effect on the nutritional value of protein except in purple rices which have poor digestibility of brown rice. Protein distribution in the endosperm depends on brown rice protein content. Preliminary studies on brown rice from crop grown in sulfur-deficient soils showed no adverse effect on sulfur containing amino acids. No high-lysine rice mutant has been identified.

Cooperative studies in preschool children on protein requirements for high-protein rice and rice-based weaning diets showed protein quality of rice may be improved by adding lysine-rich proteins particularly of animal origin at 1/3 of the dietary N.

Introduction

Rice protein is unique among cereal proteins because it is high in lysine content, low in prolamin, and low in matrix protein (Juliano, 1985). Cereal proteins are first limiting in the essential amino acid lysine for which 5.5 g/16 g N is considered adequate. Rice protein has 3.5-4.0 g lysine/16 g N.

Solubility fractions of rice protein by the Osborne classification are 5% albumin (water-soluble), 10% globulin (NaCl-soluble), 3% prolamin (alcohol-soluble), and 80% glutelin (alkali-soluble) (Juliano, 1985). In terms of amino acid composition, albumin has the highest lysine content followed by glutelin, globulin, and then prolamin. The low prolamin content helps explain the high lysine content of rice protein. Changes in protein content are mainly due to changes in the storage proteins glutelin and prolamin. Increase in protein content results in a small but significant decrease in lysine content of rice protein which was confirmed by rat studies. Feeding trials in preschool children did not show significantly lower apparent nitrogen (N) retention for high (11%) – protein rice as compared to average protein rice at identical N intakes of 240 or 250 mg N/kg body wt/day (MacLean *et al.*, 1978; Roxas *et al.*, 1979).

With other cereal proteins, protein bodies are prolamin and matrix protein is glutelin (Juliano, 1985). An exception is rice protein wherein at least 95% of endosperm protein is protein bodies. The major protein body is the large spherical type found throughout the endosperm. Small spherical and crystalline types are found only in the subaleurone layers (Bechtel and Juliano, 1980; Harris and Juliano, 1977). Crystalline protein bodies are rich in albumin-globulin (Tanaka *et al.*, 1980) and readily digested during proteolysis in vitro (Tanaka *et al.*, 1978) and during germination (Horikoshi and Morita, 1982). Cooking renders the core portion of spherical protein bodies poorly digestible (Eggum *et al.*, 1977; Tanaka *et al.*, 1978). The core fraction is rich in cystine and lipids and poor in lysine (Resurreccion and Juliano, 1981). Parboiling has a lesser effect than boiling (Eggum *et al.*, 1977). Thus, true digestibility of protein in growing rats decreases but biological value increases from cooking of rice (Eggum *et al.*, 1977). This trend in digestibility and biological value is attributed to a movement of urea from the blood to the intestines in rats, as demonstrated by the effect of dietary manipulations on N excretion pattern (Beames and Eggum, 1981).

This paper reviews recent studies on the effect on rice protein quality of (a) heat processing in view of heat lability of lysine residues through reaction of the *epsilon*-amino group, (b) pigmented pericarp and milling, (c) sulfur-deficient soils and tissue culture mutations, and (d) adding various lysine-rice proteins in the rice diet.

Methods

Amino acid analyses were performed on milled rice hydrolyzed 23 h at 110°C in 6 N HCl in tubes sealed after flushing with N_2 using a Beckman 120C amino acid analyzer with PA-35 and AA-15 resin columns. All rat balance studies were conducted at the Department of Animal Physiology and Chemistry, National Institute of Animal Science, Copenhagen, Denmark, on Wistar male growing rats weighing 65-68 g, five rats/diet. Energy value of food and feces was estimated by an IKA adiabatic calorimeter.

Energy and nitrogen balance studies in male preschool children 18-30 months old were undertaken at the Nutritional Evaluation Laboratory, Food and Nutrition Research Institute, NSTA (Intengan *et al.*, 1984; Santiago *et al.*, 1984; Cabrera-Santiago *et al.*, 1985), using the standard protocol of the United Nations University World Hunger Programme (Torún *et al.*, 1981) at 418.4 kJ/kg body wt/day energy intake. Single-level nitrogen balance studies were done at 200 or 250 mg N/kg body wt/day. Multilevel N balance was done following increasing and decreasing sequence of dietary protein intake levels of 0.75, 1.00, 1.25, 1.50, and 1.75 g/kg body wt/day (120, 160, 200, 240, and 280 mg N/kg/day). Macro Kjeldahl N was determined on composite diet and on pooled feces and urine samples. Energy content of diet and feces was determined with a Parr adiabatic calorimeter. Amino acid composition and neutral detergent fiber of diets were analysed at IRRI.

Histological examination of samples was by the methods of Bechtel and Juliano (1980).

Heat Processing

Lysine is very sensitive to thermal degradation through reaction of its *epsilon*-amino group through formation of lysinoalanine (Raymond, 1980) or Maillard reaction with reducing sugars. Although boiling, regular and pressure parboiling, noodle extrusion, pan baking, accelerated aging, and pan toasting have little or no effect on the lysine content of rice protein, stack burning or yellowing, popping, extrusion cooking, and puffing significantly reduced the lysine content of protein (Eggum *et al.,* 1977, 1984, 1985; IRRI, 1984; Khan and Eggum, 1978, 1979; Khandker *et al.,* 1985) (Table 1). Actual rat feeding trials confirmed lysine data: net protein utilization (NPU) was not affected by treatments that do not decrease lysine content, but was decreased by stack burning, extrusion cooking, and most, by puffing. A sample of commercial puffed rice had 0.1% lysinoalanine in its protein (Raymond, 1980). Spherical protein bodies were still intact in the protein

Table 1. Effect of heat treatment and processing on the lysine content and net protein utilization (NPU) of rice in growing rats (Eggum *et al.*, 1977, 1984, 1985; IRRI, 1984; Khan and Eggum, 1978, 1979; Khandker *et al.*, 1985)

Processing method	*Percent decrease in*		*Reference*[a]
	Lysine content	*NPU*	
Boiling	1-3	0	1
Pressure parboiling	0	0	2
Noodle extrusion (35% H_2O, 55°C)	0	–	3
Pan baking (220-230°C, 7-10 min)	0	0	4
Accelerated aging (100°C, 3 h, sealed)	3	–	5
Pan toasting	5	–	5
Stack burning[b] (high moisture, <100°C)	9-18	6-12	2
Induced yellowing (60°C, 4 d, sealed)			
25-26% H_2O	14-18	–	5
14% H_2O	9	–	5
Popped (207°C, 45 s)	16-17	–	5
Extrusion cooked (15% H_2O, 150°C, 45 bars)	11	7-8	5
Commercial steamed and puffed	37	–	5
Commercial rice krispies	53	41	7

[a] 1 (Eggum *et al.*, 1977); 2 (Eggum *et al.*, 1984); 3 (Khandker *et al.*, 1985); 4 (Khan and Eggum, 1978); 5 (IRRI, 1984); 6 (Eggum *et al.*, 1985); 7 (Khan and Eggum, 1979).

[b] 21% decrease in lysine for yellow grains and 18% decrease for white grains in stack burned sample.

masses of extrudates at 150°C and 15% moisture (Mosqueda *et al.*, 1985), which may explain the only slight lysine degradation in extrusion-cooked rice.

Milling

Brown rice is richer in protein, lipid, dietary fiber, ash, and B vitamins than milled rice (Juliano, 1985). Using composition data, brown rice has often been referred to as more nutritious than milled rice. Among brown rices, protein of pigmented rices have lower digestibility but higher biological value (Eggum *et al.*, 1981) (Table 2). However, NPU of red rice H4 was not significantly lower than that of IR32, but NPU of purple rice Perurutong was lower. Pigmented rices, particularly purple rice, are rich in phenolics. These differences were essentially removed by milling.

Table 2. Properties and nitrogen balance of nonpigmented and pigmented brown rices in growing rats[a] (Eggum *et al.*, 1981)

Property	*Brown rice*[a]			
	IR8	*IR32*	*H4*	*Perurutong*
Color	*None*	*None*	*Red*	*Purple*
Protein (N x 6.25)	8.0c	8.7a	8.0c	8.3b
Lysine (g/16 g N)	3.86a	3.80ab	3.68b	3.78ab
Methanol soluble phenolics (% as catechin)	0.02c	0.01c	0.25b	0.62a
True N digestibility (%)	97.1a	96.9a	83.0b	72.4c
Biological value (%)	72.7c	68.9d	80.0b	81.6a
Net protein utilization (%)	706a	66.7b	66.6b	59.1d

[a] Values for milled rices were 3.36-3.67 g lysine/16 g N, 0.01-0.07% phenolics, 96.2-99.2% true digestibility, 65.7-73.1% biological value, and 65.2-70.3% net protein utilization. Means in the same line followed by the same letter are not significantly different by Duncan's (1955) multiple range test.

Comparison of the energy and protein digestibility of brown rice and milled rice in rats showed high values for milled rice (Eggum *et al.*, 1982) (Table 3). This could be partly because of the higher phytate and fiber contents in the bran of brown rice which reduce protein digestibility. NPU was similar in brown and milled rices. In preschool children fed rice-milk diets wherein milk contributed 1/3 of dietary N, the differences in apparent protein digestibility and retention were not significant, and energy and fat absorption were higher in the milled rice diet than in the brown rice diet (Santiago *et al.*, 1984) (Table 3). The true remaining nutritional advantage of brown rice is its higher B vitamin content (Resurreccion *et al*, 1979).

Although the mineral content of brown rice is higher than that of milled rice, rats fed brown rice have lower femur zinc concentration and Ca and P deposition also appears affected (Pedersen and Eggum, 1983). Phytate and/or fiber in bran are not solely responsible for this effect.

Table 3. Relative nutritional properties of IR32 brown and milled rice in growing rats (Eggum *et al.*, 1982) and in preschool children[a] (Santiago *et al.*, 1984)

Property	*Brown rice*	*Milled rice*
	Rice in 5 growing rats	
Protein content (% N x 6.25)	8.7a	8.3b
Lysine content (g/16 g N)	3.8a	3.6b
Neutral detergent fiber (%)	2.6b	0.8a
Energy value (kJ/g)	16.3a	15.9b
True N digestibility (%)	96.9b	98.4a
Biological value (%)	68.9a	67.5a
Net protein utilization (%)	66.7a	66.4a
Digestible energy (%)	94.3b	96.6a
	Rice-milk diets in 6 preschool children	
Lysine (g/16 g N)	5.5a	5.6a
Neutral detergent fiber (%)	3.2b	1.8a
Apparent N absorbed (% of intake)	59.7 ± 8.6a	63.3 ± 3.3a
Apparent N retained (% of intake)	27.3 ± 9.4a	28.9 ± 2.4a
Apparent energy absorbed (% of intake)	90.1 ± 1.0b	92.0 ± 1.0a
Apparent fat absorbed (% of intake)	94.3 ± 1.8b	97.6 ± 1.3a

[a] Means in the same line followed by the same letter are not significantly different by Duncan's multiple range test. Mean ± s.d. for apparent absorption and retention data in children.

The slopes of protein gradient in average and high protein grains are similar (Ellis *et al.*, 1985). Milling (10% wt removal) mainly removes the pericarp, seed coat, nucellus, aleurone, and germ in rices with shallow surface ridges, with 17-18% loss of brown rice protein. Thus, milled rice protein content is readily predicted from brown rice protein content.

Sulfur-Deficient Soils

Amino acid composition of brown rice grown in sulfur (S)-deficient soil obtained by the usual acid hydrolysis procedure without performic acid oxidation was reported lower in cysteine (Ismunadji and Miyake, 1980). Because of the large area of S-deficient soils in Indonesia and Bangladesh grown to rice and the widespread use of rice-legume diets in these countries, samples of rices grown in S-deficient soils with and without S amelioration were analyzed for cysteine and methionine contents with prior performic acid oxidation. Rice contributes excess cystine-methionine and the legume contributes excess lysine to obtain a better quality dietary protein.

Analysis of a set of samples from Indonesia and Bangladesh showed that for samples with normal grain weight, S-deficiency did not decrease S amino acids cysteine and methionine in brown rice protein although yields were low (IRRI, 1983a) (Table 4). S analysis by X-ray fluorescence spectrometry at the University of Nebraska showed that amino acid S accounted for 43-46% (mean 44%) of total brown rice S (IRRI, unpublished data). A second set of field samples from Bangladesh, however, showed 5.6 g cysteine plus methionine/16.8 g N with S fertilization as compared to 4.6 g/16.8 g N without S addition (IRRI, unpublished 1985 data). The WHO (1973) reference amino acid pattern considers 3.7 g cysteine plus methionine/16.8 g N as adequate. Samples from Indonesia and Philippines are awaited for confirmatory analysis.

Table 4. Properties of brown rice from sulfur-deficient soils in Ujung Pandang, Indonesia and Bangladesh (IRRI, 1983a)

Property	*–S*		*+S*	
	Range	*Mean*	*Range*	*Mean*
	Indonesian samples			
All samples (n = 19)				
Brown rice protein (% at 12% H_2O)	6.8-12.1	9.0	6.8-12.2	9.7
Four selected samples with similar protein contents[a]				
Brown rice protein (% at 12% H_2O)	7.2-12.1	9.3	6.8-12.0	9.2
Mean grain wt (mg)	17.2-27.2	20.1	18.1-27.7	21.2
Cystine[b] (g/16.8 g N)	2.1-2.4	2.3	2.1-2.2	2.2
Methionine[b] (g/16.8 g N)	2.6-3.3	3.0	2.6-2.9	2.7
Cystine + methionine[b] (g/16.8 g N)	5.0-5.7	5.2	4.7-5.1	4.9
	Bangladesh BR3 samples			
Brown rice protein (% at 12% H_2O)	7.2; 7.6	7.4	6.9-7.7	7.3
Mean grain wt (mg)	22.0; 22.8	22.4	22.7-23.2	22.9
Cystine[b] (g/16.8 g N)	2.1; 2.2	2.2	2.1-2.3	2.2
Methionine[b] (g/16.8 g N)	2.7; 3.2	3.0	2.8-2.9	2.8
Cystine + methionine[b] (g/16.8 g N)	4.8; 5.4	5.1	5.0-5.1	5.1

[a] Gemar, IR32, IR44 and Gati.

[b] By performic acid oxidation. Cystine + methionine content in WHO (1973) standard amino acid pattern is 3.5 g/16 g N.

Rice Mutants and Hybrids

Improved rice proteins have been reported in plants regenerated from *S*-2-aminoethyl-*L*-cysteine-resistant calli from PI 353705 Assam 5 (Schaeffer and Sharpe, 1981). The M13 mutant showed increased protein content or lysine or both in the third through fifth generation progeny. Confirmatory analysis at IRRI on the M13 mutant and its parent over two generations showed lighter grains for the mutant, 10% higher percentage of lysine and 22% higher percentage of protein in brown rice than the parent but actually no difference in amount of lysine and protein per seed (IRRI, 1983a) (Table 5). The differences in percentage of protein and lysine contents were also reflected in the milled rices.

Table 5. Mean grain properties of second- and third-generation higher lysine strain MA 13 obtained from PI 353705 anther cultures screened against *S*-2-aminoethyl-*L*-cysteine and its parent (IRRI, 1983a)

	PI 353705	*MA 13*
Brown rice wt (mg/grain)	19.0	14.5
Lysine (g/16.8 g N)	4.2	4.6
Protein content (%)	8.9	10.9
Protein per seed (mg)	1.7	1.6
Lysine per seed (mg)	0.07	0.07
Milled rice lysine (g/16.8 g N)	3.9	4.5
Protein content (%)	6.8	9.6

SDS-polyacrylamide gel electrophoresis of this MA13 mutant and of brown rice of nine endosperm mutants prepared by treating fertilized eggs with *N*-methyl *N*-nitrosourea (Satoh and Omura, 1981) showed normal electrophoretic pattern of rice proteins (IRRI, 1983b). The mutants had 6.5-9.8% protein and 3.8-4.9 g lysine/16.8 g N as compared with 7.4% protein and 3.7 g lysine/16.8 g N for the Kinmaze parent (IRRI, 1983b). The shrunken mutant had 9.8% protein and 4.9 g lysine/16.8 g N which may have been due to the greater contribution of embryo and bran protein in shrunken brown rice. Because glutelin is 80% of rice endosperm protein with only two molecular weight subunits (Zhao *et al.*, 1983; Tanaka *et al.*, 1980), a mutant with more than 5% lysine probably will involve change in the structure of the protein bodies. The third molecular weight 20000 subunit of glutelin (Juliano and Boulter, 1976) is probably contaminant crosslinked prolamin.

Rice/sorghum and rice/wheat hybrids from the People's Republic of China were rechecked also for amino acid composition particularly lysine, because rice protein is richer in lysine (3.5-4%). than sorghum (1-2% lysine) and wheat (2-3% lysine) proteins (Juliano, 1985). Lysine content of four milled rice/sorghum hybrids of 3.1-3.6 g/16.8 g N was closer to rice than to sorghum (IRRI, 1980). One rice/wheat hybrid milled sample had 4.1 g lysine/16.8 g N at 10.8% protein and with SDS-polyacrylamide gel electrophoretic pattern characteristic of milled rice glutelin (IRRI, 1983a).

Protein Improvement

Attempts by conventional breeding to improve the protein content of milled rice from 7% or brown rice protein of 8% by 2 percentage points while maintaining yield had not been successful because of the low heritability of and large environmental effect on protein content (Khush and Juliano, 1984). Brown rice protein content of all promising lines in the IRRI breeding is routinely monitored by micro Kjeldahl N analysis. With the emphasis on early-maturing rices, the early-maturing lines were shown to have higher grain protein than medium-maturing varieties (IRRI, 1983b). The higher protein content may be physiological rather than genetic probably because of the higher level of vegetative N available for partitioning during grain development in the early-maturing rices.

Protein Requirements of Rice and Rice-Based Diets

Although rice protein is limiting in lysine, rice diets have been calculated to be adequate in lysine, because other protein sources in the diet are rich in lysine (Juliano, 1985). As part of the United Nations University World Hunger Programme (Torún *et al.*, 1981), the Food and Nutrition Research Institute (FNRI), NSTA, undertook studies on the protein requirements of typical rice-based weaning foods in male preschool children (Intengan *et al.*, 1984; Cabrera-Santiago, 1985). Milk, fish, or mung bean contributed 1/3 of the total dietary N.

Protein requirements for maintenance (PR_m) and for 97.5% of the population ($PR_{0.975}$) based on the method of Rand *et al.*, (1977) were low for rice-milk and rice-fish and higher for the rice–mung bean and IR58 high-protein rice diets (Intengan *et al.*, 1984; Cabrera-Santiago *et al.*, 1985) (Table 6). Rice-whole mung bean was not better than rice alone despite the higher lysine content of the rice-bean diet. Dehulling the mung bean by toasting slightly improved the protein quality of the diet. Rice-whole mung bean diet had the highest neutral detergent fiber content of the five diets. Protein quality based on $PR_{0.975}$ of 65% of milk protein for IR58 milled rice diet is close to the IR58 rice (3.5 g lysine/16 g N) NPU of 68% in growing rats (IRRI, 1984). Thus, adding lysine-rich protein foods that are fairly digestible, such as milk and fish, to rice improves its nutritive value. Legumes such as mung bean have antinutrition factors which have to be inactivated or removed to improve protein utilization. Milled rice, fortunately, has minimal content of antinutrition factors such that its nutritional value may be readily predicted from protein content.

Table 6. Protein requirements and relative quality of rice and rice diets in preschool children based on $PR_{0.975}$ and PR_m of milk as 100% (Intengan *et al.*, 1984; Cabrera-Santiago *et al.*, 1985)

Property	*Diet*				
	Rice-milk	*Rice-fish*	*Rice-toasted mung bean*	*Rice-whole mung bean*	*IR58 rice*
Children (no.)	8	7	8	6	4
$PR^a{}_m$ (mg N/kg body wt/day)	130	139	163	183	164
$PR^a{}_{0.975}$ (mg N/kg body wt/day)	177	189	215	250	231
$PR^a{}_{0.975}$ (g/kg body wt/day)	1.11	1.18	1.34	1.56	1.44
Protein quality[b] (% of milk PR_m)	75	71	58	54	60
Protein quality[b] (% of milk $PR_{0.975}$)	85	80	70	60	65
Lysine[c] (g/16 g N)	5.4 ± 0.1	6.7 ± 0.2	4.7 ± 0.2	5.2 ± 0.1	3.5 ± 0.3
Cystine + methionine[c] (g/16 g N)	3.7 ± 0.9	4.3 ± 0.3	3.5 ± 0.6	3.5 ± 0.6	4.3 ± 0.1
Neutral detergent fiber[c] (%)	3.0 ± 0.2	2.2 ± 0.3	3.5 ± 0.1	5.1 ± 0.1	2.2 ± 0.4

[a] Energy intake at 418 kJ/kg body wt/day. Allowing 10 mg N/kg body wt/day loss (Huang *et al.*, 1980) and 15 mg N/kg body wt/day for growth (Torún *et al.*, 1981).

[b] Based on PR_m of 98 mg N/kg body wt/day and $PR_{0.975\ y\ =\ 15}$ of 0.94 g/kg body wt/day for milk protein (Torún *et al.*, 1981).

[c] Mean + s = d.

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Ernesto J. del Rosario, Discussant

My brief comments on Dr. Juliano's paper shall deviate from the chemical and nutritional studies of rice protein, which have been adequately discussed in the paper, and shall instead concentrate on a frontier topic which is quite important, namely genetic engineering. The latter technique (also known as recombinant DNA technology) may be used to increase the lysine content of rice protein. This technique has tremendous potential, compared to traditional plant breeding methods, because of its direct and powerful approach towards attaining a desired objective. One specific strategy, which could be tried, is to increase the number of gene copies of lysine-rich albumin in rice protein chromosome.

Evelyn Mae Tecson-Mendoza, Discussant

Rice is consumed as a major staple food by about 1.8 billion people all over the world, 90% of whom are in Asia. Where rice is a major source of calories, rice is also an important source of proteins. Thus, studies on factors affecting the nutritional quality of rice protein are beneficial to a great number of people, and those that need these benefits most.

This paper focuses on issues that could have applications and repercussions in agriculture, the food industries as well as in the household kitchen.

Some of the more important points this paper brings out are the following:

1. Heat processing that decreases lysine content through the formation of lysinoalanine, like stack burning or yellowing, popping, extrusion cooking and puffing also decreases net protein utilization (NPU).
2. Although brown rice has higher protein and fat, milled rice has better protein and energy digestibility because of the higher phytate and fiber contents of the bran of brown rice. Thus, the only nutritional advantage of brown rice is its high vitamin B content.
3. Pigmented rices have lower protein digestibility (PD) and sometimes lower NPU due to their high phenolic contents which upon removal by milling results in an significant increase of PD and NPU to levels of white milled rice.
4. Rice-mungbean diet has lower protein quality than rice alone. This may be due to some antinutritional factors present in mungbean. Toasting which removes the phenolics and neutral detergent fiber (through the removal of the hull) increases the protein quality. This supports other local studies that dehulling improves the quality of mungbean.
5. It is important to note that although conventional breeding to improve protein content of rice has been stopped, other techniques like induced mutation by chemical means are still continued. Wide crosses between rice and sorghum and rice and wheat have been made towards this end.

6. With growing emphasis on the more efficient use of marginal lands, studies on the effect of mineral deficiency or excess similar to the study on the effect of S-deficiency on the rice protein quality should be expanded.