PROPERTIES OF STARCH AND PROTEIN RELATED TO EATING QUALITY OF MILLED RICE

By Bienvenido O. Juliano

Abstract

Although amylose/amylopectin ratio of starch is the major determinant of the texture of cooked rice, varietal differences are observed which are related to other starch properties. Among high (> 25&%) amylose rices, preferred varieties with softer texture of cooked rice (with an Instron food tester-Ottawa Texture Measuring System cell) tended to have soft gel consistency and intermediate gelatinization temperature. Among intermediate (20-25%) amylose rices with similar gel consistency, the variety C4-63G with softer cooked rice tended to have higher gelatinization temperature than harder cooked rice (BPI-121-407). Among low (8-20%) amylose Korean rices of low gelatinization temperature, harder cooked rice varieties tended to have harder gel consistency than softer rices. Waxy rices with soft cooked rice had lower gelatinization temperature and softer neutral gel consistency than waxy rices with hard cooked rice.

Protein content was shown to be a secondary factor affecting the stickiness and hardness of cooked rice. About 10-15% of cooked rice protein bodies representing the lipid-rich core portion were not digested by man and expelled as fecal protein particles. Cooking made the lipid more extractable but the protein less soluble. The major undigested protein had polypeptide MW of about 5000 and was present at about 10% of total protein UV absorption of raw milled rice protein. It had lower (< 2%) lysine content and higher cysteine and methionine contents than whole rice protein.

Introduction

An interdisciplinary approach called Genetic Evaluation and Utilization (GEU) program has been in operation in IRRI since the 1960s. Although grain quality can be improved by postharvest operations and processing, IRRI's basic philosophy is to incorporate or build in as much of the desirable characteristics into a rice variety. In GEU-Grain Quality, chemists strive to obtain a better understanding of heritable grain properties related to grain quality with an end in view of developing simple, sensitive and rapid indicators of eating quality applicable to the breeding program. A big bottleneck in our research is the lack of definitive information on relative eating quality of varieties of similar amylose contents. Our Plant Breeding department maintains a Rice Quality Laboratory, which analyzes breeding lines and selections for quality characteristics. This set-up allows the Chemistry department to devote most of its time to research. The Head of the Chemistry department, however, also oversees two chemical service laboratories, the Analytical Service Laboratory for routine analysis of elements in samples of plants, soils and solutions and the Pesticide Residue Laboratory for routine analysis of pesticide residues in plants and soils.

The major constituents of milled rice are starch and protein (Table 1). Minor constituents are fat (lipids), crude fiber (dietary fiber), and crude ash and vitamins. Starch occurs as compound

Table 1. Mean composition of waxy and nonwaxy milled rice

Constituent	*Content (wt % dry basis)*	
	Waxy (glutinous)	*Nonwaxy (nonglutinous)*
Starch	~90	~90
Amylose	1-2	12-33
Amylopectin	88-89	57-78
Protein	8	8
Fat (lipids)	1	1
Nonstarch	0.9	0.4-0.6
Starch	0.1	0.4-0.6
Crude fiber	~0.5	~0.5
Dietary fiber	~1	~1
Crude ash	~0.5	~0.5

granules in the rice endosperm 3-9 μm in size. Starch, a polymer of glucose, is the major factor affecting the texture of cooked rice, mainly the ratio of linear fraction amylose to branched fraction amylopectin (1). Amylose content is measured by its characteristic blue-colored complex with iodine. Amylose classes are: waxy (glutinous) 1-2% amylose; low amylose 12-20%; intermediate 20-25%; and high 25-33%. Waxy rice is as digestible in children as nonwaxy rice (4) and differed in composition from nonwaxy rice mainly in the lipid distribution in the endosperm (Table 1). Amylose content correlates negatively with taste panel scores for tenderness, cohesiveness, gloss, and color of cooked rice (2,3) (Table 2).

Table 2. Mean properties of milled rice and scores by a laboratory taste panel of cooked rice from low amylose and high amylose pairs from three different crosses (Juliano *et al* 1972).

Property	*Low amylose lines*	*High amylose lines*
Amylose (% dry basis)	14.2	25.3
Protein (% at 14% moisture)	10.4	10.4
Final gelatinization temp. (°C)	61.5	61.5
	Trial I (Identical water: rice ratio)	
Water: rice ratio	1.8	1.8
Tenderness[a]	7.6	4.0
Cohesiveness[a]	7.3	3.4
Color[a]	2.7	1.6
Gloss[a]	8.3	4.4
	Trial II (Adjusted water: rice ratio)	
Water: rice ratio	1.6	1.8
Tenderness[a]	6.7	4.1
Cohesiveness[a]	6.6	3.5
Color[a]	1.7	1.6
Gloss	6.9	4.2

[a]Mean of duplicate assessment by a taste panel of four judges. Numerical scores of 1 to 9 were assigned, "1" representing the least expression of the property in question and '9" highest expression.

Other starch properties we routinely measure in the breeding program are final gelatinization temperature and gel consistency. Final gelatinization temperature, measured in the program by the alkali test (5), represents the temperature of hot water at which most of the starch granules lose crystallinity and start to swell irreversibly. It is classified as low 55-69.5°C, intermediate 70-74°C, and high 74.5-80°C. Gel consistency measures the softness of a milled rice gel (100 mg) in 2 ml 0.2 N KOH by actually measuring gel length in a horizontal 13- x 100-mm test tube (6). It is classified as hard 26-40 mm, medium 41-60 mm, and soft 61-100 mm.

Since gelatinization temperature is measured by the degree of disintegration of milled rice after 23 hours at 30°C in 1.7% KOH (5), the whole grain probably reflects this starch granule property. Gelatinization temperature also correlates with resistance of the starch granules to 2.2 N HCl and α-amylolysis (7,8). Resistance of starch granules to HCl corrosion was positively correlated with gelatinization temperature, and to a lesser extent, with amylose content (8). We are checking in a cooperative study whether insects and fungi can differentiate among various brown rices differing in these starch properties.

Although protein is not a primary factor affecting eating quality of rice, rice is the principal source of dietary protein in tropical Asia (9). Proteins are polymers of amino acids joined by peptide bonds. They occur in the rice endosperm in the form of single-membraned particles called protein bodies, 0.5-4.0 μm in size (10). Cooking reduced the true protein digestibility of three milled rices in rats from 100% to 89% but improved the biological value of their protein, such that net protein utilization remained practically the same in raw and cooked rice regardless of protein content (11) (Table 3). True digestibility of rice in man is also about 85% (12) and 15% of dietary protein of man fed rice was reported to be in the feces in the form of fecal protein, with eating quality of milled rice .

Table 3. Mean nutritional properties of raw and freeze-dried cooked rice of three rice samples (Eggum *et al* 1977).

Property	*Raw milled rice*	*Freeze dried cooked milled rice*
Crude protein (% N x 5.95)	8.93	9.04
Lysine (g/16 g N)	3.60	3.51
Amino acid score[a] (%)	65.5	63.8
N balance in growing rats		
True digestibility (%)	99.7	88.6
Biological value (%)	67.7	78.2
Net protein utilization (%)	67.5	69.2
Utilizable protein (%)	6.02	6.24

[a]Based on 5.5 g lysine/16 g N as 100%.

Starch as an eating quality factor

Varietal differences in eating quality are known among rices of similar amylose content (14). For example, IR5 is considered of better quality than IR8 in the Philippines. In India, IR8 and the early semidwarf rices were considered of inferior quality to traditional rices all with high amylose content (15). Glutinous or waxy varieties also differ in quality as with Japanese rice cakes (16) and waxy rices for *pinipig* (17) and *suman sa antala* (18).

We found that the poorer quality rices such as IR8 has a stiffer cooled gel in the Amylograph and less soluble starch (particularly amylopectin) in cooking water than the softer high amylose rices such as IR5 (1). We developed in 1973 a simple micro method to differentiate among these high amylose varieties — the gel consistency test (6).

A taste panel could not readily differentiate between IR5 and IR8. We sent IR5 and IR8 samples to Instron in 1975 for measurement of stickiness and hardness of cooked rice and it sent back results which were encouraging (Table 4). We, thus, acquired an Instron Food Tester Model 1140 in 1977 and developed a modified Ottawa Texture Measuring System cell extrusion technique for hardness, and the use of the force required to lift the plunger from the platform with cooked rice sandwiched between them as a measure of stickiness (19). The use of a cooked rice mold for measuring stickiness was not very effective on low amylose rices.

Table 4. Properties and Instron data on cooked 1974 wet season IR5 and IR8 milled rice. IRRI and Instron Ltd., England, 1975.

Property	*IR5*	*IR8*
Alkali spreading value	4..0	7.0
Amylose content (% dry basis)	28.4	27.9
Gel consistency (mm)	98	36
Mean taste panel scores[a] for		
warm rice		
Tenderness	4.4	3.8
Cohesiveness	4.6	4.1
Hardness (OTMS cell, 100 mm/min.) (kg)	140	160
Cohesiveness (compression anvil and compression table, 50 mm/min.) (g)	286	227

[a]Mean of duplicate assessments by a taste panel of six. Scores based on a scale of 1-9 with the higher number indicating a greater intensity of the character (Juliano *et al* 1965).

When Instron data of hardness and stickiness of cooked rice were correlated with amylose content, correlations were highly significant (19) (Fig. 1). There was more spread in values for hardness than in stickiness at similar amylose values, suggesting that these secondary factors probably affect tenderness of cooked rice rather than stickiness.

Among intermediate amylose rices, C1-63G with intermediate gelatinization temperature had 1 kg softer cooked rice than low gelatinization temperature variety BPI-121 407 although they had similar soft gel consistency values. We tested eight milled rice samples last January 1979 with Prof. A. M. del Mundo of the Institute of Human Ecology, UPLB using a consumer panel in Barrio Bukal, Lemery, Batangas. We found inconsistent results

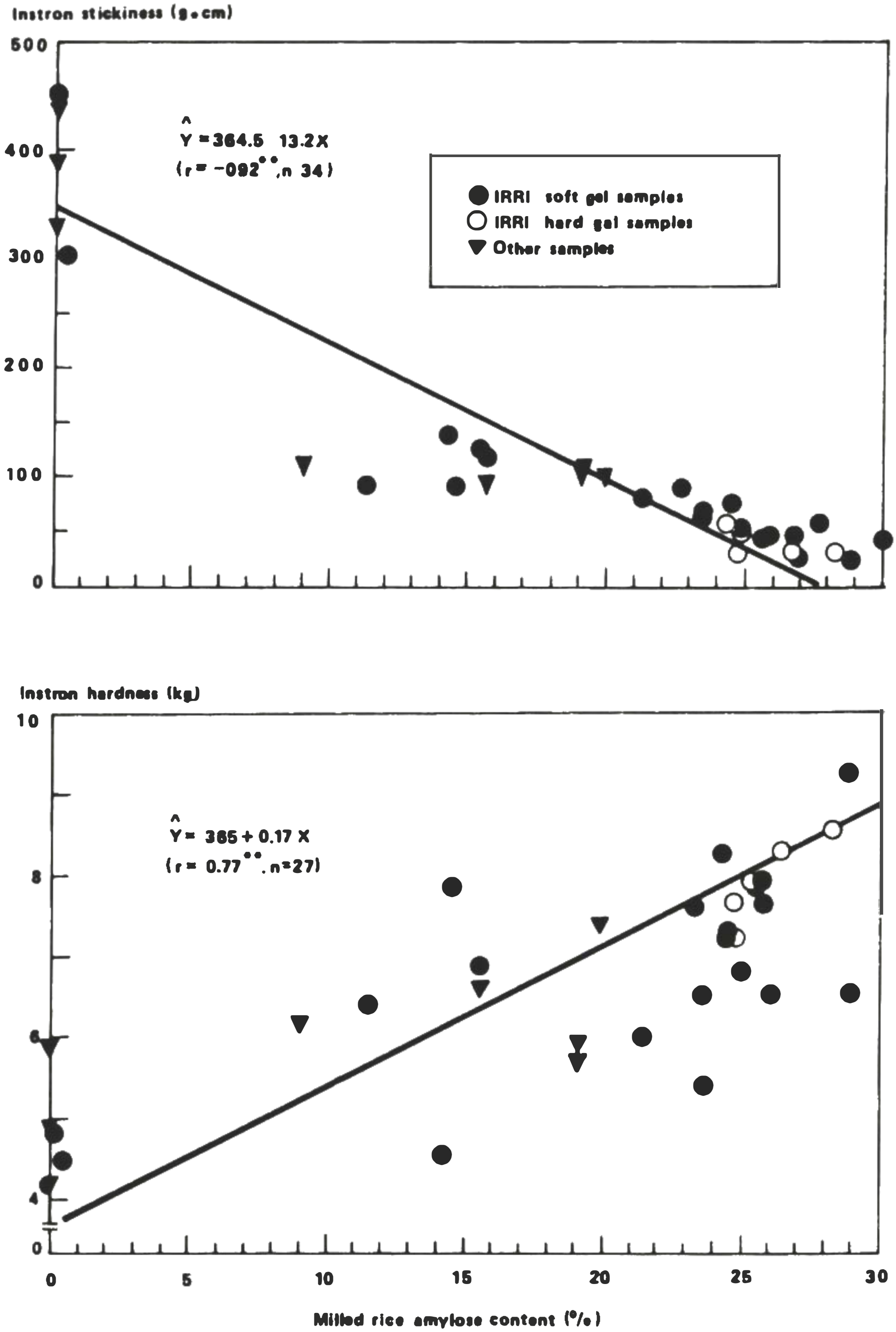

Fig. 1 Relationship between amylose content of milled rice and Instrom strickiness and hardness of rice cooked with the optinum level of water and cooled.

with BPI-121 407 and C4-63G and intermediate amylose lines derived from them (Table 5). Evidently, the panel preferred samples with minimum brokens and without a gray tinge. The results were surprising as in practice there is little price premium given to head or whole-grain rice in the country. We are repeating the test later this year using head milled rices with similar whiteness to obtain definitive results.

Among low-amylose rices, the good samples of quality differences was the first South Korean high-yielding, semi-dwarf variety, Tongil, which came from indica x japonica crossing program. Tongil was considered inferior (harder cooked rice) to traditional japonica varieties of similar amylose content and gel consistency, such as Jinheung (Table 6). We found that by increasing gel concentration from 100 mg to 120 mg/2 ml 0.2 N KOH in the gel consistency test and from 40 g to 48 g/400 g aqueous suspension in the Amylograph (19), Tongil showed harder cooled paste than Jinheung. It also showed a higher gelatinization temperature than Jinheung. Late in 1978, we tested the newer varieties from the Korean program and found them to be already similar to japonica rices in these grain properties and of improved eating quality. Egyptian low-amylose rices also differed in hardness of cooked rice (Table 5).

Table 5. Properties and preference and acceptability scores of five total milled rices used in consumer panel testing. Institute of Human Ecology, UPLB and IRRI, 1979.

Property	*IR4570-74-2*	*C4-63G (check)*	*IR9129-209-2*	*C4-63G*	*BPI-121-407*
Mean preference score[a]					
Cooked	0.247a	0.094ab	0.089ab	−0.136bc	−0.294c
Raw	0.022ab	0.178a	−0.264b	0.081ab	−0.017ab
Alkald spreading value	7.1	3.8	4.7	4.0	7.0
Amylose content (%)	24.7	23.7	21.6	24.6	23.9
Gel consistency (mm)	84	87	82	94	70
Kett Whiteness reading	40.8	43.1	37.4	40.8	38.0
Instron cooked rice					
Hardness (kg)	6.4	4.8	5.6	5.7	6.6
Stickiness (g.cm)	79	87	105	89	99

[a]Mean of 39 panelists who rated five samples each in two sets, with a common C4-63G check sample. 1st = 1.16; 2nd = 0.50; 3rd = 0; 4th = −0.50; and 5th = −1.16. Means followed by a common letter in the same line are not significantly different at the 5% level. Brokens ranged from 25-73% of total milled rice.

Table 6. Physicochemical properties of japonica and indica low amylose rices from Korea and Egypt[a], IRRI, 1978.

Variety	*Source*	*Grain type*	*Amylose content (% dry basis)*	*Gel consistency (mm)*	*Cooked rice hardness (kg)*
Jinheung	Korea	japonica	19.1	89	5.7
Akibare	Korea	japonica	17.5	90	4.6
Tongil	Korea	ind. x jap.	19.9	86	7.4
Milyang 23	Korea	ind. x jap.	19.5	92	4.7
Suweon 264	Korea	ind. x jap.	18.6	92	4.4
Giza 171	Egypt	japonica	18.4	86	5.1
Reiho	Egypt	japonica	19.6	98	5.6
IR1615-246	Egypt	indica	20.0	89	6.6
IR26-203	Egypt	indica	19.6	90	6.4

[a]All with alkali spreading value of 7.0.

Among waxy rices, those with low gelatinization temperature about 65-68°C had the softest texture of cooked processed rice products (Table 7). High gelatinization temperature rices had definitely harder cooked rice combined with harder neutral gel consistency and higher Amylograph viscosity (17,18). We had to use 200 mg flour/2 ml 0.15 N potassium acetate to obtain some degree of differentiation in gels of waxy milled rices. Among Japanese rices, very low gelatinization temperature (56-63°C) waxy rices gave inferior rice cakes to those with 65-68°C gelatinization temperature (16), although they have similar gel consistencies (20). The preferred Japanese rices have higher peak viscosity in the Amylograph (16). The secondary factors that we have found so far as indicators of eating quality among rices of similar amylose contents are summarized in Table 8.

The subject of chemical aspects of rice grain quality was reviewed in an international workshop held last October 1978 at IRRI. The proceedings of this workshop are in press. As a follow-up of this workshop, chemists are doing cooperative ring tests on five grain quality tests in order to standardize the procedures and identify sources of variation in these tests. Of particular interest is the comparison of results with various instruments measuring the texture of cooked rice.

Relationships among starch properties

Although amylose content and gelatinization temperature are independent properties of starch, not all combinations are common in the popular varieties grown in the world (Table 9). We are seeking these odd types from the IRRI world rice collection to better understand their effects on eating quality.

Table 7. Physicochemical properties of waxy rices from three countries. IRRI, 1978.

Variety or line	Processed rice quality	Alkali spreading 1.7% KOH	Alkali spreading 1.3% KOH	Final BEPT (°C)	Neutral gel consistency (mm)	Amylograph peak visc. (B.U.)	Cooked rice hardness (kg)
Philippines							
Malagkit Sungsong	good	6.4	6.0	62.5	76	440	3.5
IR29	fair	7.0	4.2	63	52	560	5.6
UPLB-Ri-1	—	6.5	4.6	64	62	400	3.3
Pampet 63	poor	2.3	2.0	77	35	870	5.9
Thailand							
Niaw San Pahtawng	good	6.8	4.8	64	94	570	4.9
RD6	good	7.0	5.0	63.5	74	450	5.9
RD4	poor	7.0	2.0	73.5	46	820	6.6
Japan							
Norin No. 1	poor	7.0	4.3	58.5	86	350	6.9
Nakatamochi	poor	7.0	4.7	63	90	350	5.9
Koganemochi	good	7.0	2.2	67.5	86	560	5.6
Hatsunemochi	good	7.0	2.8	68.5	81	595	6.0

Table 8. Summary of important secondary eating quality factors for milled rice with similar amylose content.

Amylose type and property	*Property class of milled rice*	
	Good quality (softer)	*Poor quality (harder)*
High amylose		
Gel consistency (100 mg/ 2 ml 0.2 N KOH)	soft	hard
Gelatinization temperature (°C)	intermediate	low
Amylograph peak viscosity (Brabender units)	low	high
Hot water soluble amylose (% of milled rice)	14-16	10-12
Intermediate amylose		
Gel consistency (100-110 mg/2 ml 0.2 N KOH)	soft	hard
Gelatinization temperature (°C)	intermediate ?	low ?
Low amylose		
Gel consistency (120 mg/ 2 ml 0.2 N KOH)	soft	hard
Amylograph peak viscosity (B.U.)	low	high
Waxy (glutinous)		
Neutral gel consistency (200 mg/2 ml 0.15 N potassium acetate)	soft	hard
Gelatinization temperature	low (65-68° C)	interm. to high; (≥70° C) very low (≤60° C)
Amylograph peak viscosity (B.U.)	intermediate	high; low

Among waxy rices, which is an ideal model system as their starch is essentially 98% amylopectin, molecular size as indexed by intrinsic viscosity and sedimentation constant tended to show a U-shaped correlation with gelatinization temperature with minimum at 65-68°C (21). Gel consistency also showed low values for low gelatinization temperature waxy rices than intermediate and high gelatinization temperature rices (20,21). Limited study on eight nonwaxy starches seemed to indicate that amylopectin

Table 9. Combination of amylose content and gelatinization temperature commonly found in cultivated rice varieties.

Amylose type	*Gelatinization temp. class*		
	Low	*Intermediate*	*High*
Waxy (0-2%)	++[a]	0[a]	+[a]
Very low (2-12%)	++	0	+
Low (12-20%)	++	0	0
Intermediate (20-25%)	+	++	0
High (25-33%)	++	++	0

[a]++ = most common; + = less common; 0 = rare.

contributes more to gel consistency than amylose (22) (Table 10). Gel viscosity correlates positively with molecular size of amylopectin also (22). Periodate oxidation indicated that the degree of branching of amylopectin is 4-5% or a mean chain length of 23-28 glucose units. Hence, molecular size was the major molecular property of amylopectin that was related to gel viscosity of both waxy and nonwaxy rices.

We are trying to recheck by gel filtration on agarose gel (Sepharose 2E) and by ultracentrifugation the molecular size of amylose and amylopectin and the intermediate fraction among high amylose rices differing in gel consistency. The fraction intermediate in properties between amylose and amylopectin has been demonstrated in corn starch and may help explain our

Table 10. Range of intrinsic viscosity and gel viscosity (160 mg/2 ml 0.2 N KOH) of amylose and amylopectin of seven nonwaxy rices (Juliano and Perdon 1975).

Property and fraction		*Viscosity*	
		Range	*Mean*
Intrinsic viscosity (ml/g)	Amylopectin	159-197	176.6
	Amylose	136-230	169.7
Gel viscosity (cps)	Starch	140-686	423.4
	Amylopectin	354-605	487.0
	Amylose	26- 73	39.6
	Reconstituted starch	148-240	210.4

difficulty in obtaining pure amylose and amylopectin from high amylose rices by the usual crystallization of amylose as the amylose-butanol complex (22).

Protein as an eating quality factor

Although we have studied in detail the major proteins of milled rice, glutelin (23,24), prolamin (25) and α-globulin, (26), and are studying the major albumin and the second major globulin, our discussion will be limited to the effect of boiling on protein digestibility. The 85% digestibility of milled rice protein in man is less than the 90% reported for wheat flour protein (12). In the mid 1960s, Dr. George Graham concluded that there was no advantage of rice over wheat in baby foods, and UNICEF dropped its project to use the overmilling fraction of milled rice (with 15% protein) as a baby food.

Dr. Y. Tanaka, who isolated and characterized the fecal protein particles (13) spent 8 months with us in 1977 and brought some of his samples for characterization. Our study since then indicated that the fraction rendered indigestible by boiling represented the core or center of the large spherical protein bodies of milled rice (10-15% of total protein and 80% of lipid) (28). Cooking evidently resulted in more protein-protein interaction resulting in reduced extractability of protein and increased extractability of the lipid (29). Fecal protein particles can be simulated by pepsin treatment of amylase-destarched milled rice (28,29).

The core portion had higher lipid content (~ 20%) than whole PB (7-9% lipids) and proportionately less protein (Table 11). Lipids had identical ratio of lipid fractions and fatty acid composition in the core and whole PB but protein showed drastically different composition (20). Core protein had lower lysine content but higher cysteine/methionine content of whole PB protein, which explains its insolubility by disulfide bond formation. (Table 9). These results explain why the net protein utilization of rice protein does not deteriorate on cooking as the protein rendered less digestible is of poorer quality than the whole protein. Digestibility alone is not a reliable indicator of protein quality. Rice protein is better retained in man than wheat protein because of its higher biological value (30).

In attempts to explain the source of this core protein, we fractionated the SDS-β-mercaptoethanol extract of milled rice and found 10% of its UV absorption on SDS-Sephadex G-75 gel filtration to correspond to the major core PB polypeptide (29) (Fig. 2). This fraction correspond to a polypeptide molecular weight of 5000. Evidently the core protein must have been synthe-

Table 11. Properties of whole and pepsin-treated (undigested) IR480-5-9 milled rice protein bodies.

Property	*Cooked whole protein bodies*	*Pepsin-treated cooked protein bodies*
Weight recovery (%)	100.0	34.5
Protein content (% N x 5.95)	79.1	62.4 (27.2%)[a]
Lipid content (%)	9.51	22.2 (80.1%)[a]
Lysine (g/16.8g N)	4.0	1.3
Cysteine (g/16.8 g N)	2.6	4.6
Methionine (g/16.8 g N)	3.1	4.8
Neutral lipid/glycolipid/ phospholipid ratio	92/5/3	92/5/3
Fatty acid composition of neutral lipids		
Palmitic (wt % of total)	32	34
Oleic (wt % of total)	24	24
Linoleic (wt % of total)	42	40

[a]Values in parenthesis are protein or lipid recoveries.

sized ahead of the rest of the outer PB proteins about 7-8 days after flowering in the presence of more lipids. It has escaped detection earlier due to its poor capacity to absorb protein stains on electrophoresis due to its small molecular size. Isolation of this polypeptide as the *S*-cyanoethyl derivative is in progress.

Other minor constituents as eating quality factors

Fig. 3 summarizes our current research program on grain quality and Fig. 4 summarizes our cooperative work with non-IRRI scientists.

We have studied lipids (1%) of milled rice as an eating quality factor. Waxy rice has more nonstarch lipids and less starch lipids than nonwaxy rice (31). These lipids are important in the gel consistency test as defatted rices all have soft gel values (29). Parboiling also makes gel consistency values softer, probably because of the lower oil content of milled parboiled rice as compared to raw milled rice.

Cell wall polysaccharides have been a popular research subject recently in view of the nutritional interest on "dietary fiber." Our studies in 1968-69 showed very little hemicellulose in milled rice (32). Cell walls are probably important in grain integrity and direction of expansion of rice grain during cooking. We reactivated our work on cell wall polysaccharides with the 4-month stay in Nov. 1978 - Feb. 1979 of Dr. B. A. Stone, Dept. of Biochemistry, LaTrobe University, Victoria, Australia.

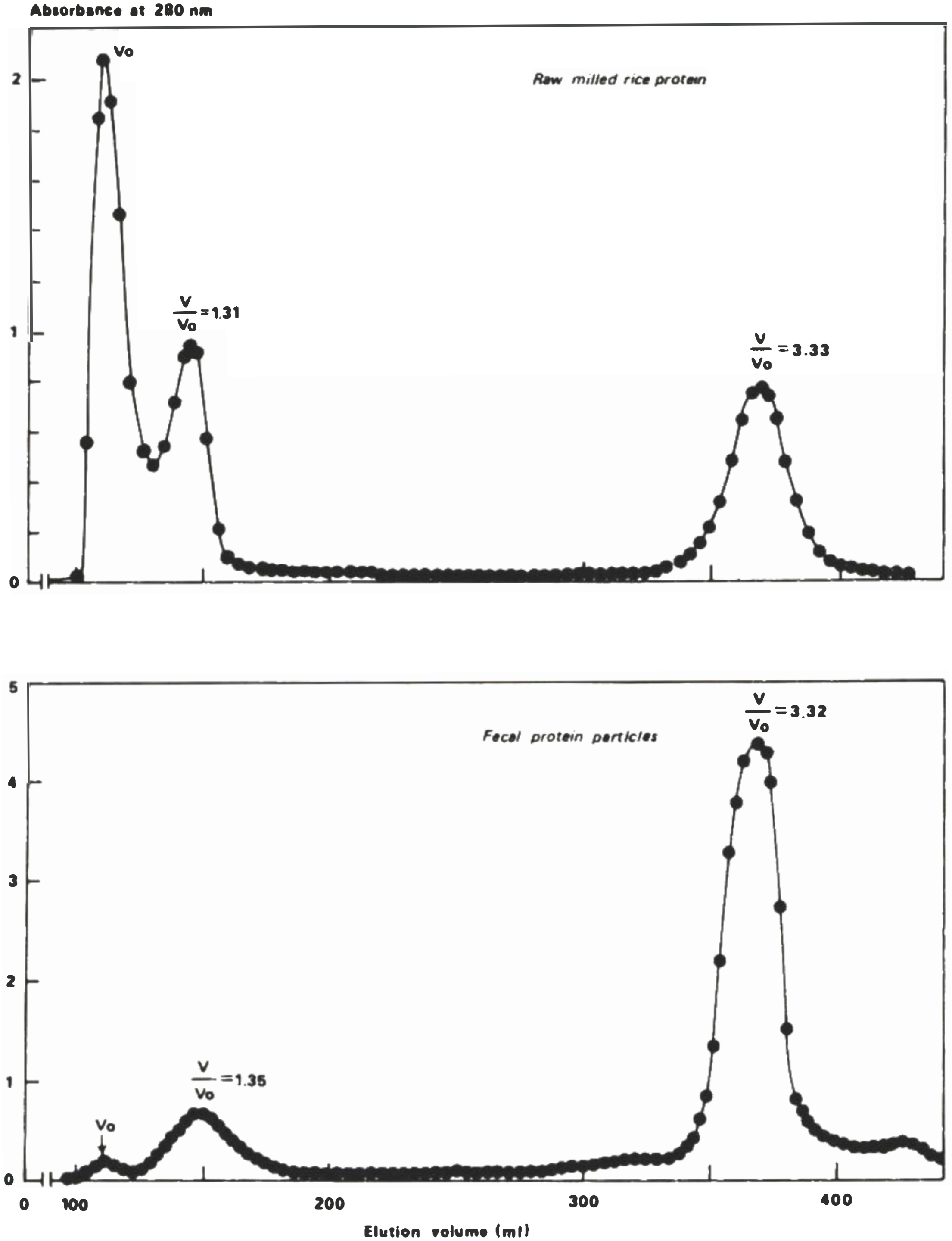

Fig. 2 SDS-Sephadex 6-75 gel filtration of total raw IR480-5-9 milled rice protein extracted by 0.5% SDS-0.6% B-mercaptoethand and of fecae protein particles from a man on an IR480-5-9 rice diet, IRRI, 1978.

4. Quality characteristics of rices grown in different countries	Researchers in 40 countries
5. International collaborative tests on rice quality methods	
a) Gel consistency	7 cooperators (5 countries)
b) Alkali test (2 methods)	9 cooperators (6 countries)
c) Amylose content (3 methods)	9 cooperators (6 countries)
d) Instrument methods for cooked rice texture (7 methods)	12 cooperators (8 countries)
6. Structural changes in developing rice grain	U.S. Grain Marketing Res. Lab., Manhattan, Kansas Mr. D. B. Bechtel
7. Lipids of starch of tropical rice	Obihiro University, Japan Dr. Y. Fujino/Mr. Y. Mano
8. Polysaccharides of intact cell walls of milled rice	LaTrobe Univ., Australia Dr. B. A. Stone

Crude ash (minerals) is also important in nutrition and may also alter the cooking characteristics of starch. The identification in the GEU program of varieties such as IR42, with tolerance to a variety content of milled rice is affected by the different soils on which IR42 can be grown without a decrease in yield.

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