Physicochemical Basis of Eating Quality of Rice

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ABSTRACT

The wide varietal difference in the eating quality of rice has been intensively studied in several laboratories around the world since the 1950s. The voluminous data initially suggested that the amyllose starch content of rice was the single largest determinant of its end-use quality; yet amyllose was not enough, additional factors seemed to be involved, among them possibly insoluble amyllose, Brabender breakdown, gel consistency, and gelatinization temperature (GT). Still, the meaning of it all was not clear. A paradigm shift occurred in the mid-1980s. A study showed that amyllopectin of rice varieties bound varying amounts of iodine, which correlated positively with rice texture. It was then found that chain lengths of rice amyllopectin varied and, more important, the proportions of the long chains differed greatly among varieties. The relative abundance of extra long chains of amyllopectin strongly correlated (positively) with the cooked-rice texture. Rheological and microscopical studies suggested that abundant long chains in amyllopectin resulted in a strong and resilient starch granule, while their absence led to a weak and fragile granule, which explained the difference in textures of cooked rice. Thus after half a century of extensive research, the end-use quality of rice has now been firmly attributed mostly to its amyllopectin chain structure, although the protein content probably plays some minor role.

Rice is the most important staple food of humankind (76). More than 90% of it is grown and consumed in South, East, and Southeast Asia. But despite this concentration, rice is actually very adaptable and versatile. It is grown in all continents (except Antarctica) and in more than 100 countries, between 40°S and 53°N latitudes, from sea level to 3,000 m in altitude, and from dry land to under 1- to 2-m-deep water. Having been thus adapted to such diverse agroclimatic conditions, rice comes in thousands of cultivars that vary greatly in their attributes, including cooking, eating, and product-making quality. Rice palatable to one cultural group or suitable for one product is, more often than not, not so for another.

A striking example of this variation is the systematic change in rice quality within the heartland of rice in Asia. There is a clear trend of textural difference in the native rice from the east to the south of Asia. Rice in the north and east of Asia (japonica) usually cooks soft and sticky, whereas that in South Asia (indica) generally cooks dry and fluffy, while that of Southeast Asia comes somewhere in between (53). However, this is only an example of the gross variation in rice quality, as there is a wide divergence between individual cultivars.

This extraordinary diversity in the quality attributes among rice cultivars, particularly in their cooking-sensory attributes (henceforth called eating quality), naturally evoked the curiosity of scientists who wanted to understand the factors behind specific quality attributes. Work on this subject was started almost a century ago and scientists have spent a substantial part of the twentieth century exploring this enigma. As a matter of fact, it may not be an exaggeration to say that this topic has been one of the most intensively researched fields in the realm of cereal chemistry. Research in the subject can be divided into three phases: initiation (pre-1950s), data accumulation (the 1950s to the mid-1980s), and exploration at the molecular level (after the mid-1980s).

INITIATION

The subject was first explored almost a century ago with the work of Warth and Darabsett (124) who studied the progressive digestion of rice grains by increasing concentrations of dilute alkali. Their work was probably the first attempt to understand the varietal difference in rice quality. Since rice is cooked by boiling it in water before consumption, water uptake by rice during cooking (grams of water absorbed per gram of rice in a definite time, say 20 min) was the issue that was first, in fact mainly, examined during this period, primarily in India (107,108,113,114). Sanjiva Rao et al. (109) concluded that “good” rice varieties had a high water uptake, besides having a high amyllose content. This assertion can be understood with hindsight. Indian rice is almost entirely indica, and fine-grained indica rice is what Indians prefer. Now indica rice, certainly Indian indica rice, contains a relatively high amount of amyllose starch (13). Secondly, being fine-grained, i.e., with small and slender grains, these rices have a relatively high surface area per unit weight. It is clearly this fortuitous association that led to the assertion mentioned above. Bhattacharya and Sowbhagya (8) showed later that water uptake by rice in boiling water was related only to the surface area per unit weight (i.e., the size and shape of the grains) and was virtually unaffected by its intrinsic property or chemistry, including amyllose content. Nonetheless, the above assertion almost set up a paradigm of rice quality lasting for decades. Rice researchers began compulsorily examining the water uptake of their samples. Until recently, most studies of rice quality carried out after the 1940s invariably contained a report on its water uptake, no matter what those data revealed. As a matter of fact, water uptake data were measured and reported even while cooking rice in an automatic cooker, where the water added to the inner vessel as well as to the outer vessel was predetermined, thus rendering the “water uptake” also virtually predetermined (59). Unfortunately, researchers also started reading more into the parameter than warranted. Forgetting that water uptake, as defined, was no more than an expression of the rate or ease of hydration (amount of water absorbed in a given time), it was often wrongly interpreted as an expression of the hydration ability of a sample, erroneously concluding therefore that varieties with high water uptake inherently absorbed more water, or had more affinity for water, than others. Needless to say, these studies during the phase of initiation shed little light on the varietal difference in rice quality.

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Systematic study on rice quality was started shortly after World War II. A massive amount of research was carried out on the subject in various laboratories around the world for almost four decades from the early 1950s to the 1980s. By a peculiar coincidence, this work was started almost simultaneously in many laboratories around the world, though it was initiated in the United States.

The work in the United States arose largely from an accident. Unlike in the heartland of tropical Asia, where rice is quintessentially a food, rice in the United States, like all other materials, is valued as much as a material for industry and commerce as an item of use. As a result, only a limited number of carefully selected varieties with clearly identified specifications are grown in the United States at any given time. A cultivar, Century Patna 231, was released in 1951 as a long grain, which turned out to be a disaster for the industry, for its cooking and processing qualities were different from the expected pattern (75). This event led to the USDA’s realization that developing indices that could predict the eating and processing qualities of rice was essential. What followed was a remarkably well-planned and well-coordinated research effort carried out simultaneously in a number of U.S. laboratories. A method was developed to obtain a quantitative estimate of the sensory attributes of cooked rice and also various physiochemical criteria (4.5). The results showed that the so-called starch iodine blue value (SIBV), i.e., the iodine-blue color of a warm-water extract of rice flour (36), the amylose starch content of rice (127), the Brabender pasting pattern of rice flour and the gelatinization temperature (GT) read therefrom (35), and the digestion score of whole rice grains in dilute alkali (72), could provide indices for successful prediction of the sensory and processing qualities of rice (6).

A clarification regarding the first two indices above may be necessary here. We know now that these two parameters are interrelated, albeit in a complex way as will be explained later, but this was hardly realized at that time (11,12,60). In fact, the SIBV was developed as a purely empirical test following a study of parboiled rice properties and, as it developed, had several inconsistencies. Nonetheless, empirically, the index gave an excellent idea about the cooking and processing quality of the small number of prevalent American rice (6).

These above indices could also easily differentiate between U.S. rice grain types (long, medium, short) as well as pick up atypical cultivars (such as Century Patna 231 among long grains and Early Prolific among medium), further proving their relevance. In general, rice with a greater amount of amylose content, a high SIBV (low transmittance), and a medium-high alkali score and paste viscosity cooked relatively dry and firm; rice with lower amylose SIBV and paste viscosity and a high alkali score cooked soft, moist, and sticky. The researchers also studied water uptake of rice and found it of little value, as it was nearly constant within a grain type and could not identify atypical varieties (4.5,36). This well-coordinated work carried out cooperatively in a few American laboratories over a period of only a few years successfully established the basic factors behind rice quality, which have stood the test of time even today. Surprisingly, after this successful conclusion, work in this area suddenly disappeared in the United States for several years.

Work in the area was taken up simultaneously or shortly thereafter in several world centers, including Japan, Germany, Italy, Spain, France, and a little later, especially in the International Rice Research Institute (IRRI) in Los Baños, Philippines, and the Central Food Technological Research Institute (CFTRI) in Mysore, India. The studies in Italy were, broadly speaking, a continuation of the water-uptake paradigm approach of the earlier Indian phase. Thus, these researchers concentrated on such measurements as the so-called Ranghino test (the time of cooking of rice) (100) and the so-called Refai test (the volume expansion of rice when cooked in water at 80°C) (101,102). The effect of amylose content, alkali score, etc., were also studied but more to examine their relationship to the above parameters (2,27,28,31). However, the Refai test did succeed to some extent in identifying desirable cultivars from the Italian perspective. This is because most Italian rice is japonica, which has low GT. Therefore, these rices expanded more in water at 80°C than imported indica rice, which had a higher GT and were thus easily identified.

Scientists at the Federal Research Centre for Cereal and Potato Processing in Detmold, West Germany, also took up research on rice quality initially to study the nature of imported rice in the aftermath of the war. Hampel (40) and Pelshenke and Hampel (87,88) first developed the “rice swelling coefficient” test, which was somewhat akin to the SIBV. They also developed an “oryzogram test” as a measure of water absorption and the softness of cooked rice. Crucially, Hampel (41) developed perhaps the first successful instrumental method to measure the texture of cooked rice using the Haake consistometer. He confirmed the American findings in general, especially the importance of amylose content as a key indicator of rice texture (42–45).

A good deal of work was also done in Spain in the late 1950s and the 1960s in the Instituto de Agroquimica y Tecnologia de Alimentos (IATA) in Valencia, Spain. Apart from studying the effect of amylose and GT, etc., as established by the American workers, the major thrust of Spanish research was in two areas: effect of proteins and sulfhydryl and disulfide groups, especially in the outer layers of the grain, on the eating quality of rice (92–95). Spanish scientists strongly believed that these parameters substantially affected cooked-rice texture. Secondly, in a series of papers, the deterioration of sensory qualities as well as other changes in rice during storage were studied in detail (3).

Japanese scientists have a long history of sustained research on the sensory quality of rice. They were pioneers in devising new instruments and new techniques. Fukuba (32) and Fukuba and Yamamoto (33) pioneered the use of the Brabender viscosimeter to study the pasting properties of rice even before the more well-known use of the technique by Halick and Kelly (35). Japanese scientists also devised and used methods to measure the viscoelastic and textural properties of cooked rice and paste long before such attempts were made elsewhere. Thus, Chikubu et al. (18,19) used an oscillating rheometer to study the dynamic viscoelasticity of rice starch paste. Later they devised a parallel plate plastometer to study the viscoelasticity of cooked rice grains (21). Kurasawa et al. (68) devised a table balance to measure the adhesiveness of cooked rice. They also devised a technique to index the sensory palatability of cooked rice, called the gross palatability index (GPI). Suzuki and Take-tomi (117) devised alkali viscosimeter as an alternative system to study rice pasting patterns.

Scientists in the National Food Research Institute (NFRI) at Tsukuba first studied the comparative properties of local and domestically disliked imported rice in the wake of the food shortage after the war (18–21,48,49). These included water uptake at 70–80 and 100°C, solids lost during cooking, volume expansion, blue value and pH of the excess cooking water, alkali digestion, amylose content, SIBV, pasting properties, alkali viscosimeter, and bound protein content of rice starch, as well as cold starch-paste rigidity and cooked-rice viscoelasticity. Chikubu (17) and Tani et al. (120) summarized these studies and found a distinct gradation in all these properties among rices from Thailand, Burma, Italy, Spain, Egypt, the United
States (California), China mainland, Taiwan (China), and Japan. There was a clear difference between indica and japonica rices: the relative water uptake at 70–80 and 100°C of the two were quite the reverse (cf. the American work earlier [35]) and indica rices cooked firm and flabby, while japonica rices were soft and sticky. Amylose content was correlated to starch-paste rigidity and Brabender cold-paste viscosity (positively) and breakdown (negatively). The starch-bound protein was proportional to the amylose content, an observation that was confirmed later by other scientists who showed that the residual starch-bound protein was a starch synthase, the waxy gene product (110,116,122,123). The GT, as revealed by Brabender visigraph, was closely correlated to the gelatination normality of alkali in alkaliviscogram.

Later, NFRI scientists studied the factors related to the palatability of their own Japanese rice (i.e., japonica) to Japanese consumers. They grew five varieties of rice in different locations in Japan and then had the palatability tested by a panel of 24 testers and repeated the work twice more (17,22,23,30). It was concluded that the palatability of Japanese rice could be measured by five properties: protein content, peak and minimum Brabender viscosities, breakdown, and SIBV.

Likewise, scientists at the Niigata University devoted attention to this subject. They obtained results broadly similar to those obtained in the NFRI (69–71). They used the table balance stickiness score and the sensory GPI score to evaluate their results. Japanese scientists continued their involvement in measuring and predicting the palatability of cooked rice using various instruments. For example, Okabe (82) used the General Foods Texturometer to construct a texturometric using the parameters of cooked rice hardness (positive peak, H) and stickiness (negative peak, –H). Zones of various levels of acceptability could be designated from this diagram on the basis of the values of H and the ratio of –H/H. Similarly, NFRI scientists used the Tensipresser to study the effect of various factors, especially amylose and protein on rice texture (83,84).

In 1961, the IRRI was established in Los Baños, Philippines. The Chemistry Department of the IRRI under the leadership of B. O. Juliano carried out the most intensive work in this area from the time of the institute’s establishment. One can say this school had left off and soon became the main focus of all rice quality research in the world. One should also note that the IRRI work, for the first time, focused on the entire cross-section of the world’s rice, including that in the heartland of tropical Asia, which had hardly been studied before.

Juliano et al. (57) first confirmed a general interrelationship between amylose content, SIBV, and amylograph setback, as well as between alkali digestion score and Brabender GT. In a study of 55 Southeast Asian rices, they concluded that amylose was the most important factor in rice quality; amylose was unrelated to Brabender peak viscosity but correlated with breakdown (negatively) and setback (positively) (58). The protein content was generally unrelated to any property. The relative contribution of amylose, protein, and GT to sensory properties of cooked rice was carefully studied using a new method of sensory analysis (59,85). The preeminent contribution of amylose was confirmed, while protein and GT were found generally unrelated to sensory quality. The importance of amylose was repeatedly confirmed (55,90).

However, IRRI scientists noticed differences in texture and product properties even in waxy rices (56,67,79,91). As these rices had no amylose, clearly other factors must have been playing a role. Similarly, cultivars having identical amylose content often showed differences in sensory properties. A stream of research papers were published during the two decades (the mid-1960s to the mid-1980s) to identify these other factors. Properties of purified rice starches were also studied, providing a wealth of useful information about rice starch (1,54,61,74,103,121).

Cagampang et al. (16) devised a gel consistency test, i.e., the mobility of a dilute alkaline rice flour gel, which seemed to correlate well with the quality of especially high-amylose rices. The scientists believed that this test was a good index of the properties of rice amyllopectin (89). Thus, the amylose content and the gel consistency were considered to complement each other and hence together provide a total picture of the sample. Nonetheless, the extraordinary diversity in tropical rice created many deviations. The parameters and indices often overlapped. A series of studies were therefore conducted to explore the matter further over many years (53,55,56,79,90,91). The general conclusion was that one or more of the three indices, GT, gel consistency, and Brabender setback, along with amylose content, could account for the properties of rice.

Scientists at the CFTRI in Mysore, India, under the leadership of the present author, also made an intensive study of the subject from the late 1960s onward. While studying the effect of amylose content, SIBV, alkali digestion score, and Brabender visigraph pattern, the CFTRI initially devoted considerable attention to improving the above tests, as well as developing new tests. In this effort, two crucial concepts emerged. One, while studying the SIBV, the authors realized that it was nothing but the amylose dissolved in hot water, from which they noted a distinct pattern of amylose solubility among the varieties. Most native Indian varieties, which were preponderant in their study samples, were high in amylose and so were the newly emerging, high-yielding crosses being developed then by breeders in India by crossing the local varieties with dwarfing-gene donors, a process then in full swing. It was noted with surprise that although the amylose content was uniformly high (greater than 26% dry basis) in all these varieties, their SIBVs differed sharply. Thus, the original Taiwanese high-amylose, low-GT, dwarfing-gene donors and their initial progenies all had an amylose solubility of no more than 35–40%; a part of the native Indian high-amylose rices showed a medium solubility value of 45–55%; and the remaining high-amylose Indian rices gave a high solubility of 55–65%. All other rices (intermediate- and low-amylose varieties studied) showed a high-amylose solubility of 55–65% (11). This property thus clearly classified the high-amylose rices into three groups and they indeed sharply differed in their textures as well (Table I). As the amylose content varied widely among rice varieties, this property was later expressed by its obverse, insoluble amylose (total amylose

<table>
<thead>
<tr>
<th>Amylose Class</th>
<th>No. of Varieties</th>
<th>Total Amylose (% db)</th>
<th>Insoluble Amylose (% db)</th>
<th>Cooked Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>5</td>
<td>28.7</td>
<td>18.4</td>
<td>1.6 151</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>28.7</td>
<td>13.0</td>
<td>2.5 88</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>28.8</td>
<td>11.9</td>
<td>3.2 48</td>
</tr>
<tr>
<td>Intermediate</td>
<td>4</td>
<td>23.8</td>
<td>9.0</td>
<td>4.6 19</td>
</tr>
<tr>
<td>Low</td>
<td>4</td>
<td>20.6</td>
<td>8.2</td>
<td>6.4 12</td>
</tr>
</tbody>
</table>

aData of Bhattacharya et al. (12). (Used with permission.)
bHaake consistometer.
minus hot-water-soluble amylose) so as to make a uniform scale among all rices (12). It was found that this parameter correlated very well with cooked-rice texture as well as other rice properties (12, 29, 77). Interestingly, Juliano et al. (60) also noted low SIBV in certain cases, but they did not pay much attention to it, explaining it away as in situ retrogradation when the amylose was very high. But Bhattacharya et al. (11) found very low to very high solubility precisely in high-amylose rices. Probably the fact that IRRI scientists dealt more with intermediate-amylose rice (native to Southeast Asia), where amylose solubility hardly differed, while those of the CFTRI dealt more with high-amylose rice (native to South Asia), where it did vary, explained the difference in observation.

The CFTRI group also devised a new system of viscography (9, 10). In this system, samples were viscosographed not at a fixed slurry concentration as is customary but at a fixed value of peak viscosity. The breakdown observed at such a fixed peak value gave an excellent inverse correlation with the cooked-rice hardness and also sharply differentiated the different rice quality types. The CFTRI then made a thorough study of 177 rice varieties representing a broad cross-section of the world’s rice using both of the above newly developed indices along with other conventional properties (14). Based on these results, rice varieties were divided into eight quality types (Table II) based primarily on the total and insoluble amylose contents as well as the viscochart “relative breakdown.” The high-amylose rices were classified into three distinct types depending on their insoluble amylose contents as already mentioned. The intermediate-amylose rices were also divided into three types, this time based on their viscochart “relative breakdown” pattern. Interestingly, about 25 samples of scented rices of India, collected from geographically widely different parts of the country, showed a surprisingly uniform and unique set of properties, for which the scented rices were assigned a distinct group (type IV). It is interesting that an isozyme polymorphism study of the world’s rice by Glaszmann (34) later showed all these aromatic rices belonged to a distinct group (his Group V). In a detailed study, these three criteria—total and insoluble amylose and viscochart breakdown pattern—were later shown to correlate very well with sensory as well as instrumental measures of cooked-rice texture for all eight rice types (112).

To summarize, a very intensive amount of work was thus conducted in various laboratories of the world, especially in the United States, Japan, IRRI, and CFTRI, to explain the physicochemical determinants of varietal differences in eating quality of rice during the period from the 1950s to the mid-1980s. Probably some 500 or more papers on the subject came out during this period. The outcome was mixed. There was a clear indication that the amylose starch content was the single most important indicator of the eating quality of rice. However, the same studies also equally emphasized that, while amylose was necessary, it was not sufficient. Other factors were also involved. Unfortunately, there was no unaniimity about these other factors. As a matter of fact, one can say that determining the identity of these other factors was the chief burden of the large research effort that went into this period of data accumulation. But no unambiguous or unanimous answer emerged. Some of the secondary factors that were explored by the different studies were SIBV, GT, alkali digestion score, Brabender viscochart pattern, alkali viscochart pattern, mobility of a dilute alkaline rice-flour gel (gel consistency), and hot-water-insoluble amylose.

All or most of these indices could, along with amylose content, no doubt provide an excellent index of the end-use quality of a sample. But there were three problems. First, none of these secondary factors applied uniformly in all cases. Some fitted the behavior of one variety; some fitted others. Second, the matter lacked a theory. Why any or all of these indices correlated with rice quality could not be explained. Third, one should note a major conceptual distinction among the above factors. Gel consistency and the pasting pattern (or alkali viscochart) could no doubt, wherever applicable, be good tests of rice quality. But these factors could not by any means be called determinants of rice behavior, for these properties themselves must have been determined by certain other causative agents, i.e., these were at best effects, not causes. On the other hand, amylose content, GT (or alkali score), and insoluble amylose (or SIBV), as some physical entities could, at least theoretically speaking, be considered not just as indices but as determinants wherever their involvement was confirmed. However, the involvement of GT was uncertain at best and the nature of the entity called insoluble amylose was unknown.

It was thus clear that this kind of incremental research had reached a dead end and further advancement required the introduction of some new tools or some new conceptual approach. Indeed, a new approach did arrive in the mid-1980s and a third phase of rice quality research ensued.

### Exploration at the Molecular Level

**Role of Amylopectin Structure**

Theories of starch structure have been going through continuous change from the structure’s very inception. No theory lasted for more than a few years or at most a decade. From the mid-1970s, evidence had been accumulating that amylopectin structure was much more complicated than had been thought and that the structure differed from species to species, perhaps even among cultivars. As an example, Biladeris et al. (15) showed that amylopectin had a trimodal branch structure and that the relative proportion of these three sets of branches differed among different legume and waxy starches. Professors Fuwa and Hizukuri in Japan also obtained many puzzling data about amylopectin structure, in particular data confirming that amylopectin from different sources all had a trimodal branch distribution but with varying proportions of its short, medium, and long branches.

At this time in the CFTRI lab, Chinnaswamy (24) obtained some results that in the background of the newly emerging information on starch chemistry initiated a paradigm shift in our approach to the

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**Table II. Quality classification of rice**

<table>
<thead>
<tr>
<th>Rice Quality</th>
<th>Amylose (%)</th>
<th>BD, (%)</th>
<th>Hardness</th>
<th>Stickiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Designation</td>
<td>Total</td>
<td>Insoluble</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>HA: Hard</td>
<td>&gt;26</td>
<td>&gt;15</td>
<td>0–5</td>
</tr>
<tr>
<td>II</td>
<td>HA: Intermediate</td>
<td>&gt;26</td>
<td>12.5–15</td>
<td>16–17</td>
</tr>
<tr>
<td>III</td>
<td>HA: Soft</td>
<td>&gt;26</td>
<td>&lt;12.5</td>
<td>31–55</td>
</tr>
<tr>
<td>IV</td>
<td>IA: Aromatic</td>
<td>22–26</td>
<td>&lt;10</td>
<td>56–81</td>
</tr>
<tr>
<td>V</td>
<td>IA: Normal</td>
<td>22–26</td>
<td>&lt;10</td>
<td>56–78</td>
</tr>
<tr>
<td>VI</td>
<td>IA: Bulu</td>
<td>22–26</td>
<td>&lt;10</td>
<td>134–157</td>
</tr>
<tr>
<td>VII</td>
<td>LA</td>
<td>15–22</td>
<td>&lt;10</td>
<td>111–153</td>
</tr>
<tr>
<td>VIII</td>
<td>WX</td>
<td>&lt;5</td>
<td>...</td>
<td>252–333</td>
</tr>
</tbody>
</table>

* Compiled from Bhattacharya et al. (14). © Institute of Food Technologists. (Used with permission.)

* Relative breakdown at a peak viscosity of 1,000 BU. Values are from Bhattacharya and Sowbhagya (10), n = 45.

* HA, high amylose; IA, intermediate amylose; LA, low amylose; and WX, waxy.
peciins had more short chains (Fig. 3). As chains, while low-IA japonica rice amylopectins of high-IA branching and fractionation by gel-permeation HPLC, amylopectins of high-IA affinity (IA). Upon debranching and fractionation by gel-permeation chromatography (GPC) into two fractions (Fig. 1). The major fraction (Fr I) eluted at the void volume, thus obviously having a very high molecular weight (MW). It was also branched, as evidenced by the fact that no material remained in the void volume after the fraction was debranched before GPC. In other words, it was amylopectin. The other smaller fraction (Fr II) that entered the gel was presumably largely amylose. Yet he noted with surprise that Fr I, despite being amylopectin, stained blue with iodine. What is more, the blue color seemed to intensify with increasing reputed amylose content of the parent rice (Fig. 1). As a matter of fact, the $\lambda_{\text{max}}$ of the iodine complex correlated very well with the reputed insoluble amylose content of the parent rice (Fig. 2). These data showed that what was being measured as amylose by iodine coloration in rice (and presumably other starch) was obviously not all so; a part of the color was contributed by the amylopectin (hence, from now on the parameter has been called apparent amylose content [AC] [118] or amylose equivalent [AE] [96]); there must have been something in the structure of amylopectin, presumably long branches, that made it bind iodine; and the so-called “insoluble amylase” must have been related to that “something.”

IRRI scientists now teamed up with the Hizukuri group (47,118,119) in Japan to study the chain length distribution of chemically separated amylopectin from eight rice varieties having low, medium, and high iodine affinity (IA). Upon debranching and fractionation by gel-permeation HPLC, amylopectins of high-IA indica varieties showed more very long chains, while low-IA japonica rice amylopectins had more short chains (Fig. 3). As the IA of the amylopectin increased, so the proportion of long chains increased and that of short chains decreased, and the intrinsic viscosity ($\eta$) increased and vice versa.

Later, Radhika Reddy et al. (96) took two to three representative varieties each from the eight rice quality types mentioned above. Amylopectins from these samples were isolated by preparatory GPC and their chain profile was studied after debranching. The results confirmed the tri-modal chain profile of amylopectin with varying proportions of long chains. More importantly, the proportion of long chains was highest in type I rice (highest insoluble AE) and least in type VIII (waxy) rice, the rest more or less in that order (Table III). Clearly the rice types, the insoluble AE, the long chains of amylopectin, and the cooked-rice texture were all correlated. What is more, when the amylopectins were first subjected to $\beta$-amyolysis (which removes the portions of the chains that are outside the branch point) and then debranched, the fall in the proportion of the long chains decreased in the same order of types I to VIII, showing that these long chains were generally located in the external part of the amylopectin molecule (Table III).

More evidence of “super-long chains” and their association with rice texture came from other laboratories (51,65,73,86, 129,130). Interestingly, differences in the amounts of long chains were found even among certain low-amylose japonica rices (65,129).

Ramesh et al. (98) in the CFTRI studied the nature of the low-MW Fr II of a Sepharose GPC mentioned above (Fig. 1). It was previously speculated from the nature of the data that this fraction was probably nothing but amylose. To their surprise, the authors found that even this fraction was overwhelmingly made up of branched molecules, i.e., amylopectin of low MW, and that these too had exactly the same chain profile as the main amylopectin in Fr I, and their long B-chains also correlated well with rice texture. Accordingly, the “true” amylose content in rice starch was actually very low, not more than 7–11%, which was later confirmed by Ramesh et al. (99) at Nottingham University by chromatographic separation. Interestingly, this...
value was even less than what Hizukuri et al. (47) had calculated.

Clearly, all these data not only threw light on rice texture but demanded a significant reconsideration of our understanding of starch structure itself. It seems amylopectin is not necessarily a very large molecule; and its chain profile varies widely among species and varieties. And amylose, if it exists at all, is only a minor component in starch. One is reminded of the words of Young (131): “Exclusion chromatography... produces fractions that raise the possibility that native starch may be, not a mixture of branched and linear molecules of D-glucopyranosyl units, but a mixture of covalently bound branched molecules having some extremely long chains, a broad degree of branching, and broad molecular weight distributions.”

Thus, after half a century of intense search, rice texture was found to be determined very largely by the chain profile of its amylopectin. The next question was: why was rice texture related to the chain profile of its amylopectin? Rheological studies carried out at the CFTRI provided some answer. Radhika Reddy et al. (97) studied the viscoelastic properties of rice-flour pastes in 15 varieties with a Bohlin rheometer. The relaxation (G) and storage (G′) moduli of the pastes increased with an increase in paste concentration in all varieties as expected, but the increase was more in high-AE than in low-AE rice (Fig. 4). Similarly the relaxation time (T0.75) of the paste was maximum in high- and low in low-AE paste. Clearly the starch granules in high-AE rice were more elastic than in low-AE rice. In agreement, when the pastes were heated at 95°C for another 60 min, the drop in G′ and T0.75 values at 60 min compared to 0 min was negligible in high-AE but high in low-AE rice (Fig. 4).

Sandhya Rani and Bhattacharya (105) pasted 26 varieties of rice of different quality types by heating them to 95°C and then continuing to heat the pastes at 95°C with stirring for an additional 60 min. The fall in paste viscosity at 60 min compared with 0 min of additional cooking was negligible in high-AE rice of type I but very high in low-AE rice of type VII, with the remaining types more or less in the same order (Table IV). Waxy rice (type VIII) broke down even before reaching 95°C. These data again showed the strength and resilience of the high-AE varieties and the weakness and fragility of the low-AE varieties. Microscopic examination of these pastes confirmed these conclusions, for the starch granules in high-AE rice remained largely intact after 1 h of heating but they got completely dispersed in low-AE rice (Fig. 5) (106).

It seemed from these data that the long chains of amylopectin by intra- and intermolecular interaction helped form a strong and resilient starch granule, while the deficiency of long chains rendered the granule weak and fragile. This difference in the strength of starch granules caused by the relative abundance of long chains in their starch molecules thus seemed to be at the root of the differences in rice texture.

### Table III. Long B-chains in rice amylopectin before and after β-amyolysis

<table>
<thead>
<tr>
<th>Rice Quality Type</th>
<th>Long B-Chains in Amylopectin (%)</th>
<th>β-Amyolysis % Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16.6</td>
<td>6.3</td>
</tr>
<tr>
<td>II</td>
<td>15.9</td>
<td>6.6</td>
</tr>
<tr>
<td>III</td>
<td>14.9</td>
<td>9.7</td>
</tr>
<tr>
<td>IV</td>
<td>10.0</td>
<td>5.5</td>
</tr>
<tr>
<td>V</td>
<td>11.3</td>
<td>7.9</td>
</tr>
<tr>
<td>VI</td>
<td>9.3</td>
<td>6.4</td>
</tr>
<tr>
<td>VII</td>
<td>7.3</td>
<td>5.5</td>
</tr>
<tr>
<td>VIII</td>
<td>2.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Data from Radhika Reddy et al. (96). (Used with permission.)

### Table IV. Paste breakdown upon cooking of rice-flour pastes

<table>
<thead>
<tr>
<th>Rice Quality Type</th>
<th>Number of Varieties</th>
<th>Paste Breakdown (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>11.7</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>17.9</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>17.7</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>34.1</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>36.4</td>
</tr>
<tr>
<td>VII</td>
<td>4</td>
<td>60.3</td>
</tr>
</tbody>
</table>

* Data from Sandhya Rani and Bhattacharya (105). (Used with permission.)

Spanish scientists from the IATA, however, gathered evidence to suggest that the protein content of rice played a significant influence on its eating quality. They showed that greater protein content in the outer layers of rice rendered it less sticky after cooking (92). In fact, they devised a colorimetric test of the protein in outer layers, called the “nitrogen index,” as a good index of its eating quality (93). In a later work, these authors observed a close relationship between the sulphydryl (-SH) concentration of the protein and the eating quality

### Fig. 4. Storage modules (G′) (left) and relaxation time (T0.75) (right) of three rice varieties pasted by heating up to 95°C and then further cooked at 95°C for 0 (top) and 60 (bottom) min. Rice variety: Jaya, quality type I; Br9, type IV; T65, type VII. (Reprinted with permission from Radhika Reddy et al. [97].)
and disulfide (-SS-) contents in the outer layers and the eating quality (94,95). The sulphydryl content was found correlated with palatability of Japanese rice as well (80,81).

More evidence has come in recent times. Yanase et al. (128) suggested that protein content of rice was inversely related to its viscographic breakdown and cooked-rice stickiness. Hamaker and Griffin (38,39) showed that the Brabender viscogram of rice flour was lowered when the slurry was treated with a reducing agent to break the -SS- bonds; the same result accrued upon treating the slurry with a protease before pasting (Fig. 6). Simultaneously, the stickiness of cooked rice also decreased.

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Kim et al. (66) have shown that protein content is negatively correlated with the protein content. Okadame et al. (83,84) have now provided evidence that while the overall hardness of starch, its surface hardness is related more to the protein content. Kim et al. (66) have shown that protein content is negatively correlated with Korean rice palatability.

**TESTING FOR RICE QUALITY**

From the time American researchers successfully came out with certain criteria (see above), various rice quality tests have been developed over the years. These tests have been routinely and traditionally employed for decades to evaluate the end-use quality of rice (6,7,50,52,125,126). The better or the more well-known among them include amylose (or AC or AE) content; alkali digestion score; Brabender or RVA pasting pattern; hot-water-insoluble amylose content; and mobility of a dilute alkaline gel (gel consistency).

A doubt may arise about their validity today in the changed context of our understanding of the basic factors behind rice behavior. One might feel that these above tests were appropriate in those days when the underlying reasons of rice quality were not known. But now that we know that the amylopectin branch profile is what primarily determines rice quality, would the same tests still be valid? The answer is, they would be. For one thing, there is no simple test yet to routinely determine the amylopectin branch profile of a large number of rice samples, nor is it really necessary. Empirically speaking, the above-mentioned tests are good enough to predict the quality of rice samples, no matter what they really measure.

The measured “amylose content”—strictly speaking apparent amylose (AC) or amylose-equivalent (AE)—does give an excellent indication of the expected behavior of rice. It is immaterial whether amylose starch actually exists or is involved in rice quality. What is material is, empirically, AE—i.e., not amylose but amylose-equivalent, whatever entity that may be—does correlate very well with the end-use quality of rice. Of course a problem does exist in this matter of amylose estimation in the sense that there has been a historical variability in the values of rice amylose content as reported by different laboratories at different times. This is because there are several sources of error: (a) variability in the quality of standard amylose procured from different sources; (b) interference by fat in the rice flour; (c) pH of the final solution; and (d) background contribution to color by the amyllopectin (more correctly, by the short and intermediate branches of the amylopectin) in the rice being analyzed. These problems and their solutions have been well documented, latest in the detailed discussion in the chapter by Bergman et al. (7). Unfortunately, historically there have been various levels of awareness of these problems, and of steps necessary to mitigate them, among laboratories engaged in breeding, selection, and quality evaluation of rice lines and varieties. The inevitable result has been a wide divergence in the reported amylose values among researchers and laboratories. Melissa Fitzgerald, IRRI, is currently engaged in a group action to address this problem, which will hopefully raise the level of awareness about the issue among all concerned.

![Fig. 5. Photomicrographs of starch granules in 12% rice-flour pastes of four varieties (types I, III, V, and VII). Flours were pasted by heating up to 95°C. (a) Light microscopy; and (b) scanning electron microscopy. For each, left column: just pasted (0 min); right column: maintained at 95°C for another 60 min. After 60 min, the granules are hardly affected in type I but are completely degraded in type VII. (Reprinted with permission from Sandhya Rani and Bhattacharya [106].)](image)

![Fig. 6. Amylograms of rice flour at 10% solids in water (a) with and without dithiothreitol added; and (b) incubated 2 h before analysis in water or a solution containing chymotrypsin, pronase, or bovine serum albumin (BSA). (Reprinted with permission from Hamaker [37].)](image)
Likewise, the hot-water-insoluble AE correlates with rice quality very well in the case of high-AE rice, for which it is an indispensable test. This parameter may not be so important in the case of intermediate- and low-AE rice. Brabender or RVA pasting pattern, especially the “relative breakdown” at a fixed-peak viscosity, is another test that gives an excellent indication of the behavior pattern of the test sample. Its only shortcoming is that the value of the parameter gradually decreases somewhat with increasing age of the rice, so a correction has to be applied from the results of a control sample of equivalent age (111).

The alkali digestion score is generally not relevant for predicting the end-use quality of a sample. However, it is otherwise very important, particularly for the industry. For example, if a rice sample is to be cooked or soaked for a process or a product, the temperature and time to be used will strongly depend on the GT of the sample; the alkali score is the simplest way to index the parameter. Finally, gel mobility (or gel consistency) is also useful. A survey of the literature, along with the author’s experience, suggests that these test results are not often as reliable an indicator of the quality as one would wish. However, it usually does provide good supplementary information.

Mention may be made that the above, along with a few other associated criteria, have been recently used in this laboratory to successfully characterize as well as to distinguish various cultivars’ derivatives/lines of the basmati group of rice in India (64).

CONCLUSION

The uniqueness of rice lies in many respects. It is not only one of the three largest grown food grains in the world, it is in fact the most important staple food of humankind. It is the most extensively grown cereal, being cultivated under the most diversified conditions and has the largest varietal diversity with various cooking, eating, and processing qualities. Study of these diversities has been one of the most challenging areas of study in cereal chemistry and has occupied a period approaching a century. This research has passed through three distinct phases, each with its own paradigm. The first was the water uptake paradigm which, though entirely misplaced, at least provided the intellectual drive for the issue. Next was the amylose paradigm, bolstered subsequently by the addition of the insoluble amylose paradigm. Interestingly, this paradigm proved to be strategically inadequate, for it has now been shown to be theoretically incorrect, but tactically successful, for it provided tools that could successfully assess and predict the end-use quality of rice. Finally came the amyllopectin paradigm, which now provides an overarching theoretical insight into the issue.

But the question remains, is it the end of the story? Unlikely. For one thing, the history of science shows that each clarification not only closes one chapter but helps to open another by raising new questions. Secondly, it is inconceivable that such wide diversity in cooking, sensory, and processing qualities of rice can be explained by this one single parameter.

The amyllopectin theory itself may need further refinement. More insight into the effect of precise starch structures and their precise manner of actions is clearly called for. The possible involvement of protein has already been mentioned. Much more work in this area seems necessary. More intensive work at the molecular level is called for. It is also necessary to study the matter in the style of what Juliano et al. (59) did in the early 1960s: using cultivars that are genetically identical other than in the parameter under study as the experimental material.

There should be other factors as well. For example, Japanese workers have shown that free sugars and free amino acids, particularly aspartic and glutamic acids and gamma-aminobutyric acid, contributed to the palatability of Japanese cooked rice (78,104,115). Then there is the question of the GT. No rice scientist feels comfortable with the assertion that GT is totally irrelevant to rice texture. Yet no study has yet unambiguously shown that GT is definitely and uniformly involved with the texture or with the sensory quality of cooked nonwaxy rice. That is excluding waxy rice, where it does seem to play a role (67,79,91). No doubt Juliano and his colleagues have repeatedly shown that GT often correlated with the texture of nonwaxy rice, too. However, the evidence is not consistent, nor is there any consistent theory of it yet. Whether these associations are fortuitous or causatively related, one has to wait and see. Further, precisely directed studies are clearly called for.

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