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The fine structure of rice-starch amylopectin and its relation to the texture of cooked rice

K. Radhika Reddy, S. Zakiuddin Ali* & K.R. Bhattacharya

Department of Grain Science & Technology, Central Food Technological Research Institute, Mysore-570 013, India

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Starches derived from 20 rice varieties containing from very low to very high total and hot-water-insoluble amylose-equivalent (AE) were fractionated by gel-permeation chromatography (GPC). Fraction I (amylopectin) and fraction II (amylose) correlated well with the insoluble-AE and soluble-AE, respectively, of the parent rice. Thus soluble-AE broadly represented the true rice amylose and insoluble-AE the iodine affinity of amylopectin. Amylopectins of eight representative varieties were therefore debranched and fractionated by GPC to study their chain profiles. Amylopectins from the highest-AE variety had the largest proportion of long B chains and the lowest proportion of short chains, while the reverse was true for waxy rice. Other varieties broadly followed this correlation between B-chain length and AE. In addition, when the eight amylopectins were first hydrolysed with β-amylase to remove the external chains and the β-limit dextrins were then debranched and fractionated, the greatest drop in the amount of long B chains occurred in the highest-insoluble-AE variety and the smallest drop (nil), in waxy rice. In other words, highest-insoluble-AE (i.e. high-iodine-affinity) amylopectin had not only the highest amount of long B chains, but the largest proportion of these chains was in the exterior region (carrying non-reducing ends), and vice versa. Difference in cooked rice texture seemed to be related to this difference in the fine structure of its amylopectin.

INTRODUCTION

Until the mid-eighties, scientists recognized that the content of amylose-equivalent (AE)* was the most important determinant of the eating quality of rice (Juliano, 1979, 1985). However, it was also recognized that AE was a necessary but not a sufficient factor, since varieties with similar or identical AE also often showed differences in quality due to certain unknown secondary factors, the understanding of which was the major quest of rice chemists during the previous two to three decades.

A variety of secondary factors were suggested, including gelatinization temperature, protein content, pasting pattern, gel consistency, and alkali degradation type. But while the roles of the former two were uncertain, to say the least, the latter three could at best be described as indices of rice texture, not its causes.

Bhattacharya *et al.* (1972, 1978, 1982) showed from this laboratory that the hot water (96°C) solubility of AE differed among varieties. The high-AE rice varieties in particular fell into three distinct groups with solubilities of their AE being around 40%, 50% and 60%, respectively, and these three groups of rice differed distinctly in all their physicochemical and textural attributes. These authors thus derived a new parameter called 'hot-water-insoluble AE' (total minus soluble AE) and proposed it as a key determinant of rice quality, for it correlated excellently with texture, and other physicochemical parameters of rice (Bhattacharya *et al.*, 1972, 1978, 1982; Bhattacharya & Sowbhagya, 1979, 1980; Sowbhagya *et al.*, 1987; Sandhya Rani & Bhattacharya, 1985, 1989). The insoluble AE was surely some entity and in that sense was not just an index but a determinant of rice quality. Unfortunately its identity was not known.

This was the background when Chinnaswamy (1985)

*To whom correspondence should be addressed.

*Recent research has established (see later) that the amount of 'amylose' in starch as estimated by the iodine reaction is always an overestimate, as the value includes a variable but substantial contribution from amylopectin. Takeda *et al.* (1987, 1989) therefore called the parameter 'apparent amylose'. However this term has been applied by many workers to the result of various approximate methods of estimating amylose. Hence the designation 'amylose-equivalent' seems more appropriate.

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from this laboratory showed, using gel-permeation chromatography (GPC) of rice starch, that the GPC amylopectin and amylose fractions of starch correlated very well with the reputed insoluble and soluble AE contents, respectively, of the parent rice. Subsequently, Takeda *et al.* (1987, 1989) and Hizukuri *et al.* (1989) showed that the iodine affinity of amylopectin varied from very low for low-AE rice to very high for certain high-AE rice, which in turn was related to the variable contents of long and short chains among different amylopectins. Taken together, these data suggested that variation in the fine structure of amylopectin was apparently at the root of variation in rice texture.

The iodine reaction of GPC-separated amylopectin, its chain profile and the chain profile of its β-limit dextrin in a number of graded-AE rice varieties are reported here.

MATERIALS AND METHODS

Rice

Bhattacharya *et al.* (1982) had classified rice varieties into eight quality types based primarily on their total and insoluble AE and viscogram pattern. The experimental samples (20 varieties, 2–3 in each quality type) were selected on this basis for convenience and identification. The experimental varieties are listed in Table 1; their AE contents can be judged from columns 3–5 of the table.

The rice was grown at the University of Agricultural Sciences Experimental Station at Nagenahalli (Karnataka, India) from laboratory stock seeds. Paddy was collected soon after harvest, dried in ambient conditions to 12–13% moisture (wet basis, w.b.), cleaned, fumigated, and stored in closed metal tins in the laboratory at room temperature (23–28°C) for three months for aging and afterwards in the cold room (4–6°C) until use.

Paddy was shelled and milled using McGill equipment under standard conditions and broken rice was discarded. Milled rice was ground successively in a Buhler laboratory grain mill (type MLI 204) and a Fritsch Pulverisette-14 to pass a 100-mesh screen. The flours were defatted using a Soxhlet apparatus for 18–20 h with 85% methanol, exposed in an air-conditioned room (27°C, 65% RH) to equilibrate moisture (about 12%, w.b.), and stored in the cold.

Table 1. GPC fractionation of rice starch

Variety		AE injected (mg)[b]			AE recovered (mg) in fraction			Fr I-iodine complex λ_{max} (nm)
No[a]	Name	Insoluble	Soluble	Total	I	II	Total	
11	Jaya	1·9	1·2	3·1	1·6	1·3	2·9	580
12	T (N) 1	2·0	1·2	3·2	1·6	1·3	2·9	575
13	IR8	1·8	1·3	3·1	1·6	1·3	2·9	575
21	Co32	1·6	1·5	3·1	1·3	1·5	2·8	575
22	S701	1·5	1·6	3·1	1·6	1·5	3·1	570
23	GEB24	1·6	1·5	3·1	1·6	1·3	2·9	575
31	Jhona20	1·5	1·8	3·3	1·4	1·8	3·2	570
32	Madhu	1·4	1·8	3·2	1·3	1·8	3·1	570
33	S317	1·4	1·8	3·2	1·3	1·8	3·1	575
41	Basmati370	1·2	1·5	2·7	1·2	1·5	2·7	565
42	Br9	1·2	1·6	2·8	1·2	1·4	2·6	565
51	Intan	1·2	1·7	2·9	1·3	1·5	2·8	565
61	Rojolele	1·1	1·7	2·8	1·2	1·5	2·7	565
62	Sukanandi	1·2	1·7	2·9	1·0	1·8	2·8	570
63	Benong130	1·2	1·7	2·9	1·3	1·5	2·8	565
71	K84	1·1	1·1	2·2	1·1	1·0	2·1	550
72	Changlei	1·2	1·3	2·5	1·1	1·1	2·2	560
73	T65	1·0	1·2	2·2	1·1	1·0	2·1	555
81	Purple puttu	0·5	0·1	0·6	0·6	0·1	0·7	525
82	Asm44	0·4	0·2	0·6	0·6	0·1	0·7	525

[a]The first digit of the variety number indicates the rice quality type. The second digit stands for the serial no. within each type. Thus No. 11 indicates that the variety belongs to quality type I, No. 21 to type II and so on.

[b]Amount of carbohydrate injected for each run was 10·0 mg. Amount of AE injected was calculated as % (d.b.) AE in rice flour × 0·112. The factor 0·112 was to account for assumed mean protein content of 8% (d.b.) and other constituents (3%) in milled rice.

Methods

Amylose estimation

Total AE (Sowbhagya & Bhattacharya, 1979) and hot-water-soluble and insoluble AE (Shanthy *et al.*, 1980) were determined by the methods described, except that liquid–liquid defatting was omitted, as the flours had already been defatted, and neutralization was carried out using acetic acid (Juliano, 1971). Potato amylose (ICN Biochemicals, USA) was used as a standard.

GPC fractionation of starch

Rice flour was directly used for GPC fractionation. To 100 mg defatted rice flour, taken in a 50-ml volumetric flask and wetted with 1 ml alcohol, was added 10 ml 1 N NaOH, the air was displaced with nitrogen and the flask was left overnight. The flask was heated under nitrogen in a boiling water bath for 15 min with occasional mixing. After cooling, the solution was neutralized with dilute HCl using phenolphthalein as an internal indicator, made up to volume and filtered through a G3 sintered glass filter. The carbohydrate recovery after chromatography was 83 (±2·5)%, which was considered fair.

For gel-permeation chromatography (GPC), an aliquot of the dispersion containing exactly 10 mg carbohydrate (dry basis, d.b.) was fractionated by ascending GPC on a Sepharose CL-2B (Pharmacia Fine Chemicals, Sweden) column (1·6 × 60 cm), operating at a flow rate of 24 ml h^{-1} using water containing 0·02% sodium azide as an eluent, 3-ml sub-fractions being collected (Chinnaswamy & Bhattacharya, 1986). The GPC was run in air-conditioned room (24°C ± 1°).

Carbohydrate (glucose × 0·9) was estimated in 0·5 ml of each sub-fraction by the phenol-sulphuric acid method (Dubois *et al.*, 1956). To the remaining 2·5 ml was added 0·2 ml of 0·2% iodine solution (2 g iodine and 20 g KI $litre^{-1}$) and the blue colour was read in the spectrophotometer at 630 nm. A standard amylose solution was simultaneously read to calculate the AE content of each sub-fraction. The iodine–polysaccharide complex was also scanned for its absorption maximum (λ_{max}).

Chain profile of amylopectin

About 8 ml of a rice dispersion containing 35–40 mg (d.b.) carbohydrate was fractionated as above. A portion of the gel-excluded fraction I (amylopectin) containing about 4 mg carbohydrate was debranched with 15 U pullulanase (Hayashibara Biochemicals Lab Inc., Japan) dispersed in 2 ml of 0·2 M, pH 5·5 acetate buffer, incubating for 24 h at 40 C (Billiaderis *et al.*, 1981). The enzyme action was stopped (boiling water bath, 10 min) and the solution was centrifuged (10,000 rpm (about 5500*g*), 20 min).

About 4·5 ml of the solution containing a little over 3·5 mg carbohydrate was fractionated by ascending GPC on a 1·6 × 53 cm Biogel P-10 (Bio-Rad Laboratories, USA) column, using azide water as an eluent, operating at a flow rate of 15 ml h^{-1}, 3-ml sub-fractions being collected. Carbohydrate was determined in 1 ml of each sub-fraction by the phenol–sulphuric acid method, recovery being 96 (±6)%. Iodine-complex absorbance and λ_{max} were determined as before in the remaining 2 ml.

The column was calibrated for the number average degree of polymerization ($\overline{dp_n}$) using potato amylopectin (Sigma Chemical Co., USA), debranched and fractionated as above (Mercier & Whelan, 1970). Each 3-ml sub-fraction was analysed for total carbohydrate (Dubois *et al.*, 1956) and reducing capacity (Nelson, 1944), the ratio of which gave the $\overline{dp_n}$. The $\overline{dp_n}$ could also be calculated from the iodine-complex λ_{max} of each sub-fraction (Banks *et al.*, 1971). This remained virtually constant in the corresponding sub-fractions for all the rice samples (including, surprisingly, even for the gel-excluded sub-fractions). These iodine reaction-based values agreed well with the values calculated from reducing capacity of the chains as above, except for the gel-excluded sub-fractions where the iodine-absorbance-based values were too high and hence unreliable.

Chain profile of β-limit dextrin

To 9 ml of GPC amylopectin fraction containing about 10 mg amylopectin was added 1040 U of β-amylase (Sigma) and the mixture was incubated at 40°C for 3 h (Atwell *et al.*, 1980). Enzyme action was stopped (boiling water bath, 15 min), the solution was centrifuged (10,000 rpm, 20 min) to remove the precipitated enzyme, and then dialysed overnight against distilled water.

The β-amylolysis limit was calculated from the carbohydrate contents of the respective amylopectins and their limit dextrins.

The limit dextrin prepared as above (about 4 mg carbohydrate) was debranched with pullulanase and the mixture (about 2 mg carbohydrate) was fractionated over Biogel P-10 as before.

All the experiments were carried out in duplicates and mean results are reported.

RESULTS

GPC fractions of rice starch

Rice starch separated into two fractions (Fr) over Sepharose CL-2B: a larger gel-excluded Fr I, now well recognized as amylopectin, and a smaller gel-included Fr II, recognized as amylose. The elution pattern of four representative varieties is shown in Fig. 1 and the characteristics of the fractions of all the 20 varieties are given in Table 1.

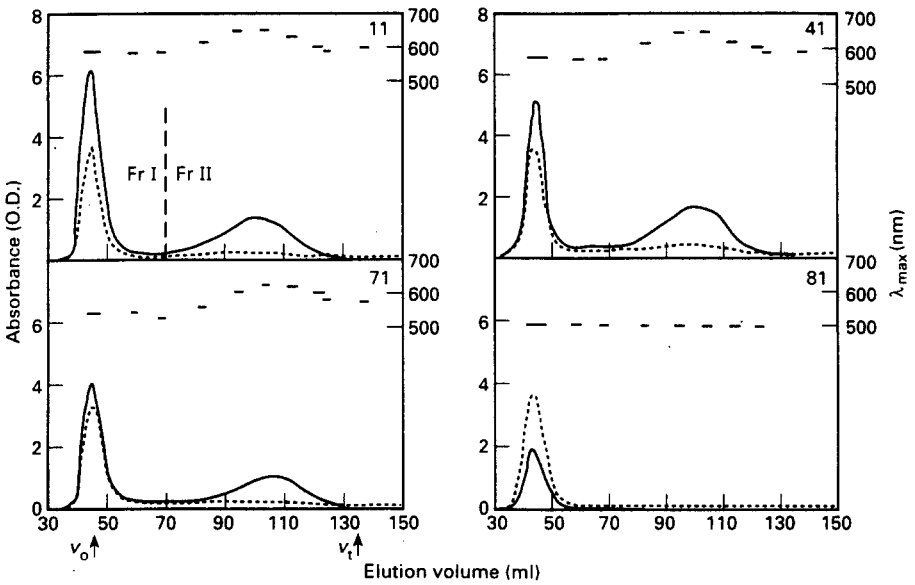

Fig. 1. Elution pattern of rice starch on Sepharose CL-2B column and characteristics of its fractions. In this and subsequent figures the rice varieties are identified by their numbers (top right corner of the graphs) as shown in Table 1. (·····) Carbohydrate; (——) iodine complex (630 nm).

The insoluble AE of the parent rice correlated very well with the iodine-complex λ_{max} of Fr I (Fig. 2, $r = 0{\cdot}895^{***}$) as also with the AE content of Fr I ($r = 0{\cdot}932^{***}$). The soluble AE of the parent rice, on the other hand, correlated well with the carbohydrate ($r = 0{\cdot}757^{***}$) as well as the AE content ($r = 0{\cdot}963^{***}$) of Fr II. The Fr II–iodine complex λ_{max}, moreover, was around 635 nm in all samples. These results confirmed those obtained by Chinnaswamy & Bhattacharya (1986) with a smaller number of samples. These results, and a comparison of the AE values loaded into and recovered from the column (Table 1), leave no doubt that soluble AE of the parent rice broadly represented the true amylose of rice starch and the insoluble AE the blue iodine-colour contribution of the amylopectin.

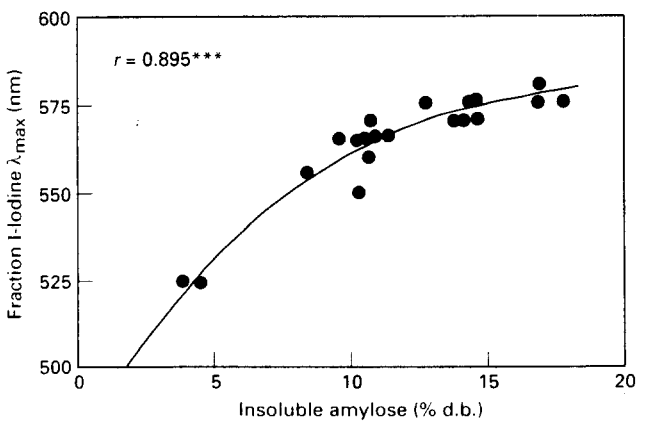

Fig. 2. Relationship between 'insoluble amylose' content as estimated in rice flour and the λ_{max} of the iodine complex of fraction I of rice starch separated on Sepharose CL-2B column.

Chain profile of amylopectin

The debranched amylopectins gave three GPC fractions (fr) each (fr. 1, 2 and 3) (Fig. 3). The chain length, iodine colour and identification of the three fractions

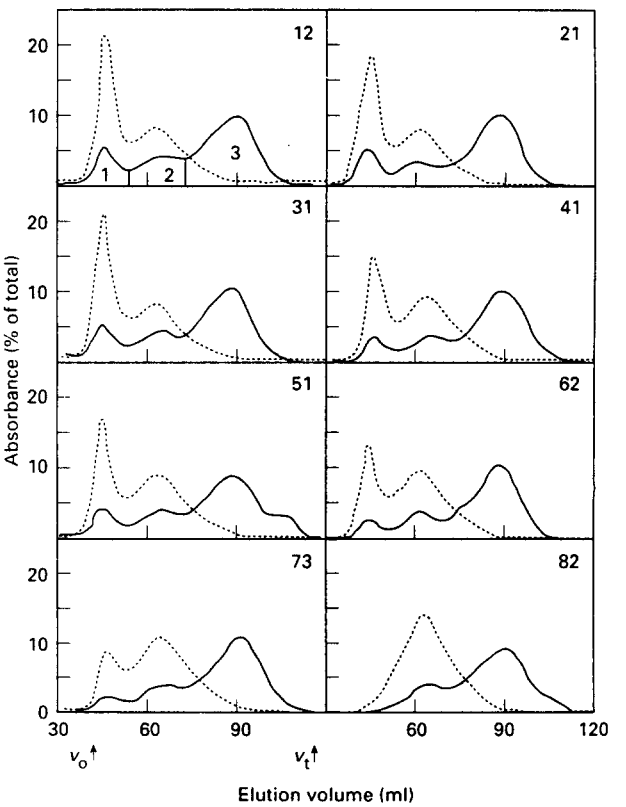

Fig. 3. Elution pattern of debranched amylopectins of rice varieties representing eight quality types on Biogel P-10 column. (——) Carbohydrate, (·····) iodine complex (630 nm).

Table 2. Characteristics of anhydroglucose chains from debranched rice amylopectin

Fraction	$\overline{dp}_n$[a]	λ_{max}[b] (nm)	Chain identification[c]
1	55–75	610–640	Long B
2	40–55	550–610	Intermediate B
3	10–25	420–530	A and short B
4[d]	2–3	nil	Maltose and maltotriose

[a]Mean number-average degree of polymerization.
[b]Of polysaccharide–iodine complex.
[c]As per literature (Hizukuri, 1985; Hizukuri *et al.*, 1989; Manners, 1989).
[d]In debranched β-limit dextrin only.

Table 3. Distribution of fractions (fr) in debranched rice-starch amylopectin

Rice[a]	Carbohydrate content (% of total)			Iodine-complex absorbance at 630 nm (% of total)		
	fr 1	fr 2	fr 3	fr 1	fr 2	fr 3
12	16·6	19·4	63·8	52	38	10
21	15·9	20·7	63·4	48	43	8
31	14·9	20·8	64·3	47	43	10
41	10·0	19·7	70·3	35	50	14
51	11·3	20·2	68·5	39	49	13
62	9·3	21·4	69·3	34	54	12
73	7·3	18·8	73·9	23	59	18
82	2·9	23·1	74·1	8	74	18

[a]See footnote *a* to Table 1.

are shown in Table 2. These are broadly similar to the values reported by Hizukuri *et al.* (1989), except that the latter authors, using a more sophisticated fractionating and detecting system, were able to further separate fraction 1 into two sub-fractions (1a and 1b), 1a showing chain lengths of up to 180 glucose residues.

The relative amounts of the three fractions and their iodine-complex absorbance values clearly differed, rather systematically, among the varieties (Fig. 3, Table 3). Type I rice (high-insoluble AE) had the largest proportion of long-B chains and the lowest proportion of short chains. Conversely, type VIII rice (waxy) had the largest proportion of short, and probably intermediate, chains and the least proportion of long chains. The other varieties broadly followed this trend. Carbohydrate in fr 1 and 3 showed a positive and a negative correlation, respectively, with the insoluble AE of the parent rice (Fig. 4(a), (b)); so did their iodine-complex absorbance values (Fig. 4(c), (d)). The carbohydrate of fr 2 was more or less the same in all the samples (Table 3), but its iodine-complex absorbance showed a negative correlation with insoluble AE ($r = -0{\cdot}961^{***}$).

Chain profile of limit dextrin

The β-amylolysis limit of the seven nonwaxy amylopectins was around 52% and that of the waxy variety 46% (Table 4).

The elution pattern of the eight debranched limit dextrins (Fig. 5) showed a rather ill-defined distribution except at the void and the total volumes. The elution profiles were divided into four fractions as per their iodine-complex λ_{max} corresponding to those of the debranched parent amylopectin (Table 2). The amounts of the four fractions are shown in Table 4. However, a direct comparison of the chains of amylopectin and its β-limit dextrin would be valid only in the case of fr 1, which alone originated from the same group of chains in either case, the origin of the other chain fractions of the limit dextrin being indeterminate.

A comparison of the amount of fr 1 in the debranched amylopectin (Table 3) and its limit dextrin (Table 4) brought out the surprising fact that its per cent fall upon β-amylolysis was directly proportional to its content (Fig. 6). Clearly, the greater the proportion of long-B chains in an amylopectin, the greater was its loss after β-amylolysis, in other words, the greater was the proportion of the number of these chains or the part of each chain that was in the exterior region (carrying the non-reducing end), i.e. outside the last branch point.

One interesting point was that the chain profile of the β-limit dextrins *per se*, i.e., after the external chains had been hydrolysed away, was more or less constant in all the varieties (Table 4). This would mean that the internal structure of the amylopectins, i.e., inside the outermost branch points, was essentially similar in all varieties. The meaning of this fact is not clear at this time.

Another somewhat intriguing finding is that the iodine-complex λ_{max} of the amylopectins, their β-limit dextrins, and the respective debranched mixtures varied somewhat randomly among the samples (Table 5). It is not easy to interpret these results at this time. Hizukuri *et al.* (1989) too had observed that the iodine λ_{max} of rice amylopectin decreased in some and remained more or less unchanged in others after β-amylolysis, which led them to conclude that the long-B chains could be either external or internal.

DISCUSSION

Meaning of amylose

Not long after Schoch (1945) had apparently settled the controversy on composition of starch by separating it into a smaller, linear molecule, amylose, and a larger, branched molecule, amylopectin, evidence started to appear of a low degree of branching in amylose on the one hand and of the presence of several unknown intermediate components — 'anomalous' amylose and amylopectin — on the other (Banks & Greenwood, 1975). The introduction of GPC to starch research (Ebermann & Schwarz, 1975; Yamada & Taki, 1976; Biliaderis *et al.*, 1979, 1981; Boyer *et al.*, 1980; Yeh *et al.*, 1981; Craig & Stark, 1984) constituted the first

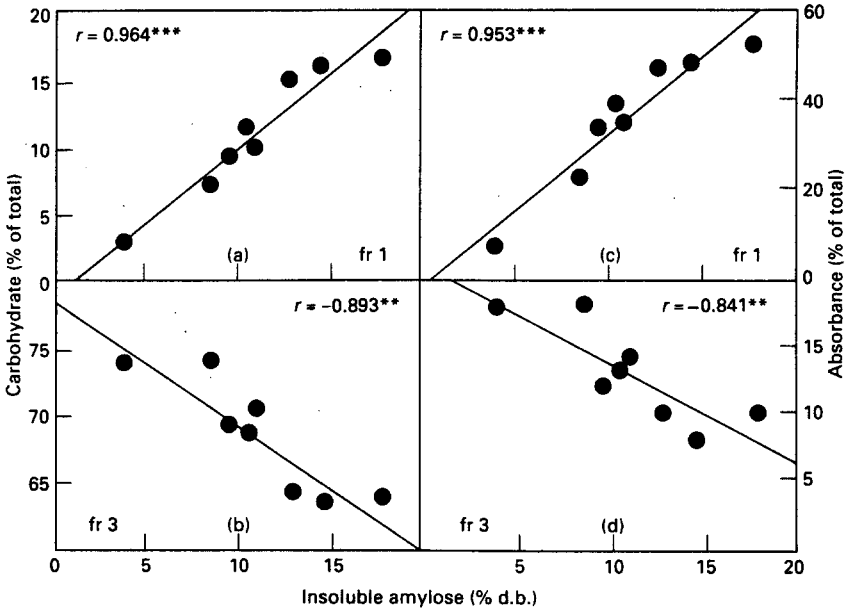

Fig. 4. Relationship between 'insoluble amylose' content as estimated in rice flour with carbohydrate contents ((a) & (b)) and iodine-complex absorbance (at 630 nm) ((c) & (d)) of fractions 1 & 3 of debranched amyolopectins of rice.

Table 4. Carbohydrate and iodine absorbance of fractions (fr) in debranched β-limit dextrin

Rice[a]	β-amylolysis limit (%)	Carbohydrate content (% of original amylopectin)					Iodine absorbance (% of total debranched amylopectin)				
		fr 1	fr 2	fr 3	fr 4	% Fall in fr 1[b]	fr 1	fr 2	fr 3	fr 4	% Fall in fr 1[b]
12	53	6·3	5·6	12·6	22·5	62	20	15	11	2	62
21	54	6·6	7·9	12·6	18·9	58	18	18	11	0	63
31	52	9·7	7·1	12·8	18·4	35	23	14	11	0	51
41	50	5·5	6·3	13·5	24·7	45	19	18	11	2	46
51	50	7·9	7·0	14·1	21·0	30	23	15	11	1	41
62	52	6·4	7 0	14·9	19·8	31	17	15	13	3	50
73	54	5·5	6·7	13·7	20·1	25	17	15	12	2	26
82	46	3·1	7·1	18·3	25·5	(−)7	6	22	23	3	25

[a]See footnote *a* to Table 1.
[b]Compared to the value in original amylopectin (Table 3).

Table 5. The iodine-complex absorption maxima of rice amylopectin, its β-limit dextrin and their debranched mixtures

Rice[a]	λ_{max}			
	Amylopectin		β-limit dextrin	
	Original	Debranched mixture	Original	Debranched mixture
12	580	588	563	586
21	582	586	579	591
31	581	594	583	602
41	565	586	577	582
51	568	580	565	589
62	566	574	547	586
73	552	569	560	586
82	521	556	523	545

[a]See footnote *a* to Table 1.

step towards clarifying this confusion, for many of these researchers noted with surprise that the GPC amylopectin fraction, and then that even chemically separated amylopectin, stained a varying shade of blue colour with iodine, and that often the blue colour seemed to intensify with the increasing reputed amylose content of the parent starch. Even earlier literature reports, e.g. that of Reyes *et al.* (1965) for rice starch, were then found to contain similar observations. While these workers did not further investigate this aspect, Yeh *et al.* (1981) made the significant observation that a part of the iodine reaction of composite starch was probably contributed by amylopectin.

Chinnaswamy & Bhattacharya (1986) not only confirmed the varying blue colour of GPC separated amylopectin–iodine complex, but showed, now amply

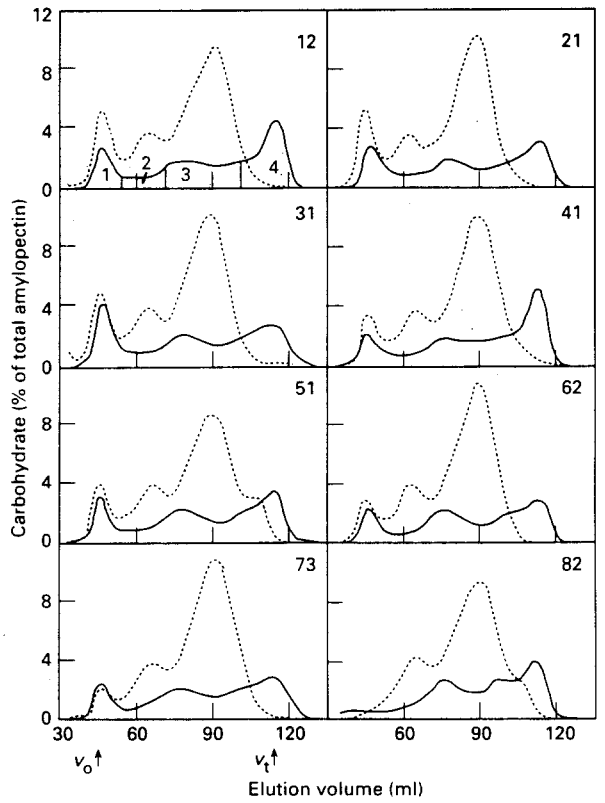

Fig. 5. Elution pattern of debranched β-limit dextrins (——) of rice amylopectins representing eight quality types in comparison to their original debranched amylopectins (·····) on Biogel P-10 column.

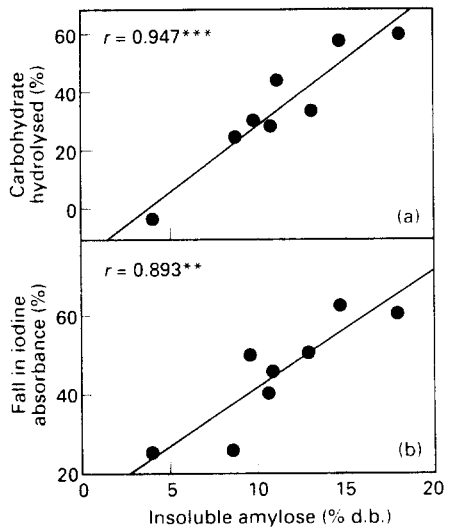

Fig. 6. Relationship between 'insoluble amylose' content as estimated in rice flour with fall (a) in amount of carbohydrate and (b) in iodine absorbance (at 630 nm) of fraction 1 of debranched amylopectin due to β-amylolysis.

confirmed in the present work, that the analytically estimated category of 'hot-water-insoluble amylose' of the parent rice represented mostly this iodine-reaction response of the amylopectin; the 'hot-water-soluble amylose' represented more or less the true amylose of the parent starch. The classical methods of estimating amylose thus always gave an overestimate; hence the term 'apparent amylose' adopted by Takeda *et al.* (1987, 1989) and, alternatively, 'amylose-equivalent (AE)' here. Until some method of directly estimating specifically amylose in starch becomes available (indirect estimation is of course possible; see Juliano *et al.*, 1987), it is inappropriate to quote an amylose content, rather the term 'amylose equivalent' should be used.

Fine structure of rice amylopectin

Why do amylopectins bind varying amounts of iodine? The answer is provided by various recent developments, taken along with the present work. Japanese starch chemists have pioneered the concept that amylopectins vary greatly in their chain profile with differing combinations of A and short-B and long-B chains. This has been demonstrated for various starches (Hizukuri, 1986), rice (Asaoka *et al.*, 1984, 1985, 1986, 1987, 1989; Takeda *et al.*, 1987, 1989; Hizukuri *et al.*, 1989), maize (Takeda *et al.*, 1988) and wheat (Hizukuri & Maehara, 1990). Takeda *et al.* (1987, 1989) demonstrated that rice amylopectins with high iodine affinity (i.a.) — which came from high-AE rice — had more long-B and less short chains than low-i.a. amylopectins — which came from low-AE rice. Indeed the amylopectin i.a. was directly proportional to long-B chains and inversely to short chains.

The present findings not only confirm the above results but throw more light. The data show that the i.a. of amylopectin — in this work measured as insoluble AE — was related not only to the long-B chains (and inversely to the short chains) of amylopectin, but also to the external–internal disposition of these chains. The greater the long-B chain content of an amylopectin, the greater was the proportion of these chains (or the greater the part of each of these chains, or both) that lay in the exterior region of the molecule, and vice versa. In other words, rice varieties with more insoluble AE not only had more long-B chains (and less short chains) but more of these long chains had uninterrupted external unbranched portions, and vice versa. This is how these amylopectin molecules bound substantial amounts of iodine.

Relation of amylopectin structure to rice texture

Rice texture was clearly related to the above chain profile. We have repeatedly shown from this laboratory for the last two decades that the texture of cooked rice — indeed most physicochemical properties of rice — correlated excellently with its insoluble AE (Bhattacharya *et al.*, 1972, 1978, 1982; Bhattacharya & Sowbhagya, 1979, 1980; Sowbhagya *et al.*, 1987). Now that the insoluble AE of rice is shown to be nothing but a

reflection of the fine structure of its amylopectin, clearly it is the latter that primarily determines the eating quality of rice. Soluble AE showed no relation to rice texture in the above studies, which means the true amylose content of rice plays little part in its quality.

Thus in a dramatic twist of history, our understanding of the etiology of rice quality has taken an 180° about-turn and shifted from amylose to amylopectin.

Interestingly, despite the above dramatic reversal of role, the importance of insoluble AE as a simple and sensitive index as well as a determinant of rice quality remains undiminished, although its original name, 'insoluble amylose', was but a misnomer, for it was not amylose at all. Since the fine structure of amylopectin is the key determinant of rice quality, and since insoluble AE is only a reflection of this fine structure, the latter is a sensitive index of rice quality. Indeed there is no other simple index at this time which reflects the amylopectin fine structure as well.

How amylopectin structure affects rice quality

The question now would be: why and how does the fine structure of amylopectin affect rice texture? The rheological and microscopic studies of Sandhya Rani & Bhattacharya (1985, 1989, 1993*a*,*b*) and additional studies on the viscoelastic properties of rice pastes just carried out by the present authors (in preparation) provide an answer. These authors have shown that high-AE rice (which would now primarily mean high-insoluble-AE rice) had rigid, elastic and strong starch granules which resisted swelling as well as disintegration when heated in water under shear. Low-AE (i.e., low-insoluble-AE) rice, on the other hand, had weak, deformable and fragile starch granules that swelled as well as tended to break down rather easily under the same conditions.

Rice insoluble AE, i.e., an index of the proportion and relative external disposition of the long-B chains of rice amylopectin, was thus related, positively, to both the strength of the starch granules and the hardness of cooked rice. All the three were thus interrelated and in this seemed to lie the key to rice quality.

Several authors (Hizukuri *et al.*, 1983; French, 1984; Hizukuri, 1985; Eliasson *et al.*, 1987; Zobel, 1988; Manners, 1989) have suggested that it is primarily the amylopectin component which contributes to the crystallinity of the starch granule. If so, the greater content and more external disposition of the long-B chains of high-insoluble-AE-rice amylopectin would be expected to make its starch granules strong and rigid through easy intermolecular interactions. The paucity of such long and external chains in low-insoluble-AE-rice amylopectin, on the other hand, would be expected to render its starch granules weak and fragile due to a low degree of intermolecular interlocking. Rigid and strong starch granules in turn would be expected to render the cooked rice hard, nonsticky and dry (for the starch would not come out of the intact granules); conversely, fragile granules would tend to disintegrate on cooking and thus render the cooked rice soft, sticky and moist.

CONCLUSIONS

The use of the iodine reaction to determine the amylose content of rice or other starches gives ambiguous results. The quantity determined consists of not only the true amylose (broadly equivalent to 'hot-water-soluble amylose') but also the iodine-complex response of amylopectin (represented by the 'hot-water-insoluble amylose equivalent [AE]'). In as much as it is the insoluble and not soluble AE that correlates with rice quality, amylopectin and not amylose is the main determinant of rice quality.

The iodine affinity of amylopectin, and varietal differences in it, follows from its fine structure. Amylopectin of high insoluble-AE rice contains a large number of long, unbranched chains in the exterior of the molecule; low-AE-rice amylopectin, on the other hand, has few long chains and even these are mostly in the interior of the molecule.

The same amylopectin fine structure seems to explain the varietal differences in rice texture. The presence of a large number of long, unbranched chains at the exterior region of the amylopectin molecule apparently leads, by their mutual entanglement and interaction, to the formation of strong and elastic starch granules and hence to firm, dry, nonsticky cooked rice. Absence of such chains, on the other hand, apparently renders the granules weak and fragile and hence makes the cooked rice soft, moist and sticky.

The insoluble AE, being a reflection of the fine structure of amylopectin, is a simple but excellent index of rice quality.

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