# Addressing the Dilemmas of Measuring Amylose in Rice

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**ABSTRACT**

Amylose content is a parameter that correlates with the cooking behavior of rice. It is measured at the earliest possible stages of rice improvement programs to enable breeders to build the foundations of appropriate grain quality during cultivar development. Amylose is usually quantified by absorbance of the amylose-iodine complex. The International Network for Quality Rice (INQR) conducted a survey to determine ways that amylose is measured, reproducibility between laboratories, and sources of variation. Each laboratory measured the amylose content of a set of 17 cultivars of rice. The study shows that five different versions of the iodine binding method are in use. The data show that repeatability was high within laboratories but reproducibility between laboratories was low. The major sources of variability were the way the standard curve was constructed and the iodine binding capacity of the potato amylose used to produce the standard. Reproducibility is much lower between laboratories using a standard curve of potato amylose alone compared with those using calibrated rice cultivars. This study highlights the need to standardize the way amylose is measured, and presents research avenues for doing so.

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Rice (*Oryza sativa* L.) is grown on every continent except Antarctica and provides ≈20% of the world's kilocalorie supply and 15% of human's protein consumption. In some regions of Asia, up to 71% of the daily energy and 70% of the protein intake comes from rice. Most rice is consumed as intact kernels that have been milled or polished, but consumers also choose whole grain (brown) rice and rice-based products such as those in Fig. 1. In almost all markets, the physical and sensory properties of the rice cultivar determine consumer acceptability and end-use.

Sensory properties of freshly cooked rice have been described by 14 parameters, 11 of which correlate strongly, either positively or negatively, with the amylose content of rice (Champagne et al 2004). Rice is also consumed as an ingredient in a multitude of foods (Fig. 1) prepared in homes and by food processing companies across the globe (Sheng 1995; Deis 1997; Bao and Bergman 2004). To meet required functional and sensory properties, rice cultivars are chosen based on specific amylose content because of the strong associations between amylose and desired properties. For example, waxy rice flour is chosen for its unique soft and sticky texture in many Asian desserts and snacks; many puffed breakfast cereals are produced using low amylose rice; intermediate amylose rice is used in canned soups; and higher amylose rice is chosen for products requiring an intact cooked product, such as extruded pasta, noodles, and retort boiled rice (Juliano and Hicks 1996). Because amylose content plays such a pivotal role in the properties of rice, it is used at early stages of breeding programs to select and discard breeding material.

The enzyme granule bound starch synthase (GBSS)l, encoded at the *Waxy* locus, is responsible for the long, essentially linear

**Fig. 1.** Rice has many uses as grains and flour.

chains that constitute amylose (Ball et al 1998). When the starch granule gelatinizes, a proportion of these long chains leach and the rest do not because they are covalently bound to amylopectin molecules (Hanashiro et al 2005). Both the free and bound long chains are the product of GBSSl activity (Aoki et al 2006), and differences in the proportion of each affect eating properties of rice (Bhattacharya et al 1978). However the objective of the present study is to improve the quantification of all the long chains, herein collectively called amylose.

Amylose content is measured by the absorbance of the amylose-iodine complex; the complexities surrounding the behavior of this complex have occupied many researchers over many decades (Bergman et al 2004; Bhattacharya 2009 and references within). Milestones in the history of measuring amylose are outlined here. McCready and Hassid (1943) proposed the first version of the iodine-binding method for rice. The standard curve was generated using mixtures of purified amylose and amylopectin. The method was later adapted to measure the amylose content of rice using a standard curve of potato amylose alone, measuring at a wavelength of 590 nm (Williams et al 1958). In 1970, a consortium of rice scientists simplified the method, which increased the reproducibility of the measure. Changes were made to the preparation of the starch solution, the chemistry of the reaction, and the wavelength was increased from 590 to 620 nm (Juliano 1971). The next iteration of the method involved the replacement of potato amylose as the standard with a set of undefatted rice cultivars calibrated at pH 10 against a standard curve made from defatted potato amylose (Perez and Juliano 1978). It was later suggested to return to the standard of 1943 and replace potato amylose alone as the standard with mixtures of potato amylose and rice amylopectin to decrease the interference of the amylopectin-iodine complex (Johnson and Webb 1976). The two types of standard curves were evaluated in an international ring test and the mixed standards were recommended (Juliano et al 1981). The International Organisation for Standardisation (ISO) approved this latest method as ISO 6647:1987, recommending the use of mixtures of amylose and amylopectin to generate the standard curve, with an option to use calibrated rice cultivars for generating standard curves for routine analyses.

The ISO 6647 method was withdrawn in 1998. However, the following year, the American Association of Cereal Chemists (AACC), approved a very similar method, AACC 61-03. Differences between AACC 61-03 and ISO 6647 are that the mixtures of amylose and amylopectin used for the standard curve are calculated on the basis of an average starch content of 90% in ISO 6647 and 70% in AACC 61-03 to account for moisture content, and defatting of the test samples is described by ISO and left optional by AACC. In 2007, ISO 6647:2007 was approved, which describes in Part 1, a method to measure the amylose content of a set of well-known cultivars of rice, using mixed standards, to a starch content of 90%, and in Part 2, the standard curve is generated from the well-known samples for routine analysis of amylose. This standard also considers wavelengths of 620 and 720 nm, but makes no specific recommendation.

Other techniques for measuring amylose have also been developed, including the concanavalin A binding method (Matheson and Welsh 1988), various near infrared (NIR) methods calibrated to various versions of the iodine binding method (Delwiche et al 1995; Himmelsbach et al 2001), differential scanning calorimetry (DSC) which measures the energy that evolves from melting of the amylose-lipid complexes (Mestres et al 1996), size-exclusion chromatography (SEC) methods (Yamada and Taki 1976; Chinnaswamy and Bhattacharya 1986; Kennedy et al 1992; Reddy et al 1993; Batey and Curtin 1996; Grant et al 2002; Hanashiro et al 2003; Radhika Takeda et al 2003; Yao et al 2005; Ward et al 2006; Zhong et al 2006; Chen and Bergman 2007) and more recently, asymmetric flow field flow fractionation ($AF^4$) (Kim et al 2007). A recent review of some of these other methods concluded that none were applicable for routine use (Zhu et al 2008). Furthermore, the different versions of the iodine method and the newer methods return different values of amylose. For example, the amylose content of Kyeema and Doongara cultivars is reported to be 14% (Champagne et al 1999) and 22% (Larkin et al 2003), respectively, using a standard curve of known rice cultivars, 19 and 24%, respectively, using a standard curve of potato amylose (NSW DPI Rice Improvement Program), 20 and 28%, respectively, using the concanavalin A binding method (Dang and Copeland 2004); Doongara is reported to be 20% amylose by SEC (Ward et al 2006) and Kyeema 11% amylose by SEC (*unpublished data*). Each different value carries with it particular expectations of sensory quality. Such differences between values make it particularly difficult when germplasm is exchanged between breeding programs, data are presented to international audiences, and associations are made between amylose values and traits or genotypes by researchers using different methods of measuring amylose.

The different values for amylose content for the same cultivar reported in different publications, and the different methods used for measuring amylose found in different research papers suggest confusion about measuring amylose at the international level. Over the decades as the iodine binding method evolved, each change to the standard, preparation of samples, or wavelength altered the amylose content by several percent (Juliano 1971, 1979; Perez and Juliano 1978; Juliano et al 1981) but technology was not sufficiently developed to enable the determination of the actual percentage of amylose in the starch, so no version or method could be validated. With the progress of technology for structural characterization of starch, particularly tools like SEC (Ward et al. 2006; Chen and Bergman 2007) and $AF^4$ (Kim et al 2007), it is timely for the measurement to be internationally re-examined, sources of variability identified, and ways to overcome the variability investigated. In 2005, the International Union of Pure and Applied Chemistry (IUPAC) commissioned a project (2004-022-3-400) to address these issues and in 2006, the International Network for Quality Rice (INQR) was established across the international rice world and is conducting the project in collaboration with ISO and AACC, harmonizing the method between the standards organizations and across all rice improvement programs. This report is for the first goal of the IUPAC project: to survey the methods that are currently in use in rice quality laboratories; to determine reproducibility of amylose quantification between the quality evaluation laboratories of the world; and to identify and explain sources of variation.

**TABLE I**
**Cultivar Names and Identifiers of 17 Samples Analyzed by 27 Laboratories**

| Sample Number | IRGC | Cultivar Name |
|---|---|---|
| 1 | INQR 1-1 | Basmati-Pakistan |
| 2 | INQR 2-1 | Doongara |
| 3 | INQR 3-1 | Goami 2 |
| 4 | INQR 4-1 | Ilpumbyeo |
| 5 | INQR 5-1 | IR 5 |
| 6 | INQR 6-1 | IR 8 |
| 7 | INQR 7-1 | IR 24 |
| 8 | INQR 8-1 | IR 29 |
| 9 | INQR 9-1 | IR 56 |
| 10 | INQR 10-1 | IR 60 |
| 11 | INQR 11-1 | IR 64 |
| 12 | INQR 12-1 | IR 68 |
| 13 | INQR 13-1 | Khao Dawk Mali 105 |
| 14 | INQR 14-2 | Koshihikari |
| 15 | INQR 15-2 | Koshihikari |
| 16 | INQR 16-1 | IRRI 123/PSBRc 82 |
| 17 | INQR 17-1 | RD 6 |

[a] International Network for Quality Rice (INQR).

## MATERIALS AND METHODS

### Amylose Analysis Around the World

To determine reproducibility between laboratories, 17 cultivars of rice (Table I) varying in amylose content were chosen. Grains of each cultivar were polished (Grainman) and ground to flour (Udy cyclone sample mill 3010-030, Fort Collins, CO). A subsample of each of the 17 samples of flour was distributed to 27 quality evaluation laboratories from many rice-growing regions of the world. Each laboratory measured the amylose content of the samples using the method currently operating in that laboratory, in duplicate on two days, and some laboratories measured the samples using several methods. Participants also provided details about the materials and chemicals used to construct the standard curve, the instrument used to measure absorbance, waiting time between addition of the last chemical and measurement, and the wavelength used to measure absorbance. Two laboratories used SEC exactly as previously described (Ward et al 2006) and one used DSC as previously described (Mestres et al 1996).

### Identifying Potential Sources of Variability

Standard curves were generated at IRRI using 1) mixtures of potato amylose (Fluka) and amylopectin (from waxy rice) assuming 90% starch as described in ISO 6647:1987 or 70% starch as described in AACC 61-03, and 2) mixed standards made from three commercial samples of potato amylose (Fluka, Kebo, and Sigma) and one sample of rice amylopectin (from a waxy cultivar). The iodine binding capacity (IBC) was determined as previously described (Schoch 1964) by Foss for all types of potato amylose used. Amylose content was determined for four of the samples (6, 7, 8 and 11) (Table I) using each curve. The standards were mixed with iodine as described in all previous methods, and absorbance at 620 nm was read in a spectrophotometer (DU 800 Beckman Coulter). Standard curves were also made from two brands of potato amylose (Calbiochem and Sigma) at INIA and amylose content of the 17 samples was quantified from both standard curves using a spectrophotometer (Milton Roy Spectronic 20D).

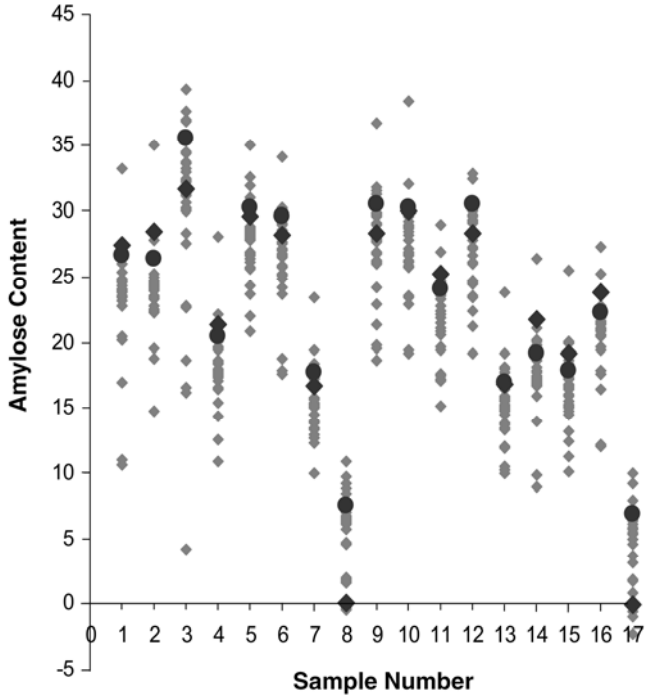


**Fig. 2.** Amylose values for 17 rice cultivars measured by 27 quality evaluation programs using current methods for measuring amylose content. Large symbols show the switching order of two laboratories.

Wavelength scans of the amylose-iodine and amylopectin-iodine complexes were collected at 450–800 nm in a spectrophotometer (DU 800 Beckman Coulter). The complexes were prepared according to the method described in AACC 61-03, resulting in final solutions of either 30% amylose or 70% amylopectin.

Samples for which the amylose classification is well known were prepared for SEC. Flour (50 mg) from samples 6, 7, 8, and 11 (Table I) was gelatinized, debranched, and analyzed by SEC (Waters Alliance 9616 and Refractive Index Detector 2414) exactly as previously described (Ward et al 2006), using an Ultrahydrogel 250 column (Waters) calibrated for molecular weight with pullulan standards (P800, P400, P200, P100, P50, P20, P10, P5) (Shodex) injected individually; the Mark-Houwink-Sakaruda equation ($K$ = 0.00126 mL/g and $\alpha$ = 0.733 for pullulan, and $K$ = 0.0544 mL/g and $\alpha$ = 0.486 for linear starch) (Castro et al 2005) and the universal calibration (Castro et al 2005; Ward et al 2006).

## RESULTS AND DISCUSSION

### Amylose Analysis Around the World

Amylose content of the 17 cultivars was measured by 27 laboratories and 34 sets of data were generated. Repeatability within each laboratory was very high, with standard deviation always <5% of the mean (data not shown). However, different laboratories reported very different values for each sample, in particular for sample 3, with values of 4–40% amylose (Fig. 2). Furthermore, the differences between laboratories were not relative to each other, as shown by the switching order of the larger black symbols representing two laboratories. Rice is classified on the basis of amylose content as waxy (0–2%), very low (3–9%), low (10–19%), intermediate (20–25%), and high (>25%) (Kumar et al 1987; Juliano et al 1990; Juliano 2001). Figure 2 shows that amylose values of all cultivars spanned at least two classes of amylose, with sample 3 (a mutant) (Kang et al 2003) spanning four. This suggests that either the method must be standardized, or new technologies must be explored to develop a new method.

Discrepancies such as those illustrated in Fig. 2 make it difficult for breeding programs to utilize amylose values to categorize cultivars into clear quality classes during development. Before 2004, germplasm was generally exchanged between rice improvement programs subject to approval at ministerial levels. The International Treaty on Plant Genetic Resources for Food and Agriculture came into force in 2004, providing a clear legal framework for germplasm exchange, thereby facilitating wider transfer of cultivars and information. Germplasm often is dispatched with information on amylose content (www.irri.org, www.ars-grin.gov) and this is used as a selection criterion to determine the hybridization program in which the cultivar is used and it generates expectations of grain quality in potential new cultivars developed from the crosses. The present study reveals that the two organizations responsible for the amylose data in material dispatched from IRRI and the USDA use different methods for measuring it, and the values they reported in this study are quite different for each sample (data identifying individual laboratories are not shown). All of the differences across the dataset raise a number of questions.

### Is Amylose Present in Waxy Rice?

The *Waxy* gene is required for the synthesis of amylose (Ball et al 1998). Samples 8 and 17 are cultivars of rice classified as waxy mutants because they carry a premature stop codon in the *Waxy* gene caused by a duplication of nucleotide bases (Wanchana et al 2003) leading to the null *waxy* allele. Theoretically, cultivars carrying this mutation are unable to produce the long chains of amylose (Mikami et al 2008) and therefore should return a reading of zero. SEC and DSC reported zero amylose for those samples, but many iodine methods did not (Fig. 2). Either amylose is present in

waxy rice or the amylopectin-iodine complex is contributing differently in each method.

All laboratories that obtained a positive reading for amylose in the waxy cultivars used commercial potato amylose to generate the standard curve. The standard curve made from potato amylose passes through zero, but curves made from mixed standards do not (Table II). The laboratories that obtained values of 0–2% amylose for the two waxy cultivars used calibration curves made from mixtures of potato amylose and waxy rice starch. Amylopectin has an IBC of ≈0.4 and the amylopectin-iodine complex absorbs at 620 nm (Davis et al 1994). Thus, a sample of gelatinized waxy rice reacted with iodine will always give a positive amylose value at that wavelength if the standard curve passes through zero.

Standard curves made from mixtures of amylose and amylopectin take account of measuring amylose within a matrix of amylopectin. This is most likely the reason why the latest publications and the ISO and AACC methods recommend making the standard curve from mixtures of amylose and amylopectin. It is therefore likely that the positive values of amylose obtained for the waxy rices are due to the use of an inappropriate standard curve and not to the presence of chains of a length consistent with being amylose.

### The Standard Curve

Each different version of the iodine binding method has led to revision of the protocol for the standard curve. The possibilities are potato amylose alone (Juliano 1971), mixtures of amylose and amylopectin (Johnson and Webb 1976) assuming 70% (AACC 61-03) or 90% (ISO 6647) total carbohydrate, and calibrated rice cultivars (either to potato amylose or to one of the curves from mixed standards) (Perez and Juliano 1978). In the present study, 15 datasets were obtained using a standard of potato amylose, 14 were from three or four calibrated rice cultivars, calibrated against potato amylose (seven) or mixed standards (seven), and two used mixed amylose and amylopectin solutions directly to generate their standard curve. This shows that half of the rice world, represented in the present study, is using the simplified method described by Juliano (1971), one quarter blends methods using the simplified method (Juliano 1971) to calibrate cultivars and then uses those for the routine method described by the standards organizations, and the other quarter uses the standard curve and routine method exactly as described by ISO 6647 2007.

Table II shows that the slope and *y*-intercept of the curve made from potato amylose differs from that made from mixed standards of potato amylose and amylopectin. Such differences in slope lead to differences in amylose values across the range of the curve (Table II). Interestingly, the variability surrounding mean values for the 17 samples is much greater for the values obtained from curves of potato amylose compared with values obtained from curves using calibrated rice or mixed standards except for samples 8 and 17 (Fig. 3). Factors that contribute to variability are moisture content of the grain, operator skill for dissolving the starch, standing time between addition of last chemical and measurement, brand of chemicals, and type of spectrophotometer. However if these were the major causes of the variability between laboratories, we would expect similar variability for both types of standard curves, which suggests that the variability must be a feature of the standard curves.

Seven different types of potato amylose were used by 15 people who constructed their standard curve from different concentrations of potato amylose. Figure 4 shows the values obtained for the 17 cultivars by one operator using standard curves constructed from two of the different brands of potato amylose (Calbiochem and Sigma). One of the problems of potato amylose is the difference in the IBC of different brands and batches of amylose.

Illustrating this, standard curves made from potato amylose differing in IBC (14.9, 18.3, and 19.5) mixed with amylopectin differed in slope and *y*-intercept and subsequently returned quite different amylose values (Table II). The difference between values from the curves differing in IBC increases as amylose content increases, with the difference being ≈8% for the high-amylose cultivar. The values in Fig. 4 are likely to reflect differences in IBC of the two brands of potato amylose. Figure 4 also shows that the difference between values from the two standard curves increases with increasing amylose content, but shows larger differences than those seen in Table II. The values in Fig. 4 were obtained from standard curves of potato amylose alone, whereas those in Table II were obtained from curves made from mixtures of amylose and amylopectin. Perhaps the presence of amylopectin attenuates the effect of IBC, which could explain the higher vari-

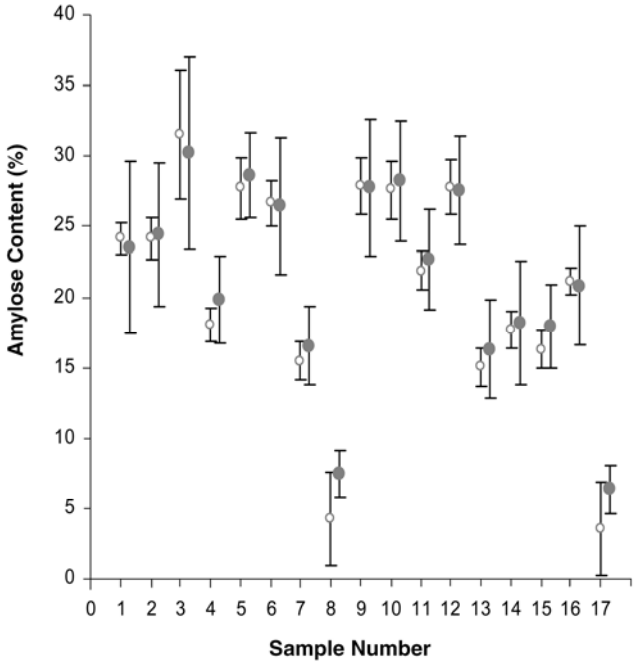


**Fig. 3.** Mean and standard deviation for amylose values from standard curves of potato amylose (solid circles) or calibrated rice cultivars (open circles). Values for potato amylose are offset by 0.3 on the *x*-axis for ease of comparison.

**TABLE II**
**Slope and *y*-Intercept of Different Types of Standard Curves, and Amylose Content of Waxy (sample 8), Low (sample 7), Intermediate (sample 11), and High (sample 6) Amylose**

| | | | Amylose Content, % | | | |
|---|---|---|---|---|---|---|
| **Standard** | **Slope** | ***y*-Intercept** | **Waxy** | **Low** | **Intermediate** | **High** |
| Potato amylose | 0.0126 | 0.0000 | 7.5 | 21.6 | 29.3 | 34.9 |
| AM+AP 70% IBC 19.5% | 0.0137 | 0.0928 | 0.1 | 13.1 | 20.1 | 25.3 |
| AM+AP 70% IBC 18.3% | 0.0148 | 0.0574 | 2.5 | 14.5 | 21.0 | 25.8 |
| AM+AP 70% IBC 14.9% | 0.0097 | 0.1138 | –2.0 | 16.3 | 26.3 | 33.6 |
| AM+AP 70% IBC >19% | 0.0084 | 0.0573 | 2.0 | 17.0 | 25.1 | 31.0 |
| AM+AP 90% IBC >19% | 0.0085 | 0.0700 | 0.5 | 15.1 | 23.0 | 28.8 |

ability between values obtained from standard curves made from the different brands of potato amylose in Fig. 3.

Another factor often considered when measuring amylose is the moisture content of the grain. Constructing the standard curve to 70% total carbohydrate takes moisture into account. Comparing values from this curve with those from a curve made to 90% starch, which is based on the dry weight of milled grain, shows that the *y*-intercept differs but the slope does not (Table II). Consistent with this is a constant difference in amylose values of two percentage points for all amylose classes. These data suggest that differences in moisture have a reasonably small effect on amylose content, whereas differences in IBC of different brands of potato amylose appear to be the largest source of variation, and this variation is augmented when standard curves are made from amylose alone.

**Wavelength**

Amylopectin has an IBC of ≈0.4% (Davis et al 1994) and the absorption spectrum of this complex overlaps with that of the amylose-iodine complex (Jarvis and Walker 1993) (Fig. 5). The wavelength first used in the amylose assay was 590 nm (Williams et al 1958). This was increased to 620 nm to lessen the strong interference of the amylopectin-iodine complex (Juliano 1971), and because the starch-iodine lambda max is closer to 620 nm (Juliano et al 1981). In the present study, three wavelengths were used by different laboratories: 600, 620, and 720 nm. However variability due to the standard curves masked any differences that could be due to wavelength.

Figure 5 shows that increasing the wavelength from 590 to 620 nm lowers the amylopectin-iodine contribution and it also shows that at 720 nm there is still substantial absorbance by the amylose-iodine complex, but almost none by the amylopectin-iodine complex. This suggests that there is more room to alter the wavelength, which could provide another means of reducing variability or obtaining truer measurements of amylose content.

**Obtaining Actual Values of Amylose**

The amylose method has been altered several times over the years, but in that time there was no method readily available to validate any version of the method by determining the actual amount of amylose present in a sample. New technology for measuring structural features of starch directly and indirectly is becoming increasingly more available and accurate.

DSC methods for determining the amylose content of rice have been reported. These methods are based on the understanding that amylose forms crystalline complexes with lipids under certain conditions (Young 1984). Specifically, they determined amylose as amylose-L-lysophosphatidylcholine (LPC) complex (Sievert and Holm 1993; Mestres et al 1996). Sievert and Holm (1993) used potato amylose-amylopectin mixtures and Mestres et al (1996) used varying amounts of potato amylose. These samples were heated along with LPC, cooled, and then reheated to quantify the melting of the amylose-LPC complexes formed during cooling. A linear relationship between the amylose content of the mixtures and the enthalpies of the amylose-lipid complexes was used to predict the amylose content of various rice flours. Amylose values determined using these DSC methods were highly associated with those found using colorimetric assays. In contrast to the colorimetric methods, the DSC procedures are not influenced by amylopectin, but DSC does not remove the variability from the colorimetric methods that accompanies the use of purified starch or amylose as the standard. For example, Mestres et al (1996) reported the exotherm for pure potato amylose to be 28.5 J/g (dmb) for Avebe amylose, while that for ICN amylose was 27.0 J/g. Furthermore, the expense of the consumables and the slow throughput exclude DSC as a method for routine analysis (Zhu et al 2008)

Size-exclusion chromatography (SEC) is a method used to separate polymers based on size. The size of amylose and amylopectin molecules differs substantially, so intuitively they could be separated by SEC. However, SEC separates on the basis of hydrodynamic volume ($V_h$), which is the volume the molecule occupies in the solvent (Ward et al 2006), and branching patterns of the molecule affect $V_h$. It was observed previously that particular SEC conditions cause 1) co-elution of amylose with smaller amylopectin molecules and 2) exclusion of large molecules of amylopectin from the pores of the instrument (Ward et al 2006).

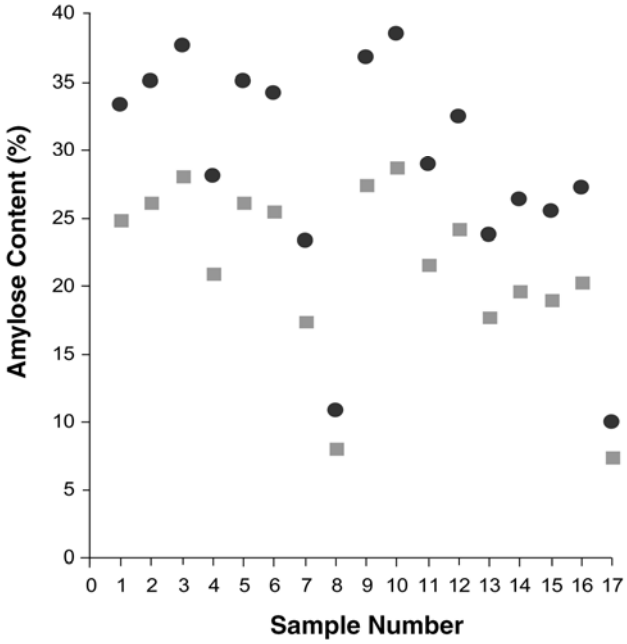


**Fig. 4.** Amylose content of the 17 rice cultivars using calibration curves generated from two different brands of potato amylose in one laboratory.

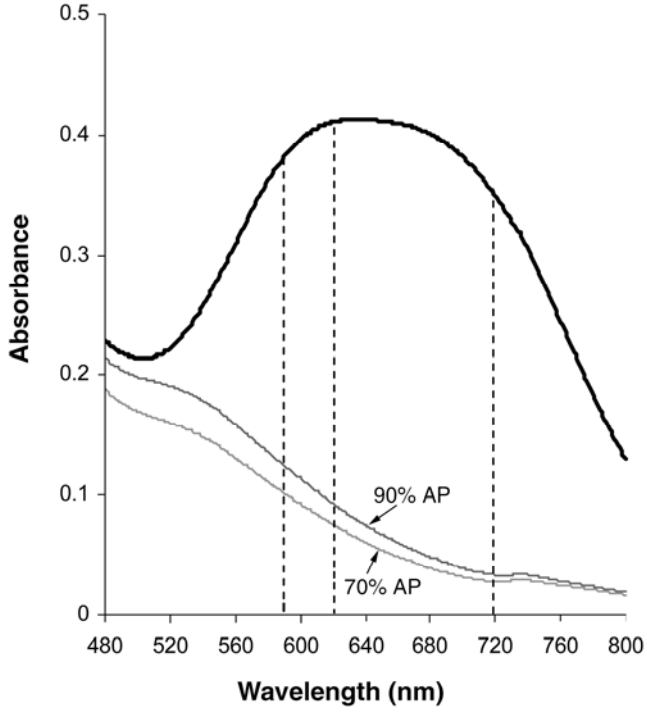


**Fig. 5.** Wavelength scans of amylose-iodine complex (thick line) and amylopectin (AP) iodine complex (thin lines). Dashed lines at 590, 620, and 720 nm.

Both factors suggest that suitable conditions are not yet available for quantifying the actual proportion of amylose in starch on the basis of different proportions of amylose and amylopectin molecules. However, linear chains of starch do not suffer the complication of exclusion from the column or co-elution based on $V_h$, because linear chains of starch are much smaller than complete molecules of starch and the $V_h$ of a linear chain in a particular solvent is directly proportional to the molecular weight of that chain (Ward et al 2006).

Amylopectin molecules are constructed from chains in a range of DP 6–100 (Takeda et al 2003) and chains synthesized by GBSSl in a range of DP 230–10,000 (Ward et al 2006). Linear chains of starch are obtained by debranching the starch (Batey and Curtin 1996) and current SEC methods can almost separate chains of the length consistent with amylose from the shorter chains belonging to amylopectin molecules (Sargeant and Wycombe 1982; Batey and Curtin 1996; Castro et al 2005; Ward et al 2006; Chen and Bergman 2007) (Fig. 6). SEC also reflects the concomitant decrease in the proportion of amylopectin chains with increasing numbers of amylose chains. SEC methods are not affected by the presence of lipids (Chen and Bergman 2007) and the proportion of amylose (or the long chain fraction) can be quantified directly from the chromatogram so a calibration curve is not required. The SEC conditions used to generate Fig. 6 do not give baseline resolution between the chains of amylose and amylopectin; this can be seen by comparing the start of the peak in the waxy sample with the nonwaxy samples. Presumably choice of column and manipulation of SEC operating conditions could optimize separation. While SEC of linear chains of starch is not appropriate for routine screening of amylose, the technique offers a method to determine the actual proportion of amylose and a potential alternative method for providing standard values within an extensive and organized network such as the INQR, once separation of families of chains is optimized.

## CONCLUSIONS

A routine method for the quantification of amylose in rice improvement programs went through rapid development in the 1970s and early 1980s, resulting in the publication of two slightly different methods by ISO and AACC. By conducting a survey of amylose methods in operation in various rice quality evaluation laboratories, as well as testing the reproducibility between laboratories, it was found that at least five different versions of the iodine method are in operation, with variability in the construction of the standard curve, standing time, and wavelength used for measurement. Reproducibility between laboratories was poor, and the data indicate that the IBC of the potato amylose used to construct the standard curve is the major source of variability. In a world of advancing technology, increasing rice research in response to decreasing global rice supply, increasing exchange of germplasm, exchange of germplasm with amylose data, and requirement for higher quality rice by consumers, it is timely and necessary for quality evaluation laboratories in rice improvement programs to revisit the method for measuring amylose and together standardize a method for quantifying actual values of amylose.

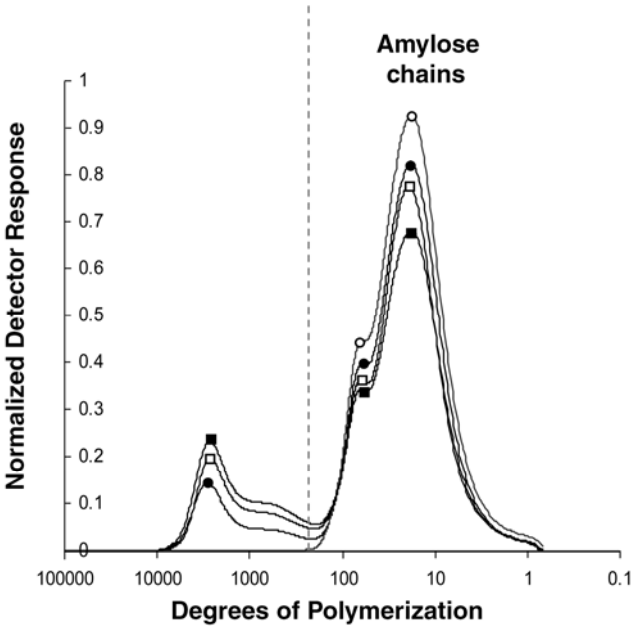


**Fig. 6.** SEC traces of debranched starch of IR8 (■), IR64 (□), IR24 (●), and IR29 (○). Dashed line indicates region between chains derived from amylose and amylopectin.


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