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The importance of amylose and amylopectin fine structure for textural properties of cooked rice grains

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Abstract

Statistically and causally meaningful relationships are established between starch molecular structure (the molecular distribution of branched starch and the chain length distribution of debranched starch) and texture (hardness and stickiness) of cooked rice grains. The amounts of amylose chains with degree of polymerization (DP) 100-20000, and of long amylopectin chains, positively correlated with hardness, while amylopectin chains with DP<70 and amylose molecular size both showed negative correlations with hardness ($p<0.05$). There was also a significant negative correlation between stickiness and the amounts of long amylopectin chains ($p<0.01$). For rices with similar amylose content, the amount of amylose chains with DP 1000-2000 positively correlated with hardness while size negatively correlated with hardness ($p<0.05$). This indicates for the first time that, regardless of amylose content, rice varieties with smaller amylose molecular sizes and with higher proportions of long amylose chains have a harder texture after cooking.

Keywords: rice; hardness; stickiness; size exclusion chromatography; amylose; amylopectin; sensory properties
1. Introduction

Rice is a major staple food world-wide. In recent years, consumer preferences have shifted towards better-quality rice, particularly towards varieties with good eating quality. Each country, and often region, prefers rice with a particular suite of quality traits (Calingacion et al., 2014). The textural attributes of cooked milled rice are of prime importance to its eating quality. Texture is a multi-parameter sensory property, with hardness and stickiness as the most commonly determined parameters for cooked rice (Patindol, Gu & Wang, 2010). In addition to sensory evaluation by human panels, textural properties of cooked rice are most commonly measured by instruments such as a textural analyser (Cameron & Wang, 2005; Champagne et al., 1998).

Cooked rice texture is affected by a wide range of factors, such as the amylose content (Juliano, Onate & Del Mundo, 1972), postharvest processing (Champagne et al., 1998), and cooking method (Leelayuthsoontorn & Thipayarat, 2006). Among these, starch structure has an important role in rice texture (Cameron et al., 2005; Ramesh, Zakiuddin Ali & Bhattacharya, 1999). Starch is a branched glucose polymer comprising two types of molecules: amylopectin (Ap) and amylose (Am). Ap molecules are highly branched with a vast number of short branches and relatively large molecular weights, ~10^{7-8}, whereas Am has a smaller molecular weight (~10^{5-6}) with a few long branches (Gilbert, Witt & Hasjim, 2013). The amylose content has been considered to be the most important determinant of the eating quality of rice since the mid-1980s (Bhattacharya & Juliano, 1985). In the mid-1990s, it was proposed that the texture of cooked rice is also related to the fine structure of amylopectin (Ramesh et al., 1999). Ong and Blanshard (1995) determined the amylose content and the amylopectin fine structure of 11 cultivars of non-waxy rices, and confirmed that the texture of cooked rice was critically controlled by the proportion of the longest and shortest amylopectin chains but not the intermediate ones. Ramesh et al. (1999) analyzed the starch structure of 7 rice varieties, concluding that the content of all long linear chains, including amylose if any, governed the texture of cooked rice.
The present study is an in-depth consideration of the mechanisms of starch structural effects on rice texture. A novel factor in the present paper is an examination of the role of the fine structure of amylose (Gilbert et al., 2013), which is a significant factor in starch digestibility (Syahariza, Sar, Tizzotti, Hasjim & Gilbert, 2013).

There are several techniques for starch fine structural analysis: fluorophore-assisted carbohydrate electrophoresis (FACE), high-performance anionic-exchange chromatography (HPAEC), and size-exclusion chromatography (SEC – sometimes termed gel-permeation chromatography or GPC) (Wu, Witt & Gilbert, 2013c). FACE is the optimal method for determining the chain-length distributions (CLDs) of amylopectin. SEC suffers from the problems of band-broadening, calibration, and inaccuracies in the Mark-Houwink relation used to relate molecular size to degree of polymerization (DP), which are all obviated with FACE. However, because of the inability to quantitatively detect chains above a relatively low DP, currently ~ 180 (Wu, Li & Gilbert, 2014), FACE and HPAEC can only give information on amylopectin chains and (for FACE) the shortest amylose chains. SEC does not suffer from the same restriction and can therefore be used for the measurement of amylose fine structure (Gilbert et al., 2013).

The objective of this study is to obtain a mechanistic understanding of the relationship between starch (Ap and Am) fine structure and textural properties (hardness and stickiness) of cooked rice grains. Since the starch granular and crystalline structures are greatly disrupted by the cooking process, only the grain composition and starch molecular structure will be analysed here. The structural features are the CLDs of the individual polymeric chains of debranched Am and Ap, and the molecular size distributions of whole (fully branched) starch (Syahariza et al., 2013). The rice varieties chosen for the present study have a wide range of Am content. Among these, 7 rice varieties were deliberately chosen to contain similar amylose content but which differ in sensory properties, in order to discover any correlations that are separate from those due to amylose content alone. The hardness and stickiness of the cooked rice were determined from texture profile analysis using a texture analyser. The
results will aid understanding of the role of starch fine structure in determining the textural properties of cooked rice grains.

2. Materials and methods

2.1. Materials

Twelve milled rice grain samples were chosen from a collection of rice varieties with known phenotypes and genotypes for quality traits (Table 1). Protease from *Streptomyces griseus* (type XIV), and LiBr (ReagentPlus) were purchased from Sigma-Aldrich Pty. Ltd. (Castle Hill, NSW, Australia). Isoamylase (from *Pseudomonas sp.*) and a D-glucose (glucose oxidase/peroxidase; GOPOD) assay kit were purchased from Megazyme International, Ltd. (Wicklow, Ireland). A series of pullulan standards with peak molecular weights ranging from 342 to $2.35 \times 10^6$ were from Polymer Standards Service (PSS) GmbH (Mainz, Germany). Dimethyl sulfoxide (DMSO, GR grade for analysis) was from Merck Co. Inc. (Kilsyth, VIC, Australia). All other chemicals were reagent-grade and used as received.

2.2. Cryogenic grinding of rice grains

Rice grains were ground into flour with a cryogenic mill (Freezer/Mill 6850; SPEX, Metuchen, NJ) in a liquid nitrogen bath as the cryogenic medium, following the procedure described by Syahariza et al. (2013).

2.3. Composition of rice grains

The starch content of the rice grains was analyzed from the ground rice flour using a GOPOD assay kit. The crude lipid content was determined by Soxhlet extraction, following AOAC method 920.39C (AOAC, 2002). The crude protein content of the rice grains was calculated from the nitrogen content of the rice flour, obtained using a LECO CNS2000 auto analyser (LECO Corporation, St. Joseph, MI) with a conversation factor of 5.95 (Jones, 1941).

2.4. Starch extraction from rice grains

All starch samples were extracted and dissolved in a DMSO solution with 0.5% (w/w) LiBr (DMSO/LiBr) at a concentration of 2 mg/mL, following a method described elsewhere...
A protease and sodium bisulfite solution was used first, followed by a centrifugation step, to remove protein from the rice flour. The treated rice flour was agitated in DMSO/LiBr and the starch then precipitated from the resulting soluble portion by adding 10 mL of ethanol; samples were then centrifuged at 4000 g for 10 min. This is better than extracting starch from rice grains using an alkaline solution, which can act as a catalyst for starch hydrolysis, especially when heating and mixing are involved (Chiou, Martin & Fitzgerald, 2002; Wu, Li & Gilbert, 2014b). The extracted starch in the DMSO/LiBr solution was stored at room temperature for subsequent analysis by SEC and debranching for CLD analysis.

2.5. Molecular size distribution of whole branched starch molecules

The structure of extracted whole starch molecules was characterized using an Agilent 1100 Series SEC system (Agilent Technologies, Waldbronn, Germany) equipped with GRAM 30 and 3000 analytical columns (PSS) and a refractive index (RI) detector (RID-10A, Shimadzu Corp., Kyoto, Japan) following a method described elsewhere (Cave, Seabrook, Gidley & Gilbert, 2009; Liu, Halley & Gilbert, 2010). The molecular size distribution of branched starch was plotted as the weight distribution, \( w_{\text{br}}(\log R_h) \), against the hydrodynamic volume \( V_h \) (the separation parameter for SEC), or the equivalent hydrodynamic radius, \( R_h \): \( V_h = \frac{4}{3} \pi R_h^3 \).

For branched starch molecules, as for any branched polymer, there is no unique relation between size and the molecular weight \( M \). The assumption of universal calibration for SEC is that the elution time of the analyte depends only on its \( V_h \) and not on its structure, whence one has for two polymers, a sample and a standard, the relation:

\[
K_{\text{standard}} M^{\alpha(\text{standard})+1} = K_{\text{sample}} M^{\alpha(\text{sample})+1} \quad (1)
\]

Pullulan standards with known peak molecular weights were used for calibration to obtain a relationship between SEC elution volume and \( V_h \) of starch molecules following the Mark-Houwink equation:

\[
V_h = \frac{2}{5} \frac{M^{1+\alpha}}{N_A} \quad (2)
\]
Here $N_A$ is Avogadro’s constant. The Mark-Houwink parameters $K$ and $\alpha$ of pullulan in DMSO/LiBr solution at 80 °C are $2.424 \times 10^{-4}$ dL g$^{-1}$ and 0.68, respectively (Cave et al., 2009).

2.6. Starch debranching and measuring the CLD of debranched starch using SEC

The extracted starch (~4 mg) was dissolved in 0.9 mL of deionized water and then mixed with 2.5 µL isoamylase (1000 U/mL), 0.1 mL acetate buffer solution (0.1 M, pH 3.5), and 5 µL sodium azide solution (0.04 g mL$^{-1}$). The mixture was incubated at 37 °C for 3 h. The debranched starch suspension was then heated in a water bath at 80 °C for 2 h after being neutralized with 0.1 M NaOH solution, and then freeze-dried overnight. The dried debranched starch was dissolved in DMSO/LiBr solution for SEC analysis.

To obtain SEC distributions of debranched starch, GRAM 100 and GRAM 1000 columns (PSS) were used, with the same pullulan standards and procedure used to calibrate the SEC for whole branched molecules. The SEC weight distribution, $w(\log X)$, obtained from the DRI signal was plotted against DP $X$, with $X$ being determined using the Mark-Houwink relationship (see Equation 1), with $M = 162.2(X-1)+18.0$ (162.2 is the molecular weight of the anhydroglucose monomeric unit and 18.0 is that of the additional water in the end group); $K$ and $\alpha$ for linear starch chains in the eluent of DMSO/LiBr at 80 °C are $1.5 \times 10^{-4}$ dL g$^{-1}$ and 0.743, respectively. For a linear polymer (such as debranched starch), the number distribution of chains (obtained by debranching), $N_{de}(X)$, is related to the corresponding SEC weight distribution by (Castro, Dumas, Chiou, Fitzgerald & Gilbert, 2005):

$$w(\log X) = X^2 N_{de}(X) \quad (3)$$

The degree of branching (DB) is obtained from the CLD using the relation $DB = 1/(\text{number average of } N_{de}(X))$.

2.7. Fitting amylopectin number CLD with a biosynthesis model

The number distribution was fitted using the Wu-Gilbert model (Wu & Gilbert, 2010; Wu, Morell & Gilbert, 2013b), which considers the CLD from a biosynthetic perspective. In this
model, the number distribution is assumed to be controlled solely by the action of three types of starch biosynthesis enzyme: starch synthase (SS), starch branching enzyme (SBE), and starch debranching enzyme (DBE). The kinetic equations of the rates of action of each enzyme determine the number distribution of branches, that is, \( N_{\text{de}}(X) \), giving the relative number of chains of the debranched starched comprising \( X \) monomer units. There are several different sets of the three types of enzymes, denoted “enzyme sets”: for example, there are four isoforms of branching enzyme, SBEI, SBEII, SBEIIa and SBEIIb, and a particular enzyme set contains only one of these four (plus one each of the various types of starch synthase and debranching enzymes). The overall \( N_{\text{de}}(X) \) is the sum of the contributions of each enzyme set. By fitting the number CLD of amylopectin with this model, a series of parameters can be obtained characterizing the enzymatic processes of the amylopectin biosynthesis. In addition, SBE can only form branches with lengths longer than a certain minimum DP, \( X_{\text{min}} \), and the length of moiety retained after branching must be more than a certain minimum DP, \( X_0 \). The activity ratios of SBE/SS and DBE/SS are denoted \( \beta \) and \( \gamma \), respectively. From the mathematical development, for given values of \( X_0 \) and \( X_{\text{min}} \), each value of \( \gamma \) is associated with a value of \( \beta \), so that \( \gamma \) is eliminated from the fitting (Wu et al., 2010).

For SEC CLD data (where some features of the fine structure are masked by band broadening), the CLD of amylopectin branches with DP \( \leq 100 \) can be fitted by three enzyme sets, denoted enzyme sets 1, 2 and 3, and the relative contributions of enzyme set 2 and 3 to enzyme set 1 are termed \( h_{2/1} \) and \( h_{3/1} \), respectively (Witt, Doutch, Gilbert & Gilbert, 2012). The role of phosphorylase in forming enzyme complexes between different enzymes and isoforms of these (Tetlow et al., 2008; Tetlow et al., 2004) is acknowledged as contributing to the action of each enzyme set. Fitting is implemented with publicly-available code (Wu & Gilbert, 2013a).

2.8. **Amylose content**

The Am content of rice starch was determined from the SEC weight distributions of debranched starch. This was taken as the ratio of the area under the curve (AUC) of Am branches (defined to have DPs \( \geq 100 \)) to the AUC of the entire distribution (including both
Ap and Am branches). This method has been shown to be more accurate than the iodine colorimetric method (Fitzgerald et al., 2009; Vilaplana, Hasjim & Gilbert, 2012).

2.9. Preparation of cooked rice

Rice (100 g, 14% moisture content) was rinsed with distilled water three times. Distilled water was then added to the rice to give a rice-to-water weight ratio of 1:1.6. The cooking process was conducted using the pre-set cooking setting of a rice cooker (Kambrook Rice Express, VIC, Australia), followed by a 10 min holding period at the warming setting. The top 1 cm layer of cooked rice and rice adhering to the sides of rice cooker were not used. Cooked rice for sampling was taken directly from the middle of each cooker, transferred to a pre-warmed (120 °C) glass bowl, and mixed thoroughly while minimizing kernel breakage. The cooked rice was then cooled to room temperature (~25 °C) for textural measurements.

2.10. Texture profile analysis (TPA)

A 1 g subsample of cooked rice grains was weighed and placed as a single layer of grains on the base plate. A two-cycle, force-versus-distance compression program was used to measure and calculate using a TA.XT-Plus Texture analyser with a 35 mm cylindrical probe attachment (Stable Micro Systems Ltd., Surrey, UK). The probe was allowed to descend at 1 mm/s, return, and then the compression cycle repeated. Compression was set to 80% strain. For each cooking replicate, texture measurements were conducted six times. Parameters recorded from the test curves were hardness (force at the peak of the first curve) and stickiness (area of the negative force curve).

2.11. Statistical analysis

For each structural measurement, duplicated analyses were performed for each sample. All data were reported as mean ± standard deviation (SD) using analysis of variance (ANOVA) with Tukey’s pairwise comparisons. Significant differences of the mean values were determined at \( p < 0.05 \). The textural measurements were analyzed in duplicate for each sample. One-way analysis of variance (ANOVA) and Pearson as well as Spearman rank
correlation methods were carried out using SPSS V. 16.0 software (SPSS Inc., Chicago, IL). The means of duplicated measurements were used for the correlation analysis.

3. Results and discussion

3.1. Rice composition

Rice compositions are presented in Table 1. The total starch content ranges from 78% to 86%, the protein content from 6.5% to 9.4%, and total lipid content is between 0.2% and 0.9%. Between these different rice samples, there are some significant differences in the total starch, protein, and lipid content. The starch, protein, and lipid content of rice samples in this study are within the ranges previously reported for rice.

3.2. Starch molecular structure

Typical SEC weight distributions, \( w_{br}(\log R_h) \), of whole branched starch from all rice grain samples are shown in Fig. 1, normalized to the peak maximum of Ap; the fully branched distribution of all rice samples display two populations of \( \alpha \)-glucans: Am (\( R_h \) up to \( \sim 100 \) nm) and Ap (\( R_h \) between 100 and 4000 nm) (Fig. 1). There is another small peak/shoulder peak at \( R_h \sim 3 \) nm, which may be residual proteins (Syahariza et al., 2010; Syahariza et al., 2013). These residual proteins possibly arise from incomplete hydrolysis by protease during the starch extraction procedure and are not relevant to this study, and this component of \( w_{br}(\log R_h) \) is not considered further. The Am component of the whole-molecule distributions is expressed as the value of \( R_h \) at the Am peak maximum and the average \( R_h \) (between 0 and 100 nm) of Am, \( \overline{R_h} \), as defined elsewhere (Vilaplana & Gilbert, 2010), while the corresponding for the Ap component is expressed as the value of \( R_h \) at the Ap peak maximum. As presented in Table 2, there are statistically significant differences in both the \( R_h \) at the Am peak maximum and \( \overline{R_h} \) of Am among different rice varieties, whereas there is little significant difference in the \( R_h \) at the Ap peak maximum between samples. As shown in Fig. 1, Hom Mali Niaow (HNM) is a waxy rice with the lowest amylase content, and thus has the lowest AUC in the amylase region (even lower than that of the residual protein); however this starch, while its amylase content is very small, has the largest molecular size in the \( R_h \) at
the Am peak maximum and $R_h$ of Am (Table 2). Given that genetically this variety cannot
produce amylose (Wanchana, Toojinda, Tragoonrung & Vanavichit, 2003), it is probable that
the polymers found in the region where amylose molecules are found are small molecules of
amylopectin that co-elute in the amylose region of the chromatogram. In contrast, high-
amylose rice starches such as SLG and SN, which have Am peaks close to or even higher
than the Ap peaks, have relatively low Am molecular sizes with a smaller $R_h$ at the Am peak
maximum and a smaller $R_h$ across the Am region (Fig. 1). It has been pointed out (Fitzgerald
et al., 2009; Vilaplana et al., 2012) that amylose content cannot be accurately measured from
the whole-molecule size distribution because of co-elution of the molecules, but is best
measured by the AUC from the debranched distribution as above.

Typical SEC weight distributions of debranched starch, $w_{de}(\log R_h)$, from all grain samples are
presented in Fig. 2A. The same information is presented in Fig. 2B as the CLD, in terms of
the number distribution $N_{de}(X)$; those different representations of the same data bring out
different features of the distribution. All weight and number distributions are normalized to
the highest Ap branch peak. The components with $X < 100$ are defined as Ap chains, while
those with $X \geq 100$ are defined as amylose chains (Vilaplana et al., 2012). The SEC weight
distributions of debranched starch from all rice grain samples show the usual features. There
are two large peaks of Ap branches and one smaller peak of Am branches. The first peak
(denoted Ap1) is the global maximum, which comprises the shorter amylopectin branches with
lengths up to a DP of 30 ($R_h \sim 0.5–2$ nm); these are confined to one amorphous/crystalline
lamella. The second peak or shoulder (denoted Ap2) are longer amylopectin branches with
DPs ranging from 30 to 99 ($R_h \sim 2–4$ nm), which span more than one crystalline lamella. The
amylose CLDs have DPs ranging from 100 to 20000 and an $R_h$ ranging from 4 to 300 nm. As
seen elsewhere (Syahariza et al., 2013; Wang, Hasjim, Wu, Henry & Gilbert, 2014; Ward,
Gao, de Bruyn, Gilbert & Fitzgerald, 2006), there are significant differences in these Am
peaks between different rice varieties. These differences are probably due to differences in
potentially discrete enzymatic processes in plant starch biosynthesis.
To compare the fine structure of the various starches, in addition to fitting with the Wu-Gilbert model (which only is applicable to Ap), a set of empirical parameters was used as defined previously (Syahariza et al., 2013). These are the DP at the maximum of each peak, donated $X_{Ap1}$, $X_{Ap2}$, and $X_{Am}$, and the height ratio of each maximum relative to that of Ap1, $h_{Ap2/Ap1}$ and $h_{Am/Ap1}$. The DP at the maximum of each peak reflects the relative size of chains in each group of branches, while the height ratio of each peak maximum relative to Ap1 represents the relative amount of chains in each group of branches. Because of SEC band broadening (Gilbert et al., 2013), the two peaks/shoulders from the shorter and longer amylopectin branches and the two peaks from the longer amylopectin branches and the shorter amylose branches overlap. To gain more information on differences in the amylose fine structure between samples and its responsible properties, the $X$ range of amylose is further subdivided into 3 different fractions, $100 \leq X < 1000$, $1000 \leq X < 2000$, and $2000 \leq X < 20000$. The percentage of the AUC for each fraction was also calculated (Vilaplana et al., 2012).

Amylose is synthesized through the Waxy ($Wx$) gene, which encodes granule-bound starch synthase. Different haplotypes of the $Wx$ gene are defined by single nucleotide polymorphisms (SNPs) at exon 1 and 6, which affect the amount of amylose accumulated (Chen, Bergman, Pinson & Fjellstrom, 2008). Waxy varieties contain a duplication in exon 2 of the $Wx$ that completely disables transcription, so waxy varieties produce no amylose (Wanchana et al., 2003). The varieties used in the present paper have previously been genotyped at the $Wx$ locus (Calingacion et al., 2014). As shown in Table 2, all rice varieties containing amylose can be divided into 3 categories which agree with the $Wx$ haplotype, defined by functional SNPs at exons 1 and 6 of the $Wx$: low-amylose rice which all contain T at exon 1 (TJ, PRD, SMG, LG, GW, KG, V8, and KM amylose content~0-19%); one variety, BM, with haplotype G-C of the $Wx$ gene with intermediate amylose (amylose content~20-25%); and high amylose rice, with $Wx$ haplotype G-A (SLG, and SN, amylose content >25%). There are significant structural differences between these 3 categories of rice. Compared to $X_{Ap1}$ and $X_{Ap2}$, $X_{Am}$, which measures the DP at the peak maximum, varies much more
significantly (Table 2). For rice varieties with intermediate and high amylose content, and with G at exon 1 of the Wx gene, XAm tends to be smaller than for those with low amylose and T at exon 1 of the Wx. This could indicate that rice with a functional allele of Wx contains more short branches. It would be interesting to explore whether this is a characteristic of all high amylose rices, which could provide insight into functional differences between the Wx haplotypes.

The amylopectin number CLDs (Fig. 2B) were fitted with the amylopectin biosynthesis model (Wu et al., 2013b), with all the features reproduced well in the fitted number CLDs for all rice samples (see Figure S1 of the Supporting Information). The model provides information on the activities of the core starch-synthesizing enzymes and gives insights into starch biosynthesis (Wang et al., 2014). As shown in Fig. 2C, the group of amylopectin chains of X<34, which are confined to one crystalline lamella (single-lamella), was dominated by enzyme set 1, while enzyme set 2 dominated DPs in the range between 34 and 70, which are trans-lamellar branches that span one crystalline lamella and the adjacent amorphous lamella. Correspondingly, enzyme set 3 was largely responsible for synthesizing the branches from DP 70 to 100 (Wu et al., 2013b). From the model fitting, three \( \beta \) values \( (\beta_{(i)}, \beta_{(ii)}, \text{and } \beta_{(iii)}) \), each representing the relative activity of SBE to SS within each enzyme set, and another set of parameters \( h_{2/1} \) and \( h_{3/1} \) reflecting the relative contributions of enzyme sets 2 and 3 to that of enzyme set 1 were obtained. As shown in the “model fitting” section of Table 2, the \( \beta \) values of rice starches between different rice varieties were not significantly different, while \( \beta_{(ii)}, \beta_{(iii)}, h_{2/1}, \text{and } h_{3/1} \) differed significantly. This indicated that the effects of enzyme sets 2 and 3 on the number CLDs are more significant than those of enzyme set 1, suggesting that the differences in the proportion of longer amylopectin branches between all starch samples, as observed from the SEC weight CLDs (Fig. 2A), are mainly due to the differences in the reaction rates of enzyme sets 2 and 3. As indicated from Table 2, high and intermediate amylose rices tend to have higher values of \( h_{2/1} \) and \( h_{3/1} \), and smaller values of \( \beta_{(ii)} \) and \( \beta_{(iii)} \), suggesting that enzyme sets 2 and 3 have a lower SBE activity, and/or a higher SS activity, consequently causing a higher proportion of long amylopectin branches. These
three varieties are known to carry haplotype 1 of SSIIa, (G/G/GC), which is a more active form of the enzyme ( Cuevas et al., 2010a ), therefore suggesting that SS activity explains the values. This method of obtaining statistically useful information by fitting to the biosynthesis-based model is very much to be preferred over the older method of dividing the CLD into arbitrarily chosen DP ranges and using the proportions of each; this older method is empirical, and different results can be obtained if different ranges are chosen.

3.3. Textural properties of cooked rice grains

During cooking, rice granules absorb water and swell to much more than their original size. This granule expansion causes ruptures in the grain, leading to a decrease in the hardness. Furthermore there is well-documented evidence that amylose and amyllopectin molecules leach into the surrounding water above the gelatinization temperature ( Cuevas, Gilbert & Fitzgerald, 2010b ). These leached amylose and amyllopectin molecules are likely to contribute to the stickiness of cooked rice ( Leelayuthsoontorn et al., 2006 ).

In this study, all rice varieties are cooked in the same rice/water ratio to avoid the effect of water content on the textural properties of cooked rice, as it has been shown that greater amounts of water will decrease the rice’s hardness ( Bett-Garber, Champagne, Ingram & McClung, 2007 ). As shown in Fig. 3A and 3B, cooked rice grains from different rice varieties exhibit significant differences in their hardness and stickiness. It is noteworthy that, for these rice varieties, hardness is negatively correlated with stickiness ( Fig. 3C ). Juliano et al. (1981) measured the texture of 10 milled cooked rices using instrumental methods from 11 laboratories. They also found that hardness showed significant negative correlation with stickiness, showing that hardness was positively correlated with amylose content, whereas stickiness was negatively correlated with amylose content ( Juliano et al., 1981 ). This is consistent with other reports ( Cameron et al., 2005 ; Patindol et al., 2010 ).

3.4. Structure - texture relations

The coefficients from Pearson’s and Spearman’s rank correlation tests between the textural properties (hardness and stickiness) and the starch structural parameters of all samples are
summarized in Table 3. Pearson’s correlation test reflects linear correlations, while Spearman’s rank correlation test is able to detect non-linear correlations. The correlations of rice samples with similar amylose content (rice category with low amylose (PRD, SMG, LG, GW, KG, V8, and KM)) are also presented in Table 3 to demonstrate statistically significant differences in the correlations when a narrow range of Am contents was used.

The influence of starch fine structural features on the texture of cooked rice was also investigated. This is the first such examination of these effects, especially in regards to the fine structure of amylose. Among these starch structural parameters, eleven independent structural variables were used to describe the fine molecular structure of whole and debranched starch. These were: $R_h$ at the Am peak maximum; the $R_h$ of the Am component; the height ratio of amylose to amylopectin peak, $h_{\text{Am/Ap}}$ in the SEC weight distributions of whole starch; three branch-chain lengths ($X_{\text{Ap1}}$, $X_{\text{Ap2}}$, and $X_{\text{Am}}$); two height ratios ($h_{\text{Ap2/Ap1}}$ and $h_{\text{Am/Ap1}}$) of the peak maxima of debranched starch; and the proportions of chains in the three subdivided sections of the CLDs (100≤$X$<1000, 1000≤$X$<2000, and 2000≤$X$<20000). Five model fitting parameters were used to describe the structure of amylopectin branches from the insights of starch biosynthesis: three enzymatic activity ratios of SBE/SS ($\beta_{(i)}$, $\beta_{(ii)}$ and $\beta_{(iii)}$), and two relative contributions of enzyme sets 2 and 3 to enzyme set 1 ($h_{2/1}$ and $h_{3/1}$); details of the fitting are given in Figure S1 of the Supplementary Data.

Among these structural parameters, $h_{\text{Am/Ap1}}$, 100≤$X$<1000, 1000≤$X$<2000, 2000≤$X$<20000 and $h_{\text{Am/Ap}}$ are all directly related to the amylose content. For the correlation of all rice samples, all of these parameters, along with the amylose content, show similar and significant positive correlations with hardness and negative correlations with stickiness. This is consistent with past conclusions that found that the amylose content is the most important determinant of rice textural quality (Juliano et al., 1972). Additionally, both Pearson and Spearman correlation tests show that the parameters of 100≤$X$<1000 and 1000≤$X$<2000 have higher correlation coefficients, especially for 1000≤$X$<2000. This indicates that rices with higher amylose contents, especially higher proportions of Am branches ranging from 1000 to 2000 DP, yield harder texture after cooking. Correspondingly, the parameters $\beta_{(i)}$, $\beta_{(ii)}$, $\beta_{(iii)}$. 
$h_{2/1}$ and $h_{3/1}$ represent the content of amylopectin chains. Both $\beta_{(ii)}$ and $\beta_{(iii)}$ significantly and positively correlated with stickiness while $h_{3/1}$ showed strong and negative correlation with stickiness, indicating that rices with more Ap short chains and less Ap trans-lamella chains tend to be more sticky. As expected, $h_{3/1}$, reflecting the proportion of long trans-lamella chains with DP $70 \leq X < 100$, shows a significant and positive correlation with hardness, which is also consistent with other reports (Ong & Blanshard, 1995). On the other hand, $R_h$ at the Am peak maximum and $\bar{R}_h$ of the amylose region are both parameters reflecting the molecular sizes of whole amylose molecules. As summarized in Table 3, the amylose molecular size is significantly and negatively correlated with hardness and positively correlated with stickiness. Because the amylose content correlates so strongly with the texture and structure of cooked rice, many of the observed correlations may simply be due to amylose content. Therefore in order to find correlations that are independent of the amylose content, 7 varieties with similar amylose contents were selected from all of the varieties and statistically re-analyzed using Pearson and Spearman correlation tests.

For rice samples with similar amylose contents, as expected there was no significant correlation between the texture of the cooked rice and the amylose content (Table 3). However, the whole amylose molecular size parameters ($R_h$ at Am peak maximum and Am $\bar{R}_h$) and the proportions of amylose branches ranging between 1000 and 2000 DP still correlated significantly with hardness (Table 3). This indicates that, independent of the amylose content, rice varieties with higher proportions of amylose branches ranging from 1000 to 2000 and with smaller whole amylose molecules are harder. Furthermore, although the stickiness of these rice samples with similar amylose content was significantly different (Fig. 3B), there was no significant correlation between stickiness and any of the structural parameters (Table 3). These new understandings of the fine structure of amylose content pave the way for a much deeper understanding of the important properties of rice, such as gel consistency, they offer new and significant phenotypes for understanding the eating quality of rice, and they could enable scientists to unravel the genetic and biochemical pathways that lead to high quality rice.
4. Conclusions

This study gives a new perspective on the relationship between the fine structure of amylose and amylopectin and the texture of cooked rice. The correlations found here support past studies that have found the Am content to be important for the texture of cooked rice. Our study also shows, for the first time, that the whole amylose molecular size and the proportion of amylose branches ranging from 1000 to 2000 DP have significant effects on the hardness of cooked rice. A smaller amylose molecular size and a higher proportion of amylose branches with DP from 1000 to 2000 were found in the varieties with intermediate and high amylose, and these also led to an increase in hardness. How these structural features affect amylose leaching during cooking, and/or the degree of starch granule swelling during heating, may help explain the mechanism for this increase in hardness. Additionally, the amylopectin content and short chains of Ap are significantly and positively correlated with the stickiness of cooked rice samples with a wide range of amylose content. This study provides valuable information for further research to progress our understanding of (i) the relationship between the fine structure of starch and the sensory properties of rice, and (ii) the genetic regulation of the starch biosynthetic pathway.

Acknowledgements

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References


Figure captions

Figure 1: SEC weight distributions of whole starch, \(w_{wh}(\log R_h)\), extracted from all rice grain samples, and normalized to the amylopectin peak.

Figure 2: (A) SEC weight and (B) number CLDs of debranched rice starches. All distributions are normalized to the highest amylopectin peak. (C) Plot of the experimental results from SEC (in blue) and the model fitting (in red) of number CLD of amylopectin branches for sample HMN. The total CLD, \(N_{de}(X)\), is the sum of the components from enzyme sets 1 and 2 (green and purple, respectively); note that the plot has a logarithmic scale.

Figure 3: A) Hardness of all rice varieties; B) Stickiness of all rice varieties; C) Scatter plot between hardness and stickiness with a significant coefficient of -0.753 (\(p<0.05\)). Different letters above the column represent the significant difference with \(p<0.05\).
Table captions

Table 1: Chemical composition of rice samples

Table 2: Starch molecular parameters extracted from SEC and model fitting parameters for all rice samples

Table 3: Correlation coefficients between textural properties (hardness and stickiness) and the structural attributes
Figure 1

SEC weight distributions of whole starch, $w_{br}(\log R_h)$, extracted from all rice grain samples, and normalized to the amylopectin peak.
(A) SEC weight and (B) number CLDs of debranched rice starches. All distributions are normalized to the highest amylopectin peak. (C) Plot of the experimental results from SEC (in blue) and the model fitting (in red) of number CLD of amylopectin branches for sample HMN. The total CLD, Nde(X), is the sum of the components from enzyme sets 1 and 2 (green and purple, respectively); note that the plot has a logarithmic scale.
Figure 3

A) Hardness of all rice varieties; B) Stickiness of all rice varieties; C) Scatter plot between hardness and stickiness with a significant coefficient of -0.753 ($p<0.05$). Different letters above the column represent the significant difference with $p<0.05$. 
Table 1

Chemical composition of rice samples*

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Abbreviation code</th>
<th>Sample collection</th>
<th>Country of origin</th>
<th>Total starch (%)</th>
<th>Total protein (%)</th>
<th>Total lipid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hom Mali Niaow</td>
<td>HMN</td>
<td>Lab collection</td>
<td>Australia</td>
<td>81.1±0.4b</td>
<td>8.4±0.1b</td>
<td>0.3±0.0a.c</td>
</tr>
<tr>
<td>Tailand Jasmine</td>
<td>TJ</td>
<td>Supermaket</td>
<td>Thailand</td>
<td>81.1±1.4b</td>
<td>6.9±0.0b</td>
<td>0.9±0.2f</td>
</tr>
<tr>
<td>Kangaroo</td>
<td>KG</td>
<td>Lab collection</td>
<td>Australia</td>
<td>81.2±1.3b</td>
<td>7.3±0.0d</td>
<td>0.7±0.0d.e</td>
</tr>
<tr>
<td>Phka Rum Duol</td>
<td>PRD</td>
<td>Lab collection</td>
<td>Australia</td>
<td>78.0±1.1a</td>
<td>9.4±0.0f</td>
<td>0.2±0.0a</td>
</tr>
<tr>
<td>Kyeema</td>
<td>KM</td>
<td>Lab collection</td>
<td>Australia</td>
<td>81.1±1.4b</td>
<td>8.2±0.0e</td>
<td>0.8±0.0e.f</td>
</tr>
<tr>
<td>LanGI</td>
<td>LG</td>
<td>Lab collection</td>
<td>Australia</td>
<td>80.7±1.0b</td>
<td>8.2±0.0e</td>
<td>0.5±0.0b.d</td>
</tr>
<tr>
<td>Sunrice Medium Grain</td>
<td>SMG</td>
<td>Supermaket</td>
<td>Australia</td>
<td>82.9±0.2b.c</td>
<td>7.0±0.0b</td>
<td>0.3±0.1a.b</td>
</tr>
<tr>
<td>Golden way</td>
<td>GW</td>
<td>Lab collection</td>
<td>Australia</td>
<td>85.7±0.5c</td>
<td>7.2±0.0b.c</td>
<td>0.6±0.0d.e</td>
</tr>
<tr>
<td>Viet 8</td>
<td>V8</td>
<td>Lab collection</td>
<td>Australia</td>
<td>79.0±1.1b</td>
<td>7.4±0.1d</td>
<td>0.5±0.1b.d</td>
</tr>
<tr>
<td>Basmati</td>
<td>BM</td>
<td>Supermaket</td>
<td>India</td>
<td>79.0±1.2b</td>
<td>8.3±0.1a.f</td>
<td>0.3±0.1a.c</td>
</tr>
<tr>
<td>Sunrice Long grain</td>
<td>SLG</td>
<td>Supermaket</td>
<td>Thailand</td>
<td>86.1±1.3c</td>
<td>6.5±0.1a</td>
<td>0.5±0.1c.d</td>
</tr>
<tr>
<td>Swarna</td>
<td>SN</td>
<td>Lab collection</td>
<td>India</td>
<td>79.7±0.9a.b</td>
<td>8.6±0.0f</td>
<td>0.6±0.1d.e</td>
</tr>
</tbody>
</table>

*Mean ±SD is calculated from duplicates. Values with different letters in the same column are significantly different with p < 0.05.
Table 2

Starch molecular parameters extracted from SEC and model fitting parameters for all rice samples.*

<table>
<thead>
<tr>
<th>Rice varieties</th>
<th>Branched starch parameters</th>
<th>Debranched starch parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_d$/nm at Am peak maximum</td>
<td>$R_d$/nm at Ap peak maximum</td>
</tr>
<tr>
<td></td>
<td>Average $R_d$/nm for Am</td>
<td></td>
</tr>
<tr>
<td>HMN</td>
<td>4.92 ± 0.01 $^a$</td>
<td>41.2 ± 2.2 $^f$</td>
</tr>
<tr>
<td>TJ</td>
<td>4.43 ± 0.10 $^a$</td>
<td>27.6 ± 1.5 $^c$</td>
</tr>
<tr>
<td>KG</td>
<td>4.34 ± 0.00 $^a$</td>
<td>24.0 ± 0.2 $^{c,e}$</td>
</tr>
<tr>
<td>PRD</td>
<td>4.62 ± 0.06 $^a$</td>
<td>27.8 ± 0.8 $^e$</td>
</tr>
<tr>
<td>KM</td>
<td>4.61 ± 0.33 $^a$</td>
<td>24.6 ± 1.6 $^{c,e}$</td>
</tr>
<tr>
<td>LG</td>
<td>4.89 ± 0.07 $^a$</td>
<td>23.8 ± 0.2 $^{b,c}$</td>
</tr>
<tr>
<td>SMG</td>
<td>4.63 ± 0.17 $^a$</td>
<td>25.1 ± 0.7 $^{d,e}$</td>
</tr>
<tr>
<td>GW</td>
<td>4.86 ± 0.12 $^a$</td>
<td>22.8 ± 1.3 $^{a-d}$</td>
</tr>
<tr>
<td>V8</td>
<td>4.69 ± 0.41 $^a$</td>
<td>20.8 ± 0.7 $^{a-d}$</td>
</tr>
<tr>
<td>BM</td>
<td>4.65 ± 0.11 $^a$</td>
<td>20.4 ± 0.9 $^{a-c}$</td>
</tr>
<tr>
<td>SLG</td>
<td>4.57 ± 0.30 $^a$</td>
<td>18.8 ± 0.7 $^a$</td>
</tr>
<tr>
<td>SN</td>
<td>4.50 ± 0.00 $^a$</td>
<td>19.6 ± 0.0 $^{a,b}$</td>
</tr>
</tbody>
</table>

* Values with different superscript letters indicate significant differences.
<table>
<thead>
<tr>
<th>Amylose content</th>
<th>DP (100-20000)</th>
<th>Model fitting parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100&lt;X&lt;1000</td>
<td>1000&lt;X&lt;2000</td>
</tr>
<tr>
<td>1.40 ± 0.01</td>
<td>0.85 ± 0.30</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>13.34 ± 0.01</td>
<td>5.02 ± 0.54</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>18.21 ± 0.00</td>
<td>6.25 ± 0.02</td>
<td>1.42 ± 0.05</td>
</tr>
<tr>
<td>18.38 ± 0.00</td>
<td>5.82 ± 0.25</td>
<td>1.40 ± 0.02</td>
</tr>
<tr>
<td>18.99 ± 0.02</td>
<td>6.76 ± 1.09</td>
<td>1.32 ± 0.04</td>
</tr>
<tr>
<td>20.18 ± 0.00</td>
<td>6.46 ± 0.24</td>
<td>1.67 ± 0.00</td>
</tr>
<tr>
<td>20.94 ± 0.01</td>
<td>6.71 ± 0.37</td>
<td>1.70 ± 0.09</td>
</tr>
<tr>
<td>21.78 ± 0.01</td>
<td>7.34 ± 0.58</td>
<td>1.76 ± 0.06</td>
</tr>
<tr>
<td>21.88 ± 0.01</td>
<td>7.31 ± 0.42</td>
<td>1.84 ± 0.01</td>
</tr>
<tr>
<td>24.95 ± 0.00</td>
<td>9.03 ± 0.44</td>
<td>2.09 ± 0.00</td>
</tr>
<tr>
<td>29.94 ± 0.00</td>
<td>13.52 ± 0.38</td>
<td>2.43 ± 0.05</td>
</tr>
<tr>
<td>29.45 ± 0.01</td>
<td>12.53 ± 0.20</td>
<td>2.41 ± 0.03</td>
</tr>
</tbody>
</table>

Mean ± SD is calculated from duplicate measurements. Values with different letters in the same column are significantly different with p < 0.05.
Table 3 Correlation coefficients between textural properties (hardness and stickiness) and the structural attributes.

<table>
<thead>
<tr>
<th>Structureal attributes</th>
<th>All rice samples</th>
<th>Rice samples with similar amylose content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson</td>
<td>Spearman</td>
</tr>
<tr>
<td></td>
<td>Hardness</td>
<td>Stickiness</td>
</tr>
<tr>
<td>Grain composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch (%)</td>
<td>0.295</td>
<td>0.109</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>-0.473</td>
<td>0.059</td>
</tr>
<tr>
<td>Lipid</td>
<td>0.316</td>
<td>0.052</td>
</tr>
<tr>
<td>Fine starch molecular structures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Am content</td>
<td>0.811**</td>
<td>-0.905**</td>
</tr>
<tr>
<td>X_{AP1}</td>
<td>0.184</td>
<td>0.044</td>
</tr>
<tr>
<td>X_{AP2}</td>
<td>-0.209</td>
<td>0.462</td>
</tr>
<tr>
<td>X_{Am}</td>
<td>-0.47</td>
<td>0.241</td>
</tr>
<tr>
<td>h_{Ap2}/h_{Ap1}</td>
<td>0.15</td>
<td>0.269</td>
</tr>
<tr>
<td>h_{Am}/h_{Ap1}</td>
<td>0.718*</td>
<td>-0.800**</td>
</tr>
<tr>
<td>h_{2/1}</td>
<td>0.188</td>
<td>-0.025</td>
</tr>
<tr>
<td>h_{3/1}</td>
<td>0.695*</td>
<td>-0.689*</td>
</tr>
<tr>
<td>β_{(i)}</td>
<td>-0.001</td>
<td>-0.256</td>
</tr>
<tr>
<td>β_{(ii)}</td>
<td>-0.738**</td>
<td>0.720**</td>
</tr>
<tr>
<td>β_{(iii)}</td>
<td>-0.654*</td>
<td>0.821**</td>
</tr>
<tr>
<td>100&lt;X&lt;1000</td>
<td>0.817**</td>
<td>-0.857**</td>
</tr>
<tr>
<td>1000&lt;X&lt;2000</td>
<td>0.820**</td>
<td>-0.922**</td>
</tr>
<tr>
<td>2000&lt;X&lt;20000</td>
<td>0.605*</td>
<td>-0.785**</td>
</tr>
<tr>
<td>Whole starch molecular structures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB</td>
<td>-0.427</td>
<td>0.279</td>
</tr>
<tr>
<td>R_{n/m} at Am peak maximum</td>
<td>-0.843**</td>
<td>0.892**</td>
</tr>
<tr>
<td>Average Am R_{n/m}</td>
<td>-0.878**</td>
<td>0.827**</td>
</tr>
<tr>
<td>h_{Am}/Ap</td>
<td>0.756**</td>
<td>-0.721**</td>
</tr>
</tbody>
</table>

* Correlations are significant at $p < 0.05$; ** Correlations are significant at $p < 0.01$. 
Highlights:

1. Starch fine structures were measured by size-exclusion chromatography
2. Amylopectin chain-length distribution was fitted in a biosynthesis model
3. Hardness and stickiness of cooked rice grains were tested by texture analyzer
4. Statistical correlation found between structural characteristics and rice texture