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The importance of amylose and amylopectin fine structures for starch digestibility in cooked rice grains

Z.A. Syahariza [a,b], Seila Sar [a], Jovin Hasjim [a], Morgan J. Tizzotti [a], Robert G. Gilbert [a,*]

[a] The University of Queensland, Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD 4072, Australia
[b] School of Industrial Technology, Universiti Sains Malaysia, Gelugor, Penang 11800, Malaysia

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ABSTRACT

Statistically and causally meaningful relationships are established between starch molecular structures (obtained by size-exclusion chromatography, proton NMR and multiple-angle laser light scattering) and digestibility of cooked rice grains (measured by *in vitro* digestion). Significant correlations are observed between starch digestion rate and molecular structural characteristics, including fine structures of the distributions of branch (chain) lengths in both amylose and amylopectin. The *in vitro* digestion rate tends to increase with longer amylose branches and smaller ratios of long amylopectin and long amylose branches to short amylopectin branches, although the statistical analyses show that further data are needed to establish this unambiguously. These new relationships between fine starch structural features and digestibility of cooked rice grains are mechanistically reasonable, but suggestive rather than statistically definitive.

1. Introduction

Rice (*Oryza sativa* L.) is one of the mostly grown food crops in the world and an important staple food for more than half of the world population. A better understanding on the digestibility of starch in cooked rice grains is important because of the rapid rise of diet-related health complications, particularly obesity, type 2 diabetes, and colorectal cancers. Foods containing starch that is slowly digested to glucose, and where significant quantities reach the lower gut ("resistant starch"), can mitigate, and also prolong the onset of, these diseases.

Starch structure has a bearing on starch digestibility. Starch comprises two types of molecules: amylopectin (Ap) and amylose (Am). Ap is a group of highly branched glucose polymers with a vast number of short branches and molecular weight $\sim 10^{7-8}$, whereas Am has a smaller molecular weight ($\sim 10^{5-6}$) with few long branches. Several studies have demonstrated that the digestibility of rice starch (both isolated starch and that in the grains) is associated with Am content (Chung, Liu, Wang, Yin, & Li, 2010; Frei, Siddhuraju, & Becker, 2003; Zhu, Liu, Wilson, Gu, & Shi, 2011). These studies used rice starches with wide ranges of Am contents (e.g. 1.7% to 55.4% Am), allowing the relationship between starch digestibility and Am content to be easily observed, but not the fine structures; there do not seem to be any literature data on the effects of Am fine structures (branching structure and molecular size) on starch digestibility. The digestibility of starch in grains may also be affected by non-starch components, such as protein and cell-wall matrices which can entrap starch granules, and lipids which can form complex with Am.

The objective of this study is to obtain a mechanistic understanding of the relationship between starch structures and digestibility of cooked rice grains. Since the starch granular and crystalline structures are greatly disrupted by the cooking process, only grain composition and starch molecular structures will be considered here. The molecular structures are the molecular size distributions of individual branches (i.e. debranched starch) – generally termed the chain-length distribution (CLD) – of Am and Ap, the molecular size distributions of whole (fully branched) starch, degree of branching (DB) of starch molecules, and the average molecular size of whole starch. Structural characterisation was performed using size exclusion chromatography (SEC) with refractive index (RI) detection, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and offline multi-angle laser light scattering (MALLS) (i.e. without size separation). Offline MALLS analysis is necessary because shear scission of whole Ap molecules is unavoidable during SEC separation (Cave, Seabrook, Gidley, & Gilbert, 2009). The rice grain varieties chosen for the present study mainly have a narrow range of Am contents, allowing correlations with fine molecular structures that are normally concealed when other structural differences, such as Am content, are much more pronounced. Digestibility of cooked rice grains is determined from a common

* Corresponding author. Tel.: +61 7 3365 4809; fax: +61 7 3365 1188.
E-mail address: b.gilbert@uq.edu.au (R.G. Gilbert).

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in vitro method and the digestion profile is fitted to first-order kinetics to obtain a digestion rate coefficient. The results can be used to understand the importance of starch structures in determining various nutritional properties of cooked rice grains.

2. Materials and methods

2.1. Materials

Fourteen polished rice grain samples, which were four Malaysian and ten Cambodian rice varieties, were chosen based on the consumer popularity in both countries. The use of a large number of rice varieties is necessary to obtain the correlations between starch structures and digestibility that are statistically valid and reliable. Since the rice grain samples were collected from various locations in both countries, they allow the observation of the effects of different geographical and environmental factors on the starch structures and digestibility. The complete list of rice samples with their origins and suppliers is summarised in Table 1.

Dimethyl sulfoxide (DMSO, GR grade for analysis) was purchased from Merck Co. Inc. (Kilsyth, VIC, Australia). LiBr (ReagentPlus), DMSO-d_6 (99.5% atom D), and trifluoroacetic acid-d_1 (TFA-d_1, 99% atom D) were purchased from Sigma–Aldrich Pty Ltd. (Castle Hill, NSW, Australia). Total Starch (AA/AMG) assay kit and isoamylase from *Pseudomonas* sp. were purchased from Megazyme International Ltd. (Bray, Ireland). A series of pullulan standards with peak molecular weights from 342 to 2.35×10^6 were from Polymer Standards Service GmbH (PSS, Mainz, Germany). Other chemicals were reagent grade and used as received.

2.2. Cryogenic milling of rice grains

Rice grains were ground into flour using a cryogenic mill (Freezer/Mill 6850; SPEX, Metuchen, NJ) in a liquid nitrogen bath as the cryogenic medium. Ground rice flour was used to analyse the chemical composition of rice grains and to characterise the molecular structures of native starch in the rice grains. The mill was set to run at 10 s^{-1} and the milling process was carried out in 2 cycles of 5-min grinding with a 2-min re-cooling break in between. The resulting flour was sieved through a 250-μm screen and kept in a desiccator for subsequent analyses.

2.3. Composition of rice grains

The starch content of rice grains was analysed from the ground rice flour using the Megazyme Total Starch (AA/AMG) assay kit. The crude protein content of rice grains was calculated from the nitrogen content of ground rice flour, obtained using a LECO CNS2000 auto analyzer (LECO Corporation, St. Joseph, MI) (Jung et al., 2003), with a conversion factor of 5.95 (Breese, 1941). The crude lipid content was measured by Soxhlet extraction, following AOAC method 920.39C (AOAC, 2002). All measurements were performed in duplicate.

2.4. Starch extraction from rice grains

The extraction and dissolution of starch molecules from ground rice flour was performed following a method described elsewhere (Syahariza, Li, & Hasjim, 2010; Tran et al., 2011), which uses a combination of protease, sodium bisulfite, DMSO with 0.5% w/w LiBr (DMSO/LiBr), and ethanol solutions to completely dissolve starch molecules and remove non-starch components, i.e. proteins, lipids, and non-starch polysaccharides, with no or minimal degradation of starch molecules. This method is a better alternative to the starch extraction from rice grains using alkaline solution, as basic pH is a catalyst for starch hydrolysis, especially when heating and mixing are involved (Han & Lim, 2004; Kim, Huber, & Higley, 2006). The extracted starch in DMSO/LiBr solution was stored at room temperature for subsequent analysis by SEC and offline MALLS detector. For NMR analysis, DMSO-d_6 was used instead of DMSO/LiBr solution to dissolve the freeze-dried starch sample, and TFA-d_1 was added to the sample medium right before the NMR analysis to improve ^{1}H signals (Tizzotti, Sweedman, Tang, Schaefer, & Gilbert, 2011).

2.5. Molecular size distributions of whole branched and debranched starches and amylose content

The structure of extracted whole starch molecules was characterised using an Agilent 1100 Series SEC system (Agilent Technologies, Waldbronn, Germany) equipped with GRAM 30 and 3000 analytical columns (PSS) and an RI detector (RID-10A, Shimadzu Corp., Kyoto, Japan) following the method described elsewhere (Cave et al., 2009; Liu, Halley, & Gilbert, 2010). The structure of starch enzymatically debranched using isoamylase, as described elsewhere (Hasjim, Lavau, Gidley, & Gilbert, 2010; Tran et al., 2011), was also characterised using the same SEC system, but using GRAM 100 and 1000 analytical columns (PSS).

The molecular size distribution of whole starch was plotted as weight distribution, $w_{br}(\log R_h)$, against hydrodynamic volume, V_h (the separation parameter for SEC), or the equivalent hydrodynamic radius, R_h. For whole starch molecules, as for any branched

Table 1
Sources and chemical compositions of rice grain samples.*

Variety	Abbreviation code	Supplier/Province	Country of origin	Total starch (%)	Total crude protein (%)	Total crude lipid (%)
MR84	84	MARDI	Malaysia	81.4 ± 0.8^{a}	8.80 ± 0.6^{a,c}	0.34 ± 0.2^{b,c}
MRQ74	74	MARDI	Malaysia	77.6 ± 1.4^{a}	10.26 ± 0.3^{a}	0.16 ± 0.1^{c}
MR220	220	MARDI	Malaysia	79.1 ± 3.3^{a}	6.61 ± 0.1^{c,d}	0.26 ± 0.0^{b,c}
MR219	219	MARDI	Malaysia	76.3 ± 2.3^{a}	7.16 ± 0.1$^{a–d}$	0.43 ± 0.1^{b,c}
Phka Kagney	PK	CARDI	Cambodia	86.4 ± 2.8^{a}	7.28 ± 0.3$^{b–d}$	0.41 ± 0.2^{b,c}
Phka Malis	PM	CEDAC	Cambodia	81.7 ± 2.9^{a}	6.71 ± 0.9^{c,d}	0.54 ± 0.1^{b,c}
Neang Minh	NM	Battambang	Cambodia	79.1 ± 0.2^{a}	7.40 ± 0.0$^{b–d}$	0.39 ± 0.0^{b,c}
Neang Khon	NK	Battambang	Cambodia	81.5 ± 0.7^{a}	6.72 ± 0.1^{c,d}	0.24 ± 0.0^{b,c}
Somali	SM	Battambang	Cambodia	79.0 ± 1.5^{a}	9.13 ± 0.2^{a,c}	0.47 ± 0.1^{b,c}
Sen Pidoa	SP	Battambang	Cambodia	80.8 ± 0.4^{a}	8.95 ± 0.6^{a,c}	0.46 ± 0.1^{b,c}
IR66	IR66	Kampot	Cambodia	81.1 ± 4.0^{a}	6.08 ± 0.1^{d}	0.21 ± 0.0^{b,c}
CAR9	CAR9	Kampot	Cambodia	75.5 ± 1.6^{a}	9.33 ± 1.6^{a,b}	1.01 ± 0.0^{a}
Bei Katam	3K	Kampong Speu	Cambodia	82.5 ± 0. 3^{a}	8.06 ± 0.8$^{a–d}$	0.32 ± 0.2^{b,c}
Phka Rumduol	PR	CARDI	Cambodia	76.7 ± 8.1^{a}	8.03 ± 0.4$^{a–d}$	0.61 ± 0.1^{a,b}

MARDI, Malaysian Agricultural Research and Development Institute; CARDI, Cambodian Agricultural Research and Development Institute; CEDAC, Centre d'Etude et de Développement Agricole Cambodgien.

* Mean ± SD is calculated from duplicate measurements. Values with different letters in the same column are significantly different with $p < 0.05$.