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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) 100–20,000, 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.

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 analyzer (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 & Wang, 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, $\sim 10^{7-8}$, whereas Am has a smaller molecular weight ($\sim 10^{5-6}$) 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

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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, 2013). 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 analyzed 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 analyzer. 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 2.35×10^6 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 analyzer (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 (Syahariza, Li, & Hasjim, 2010; Tran et al., 2011). 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 et al., 2014). 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

Table 1
Chemical composition of rice samples.*

Varieties	Abbreviation code	Sample collection	Country of origin	Total starch (%)	Total protein (%)	Total lipid (%)
Hom Mali Niaow	HMN	Lab collection	Australia	$81.1 \pm 0.4^{a,b}$	8.4 ± 0.1^{f}	$0.3 \pm 0.0^{a-c}$
Thailand Jasmine	TJ	Supermaket	Thailand	$81.1 \pm 1.4^{a,b}$	6.9 ± 0.0^{b}	0.9 ± 0.2^{f}
Kangaroo	KG	Lab collection	Australia	$81.2 \pm 1.3^{a,b}$	$7.3 \pm 0.0^{c,d}$	$0.7 \pm 0.0^{d,e}$
Phka Rum Duol	PRD	Lab collection	Australia	78.0 ± 1.1^{a}	9.4 ± 0.0^{g}	0.2 ± 0.0^{a}
Kyeema	KM	Lab collection	Australia	$81.1 \pm 1.4^{a,b}$	8.2 ± 0.0^{e}	$0.8 \pm 0.0^{e,f}$
LanGI	LG	Lab collection	Australia	$80.7 \pm 1.0^{a,b}$	8.2 ± 0.0^{e}	$0.5 \pm 0.0^{b-d}$
Sunrice medium grain	SMG	Supermaket	Australia	$82.9 \pm 0.2^{b,c}$	7.0 ± 0.0^{b}	$0.3 \pm 0.1^{a,b}$
Golden way	GW	Lab collection	Australia	85.7 ± 0.5^{c}	$7.2 \pm 0.0^{b,c}$	$0.6 \pm 0.0^{d,e}$
Viet 8	V8	Lab collection	Australia	$79.0 \pm 1.1^{a,b}$	7.4 ± 0.1^{d}	$0.5 \pm 0.1^{b-d}$
Basmati	BM	Supermaket	India	$79.0 \pm 1.2^{a,b}$	$8.3 \pm 0.1^{e,f}$	$0.3 \pm 0.1^{a-c}$
Sunrice long grain	SLG	Supermaket	Thailand	86.1 ± 1.3^{c}	6.5 ± 0.1^{a}	$0.5 \pm 0.1^{c,d}$
Swarna	SN	Lab collection	India	$79.7 \pm 0.9^{a,b}$	8.6 ± 0.0^{f}	$0.6 \pm 0.1^{d,e}$

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