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Physicochemical properties and digestibility of endosperm starches in four indica rice mutants

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ABSTRACT

Structural, physicochemical characteristics and digestibility of endosperm starches from four mutants (GM01, 03–05) and their parent Guangluai 4 (GLA4) were characterized. GM05 had a little higher apparent amylose content (AAC) and showed little difference in starch and digestion properties from GLA4. However, GM03 and GM04 with increased amount of chalkiness and partial translucent region in the endosperm displayed a distinct starch and digestion properties, which had the RS of 7.9 and 8.4%, respectively. GM03 and GM04 had higher AAC (33–35%), lower degree of crystallinity, lower gelatinization temperature and pasting viscosities, and more amount of B1 (DP 12–24) and B2 (DP 25–36) chains and less amount of B3 chains (DP ≥ 37) in amylopectin. AAC and the amount of B1 chains had positive correlation with RS, but the amount of B3 chains had negative correlation with RS. The results of this study may be applied to design RS by selecting rice germplasm with high AAC and high amount of B1 chains (DP 12–24) of amylopectin.

1. Introduction

Rice (*Oryza sativa* L.) is one of most important crops in China. The eating quality is one of the most important traits that affect consumers' acceptability. However, nowadays, consumers are increasingly concerned about the health benefit of their foods. Starch is the most abundant storage reserve in rice grains. According to the rate and extent of its digestibility, starch is generally classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). Englyst, Vinoy, Englyst, and Lang (2003) reported that RDS content is positively correlated to the glycemic index (GI) of starchy foods. High SDS and RS content in foods may be beneficial to health (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996; Zhang & Hamaker, 2009) and hence, many efforts have been made to develop foods with high levels of SDS and RS which may confer foods with a low GI (Shu, Jia, Ye, Li, & Wu, 2009; Zhou et al., 2016; Zhu, Liu, Wilson, Gu, & Shi, 2011).

The RS content of rice varies with different rice genotypes. Bao, Zhou, Xu, He, and Park, (2017) reported that the RS content ranges from 0.3% to 2.42% among 105 rice accessions. Chen, Bergman, McClung, Everette, and Tabien (2017) evaluated 40 high amylose rice varieties for RS content in cooked rice and found the RS content ranging from 2.33% to 4.46%. Conventional breeding seems difficult to further increase the RS content by using the normal rice germplasm. However, mutation breeding has been successfully employed to isolate mutants high in RS content (Yang et al., 2006, 2011; Zhou et al., 2016). Amylose extender (*ae*) mutants or high resistant starch mutants have been isolated and their digestion properties have been characterized. For example, Yang et al. (2006) reported that the RS content in mutant RS111 in cooked form was 8.17%, whereas two normal rice R7954 and ZHONG9B had 3.09–2.79% of RS, respectively. The physicochemical properties in relation to the RS content have also been characterized (Kubo et al., 2010; Yang et al., 2006; Zhu et al., 2011). The high-RS mutant was characterized with significantly higher apparent amylose content (AAC) and crude lipid content, higher percentage of oval-shaped granules and bigger oval size, reduced paste viscosity, and low onset temperature, peak temperature, final temperature, enthalpy of gelatinization, and crystallinity (Yang et al., 2006). Kubo et al. (2010)

Abbreviations: AAC, apparent amylose content; ACL, average chain length of amylopectin; BD, breakdown viscosity; CLD, chain length distributions; CPV, cold paste viscosity; DP, degree of polymerization; FACE, fluorescence labeling for fluorophore-assisted capillary electrophoresis; HPV, hot paste viscosity; PV, peak viscosity; RC, Relative crystallinity; RDS, rapid digestible starch; RS, resistant starch; SB, setback viscosity; SDS, slowly digestible starch; SEC, size-exclusion chromatography; Tc, conclusion gelatinization temperature; To, onset gelatinization temperature; T_p, peak gelatinization temperature; △H, gelatinization enthalpy

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reported that *wx ae* double mutant displayed a higher pasting temperature and peak viscosity, and a slow increase of blood glucose in rats when fed with *wx ae* starch. However, the structural basis of mutant starch with high RS content is less understood.

We previously reported the physicochemical properties of two stable mutants isolated from a high amylose indica rice, Guangluai 4 (GLA4) by mutation breeding (Kong et al., 2014). We further surveyed the mutant population and identified additional four mutants which were defective in the grain size and starchy endosperm. It is widely accepted that most of genomic background are the same except for the defective genes between the mutants and their parent. Therefore, the mutants can be regarded as near isogenic lines of their parent. The objective of this study is to characterize the structural, physicochemical properties and starch digestion properties of four mutants isolated from GLA4, and to understand the structural basis of resistant starches in indica rice mutants.

2. Materials and methods

2.1. Materials

The indica rice Guangluai4 (GLA4) and its four endosperm and grain mutants were used in the study. GLA4 is a well-known high amylose indica rice cultivar in China. The four mutants were isolated after 60-Cobalt gamma-ray mutagenesis of GLA4 which had different grain size and chalky endosperm. The rice plants were grown under field conditions at the experimental station of Zhejiang University in Hangzhou, China, in June 2015. The seeds were harvested in late September.

2.2. Preparation of rice starch

Starch was extracted by using the alkaline steeping method according to the method described by Kong, Zhu, Sui, and Bao (2015).

2.3. Scanning electron microscopy (SEM)

Starch powders were mounted on the specimen stub and sputter coated with gold before viewing with an environmental scanning electron microscope (ESEM, Philips XL-3, Netherlands).

2.4. Apparent amylose content (AAC)

Determination of AAC was carried out using the iodine staining method (Bao, Shen, Sun, & Corke, 2006). The absorbance of the solution was measured at 620 nm against the blank solution using a spectrophotometer. AAC was calculated using a standard curve made from five rice samples with known AAC.

2.5. In vitro starch digestibility

The contents of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in rice starch samples were determined according to the procedure of Englyst method (Englyst et al., 1992) with slight modifications (Kong, Chen et al., 2015).

2.6. X-ray diffraction (XRD)

The X-ray patterns of starch were analyzed by an X-ray diffractometer (D5005, Siemens, Bruker AXS, Karlsruhe, Germany) operated with Cu-Kα radiation. The starch samples were scanned through 2θ ranging from 5 to 40° under an accelerating voltage and current of 30 kV and 30 mA conditions. Relative crystallinity (RC) was quantitatively calculated following the method of Nara and Komiya (1983) by using MDI-Jade 6.0 software.

2.7. Pasting viscosity

Rice pasting properties were determined using a Rapid Visco Analyser (RVA, Model 3-D, Newport Scientific, Warriewood, Australia). Starch (3 g, 12% m.b.) was mixed with 25 g of ddH_2O in the RVA sample can. The RVA was run using Thermocline for Windows software (Version 1.2). The Rice Method 1 program was used with heating and cooling cycle set as (1) holding at 50 °C for 1 min, (2) heated to 95 °C in 3.8 min, (3) holding at 95 °C for 2.5 min, (4) cooling to 50 °C in 3.8 min, (5) holding at 50 °C in 1.4 min. The RVA paddle speed was at 960 rpm for the first 10 s of the test, after which the speed was at 160 rpm. The peak (PV), hot paste (holding) (HPV), cool paste (final) (CPV) viscosities were recorded. The breakdown and setback viscosities were calculated by PV-HPV and CPV-PV, respectively. The viscosities were measured in Rapid Visco Units (RVU).

2.8. Thermal properties

Thermal properties were analyzed using a Differential Scanning Calorimeter (DSC) model Q20 (TA Instruments, Newcastle, DE, USA) equipped with DSC standard and dual sample cells. Rice starch (2.0 mg, d.b.) was weighed into an aluminum pan and 6 μL of distilled water was added. The pan was hermetically sealed and then heated at a rate of 10 °C/min from 30 °C to 110 °C. A sealed pan with 12 μL of distilled water was used as a reference. Onset (T_o), peak (T_p), conclusion (T_c) temperature, width at half peak height ($\Delta T_{1/2}$) and enthalpy (ΔH) of gelatinization were calculated by a TA Instruments Universal Analysis 2000 program, Version 4.4A (TA instruments, Newcastle, DE, USA).

2.9. Starch structure characterization using SEC

Native starch granules (about 6 mg) were dissolved in DMSO/LiBr solution and debranched using isoamylase in acetate buffer (pH 3.5), following the method of Li, Prakash, Nicholson, Fitzgerald, and Gilbert (2016). The weight distributions of debranched starch molecules were analyzed in duplicate using size-exclusion chromatography (SEC) (Agilent 1260 series, Agilent Technologies, USA) equipped with a refractive index detector (Optilab T-rEX, WYATT Corp., USA), and a differential pressure detector (Viscostar-II, WYATT Corp., USA) as described by Wang, Hasjim, Wu, Henry, and Gilbert (2014). The weight chain length distribution (CLD) of the debranched chains with degree of polymerization (DP) X, denoted by $w(\log \mathbf{X})$, obtained from the DRI signal, was plotted against X, which is related to the molecular weight (M) for starch by $M = 162.2(X - 1) + 18.0$ (where 162.2 is the molecular weight of the anhydroglucose monomeric unit and 18.0 is that of the additional water in the end groups).

2.10. Determination of amylopectin chain length distribution using FACE

The freeze-dried debranched starch was labelled using 8-aminopyrene-1,3,6, trisulfonic acid (APTS) following the method of Wang, Wambugu et al. (2015). The APTS labelled debranched starch molecules were separated using an N-CHO coated capillary (50 m diameter, 40 cm in length) in a PA-800 Plus System (Beckman-Coulter, Brea, CA, USA), coupled with a solid-state laser-induced fluorescence detector using an argon-ion laser as the excitation source. The molar-based chain-length distribution of the debranched amylopectin was reported.

2.11. Statistical analysis

All analyses were determined at least in duplicate. Analysis of variance (ANOVA) and correlation were conducted using SAS program version 8 (SAS Institute Inc., Cary, NC). The least significant difference (LSD) multiple range test was conducted for comparison of mean of samples at $p < 0.05$.