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Resistant starch: Variation among high amylose rice varieties and its relationship with apparent amylose content, pasting properties and cooking methods ☆

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ABSTRACT

Resistant starch (RS), which is not hydrolyzed in the small intestine, has proposed health benefits. We evaluated 40 high amylose rice varieties for RS content in cooked rice and a 1.9-fold difference was found. Some varieties had more than two-fold greater RS content than a US long-grain intermediate-amylose rice. The high amylose varieties were grouped into four classes according to paste viscosity and gelatinization temperature based on genetic variants of the *Waxy* and *Starch Synthase* IIa genes, respectively. RS content was not different between the four paste viscosity-gelatinization temperature classes. Multiple linear regression analysis showed that apparent amylose content and pasting temperature were strong predictors of RS within each class. Two cooking methods, fixed water-to-rice ratio/time and in excess-water/minimum-cook-time, were compared using six rice varieties that were extremes in RS in each of the genetic variant classes, no difference in RS content due to cooking method was observed.

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1. Introduction

Resistant starch (RS) is defined as a portion of starch that resists digestion by human pancreatic amylase and brush border glycosidases in the small intestine of healthy humans and reaches the colon becoming available for fermentation by the microbiota (Birt et al., 2013; Englyst, Kingman, & Cummings, 1992). Physiologically, RS is considered similar to soluble, fermentable dietary fiber. Its consumption, reportedly, improves gut health, adiposity and insulin resistance, and decreases cardiovascular disease risk factors (Keenan et al., 2015). Colon cancer risk is also lessoned with the consumption of RS (Birt et al., 2013), and was attributed to partially the production of butyrate, one of the short chain fatty acids produced from the fermentation of RS by, e.g. *Bifidobacterium* spp. in the colon (Hamaker & Tuncil, 2014). There are five types of RS (Birt et al., 2013). RS1 is starch in cell or tissue structures that is physically protected and is inaccessible for enzymatic hydrolysis in the small intestine. RS2 is found in raw starch granules that are relatively dehydrated and have a compact structure that limits digestive enzymes ability to access the starch. RS3 is retrograded starch, primarily formed from amylose that has leached from starch granules after hydration. RS3 from tuber, cereal and amylomaize starch have high melting temperatures and medium length polymers; on average 150 °C and 35 glucose units, respectively (Eerlingen & Delcour, 1995; Gidley et al., 1995). RS4 is chemically modified starch that resists enzymatic hydrolysis. RS5 is a helical amylose structure complexed with fatty acids that prevents amylase digestion (Birt et al., 2013).

Rice is consumed after cooking in water, thus the starch granules are hydrated prior to consumption. Consequently, the RS in

Abbreviations: AAC, apparent amylose content; ASV, alkali spreading value; BD, breakdown viscosity; GT, gelatinization temperature; HPV, hot-paste viscosity; PFCK, percent fully cooked kernels; PT, pasting temperature; PV, peak viscosity; RDP1, Rice Diversity Panel 1; RS, resistant starch; RVU, rapid viscosity unit; SB_HPV, setback viscosity from HPV (=CPV-HPV); SB_PV, setback viscosity from PV (=CPV-PV); SI, strong pasting properties and intermediate GT haplotype; SL, strong pasting properties and low GT haplotype; *SS*IIa, *starch synthase* IIa; WI, weak pasting properties and intermediate GT haplotype; WL, weak pasting properties and low GT haplotype.

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cooked rice is primarily the RS3 type, leached and retrograded amylose. The double helical structure of retrograded amylose losses its water binding capacity and does not fit into the binding site of amylase, thus resisting digestion (Birt et al., 2013). A portion of RS in cooked rice is attributed to amylose-lipid complexes, the RS5, since the V-type (the amylose-lipid complexes) along with B-type crystalline structure, were detected by X-ray diffraction analysis in RS isolated from the cooked rice (Shu et al., 2006). Potential RS1 in cooked rice might be present based on study by Tamura, Singh, Kaur, and Ogawa (2016), who detected the aleuron layers of the endosperm of the intact cooked rice acting as a structural barrier and slowing the starch digestibility.

Rice is considered low in RS. From the health point of view, further improvement of RS content in cooked rice is desirable. Based on the studies using AOAC Method 2002.02 for RS determination, cooked rice of wild type varieties contained RS ranging from 0.6% to 1.21% (Butardo et al., 2012; Yang et al., 2006). Multiple studies covering rice with amylose content (AAC) ranging from 0% to 28% reported that RS in cooked rice is positively correlated with AAC (Kong et al., 2015; Patindol, Guraya, Champagne, & McClung, 2010). These results suggest that to identify higher RS rice in the diverse rice accessions high-amylose types should be the focus.

Rice flour paste viscosity characteristics, as determined by the rapid visco analyzer (RVA), have been used to predict physicochemical and functional properties of rice and correlate with amylopectin structure (Bao, 2008; Chen, Bergman, Pinson, & Fjellstrom, 2008a; Horibata, Nakamoto, Fuwa, & Inouchi, 2004). Nakamura, Katsura, Kato, and Ohtsubo (2016) used RVA identifying viscosity traits, not AAC, associated with RS in a set of low/very low amylose *Japonica* rice varieties. Additionally, among high amylose rice varieties there were two types of paste viscosity as classified by the genetic variants in *Waxy* exon 10 (Chen, Bergman, Pinson, & Fjellstrom, 2008b) and two types of gelatinization temperature distinguished by genetic variants in *Starch Synthase* IIa (*SS*IIa) gene (Umemoto & Aoki, 2005) identified. How these genetic variants or functional traits relate to RS has not been determined.

There remains some controversy over the relationship between the RS content of rice and the cooking method used during preparation, as well as the correlation between RS content and various physicochemical properties. Studying three high amylose rice cultivars, Panlasigui et al. (1991) found that AAC and various physicochemical properties, such as gelatinization temperature, were associated digestibility and glycemic index. After adjusting the cooking method used based on each rice cultivar's physicochemical properties, these authors found no difference in digestibility or glycemic index. Yang et al. (2006) cooked rice using water to rice ratio ranging from 1.0 to 2.1 and found that increasing water to rice ratio increased RS content; yet raw milled rice has higher RS than the cooked rice. RS in raw milled rice is most likely composed of RS1, 2 and 5.

The objectives of this study were to investigate the variation of RS content in cooked rice of forty high amylose diverse rice varieties, and to determine the relationship of RS with paste viscosity parameters and physicochemical properties. Additionally, two commonly used cooking methods were compared to determine the impact on RS content among high amylose varieties.

2. Materials and methods

2.1. Rice varieties

Forty high amylose rice genotypes were used for this study. Thirty-nine of those were selected from two germplasm collections, the Rice Diversity Panel 1 (RDP1) (Eizenga et al., 2013) and the USDA rice core collection (Core) (Agrama et al., 2009). These selected genotypes were all classified as high amylose type based on genetics of *Waxy* gene and were among the highest AAC, chemically determined, from these two germplasm collections (Chen et al., 2008a; Eizenga et al., 2013; and unpublished data for the Core). Three US varieties, Bengal, Presidio, and Dixiebelle with low, intermediate and high AAC, respectively, were also included for comparison purposes. The ranges of AAC for low, intermediate, and high amylose classes were 10–19%, 20–24% and >24%, respectively (Bergman, Bhattacharya, & Ohtsubo, 2004).

These 42 rice varieties (40 high, 1 intermediate, and 1 low apparent amylose) were grown in 2014 and 2015 at Stuttgart, AR and 2015 at Beaumont, TX using non-replicated trials and common cultural management practices for these locations. Rough rice samples were harvested at approximately 20% moisture and dried to 12%. Whole milled kernels (head rice) were obtained from 100 g rough rice using a laboratory rice mill model PAZ-1 DTA (Zaccaria USA, Anna, TX), which removes the hulls/husks from rough rice first by rubber rolls, and then the bran layers are removed from the brown rice through a whitening step by friction between an abrasive ring and a rubber brake. Rice flour was obtained by grinding whole milled kernels in a Cyclone Sample Mill, which uses a high velocity air-flow, an abrasive tungsten carbide surface, and centrifugal forces to grind milled rice kernels, and then the ground flour is passed through a 0.5 mm screen (UDY Corp., Fort Collins, CO).

2.2. Cooking method

The cooking method used to prepare all rice accessions for RS determination was standardized based on the results achieved by determining the cooking time required for a sample of Presidio and a commercial rice sample (purchased in Stuttgart, AR). The rice to water ratio used was 1:2 (w:v) as described in Patindol et al. (2010). Five grams of milled rice and 10 mL of water were added to a 50-ml beaker. The beaker was covered with an aluminum foil cap and the rice was soaked for 15 min, at room temperature. After soaking, the rice was cooked by placing the beaker in a boiling water bath covered with a glass lid. Starting at 17 min and every minute thereafter, one beaker of rice was removed from the boiling water bath and allowed to cool for 5 min before pressing 10 cooked rice kernels between two pieces of glass. The minimum cooking time was determined when at least 9 out of 10 kernels losing its opaque central core and being fully translucent (Panlasigui et al., 1991). Both rice samples had a minimum cooking time of 20 min, consequently this was the time used for this studies' standard cooking method. The percent fully cooked kernels (PFCK) were determined by pressing 10 cooked rice kernels between two pieces of glass and recording number of kernels without opaque central core.

A second cooking method (Optimum) was performed on six rice varieties that were cooked in excess water using the variety's minimum cooking time. The minimum cooking time was determined as follows: 5 grams whole milled rice was poured into excess boiling water (200 ml) and 10 kernels were removed starting at 14 min and every minute thereafter; the minimum cooking time was determined when at least 9 out of 10 kernels had lost the opaque central core as described above. Then, according to the individual variety's minimum cooking time, 5 g of rice were cooked in excess boiling water (200 ml). The cooked rice was drained in a strainer and excess water was blotted with a paper towel before further analyses. The PFCK was determined as described above.

2.3. RS and total starch

The concentration of RS in cooked rice was determined via the AOAC Method 2002.02 (AOAC International, 2007) using the RS