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Alterations of starch structure lead to increased resistant starch of steamed rice: Identification of high resistant starch rice lines

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ABSTRACT

The development of low calorie foods to aid obesity control is a growing area of research. Among these, resistant starch (RS) in cereals has beneficial effects on reducing colon cancer rate and better fatty acid composition as well as lowering calories. Rice contains digestible starch and indigestible RS, the proportions of which are affected by starch biosynthetic enzymes. Rice lines that carry mutations in starch biosynthesis genes may have high levels of RS. In this study, percentage RS and non-digested component values were determined in steamed rice from several rice lines. These include several high-amylose indica rice cultivars, and lines carrying mutations in the starch synthase (SS) IIIa and/or branching enzyme (BE) IIb genes. High RS rice lines contained high levels of non-digested component. RS values $\leq$ 4% correlated with apparent amylose content. RS values of BEIIb-deficient mutant lines were particularly high (15 $\leq$ RS $\leq$ 35%), and were correlated with the amount of amylopectin long chains. Among rice lines used in this study, *be2b* mutant lines having large portions of amylopectin long chains were the best candidates for high RS rice foods.

1. Introduction

Resistant Starch (RS) is defined as the portion of starch or starch products that resist digestion as they pass through the small intestine (Englyst et al., 1993). The calorific value of RS is minimal due to its limited absorption as glucose in the small intestine. Foods with high RS content may therefore be of value in the prevention of diabetes. After RS reaches the large intestine, RS-like dietary fiber is digested and fermented by bacteria. This fermentation produces short-chain fatty acids that are thought to protect against large bowel cancer (Topping and Clifton, 2001) and activate G protein coupled receptor 41 (GPR41) system to increase energy expenditure (Hara et al., 2014).

Several methods are available for the estimation of RS levels in food; however, in all methods, digestible starch must first be removed using starch hydrolyzing enzymes. RS estimation by the Prosky method [Association of Official Analytical Chemists (AOAC) 985.29] uses gravimetric determination of dietary fiber after thermo-resistant α-amylase, protease, and glucoamylase digestion (Prosky et al., 1985). The McCleary method (AOAC 2009.01; McCleary and Monaghan, 2002), which is employed in a commercially available RS assay kit (Megazyme), is the official method of the American Association of Cereal Chemists (AACC) and the AOAC. Briefly, mashed food is digested by pancreatic α-amylase and amyloglucosidase at 37 °C for 16 h. Non-digestible starch is precipitated and subsequently solubilized using 2 M KOH. RS percentage can then be calculated from the RS and non-RS contents (Fig. 1).

It is thought that high-amylose starches exhibit high RS values as a result of their resistance to digestion and high retrogradation after cooking. Previously, we developed several rice lines with mutations that affected starch biosynthetic enzymes (Fujita, 2012, 2014). Starches in the mutant rice lines contained higher levels of amylose than those in standard rice cultivars, and the mutant lines might therefore be expected to contain fewer

Abbreviations: BE, starch branching enzyme; GBSS, granule-bound starch synthase; RS, resistant starch; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SS, starch synthase; WT, wild type.

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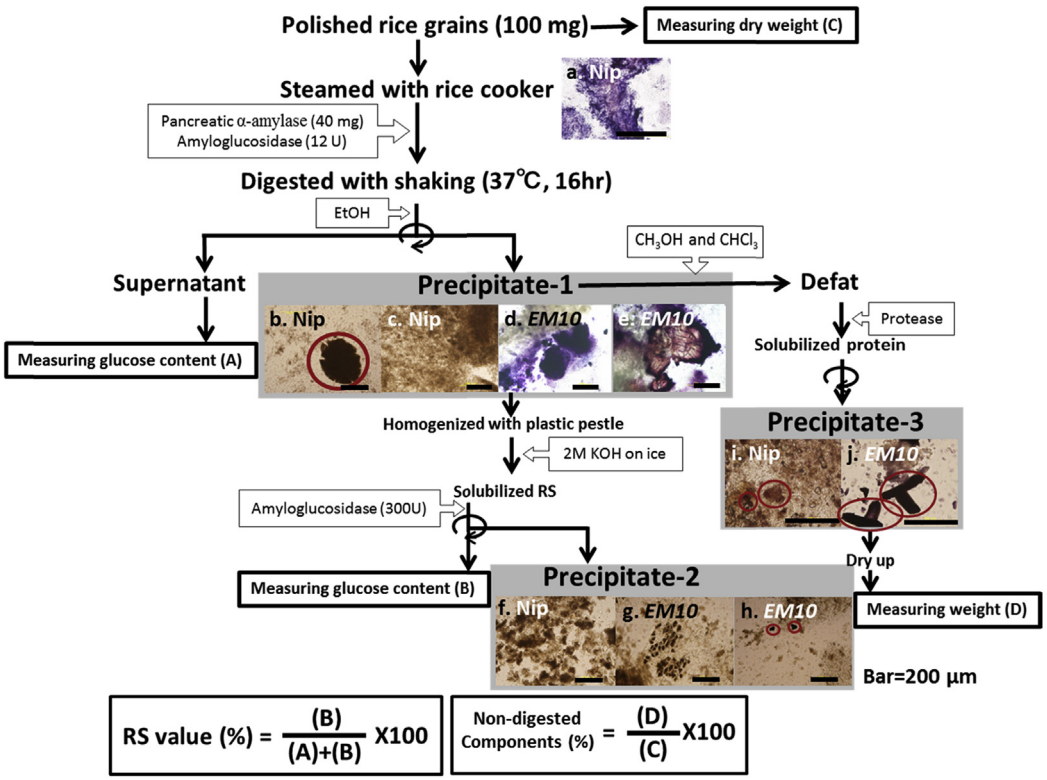

Fig. 1. Procedure of estimation of percentage RS and non-digested component values. Samples of precipitates-1–3 and steamed rice were iodine stained and examined by light microscopy (photographs a-h). Photographs a, b, c, f and i were from wild type (Nipponbare). Photographs d, e, g, h and j were from *be2b (EM10)*. Purple or red-purple (red circles) staining indicates starch (a) or RS (b, h, i and j). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

bioavailable calories. One of the rice mutant lines, deficient in starch synthase (SS) IIIa (*ss3a*) line, had elevated expression levels of granule-bound starch synthase (GBSSI), which synthesizes amylose and resulted in the accumulation of 1.5-fold higher amylose starch in the endosperm than in the wild type (Fujita et al., 2007). The branching enzyme (BE) IIb-deficient mutant (*be2b*) accumulated B-type crystalline starch (Tanaka et al., 2004; Abe et al., 2013) that displayed RS2 (ungelatinised resistant granules with B type crystallinity) characteristics similar to those of amylomaize in maize and potato starch (Brown et al., 1995). Furthermore, the proportions of amylopectin short-chain in *be2b* endosperm were significantly lower than those of the wild type. Finally, because amylopectin synthesis was limited, the apparent amylose content was higher in *be2b* than in the wild type (Nishi et al., 2001; Abe et al., 2014; Asai et al., 2014). Therefore these endosperm starches would also be expected to have high RS contents.

In this study, we modified a method to estimate RS and non-digested components of steamed rice using the Megazyme RS assay kit. As candidates for high RS containing rice lines, *ss3a*, *be2b*, and their double mutant lines as well as high-amylose indica rice cultivars were assessed. Finally, the relationship between RS values and starch structure was examined.

2. Experimental

2.1. Plant materials

Mutant rice lines were as follows: single mutants *ss3a* (*e1*; Fujita et al., 2007) and *be2b* (*EM10*; Nishi et al., 2001) and double mutants $ss1^{L}$/*ss3a* (*#6002*; Fujita et al., 2011; Hayashi et al., 2015), *ss3a/be1* (*#4012*; Fujita et al.,in preparation), *ss3a/ss4b* (*#2012*; Toyosawa et al., 2016), *ss3a/be2b* (*#4019*; Asai et al., 2014), and $ss1^{L}$/*be2b* (*#4017*; Abe et al., 2014). Nipponbare (Nip, japonica cultivar) was used as a wild-type control and Yumetoiro (Horibata et al., 2004), Kasalath, and Thai white rice, which express the Wx^{a} allele of *GBSSI*, were used as high-amylose comparator lines. The apparent amylose content of these lines is shown in Table 1.

2.2. Preparation of steamed rice

Approximately 100 mg of polished rice (5–7 grains) was washed twice with distilled water in a 15 mL test tube. After washing, 1.5 volumes of water (with respect to the dry weight of the rice grains) were added. Tubes were placed in 200 mL water and cooked using a normal program in a rice cooker (NS-WF10, Zojirushi, Japan).

2.3. Estimation of RS

RS of steamed rice was determined using an RS assay kit (Megazyme, Ireland), with some modifications. Briefly, pancreatic α-amylase and amyloglucosidase were added directly to steamed rice grains in 15 mL test tubes, and tubes were incubated at 37 °C for 16 h with shaking. After addition of ethanol and centrifugation, the supernatant was removed and the precipitate (precipitate-1, Fig. 1) was homogenized using a plastic pestle. To solubilize RS, 2 M KOH was added to the homogenized precipitate on ice. Sodium acetate buffer (1.2 M, pH 3.8) was added and incubated with