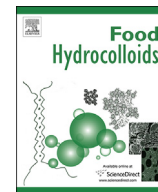




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## Changes in kernel morphology and starch properties of high-amylose brown rice during the cooking process

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## ABSTRACT

Rice is principally consumed as cooked kernels. A high-amylose rice line, TRS, has a potential to positively impact human health. Though the properties of starch isolated from TRS kernels have been studied, the properties of cooked kernel remain to be resolved. In this study, the changes in kernel morphology and starch properties of brown TRS were investigated during cooking in boiling water. TRS kernel, which had polygonal, aggregate, elongated, and interior hollow starches from the inner to the outer of endosperm, showed different gelatinization properties during cooking. The polygonal starch was gelatinized most completely and fastest, the aggregate starch was partly gelatinized, and the elongated and interior hollow starches kept almost their intact status. The molecular weight distribution of starch in kernel did not show significant change, but starch crystalline structure was not wholly disrupted during the cooking process. The starch in cooked kernel had a very high resistance to digestion.

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### 1. Introduction

Cereal endosperm starch is a major source of nourishment for humans, and consists of two main components: amylose and amylopectin. The amylose content has a pronounced effect on the properties and applications of starch. High amylose content increases the proportion of resistant starch (RS), which cannot be digested in the upper gastrointestinal tract, but functions as a substrate for bacterial fermentation in the large intestine (Nugent, 2005). RS enriched food can lower the glycemic and insulin responses and reduce the risk for developing type II diabetes, obesity, and cardiovascular disease (Nugent, 2005). Therefore, there is a growing interest in cultivating high-amylose cereal crops due to their health benefits for humans (Carciofi et al., 2012; Man et al., 2012; Regina et al., 2006; Slade et al., 2012; Zhu et al., 2012).

A transgenic RS rice line (TRS), which possesses about 60% amylose content, has been developed from an *indica* rice cultivar Te-qing (TQ) in our laboratory (Wei, Xu et al., 2010; Zhu et al., 2012). TRS kernel weight (12.7 mg/kernel) is significantly lower than TQ kernel weight (20.5 mg/kernel), mainly because of the change of

kernel appearance. The length, width and thickness of brown kernel is 5.5, 2.5 and 1.8 mm for TQ, and 5.7, 2.3 and 1.4 mm for TRS, respectively (Zhu, 2009; Zhu et al., 2012). The contents of total protein, starch, dietary fiber and soluble sugar in flour are 11.2, 82.7, 6.8 and 0.2% for TQ, and 12.4, 75.9, 15.2 and 1.8% for TRS, respectively (Zhu, 2009). TRS kernels contain abundant RS and have shown significant potential to improve the health of the large bowel in rats (Zhu et al., 2012). The starch isolated from TRS kernels has a high resistance to heating and digestion (Man et al., 2012; Wei et al., 2011). Four differently morphological (heterogeneous) and regionally distributed starch granules have been identified in TRS kernels. They are polygonal, aggregate, elongated, and interior hollow starch granules, and distributed in endosperm cells from the inner to the outer of kernel (Cai, Huang et al., 2014; Wei, Qin et al., 2010). Significantly different structural and functional properties have also been detected in these heterogeneous starches isolated from TRS kernels (Huang et al., 2016; Man et al., 2014).

Unlike other cereal grains, which are milled into flour before cooking, rice is principally consumed as cooked kernels in many countries. Starch is the principal component in rice kernel. During cooking, the kernel is converted into a palatable and digestible form due to the gelatinization of starch (Yadav & Jindal, 2007). The morphological change of kernel and the gelatinization properties of starch during cooking affect the grain texture, e.g. firmness and stickiness, and the starch digestion. The changes in kernel

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morphology of cooked normal rice have been reported in previous literature (Ogawa, Glenn, Orts, & Wood, 2003; Tamura & Ogawa, 2012; Tamura et al., 2014). In these reports, the cooked kernels are embedded in paraffin, and their sections are only suitable for observation of histological structure, such as cell outline. Usually, the thickness of paraffin section is 8–10  $\mu\text{m}$ . It is no way to obtain the section with thickness below 6  $\mu\text{m}$  for the reason of paraffin quality. The thick section has low resolution and can not be used to observe the fine structure of starch granule. Therefore, the detailed morphological changes of starch granules in cooked kernel can not be investigated using paraffin section method. Additionally, the morphological changes of high-amylose rice kernel are unclear during cooking. Though the properties of high-amylose starch isolated from TRS have been investigated in our previous studies (Huang et al., 2016; Man et al., 2012, 2014; Wei, Qin et al., 2010; Wei, Xu et al., 2010; Wei et al., 2011), the properties of starch in TRS cooked kernel remain to be resolved.

The poor kernel filling of TRS leads to the breakage of kernel during milling, and makes it difficult to obtain the whole milled kernels. In addition, brown rice, which includes embryo, aleurone and pericarp, contains abundant essential nutrients, and is more nutritious than milled rice (Briffaz et al., 2014). In this study, we investigated the changes in kernel morphology and starch properties of high-amylose brown rice during the cooking process. The objective of this study was to reveal the variation of morphology and properties of high-amylose starch granules during kernel cooking, and provide important information for the applications of TRS kernels in food industry.

## 2. Material and methods

### 2.1. Plant materials

The transgenic resistant starch rice line (TRS) was generated from an *indica* rice cultivar Te-qing (TQ) after transgenic inhibition of both starch branching enzyme I and IIb through an antisense RNA technique (Wei, Qin et al., 2010; Zhu et al., 2012). TQ and TRS were cultivated in the transgenic close experiment field of Yangzhou University, Yangzhou, China. Mature kernels were used as plant materials.

### 2.2. Cooking rice kernel

Excess water method was used to cook rice kernels according to the methods of Yadav and Jindal (2007) and Zhou, Robards, Helliwell, and Blanchard (2007) with some modifications. Twenty brown rice kernels were randomly selected and immersed in 10 mL distilled water in a glass test tube (13 mm in diameter and 150 mm

long) at room temperature for 2 h. The test tube was immersed in a boiling water bath for cooking, and then transferred from the boiling water into an ice bath at 10 min interval and allowed to cool for 2 min to prevent further cooking of rice kernels. The excess residual cooking water was withdrawn using pipette and the cooked kernels were immediately washed once with distilled water. The combined cooking and washing water was used for determination of leached materials. Some wet kernels were drained and excess surface water was carefully removed with a paper towel for morphology, volume, and weight analysis. Some kernels were freeze-dried, ground into powder, and passed through a 100-mesh sieve for preparation of kernel flour.

### 2.3. Cooking quality analysis of brown rice

For analyzing the cooking quality (cooking time, weight and volume of kernel, and leached material in residual cooking water), the cooked kernels were removed from the kernel-water mixture. The cooking time was determined by pressing the cooked kernel between two glass slides until no opaque core or uncooked centre was left (Vidal et al., 2007). For measuring the weight and volume of cooked kernel, the kernels were blotted with filter paper to remove surface water and weighed, and then their volume was measured using the volume displacement method by draining water. The residual cooking water which included soluble and insoluble leached materials was dried at 110 °C to constant weight. The resultant dry matter was weighed accurately and expressed as mg/20 kernels.

### 2.4. Morphology analysis of cooked rice kernel

The cooked rice kernels were directly photographed using a camera. The whole kernels were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and embedded in LR White Resin following the method of Cai, Huang et al. (2014). The semithin sections of 2  $\mu\text{m}$  thickness were cut on a Leica Ultrathin Microtome (EM UC7), stained with iodine solution, and observed with an Olympus BX53 light microscope equipped with a CCD camera.

### 2.5. Molecular weight distribution analysis of starch in cooked kernel

Starch was deproteinized and debranched following the methods of Tran et al. (2011) and Li, Hasjim, Dhital, Godwin, and Gilbert (2011) with some modifications. Briefly, kernel flour was deproteinized with protease and sodium bisulfite, then was defatted and dispersed in DMSO solution including 0.5% LiBr (w/v) at 80 °C for overnight using a ThermoMixer with continuous shaking

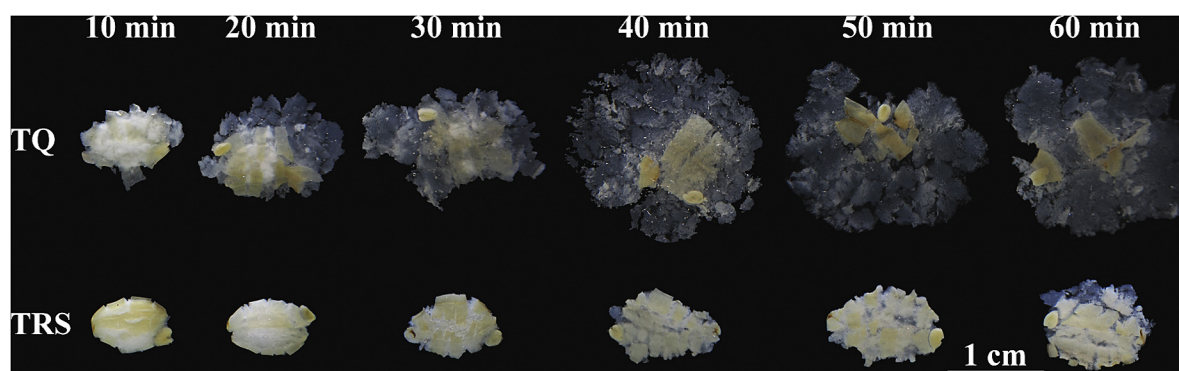


Fig. 1. Morphological change of cooked kernel pressed between two glass slides during the cooking process.

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