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Structural and functional properties of alkali-treated high-amylose rice starch

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ABSTRACT

Native starches were isolated from mature grains of high-amylose transgenic rice TRS and its wild-type rice TQ and treated with 0.1% and 0.4% NaOH for 7 and 14 days at 35 °C. Alkali-treated starches were characterised for structural and functional properties using various physical methods. The 0.1% NaOH treatment had no significant effect on structural and functional properties of starches except that it markedly increased the hydrolysis of starch by amylolytic enzymes. The 0.4% NaOH treatment resulted in some changes in structural and functional properties of starches. The alkali treatment affected granule morphology and decreased the electron density between crystalline and amorphous lamellae of starch. The effect of alkali on the crystalline structure including long- and short-range ordered structure was not pronounced. Compared with control starch, alkali-treated TRS starches had lower amylose content, higher onset and peak gelatinisation temperatures, and faster hydrolysis of starch by HCl and amylolytic enzymes.

1. Introduction

Native starch is produced by plants where it is stored as discrete semicrystalline granules, and consists of two main components: mainly linear amylose and highly branched amylopectin. According to X-ray powder diffraction (XRD) patterns, there are three types of starch reported, known as A-, B-, and C-type (Cheetham & Tao, 1998). Based on the ratio of amylose and amylopectin, starch can be separated into waxy, normal, and high amylose starch. For nutritional purposes, starch is classified into three types: rapidly digestible starch, slowly digestible starch, and resistant starch (RS). RS is a portion of starch that cannot be hydrolysed in the upper gastrointestinal tract and functions as a substrate for bacterial fermentation in the large intestine (Englyst, Kingman, & Cummings, 1992). RS has been reported to provide many health benefits for humans. In general, B- and C-type starches are rich of RS, and RS content of native starch is positively correlated with the amylose content.

Endosperm starch is the major component of cereal grains and a major source of nourishment for humans. Normally, waxy and normal cereal endosperm starches show A-type crystalline, and play an important role in meeting energy requirement and nutrient intake, but the content of RS is low (Frei, Siddhuraju, & Becker, 2003). In view of the current concept of nutrition, cereal endosperm with a higher content of digested starch and a lower content of RS is not the fittest food for health. High-amylose cereal endosperm starches usually show B- or C-type crystalline, and have high RS contents (Jiang, Lio, Blanco, Campbell, & Jane, 2010; Wei et al., 2010c). In addition, high-amylose starch is in great demand by the starch industry for its unique functional property. Therefore, high-amylose cereal crops are attracting considerable attention because of their potential health benefits, along with their industrial uses (Butardo et al., 2011). Many high-amylose cereal crops have been developed via mutation or transgenic breeding approaches (Jiang et al., 2010; Regina et al., 2006; Wei et al., 2010c).

Starch branching enzymes (SBEs) are responsible for the production of the α-1,6-glucosidic linkages in amylopectin. Three classes of SBEs (SBE I, SBE IIa, and SBE IIb) are known in cereal

Abbreviations: AAG, *Aspergillus niger* amyloglucosidase; ATR-FTIR, attenuated total reflectance–Fourier transform infrared; ^{13}C CP/MAS NMR, ^{13}C cross-polarisation magic-angle spinning nuclear magnetic resonance; DSC, differential scanning calorimetry; PPA, porcine pancreatic α-amylase; RS, resistant starch; SAXS, small-angle X-ray scattering; SBE, starch branching enzyme; SEM, scanning electron microscope; TQ, Te-qing (wild type rice cultivar); TRS, transgenic rice line; XRD, X-ray powder diffraction.

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crops. Many high-amylose cereal crops with suppression or mutation of SBEs have been proven to contain a high level of RS and have potential health benefits (Regina et al., 2006, 2010). A high-amylose transgenic rice line (TRS) has been developed by antisense RNA inhibition of both SBE I and SBE IIb in our laboratory, which yields a starch with an amylose content of about 60% (Wei et al., 2010c; Zhu et al., 2012). TRS starch is identified as having C-type crystalline (Wei et al., 2010c). TRS grains are rich in RS, are as safe as the conventional nontransgenic rice for rat consumption, and have shown significant potential to improve the health of the large bowel in rats (Zhu et al., 2012).

Native starch is often modified with various chemical reagents or through acid, alkali, enzymatic or hydrothermal treatments for use in food and nonfood industries. Alkali treatment by reagents such as sodium hydroxide is widely used in the production of many traditional food products, e.g., tortillas, waxy rice dumplings and yellow alkaline noodles, to enhance quality characteristics of color, flavor, and texture (Nor Nadiha, Fazilah, Bhat, & Karim, 2010). However, the effects of alkali on starch structure and properties have received relatively less attention compared to other methods of starch modification (Wang & Copeland, 2012). Recent studies of alkali treatment have mainly focused on the structural and functional properties of starches from sago, corn, potato, pea, and pinhão (Karim et al., 2008; Nor Nadiha et al., 2010; Thys et al., 2008; Wang & Copeland, 2012). The composition and structure of starch granules vary considerably between different plants, thus affecting the properties and functions of starches from different plants (Nor Nadiha et al., 2010). The effect of alkali treatment on different starches needs to be investigated to elucidate the action of alkali on starch structure and function.

High-amylose cereal starches have important applications in food and nonfood industries. To date, the effects of alkali on the characteristics of high-amylose cereal starches have not been studied as extensively as other types of starch modification have, such as acid and enzyme hydrolysis. Compared with wild-type rice cultivar Te-qing (TQ), high-amylose TRS rice starch has significantly higher resistances to acid hydrolysis, amylase hydrolysis, and heating (Man et al., 2012; Wei et al., 2010c, 2011). We suspect that TQ and TRS starches have different susceptibilities to alkali treatment. In this paper, starches were isolated from mature grains of TQ and TRS and treated with 0.1% and 0.4% NaOH for 7 and 14 days. The structural and functional properties of alkali-treated starches were investigated by scanning electron microscope (SEM), XRD, attenuated total reflectance–Fourier transform infrared (ATR-FTIR), ^{13}C cross-polarisation magic-angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR), small-angle X-ray scattering (SAXS), and differential scanning calorimetry (DSC). The aim of this work was to investigate the effect of alkali treatment on structural and functional properties of normal and high-amylose rice starches, and further understand the mechanism underlying the action of alkali on starch structure and functionality. The results would be useful for the applications of high-amylose TRS rice in the food and nonfood industries.

2. Materials and methods

2.1. Plant materials

An *indica* rice cultivar Te-qing (TQ) and its transgenic line (TRS) with high amylose and RS contents were used in this study. TRS was generated from TQ after transgenic inhibition of both SBE I and SBE IIb through an antisense RNA technique and was homozygous for the transgene. The expressions of SBE I and SBE IIb were completely inhibited in TRS grains (Zhu et al., 2012). TQ and TRS were cultivated in the transgenic close experiment field of Yangzhou University, Yangzhou, China, in 2011, and mature grains were used to isolate starches.

2.2. Isolation of native starches

Native starches were isolated from mature grains as previously described (Wei et al., 2010c).

2.3. Preparation of alkali-treated starch

The alkali-treated starch was prepared according to the methods of Nor Nadiha et al. (2010) and Wang and Copeland (2012) with some modifications. Triplicate samples of rice starches (2 g dry weight) were each suspended in 50 ml of 0.1% or 0.4% (w/v) NaOH solution containing 0.1% (w/v) sodium azide. Control starches were suspended in 0.1% sodium azide solution without NaOH. The starch slurries were left at 35 °C for 7 and 14 days with shaking thrice a day by hand to resuspend the sedimented starches. After hydrolysis, undissolved residues were quickly obtained by centrifugation (3000*g*, 10 min), and the supernatant was used for measurement of the solubilised carbohydrates to quantify the hydrolysis degree by the anthrone-H_2SO_4 method. The residues were subsequently washed four times with double-distilled water and twice with anhydrous ethanol, and dried at 40 °C for 2 days. The dried starches were ground into powders in a mortar with pestle and passed through a 100 mesh sieve for structural analyses.

2.4. SEM observation

Starches were suspended in anhydrous ethanol. One drop of the starch–ethanol suspension was applied to an aluminum stub using double-sided adhesive tape, and the starch was coated with gold before viewing with an environmental SEM (Philips XL-30).

2.5. XRD analysis

XRD analysis of starch was carried out on an XRD (D8, Bruker, Germany), and the relative crystallinity was measured following the method described by Wei et al. (2010a).

2.6. ATR-FTIR analysis

ATR-FTIR analysis of starch was carried out on a Varian 7 000 FTIR spectrometer with a DTGS detector equipped with an ATR single-reflectance cell containing a germanium crystal (45° incidence-angle) (PIKE Technologies, USA) as previously described (Wei et al., 2010c). Spectra were corrected by a baseline in the region from 1200 to 800 cm^{-1} before deconvolution was applied using Resolutions Pro. The assumed line shape was Lorentzian with a half-width of 19 cm^{-1} and a resolution enhancement factor of 1.9. Intensity measurements at 1045 and 1022 cm^{-1} were performed on the deconvoluted spectra by recording the height of the absorbance bands from the baseline using Adobe Photoshop 7.0 image software.

2.7. Solid-state ^{13}C CP/MAS NMR analysis

High-resolution solid-state ^{13}C CP/MAS NMR analysis of starch was carried out at B_0 = 9.4T on a Bruker AVANCE III 400 WB spectrometer as described previously (Wei et al., 2010a). Amorphous starch was prepared by gelatinising native starch following the method of Atichokudomchai, Varavinit, and Chinachoti (2004).