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Morphologies and gelatinization behaviours of high-amylose maize starches during heat treatment

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ABSTRACT

The granule morphologies and gelatinization behaviours of high-amylose maize starches during heating treatment were investigated by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). Maltese crosses demonstrated that the high-amylose maize starches maintained a granular structure even at 120 °C. The granules of high-amylose maize starches swelled slightly at 100 °C and swelled remarkably at approximately 120 °C. The destruction of the starch structure began at the centre and expanded rapidly to the periphery. The intense fluorescence of high-amylose maize starch granules gradually became feeble, and the darker region spread outward during heating at 130 °C for 30 min, indicating that the amylose component may have been damaged and shifted. The starch granules treated at 140 °C were substantially destroyed, and the CLSM, normal light microscopy (NL) and SEM images displayed no discernible granules, which indicated that the original starch granules formed a continuous integrated matrix.

1. Introduction

Starch is stored as discrete semi-crystalline granules and consists of two main biopolymers: linear amylose (20–30%) and highly branched amylopectin (70–80%) (Zhou et al., 2015). The amylose content and amylopectin structure of starch strongly influence its physicochemical properties and applications (Chung, Jeong, & Lim, 2003; Lin et al., 2016).

Starch with a high amylose content is widely applied in the areas of support films, foods, paper making, medical treatments, and electronic chips because it has a high level of resistance to gelatinization and hydrolysis (Lin et al., 2016; Tan et al., 2015). Furthermore, high-amylose starch is especially suitable for producing thermoplastic materials because amylose can easily form crystallites and entanglements (Koch et al., 2010). However, high-amylose maize starches' resistance to processing or treatment creates a substantial problem when they are used as raw materials because complete destruction of the original starch supramolecular structures is required to form a continuous integrated phase (Yang et al., 2016a, 2016b). That is, because of its compact granule structure, native high-amylose starch exhibits strong resistance to being hydrolysed or disintegrated by small molecules such as water and enzymes, which limits its applications.

For normal starches with excess water, the gelatinization endotherm can usually be observed during differential scanning calorimetry (DSC) temperature scanning in the low temperature range (54–73 °C) (Liu et al., 2011). High-amylose maize starches are well known to exhibit higher gelatinization temperatures, a wider gelatinization range and lower gelatinization enthalpy than normal starches (Qiu et al., 2016). However, although much work has been reported on the thermal properties of various starches, few reports have addressed the gelatinization behaviours of high-amylose maize starches under temperatures greater than 100 °C (Cai et al., 2014a, 2014b; Mira, Villwock, & Persson, 2007). In particular, Chen et al. (2011) studied the internal structures and phase transitions of high-amylose starches below the gelatinization temperature, which was instructive in the exploration of the gelatinization mechanism to some extent (Chen et al., 2011).

In this paper, the morphology, structure and gelatinization characteristics of high-amylose maize starches were investigated, with the objective of elucidating the granule morphologies and gelatinization behaviours of high-amylose maize starches during

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heating treatment, especially in the temperature range from 100 °C to 140 °C.

2. Materials and methods

2.1. Materials

High-amylose maize starches with amylose contents of 70% were kindly supplied by the National Starch and Chemical Co. (Bridgewater, NJ, USA). HPLC-grade fluorescein 5-isothiocyanate (FITC) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Starch paste preparation

To achieve temperatures greater than 100 °C, an autoclave was used. Starch paste was prepared for all the experiments, as previously described by Savary, Handschin, Conde-Petit, Cayot, and Doublier (2008) with some modifications. Four hundred grams of 8.0% (w/w) starch suspensions were stirred at a paddle speed of 300 rpm. The starch suspension was heated from room temperature to a preset temperature (100 °C, 110 °C, 120 °C, 130 °C or 140 °C) at a constant rate of 2 °C/min. The suspension was maintained at the preset temperature for 30 min, after this duration, the starch paste was decreased to 100 °C. Subsequently, the starch paste was poured into a beaker immediately with a water bath maintained at 80 °C.

2.3. Confocal laser scanning microscopy and light microscopy

An Olympus FV10 (Tokyo, Japan) confocal laser scanning microscope equipped with an inverted microscope was used for observing the changing trends of starch granules during gelatinization. A stock solution of fluorescein 5-isothiocyanate (FITC) was prepared by dissolving 0.2 g of FITC in 100 mL of distilled water. Starch paste (100 μL) was stained by mixing with 20 μL of FITC stock solution (Qiu et al., 2016; Zhou et al., 2015). A drop of stained starch paste (approximately 10 μL) was deposited onto a concave slide, cooled to room temperature and observed within 15 min. The excitation wavelength was 488 nm, and the emission maxima were within 500–525 nm (Nagano, Tamaki, & Funami, 2008; Zhou et al., 2014). Each line was scanned four times and averaged to reduce noise.

Light micrographs were obtained using an Olympus FV10 confocal scanning laser microscope (Tokyo, Japan) with normal light microscopy and polarized light microscopy to investigate the morphology of the unstained paste. A drop of starch paste was placed on the microscope slide with a glass coverslip. The specimen was viewed first under normal light microscopy. The same field was then viewed under polarized light microscopy. The observations were carried out in triplicate. The diameters of starch granules were measured and analyzed using Nano Measurer1.2.0 statistically software (Fudan Univ., Shanghai, China).

2.4. Scanning electron microscopy (SEM)

The starch paste was stored in an electro- thermostatic blast oven at 25 °C to be dried into a 'solid state', followed by drying at 105 °C for 10 h. Evaporating the water slowly and slightly was key to ensuring that the samples had nearly identical moisture contents and to minimizing the influence of water loss on the intrinsic structure of the starch paste. The dried starch paste was affixed to a specimen holder using an aluminium plate, and was subsequently coating it with gold in a vacuum evaporator. The cross-sections were then observed by SEM on an electron microscope (S-4800, Hitachi, Japan) operated at an accelerating voltage of 3.0 kV.

2.5. Differential scanning calorimetry

A PerkinElmer DSC Diamond-8000 equipped with a refrigerated cooling system was used to conduct DSC experiments, and nitrogen was used as the purge gas. High-pressure stainless steel pans (PE No. JYL0073) with a heat-resistant rubber ring were used because of the higher temperatures required. High-amylose maize starches and distilled water were mixed at the specific percentage of 8.0% (w/w) in a glass vial, then sealed and stored for 24 h for the water to reach equilibrium. Thereafter, the components (approximately 8 mg) were transferred to the stainless steel pan using a syringe. After the pan was sealed, it was frequently shaken to ensure that the starch granules were well immersed. The mixture was equilibrated at room temperature for 2 h before measurements. The DSC samples were heated from 40 °C to 200 °C at a rate of 10 °C/min.

2.6. X-ray diffraction (XRD)

X-ray analytical instrumentation (TTR-III, Rigaku, Japan) was used for detecting the crystalline structure of high-amylose maize starches and starch paste. The starch pastes were firstly dried at 25 °C for almost two days; they were then milled into powder before absolute drying. The powder of the starch paste was sieved through 200 mesh and exposed to the X-ray beam at 200 mA and 40 kV. The scanning region of the diffraction angle (2θ) was from 3° to 50° with a step size of 0.02°.

3. Results and discussion

3.1. Morphological structure of starches

The native high-amylose maize starch granules with typical Maltese crosses under polarized light microscopy are shown in Fig. 1A; granular birefringence of the small granules appeared somewhat weaker than that of the large granules when compared under the same background. The large granules with bright Maltese crosses disappeared when the samples were heated to 100 °C, indicating that large granules exhibited less resistance to being gelatinized. This higher resistance stems from the amylose double helices in the small granules requiring a higher temperature and energy input to dissociate completely than the shorter double helices in the large granules (Cai et al., 2014a, 2014b; Lin et al., 2016; Naguleswaran, Vasanthan, Hoover, & Bressler, 2016).

Despite the starch granules being remarkably gelatinized, a few Maltese crosses were still maintained, especially in the small granules, after the loss of birefringence when the granules were heated to 120 °C. The starch granules tended to be smaller when the temperature was further increased to 130 °C, where Maltese crosses could barely be discerned in the field of polarized light microscopy. The aforementioned phenomena reveal that high-amylose maize starches require temperatures greater than 130 °C to be completely gelatinized (Ocloo, Minnaar, & Emmambux, 2016; Lin et al., 2016).

Of particular interest, partial fragmentation with polarized light occurred via the rupture of high-amylose maize starches during gelatinization. As the temperature was increased, high-amylose maize starch granules were gradually broken into small fragments whose surface and internal structures were effectively destroyed, accompanied by the absence of Maltese crosses. Notably, the partial fragmentation that resulted from the fusion of high-amylose maize starch granules created particles that were highly bright, which may result from re-aggregation of small fragments.

CLSM was used to study the effects of heating treatment on granule morphologies and gelatinization behaviours of high-amylose maize starches (Fig. 2). Native high-amylose maize starches showed greater fluorescence intensity in the centre of the granules. The