Effects of heat stress during grain filling on the structure and thermal properties of waxy maize starch

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ABSTRACT

Clarifying the waxy maize starch physicochemical characteristics response to heat stress could modify starch quality. The effects of heat stress during grain filling (1–40 day after pollination) on starch structure and thermal properties of four waxy maize varieties were investigated. The mean day/night temperature during grain filling for heat stress and control treatments was 35.2/16.1 °C and 27.4/15.6 °C, respectively. Heat stress during grain filling increased the starch average granule size and the proportion of long chains in amylopectin. Starch granules under heat stress presented more pitting or uneven surfaces. X-ray peak intensities in response to heat stress were variety dependent. Heat stress during grain filling decreased the swelling power and increased the gelatinization temperature and retrogradation percentage, while the gelatinization enthalpy was not affected. In conclusion, heat stress during grain filling significantly affected structural characteristics of waxy maize starch and consequently, changed its swelling and thermal properties.

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1. Introduction

Maize starch is the primary starch resource and accounts for the majority (80%) of the global market share (Jobling, 2004). Maize starch can be classified as waxy (0%), normal (~25%), high amylopectin (50–70%), and sugary based on the amylose content (Singh, Inouchi, & Nishinari, 2006; Singh, Sandhu, & Kaur, 2005). Among them, waxy maize starch has high viscosity, low retrogradation tendency, and good clarity. Waxy maize starch is easy to digest and has advantages to produce stabilizer, thickener and adhesives, etc.

Temperature is an important environmental factor affecting maize yield and starch quality. Optimum temperature during maize grain filling is between 27 and 32 °C (Commuri & Jones, 1999). However, the growing temperature at this stage is often higher than 32 °C, which results in loss of grain yield especially in tropical and moderate zones (Cairns et al., 2012). The global temperature was predicted to increase by 1.8–4.0 °C at the end of this century (IPCC, 2007) and an increase of 2 °C could cause more than 10% maize yield loss (Lobell & Burke, 2010).

Heat stress not only decreases maize grain yield, but also changes starch quality. Lu, Jane, Keeling, and Singletary (1996) observed that heat stress from 15 days after pollination to maturity decreased the starch granule size, amylose content and short branch-chains and increased gelatinization temperature. Planting conditions and sowing dates were reportedly exerting significant effects on maize starch structure and properties (Lu, Guo, Dong, & Lu, 2010; Lu, Wang, Zhao, & Lu, 2009; Medic, Abendroth, Elmore, Blanco, & Jane, 2010). However, little is known on the effects of heat stress under controlled conditions on the structure and physicochemical properties of waxy maize starch. Starch quality variability necessitates continuous adjustments of many industrial processing parameters and carries over a potential quality control problem in various products (Lu et al., 1996). This study aimed to clarify the effects of heat stress during grain filling on the starch structural characteristics that may be related to the potential for variations in starch swelling and thermal properties of waxy maize.

2. Materials and methods

2.1. Samples

Four genetically unrelated waxy maize varieties, Suyunuo5, Yunnuo7, Lainongnuo11 and HuaikenuoO3, were produced in Yangzhou University, Yangzhou, China. Seeds were sown on 1 July and transplanted to plastic pots on 5 July. Two plants were placed in each pot. One plant was retained at the jointing stage. Each plastic pot measured 42 cm in height and 38 cm in diameter and was loaded with 30 kg of sieved sandy loam soil. The plants were provided a basal dressing of 10 g per pot (commercial fertiliser, N-P$_2$O$_5$:K$_2$O = 15%:15%:15%) at transplanting and a top dressing of 6 g per pot (commercial urea, N concentration = 46%) at the jointing stage.
Ten plants of each variety were grown in an environmentally controlled greenhouse with a temperature about 35 °C from 6:00 to 18:00 after pollination to maturity (1–40 day after pollination), with plants grown in the natural environment taken as control. Everyday from 18:00 to 6:00, the door and windows of the greenhouse were left open so that the indoor temperature during the night was the same as that outdoors. The average day/night temperature in the greenhouse was 35.2/16.1 °C. The outdoor condition was 27.4/15.6 °C.

2.2. Starch isolation

The plants were harvested at maturity (40 days after pollination). The grains (100 g) were steeped in 500 mL of distilled water containing 1 g/L sodium hydrogen sulfite (SO₂) for 48 h at room temperature. Starch was isolated following a method described previously (Lu & Lu, 2012). The samples were rinsed with distilled water, and then ground using a blender for 2.5 min. The suspensions were passed through a 100-mesh sieve. The materials left on the screen were again homogenised for 1.5 min, and then passed through the same sieve. The starch–protein slurry was collected in a 1000 mL wide-neck flask and allowed to stand for 4 h. The supernatant was removed through suction and the settled starch layer was collected in 50 mL centrifuge tubes and centrifuged at 3000 g for 10 min. The upper non-white layer was scooped. The white layer was resuspended in distilled water and stirred for 30 min before centrifugation. The isolation procedures were repeated three times. The starch was then collected and dried in an oven at 40 °C for 48 h.

2.3. Granule morphology

Starch granules were mounted on circular aluminium stubs with double sticky tape, coated with gold, examined by scanning electron microscopy (SEM, XL-30 ESEM, Philips, Amsterdam, Netherlands) at an accelerating potential of 20 kV, and then photographed (Lu & Lu, 2012).

Fig. 1. A–D refer to Suyunuo5, Yunuo7, Lainongnuo11, and Huaikenuo3, respectively. Capital and lowercase alphabet are control and heat stress treatments, respectively. Effects of heat stress during grain filling on the morphology of waxy maize starch.
2.4. Granule size distribution

The particle size of the starches was analysed by a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, England). Instrument accuracy was verified with Malvern standard glass particles. The instrument operates based on the principle of laser light scattering and can measure sizes between 0.1 and 2000 μm. The disperse phase was absolute ethyl alcohol. The size distribution was expressed in terms of the volume of equivalent spheres. The average granule size was defined as the volume weighted mean.

2.5. Iodine staining

The \( \lambda_{\text{max}} \) and blue value of the starches were measured according to the method described by Fiedorowicz and Rebilas (2002) with minor modifications. Starch (40 mg) was dispersed in 10 ml of DMSO containing 10% of 6 M urea. A 1.0 ml aliquot of each sample was placed in a 100 ml volumetric flask, to which 95 ml of deionised water and 2 ml of an aqueous I\(_2\)-KI solution was added. The latter solution was prepared with 200 mg of I\(_2\) and 2 g of KI in 100 ml of distilled water. The mixture was made up to 100 ml with deionised water and mixed immediate. Blank solutions that were prepared identically did not contain starch. Spectra ranging from 700 to 500 nm were obtained from all of the samples using a UV–Vis spectrophotometer. The blue value of the samples was defined as the absorbance at 635 nm, and the \( \lambda_{\text{max}} \) was designated as the peak absorbance value over the range of wavelengths examined. The iodine binding capacity of the starches was defined as the ratio of absorbance at 635 nm to that at 520 nm.

2.6. X-ray diffraction pattern

X-ray diffraction (XRD) patterns of the starches were obtained using an X-ray diffractometer (D8 Advance, Bruker-AXS, Karlsruhe, Germany). The diffractometer was operated at 200 mA and 40 kV. The scanning region of the diffraction angle (2\( \theta \)) ranged from 5\(^\circ\) to 40\(^\circ\) at a step size of 0.04\(^\circ\) with a count time of 0.6 s.

2.7. Swelling power

The swelling power was determined following a method described previously (Lu & Lu, 2012). Starch (0.1 g) was weighed in a centrifuge tube with coated screw cap to which 10 ml distilled water was added. The tube was heated at 90 °C in a shaking water bath for an hour. The tube was cooled to room temperature in an iced bath and centrifuged at 4000 g for 20 min. The supernatant was discarded. The materials that adhered to the wall of the centrifuge tube were considered as sediments and weighed ([W1]/C) before heating in the DSC. The DSC analyzer was calibrated using an empty aluminium pan as a reference. Sample pans were heated at a rate of 10 °C/min from 20 to 100 °C. Thermal transitions of starch samples were defined as \( T_o \) (onset temperature), \( T_p \) (peak of gelatinization temperature) and \( T_c \) (conclusion temperature) and \( \Delta H_{\text{gel}} \) referred to the gelatinization enthalpy. Enthalpies were calculated on a starch dry weight basis.

2.8. Thermal properties

The thermal characteristics of the starches were studied by differential scanning calorimetry (DSC, Model 200 F3 Maia, NETZSCH, Bavaria, Germany) according to a method described previously (Lu & Lu, 2012). Each sample (5 mg, dry weight) was loaded into an aluminium pan (25/40 ml, D = 5 mm) and distilled water was added to achieve a starch–water suspension containing 66.7% water. Samples were hermetically sealed and allowed to stand for 24 h at 4 °C before heating in the DSC. The DSC analyzer was calibrated using an empty aluminium pan as a reference. Sample pans were heated at a rate of 10 °C/min from 20 to 100 °C. Enthalpies were calculated on a starch dry weight basis.

The results are the means of two replications. Data entries in the same column followed by different letters differ significantly (\( P < 0.05 \)).
100 °C to measure retrogradation. The retrogradation enthalpies ($\Delta H_{\text{ret}}$) were evaluated and retrogradation percentage ($\% R$) was calculated as $\% R = 100 \times \Delta H_{\text{ret}}/\Delta H_{\text{gel}}$.

### 2.9. Statistical analyses

The data reported in all of the tables are expressed as the average of two repeated observations. Data were subjected to analysis of variance using the least significant difference test at the 5% probability level using the data processing system (DPS 7.05) (Tang & Feng, 2007).

### 3. Results and discussion

#### 3.1. Granule morphology

The waxy maize starch samples under heat stress and control treatments showed large and small granules with angular and spherical shapes. Most surfaces of granules under control treatment were smooth but a few were uneven (Fig. 1). Heat stress during grain filling significantly changed the starch morphology. The numbers of granules with uneven surfaces increase (obviously for Lainongnuo11) or granules became more pitted (Suyunuo5). A similar result was also observed in wheat starch by Hurkmans and Wood (2011). They believe that the pitting of starch granules is common in endosperm cells of shriveled grain and is a process accompanied by large increments in $\alpha$-amylase activity.

#### 3.2. Granule size distribution

Heat stress significantly affects the volume distribution of starch granules of waxy maize (Fig. 2). The average granule size of all the varieties was significantly increased by heat stress except for Huaikenuo3. The proportion of large granules was increased by heat stress, and the increment was more evident for Suyunuo5 (Fig. 2). The increase in average granule size was observed when plants were exposed to high temperature in normal maize (Lu et al., 1996), barley (Tester, 1997) and wheat (Hurkmans & Wood, 2011). Lindeboom, Chang, and Tyler (2004) reported that high temperature during grain filling reduce the granule size and number of small granules. They also found that the large granules decreased to a proportionally lesser extent than the small ones. Heat stress suppresses small granule initiation, and the available substrate may be diverted toward pre-existing large granules. Consequently, these granules enlarge and the ratio of large-to-small granules increase (Hurkmans et al., 2003).

#### 3.3. Iodine staining

Heat stress during grain filling increase the starch blue value, $k_{\text{max}}$ and iodine binding capacity of all varieties (Table 1), indicating that the proportion of long chains in amylepectin increased (Fiedorowicz & Rebilas, 2002). Similar results were reported on normal maize (Lu et al., 1996), wheat (Matsuki, Yasui, Kohyama, & Sasaki, 2003), rice (Aboubacar, Moldenhauer, McClung, Beighley, & Hamaker, 2006), and barley (Tester, 1997). The high proportion of long chains in amylepectin under heat stress may be due primarily to the low activity of starch branching enzymes, which reduce the branching frequency of amylepectin (Thitisaksakul, Jimenez, Abias, & Beckles, 2012). Lindeboom et al. (2004) reported that large granules contain more abundant long chains in amylepectin than small ones from the same variety. Therefore, the high proportion of long chains in amylepectin under heat stress may be linked to the increased average granule size. The $k_{\text{max}}$ values ranged from 530.6 to 538.7 nm, and the iodine binding capacity ranged from 0.506 to 0.578 as reflected by its waxy character (Fiedorowicz & Rebilas, 2002).
Effects of heat stress during grain filling on thermal properties of waxy maize starch.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>$\Delta H_{gel}$ (J/g)</th>
<th>$T_g$ (°C)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_{gel}$ (J/g)</th>
<th>%R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suyunuo5</td>
<td>Control</td>
<td>14.0$^{a}$</td>
<td>65.9$^{d}$</td>
<td>72.2$^{h}$</td>
<td>80.1$^{bc}$</td>
<td>6.0$^{b}$</td>
</tr>
<tr>
<td></td>
<td>Heat stress</td>
<td>13.4$^{a}$</td>
<td>69.8$^{a}$</td>
<td>74.6$^{a}$</td>
<td>82.1$^{a}$</td>
<td>7.7$^{b}$</td>
</tr>
<tr>
<td>Yunuo7</td>
<td>Control</td>
<td>13.1$^{b}$</td>
<td>66.0$^{b}$</td>
<td>71.2$^{d}$</td>
<td>79.5$^{b}$</td>
<td>4.7$^{a}$</td>
</tr>
<tr>
<td></td>
<td>Heat stress</td>
<td>12.4$^{c}$</td>
<td>67.2$^{c}$</td>
<td>72.3$^{b}$</td>
<td>80.3$^{b}$</td>
<td>6.8$^{b}$</td>
</tr>
<tr>
<td>Lainongnuo11</td>
<td>Control</td>
<td>12.3$^{c}$</td>
<td>63.8$^{c}$</td>
<td>71.3$^{d}$</td>
<td>79.7$^{d}$</td>
<td>6.5$^{b}$</td>
</tr>
<tr>
<td></td>
<td>Heat stress</td>
<td>12.1$^{b}$</td>
<td>66.7$^{bc}$</td>
<td>72.1$^{bc}$</td>
<td>80.3$^{b}$</td>
<td>8.3$^{a}$</td>
</tr>
<tr>
<td>Huaikenuo3</td>
<td>Control</td>
<td>12.4$^{c}$</td>
<td>63.8$^{c}$</td>
<td>70.6$^{d}$</td>
<td>78.8$^{a}$</td>
<td>6.7$^{b}$</td>
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<tr>
<td></td>
<td>Heat stress</td>
<td>12.8$^{d}$</td>
<td>66.3$^{d}$</td>
<td>71.8$^{b}$</td>
<td>80.1$^{bc}$</td>
<td>6.8$^{b}$</td>
</tr>
</tbody>
</table>

The results are the means of two replications. Data entries in the same column followed by different letters differ significantly (P < 0.05).

3.4. X-ray diffraction

The starch X-ray diffraction patterns of the four varieties under control and heat stress treatments showed a typical A-type pattern (Fig. 3). However, the peak intensities of starch changed with the variety and temperature. Peak intensities at different angles (2θ = 15°, 17°, 18°, and 23°) were similar between the two temperature treatments for Yunuo7 and Lainongnuo11 but were increased by heat stress during grain filling for Suyunuo5 and Huaikenuo3 (Fig. 3). The high peak intensities under heat stress was because large granules were relatively more crystalline than small ones (Chiotelli & Le Meste, 2002). In addition, given that the peak intensity at 20° was primarily for amylose-lipid complex, the intensity was low for all samples because waxy maize starch is composed of 100% amyllopectin (Singh et al., 2006).

3.5. Swelling power

Swelling power can be used to assess the extent of interaction among starch chains within the amorphous and crystalline domains of starch granules (Singh, Singh, Isono, & Noda, 2010). Tester (1997) observed that starch swelling power of barley grown at 20 °C was lower than that of barley grown at 10 or 15 °C. In the present study, the swelling power of starch in the four varieties was decreased by heat stress during grain filling, and the decrement was the highest for Yunuo7 and lowest for Suyunuo5 (Fig. 4). The high swelling power under control was because the small granules have higher water affinity, earlier hydration and swelling than large ones (Chiotelli & Le Meste, 2002). Moreover, starch with a higher degree of crystallinity had a lower swelling power (Singh et al., 2010). The swelling power was significantly different among the four varieties under both treatments.

3.6. Thermal properties

Heat stress during grain filling did not affect $\Delta H_{gel}$ for all varieties but increase the gelatinization temperature (onset, peak, and conclusion temperature) (Table 2). Increases in the gelatinization temperature were also observed in waxy maize (Lu et al., 2010), normal maize (Lenihan, Pollak, & White, 2005; Lu et al., 1996), wheat (Matsuki et al., 2003), rice (Aboubacar et al., 2006), and barley (Tester, 1997), whereas $\Delta H_{gel}$ response to heat stress was variety dependent. Campbell, Li, Berke, and Glover (1996) and Singh et al. (2010) reported that starches with large granule size and high ratio of long chains in amyllopectin have a high gelatinization temperature. Heat stress during grain filling increases the granule size and the ratio of long chains in amyllopectin, resulting in high gelatinization temperature.

After the gelatinized samples storing at 4 °C for 7 days, recrystallization of starch molecules occurred in a more-ordered structure. $\Delta H_{gel}$ provides a quantitative measure of the energy transformation that occurs during the melting of reassociated amyllopectin, and a higher $\Delta H_{gel}$ of starch corresponds to a higher tendency to retrograde (Singh et al., 2010). The sample showed higher $\Delta H_{gel}$ and %R under heat stress treatment for all varieties except Huaikenuo3, which had similar $\Delta H_{gel}$ and %R between control and heat stress treatments (Table 2). Researchers also reported maize growth in a warm environment, in the presence of high %R (Lenihan et al., 2005) or dates (Lu et al., 2010). The high %R under heat stress may be due to the starch with large granule size and high proportion of longer chains (Lenihan et al., 2005; Shi & Seib, 1995). In addition, the starch with high peak intensity and low swelling power was easy to form a stronger crystalline network within the starch granules (Singh et al., 2010). Those factors induced the higher %R under heat stress. Genotypic differences in %R were also observed among the four varieties. The lower %R for Yunuo7 under control treatment and for Huaikenuo3 under heat stress treatment was advantageous when the product required low retrogradation tendency.

4. Conclusions

Heat stress during grain filling increase the starch average granule size and make granule surfaces more pitting and uneven. The starch iodine binding capacity value was significantly increased by heat stress. Heat stress during grain filling did not change the X-ray diffraction pattern, and the peak intensities at 20° = 15°, 17°, 18°, and 23° in response to heat stress were variety dependent. It was similar between the two temperature treatments for Yunuo7 and Lainongnuo11 but increased by heat stress during grain filling for Suyunuo5 and Huaikenuo3. The large average granule size, high proportion of long chains in amyllopectin and peak intensities of starch under heat stress induced a low swelling power and high gelatinization temperature, $\Delta H_{gel}$ and %R. The structure and thermal properties of starch also differed among the four genotypes. High temperature during grain filling in modifies starch quality.

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References


