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Effects of high temperature during grain filling under control conditions on the physicochemical properties of waxy maize flour

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ABSTRACT

The effects of high temperature during different grain filling stages (1–15 d and 16–30 d after pollination) on the physicochemical properties of four varieties of waxy maize grain were studied. Heat stress during grain filling decreased grain weight and starch deposition, while it increased protein content, starch granule size, abnormal granule numbers and iodine binding capacity. These effects were more severe when heat stress was introduced at early development stage than at late grain filling stage. The peak intensities and crystallinities were decreased when plants were exposed to high temperature at early development stage. By contrast, responses to high temperature at late development stage were variety-dependent. High temperature during grain filling decreased the peak and breakdown viscosities and increased the gelatinization temperature and enthalpy, and retrogradation percentage of flours, especially during early development stage. In conclusion, high temperature during grain filling changed the grain proximate and starch structure, resulting in the deterioration of pasting and thermal properties.

1. Introduction

Crop production is influenced by genotype, environment, and crop management (Damata, Grandis, Arenque, & Buckeridge, 2010; Thitisaksakul, Jimenez, Abias, & Beckles, 2012). Given recent improvements in germplasm and cultivation measures, the growth environment, including amount of sunshine, temperature, rainfall, among others, is known to play a key role in determining grain yield and quality. Temperature is one of the most important factors that affects the crop growth and development. Projections to the end of this century have suggested that global temperatures will increase by 1.8–4.0 °C, depending on the greenhouse gas emission scenario (IPCC, 2007), and the temperature during growing season may exceed the most extreme seasonal temperature recorded in last century (Battisti & Naylor, 2009). Lobell and Burke (2010) suggested that an increase in temperature of 2 °C would result in greater reductions in maize (*Zea mays* L.) yield than a decrease in precipitation by 20%.

The optimum temperature for grain development in maize is between 27 and 32 °C (Commuri & Jones, 1999). An average temperature of 32 °C or higher during the reproductive development stage is common across many regions, especially in tropical and temperate zones. High temperature during grain filling abort some kernels and decrease grain weight, resulting in the reduction of grain yield (Barnabas, Jagerm, & Feher, 2008; Commuri & Jones, 2001; Duke & Doehlert, 1996; Engelen-Eigles, Jones, & Phillips, 2000). And the effect of heat stress during early grain development on grain yield was higher than late grain development (Commuri & Jones, 1999, 2001).

Heat stress changes the grain quality of crops. Several reviews have shown that heat stress during grain filling generally decreases starch contents, increases protein contents, enlarges starch granules, and causes starch granules to become abnormal and pitted (Barnabas et al., 2008; Cairns et al., 2012; Hedhly & Hormaza, 2008; Tester & Karkalas, 2001; Thitisaksakul et al., 2012; Wang & Frei, 2011). Compared with heat stress application during late grain development, high temperature during early grain development play a more significant role in influencing the starch pasting properties, crystalline structure, and granule size of cereal starches (Dong et al., 2011; Liu et al., 2011; Wang et al., 2008; Yan et al., 2008).

Waxy maize is a special type of maize (starch is composed of 100% amylopectin) with low hardness and high digestibility, clarity, and viscosity (Liu, Ramsden, & Corke, 1997). These characteristics contribute to the important role of the crop in the food industry. Waxy maize flour can directly use in food to replace waxy rice flour to make dumplings (glutinous maize balls), add in the frozen-food to avoid retrogradation, and mix with wheat flour to improve eating quality. Previous studies have reported that the starch pasting properties and crystalline structures of waxy maize differ according to the sowing time (Lu, Wang, Zhao, & Lu, 2009). Heat stress during grain filling has been found to decrease the table quality of fresh waxy maize (Lu & Lu, 2013), and change the starch structure

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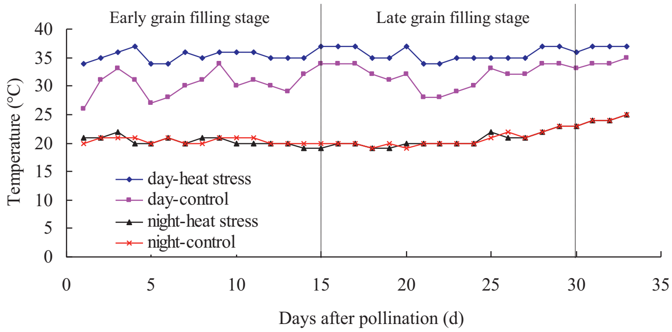

Fig. 1. Temperatures (day and night) recorded during grain filling.

and physicochemical properties of normal maize (Lu, Jane, Keeling, & Singletary, 1996). However, variations in the physicochemical properties of flour harvested at maturity in response to heat stress at different grain filling stages remain unknown. The present study investigates the physicochemical properties of waxy maize flour under heat stress during different grain filling stages. The results of this study may provide a reference for the anti-adversity cultivation of waxy maize in tropical and temperate zones.

2. Materials and methods

2.1. Plant material and growth conditions

The experiment was conducted using four waxy maize varieties: Suyunuo5 (heat resistant), Lainongnuo11 (heat resistant), Yunuo7 (heat sensitive), and Huaikenuo3 (heat sensitive), provided by Jiangsu Yanjiang Institute of Agricultural Sciences, Qingdao Agricultural University, Chongqing Academy of Agricultural Sciences, and Huaibei Kefeng Seed Limited Company, respectively, grown at the farm of Yangzhou University, Yangzhou, China.

Seeds were sown on the 13th of March, 2012 and transplanted to plastic pots on the 25th of March, 2012 (2 plants per pot and 1 plant left at the jointing stage). The plastic pots were 42 cm in height, 38 cm in diameter, and loaded with 30 kg of sieved sandy loam soil. Plants were given 10 g of basal dressing per pot (commercial fertilizer, N:P_2O_5:K_2O = 15%:15%:15%) during transplantation and 6 g of top dressing per pot (commercial urea, N concentration = 46%) at the jointing stage (15 May 2012). During maize growth, the rainfalls were avoided by canopy (4.5 m height), the weeds were removed at seedling stage, the corn borers were prevented by biological agent and soil moisture status was controlled to optimum.

After artificial pollination, plants were moved to the greenhouse for high temperature treatment. The plants were exposed to high temperature from 1 to 15 d or from 16 to 30 d after pollination (DAP). Each treatment included 10 pots as replicates. The daily average temperature of the greenhouse was 35.6 °C/20.8 °C (day/night). Outdoor conditions with temperatures of 31.4 °C/20.9 °C (day/night) were taken as the control treatment (Fig. 1). From 18:00 of the previous day to 06:00 of the next day, the door and windows of the greenhouse were left open to ensure that the indoor temperature at night was identical to that outdoors.

2.2. Production of flour

The grains were harvested at 40 DAP and oven dried at 40 °C for 5 days, and the final moisture content was about 5%. The grain dry weight = The final grain weight × 0.95. The grains were ground using a grinder and passed through a 100-mesh (0.149 mm) sieve for sample determination.

2.3. Starch isolation

The grains (100 g) were steeped in 500 ml of distilled water containing 1 g/l sodium hydrogen sulfite (1 g/l SO_2) for 48 h at room temperature. Starch was isolated from the grains following a method described previously (Lu & Lu, 2012).

2.4. Proximate analysis

The nitrogen content was determined using the Kjeldahl method (Method No. 46-10.01, AACC, 1990). The protein content was evaluated by measuring the nitrogen content in the flour (protein content = nitrogen content × 5.65) (Mosse, 1990).

The starch content in the flour was determined based by the hydrochloric acid dissolution method (Method No. 123/1, ICC, 1994). The soluble, optically active compound accompanying the substance under examination are extracted with 10% ethanol and removed by centrifugation.

2.5. Granule size distribution

The granule size of the starches was analyzed 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 size distribution was expressed in terms of the volume of equivalent spheres.

2.6. Granule morphology

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

2.7. X-ray diffraction pattern

X-ray diffraction (XRD) patterns of the flours were obtained using an X-ray diffractometer (D8 Advance, Bruker-AXS, Germany). The diffractometer was operated at 200 mA and 40 kV. The scanning region of the diffraction angle (2θ) ranged from 3° to 40° at a step size of 0.04° with a count time of 0.6 s. The relative crystallinity (%) was calculated as the percentage ratio of the sum of total crystalline peak areas to that of the total diffractogram (sum of total crystalline and amorphous peak areas) by software (MDI Jade 6).

2.8. Iodine staining

The λ_{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 flash, 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 500 to 700 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 λ_{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.

2.9. Pasting properties

The pasting properties (28 g total weight; 10%, w/w, dry basis) of the flours were evaluated using a rapid viscosity analyzer (Model 3D, Newport Scientific, Australia) following a method described previously (Lu & Lu, 2012). A sample suspension was equilibrated at 50 °C for 1 min, heated to 95 °C at 12 °C/min, maintained at 95 °C for 2.5 min, cooled to 50 °C at 12 °C/min, and then maintained at 50 °C for 1 min. The paddle speed was set at 960 rpm for the first 10 s and then decreased to 160 rpm for the rest of the analysis.

2.10. Thermal properties

The thermal characteristics of the flours were studied by differential scanning calorimetry (DSC, Model 200 *F*3 Maia, NETZSCH, Germany) according to a method described previously (Lu & Lu, 2012). Each sample (5 mg, dry weight) was loaded into an aluminum 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 aluminum 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 gelatinization temperature) and T_c (conclusion temperature) and ΔH_{gel} referred to the gelatinization enthalpy. Enthalpies were calculated on a starch dry weight basis. After conducting thermal analysis, the samples were stored at 4 °C for 7 days for retrogradation studies. The sample pans containing the starches were reheated at the rate of 10 °C/min from 20 to 100 °C. The retrogradation enthalpies (ΔH_{ret}) were evaluated automatically and retrogradation percentage (%*R*) was calculated as $\%R = 100 \times \Delta H_{ret/}\Delta H_{gel}$.

2.11. 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. Grain weight

Heat stress during grain filling decreased the cereal grain yield significantly (Cairns et al., 2012; Damata et al., 2010). The grain weight was most sensitive to heat stress applied at early days after pollination and became progressively less sensitive throughout grain filling (Stone & Nicolas, 1995). In the present study, the grain weights of Suyunuo5, Yunuo7, Lainongnuo11, and Huaikenuo3 at the early grain filling stage were decreased by 35.8%, 15.8%, 37.5%, and 27.7%, respectively, after application of heat stress. The grain weights of Suyunuo5, Yunuo7, Lainongnuo11, and Huaikenuo3 were decreased by 5.7%, 10.0%, 12.7%, and 8.1%, respectively, under heat stress at late grain filling stage (Fig. 2a). These results indicate that high temperature during the early grain filling stage have a severe effect on grain development and final grain weights because heat stress affects endosperm cell division and starch biosynthesis and can reduce subsequent dry matter accumulation within the kernels (Cairns et al., 2012). Similar results have been reported in normal maize (Commuri & Jones, 1999), wheat (Liu et al., 2011), and rice (Cao et al., 2009).

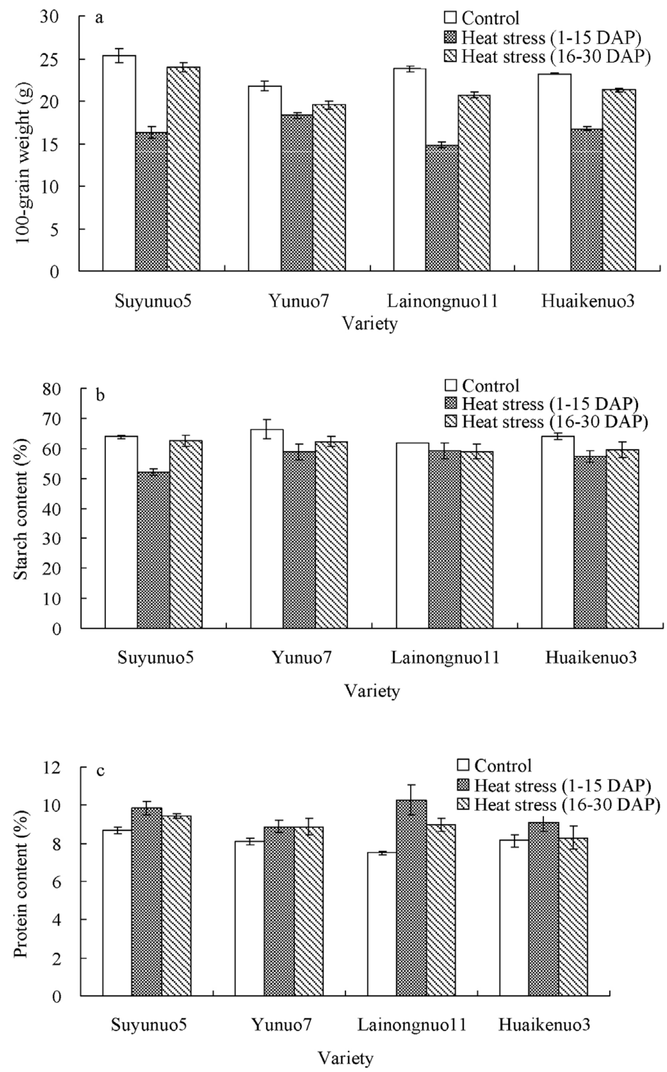

Fig. 2. Effects of heat stress on the grain weight (a) and starch (b) and protein (c) contents of waxy maize.

3.2. Starch content

Heat stress decreases the starch content of barley, rice, wheat, and maize (Wang & Frei, 2011). The amylopectin content of wheat is more sensitive to heat stress than its amylose content (Wang et al., 2008). Heat stress during different grain filling stages decreased the starch content of the grains and this decrement was generally more severe when the plants were exposed to high temperature at the early grain filling stage than at the late grain filling stage. A noteworthy exception was Lainongnuo11, the starch contents of which were similar between the two heat stress treatments (Fig. 2b). The different behave may be because the response of starch content to heat stress was variety-dependent, as reported in normal maize (Lu et al., 1996) and fresh waxy maize (Lu & Lu, 2013). The effects of heat stress on starch content were similar to those on grain yield because starch was the main component of grain and yield loss was mainly due to decrements in starch deposition (Barnabas et al., 2008). The severe loss of starch content upon application of heat stress during the early grain filling stage may be explained by high temperature at this stage disturbing endosperm cell division, reducing amyloplast numbers, deforming starch granules, restricting starch granule formation; high temperature at the late grain filling stage only moderately affect seed storage process (Commuri & Jones, 1999, 2001).

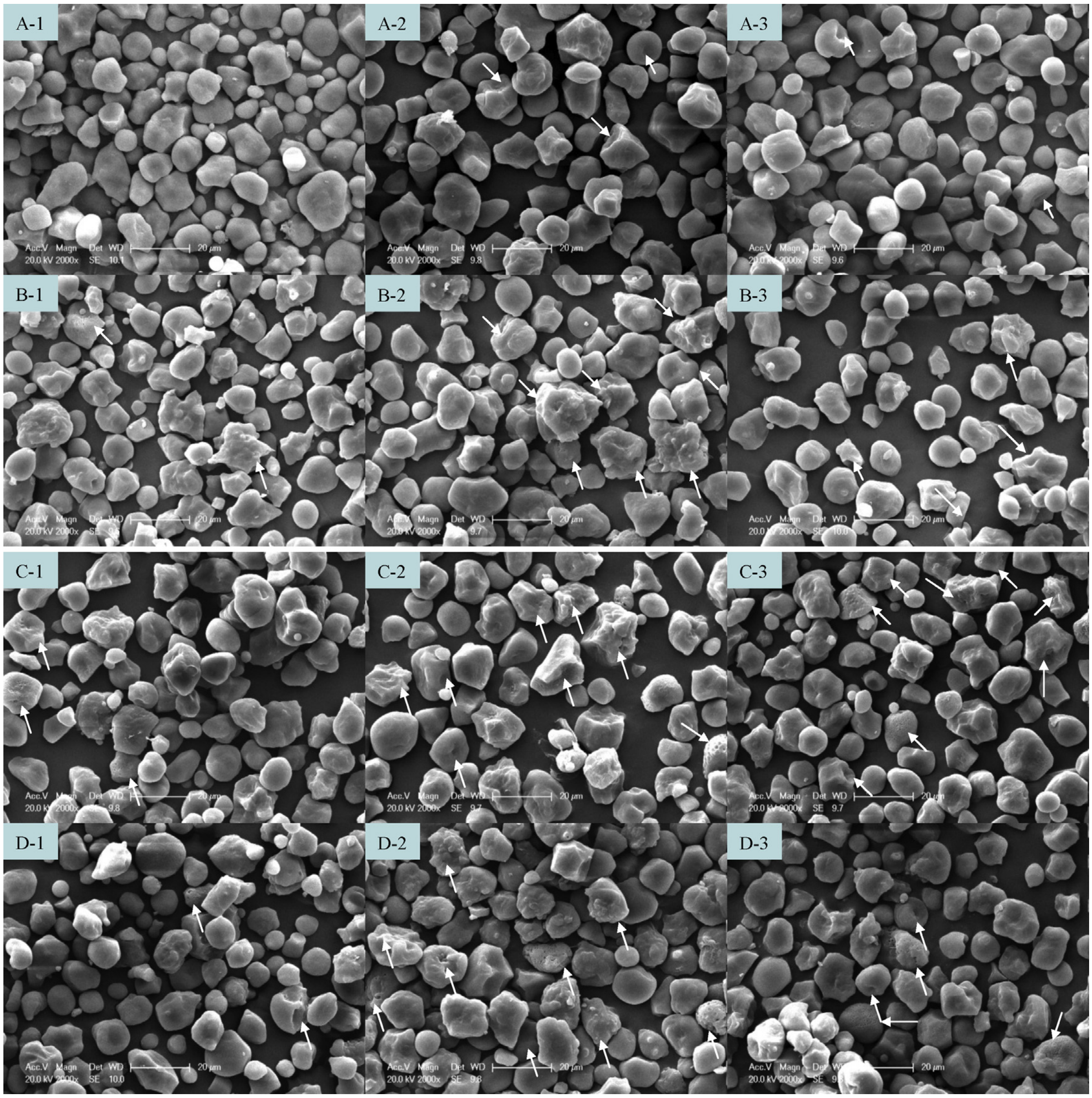

Fig. 3. Waxy maize starch morphology of Suyunuo5 (A), Yunuo7 (B), Lainongnuo11 (C) and Huaikenuo3 (D) under control (1), heat stress at 1–15 DAP (2) and heat stress at 16–30 DAP (3).

3.3. Protein content

Several reviews have indicated that heat stress increases protein contents (Cairns et al., 2012; Damata et al., 2010; Thitisaksakul et al., 2012; Wang & Frei, 2011). In the present study, heat stress increased the protein contents of grains for all the maize varieties, and the increments were higher during the early grain filling stage than during the late grain filling stage. Only protein content in Yunuo7 was similar between the heat stress treatments at early and late stages of grain filling (Fig. 2c). Similar results have been observed in rice (Zhang et al., 2006). Higher protein contents were accompanied by lower grain weights and decreased starch accumulation. The average grain weight, protein and starch quantity (mg/kernel) under control, heat stress at early and late grain filling stages were 235 mg, 166 mg, 214 mg, and 19.1 mg/kernel, 15.7 mg/kernel, 19.1 mg/kernel, and 150.3 mg/kernel, 94.2 mg/kernel, 130.3 mg/kernel, respectively (Fig. 2). The results indicated that the increase in the protein content may be due primarily to the reduction in the starch (37.3%), which was more severe than in the protein (17.8%). Our former study also observed that the increase in the protein content for fresh waxy maize grain was only a concentration effect (Lu & Lu, 2013).

3.4. Starch granule morphology

The morphology of waxy maize starch granules was observed by SEM (Fig. 3). The starch granules showed irregular polygonal shapes. Starch granules exposed to heat stress were larger than those of the control and showed higher percentages of pitting and abnormal shapes. Similar results have been reported in maize (Badenhuizen, 1969; Commuri & Jones, 1999) and wheat (Hurkman & Wood, 2011). The percentage of large, pitted, and abnormally shaped granules was higher under heat stress during the early grain filling stage than that during the late grain filling stage. This result indicates the newly developed endosperm cells and starch granules were easier affected by the heat stress. Liu et al. (2011) also observed that the wheat starch granules were easily affected by heat stress at 6–8 DAP than at other stages.

3.5. Starch granule size distribution

Several studies have observed that heat stress increases the ratio and size of large granules (Hurkman & Wood, 2011; Hurkman et al., 2003; Liu et al., 2011). In the present study, the starch granule size

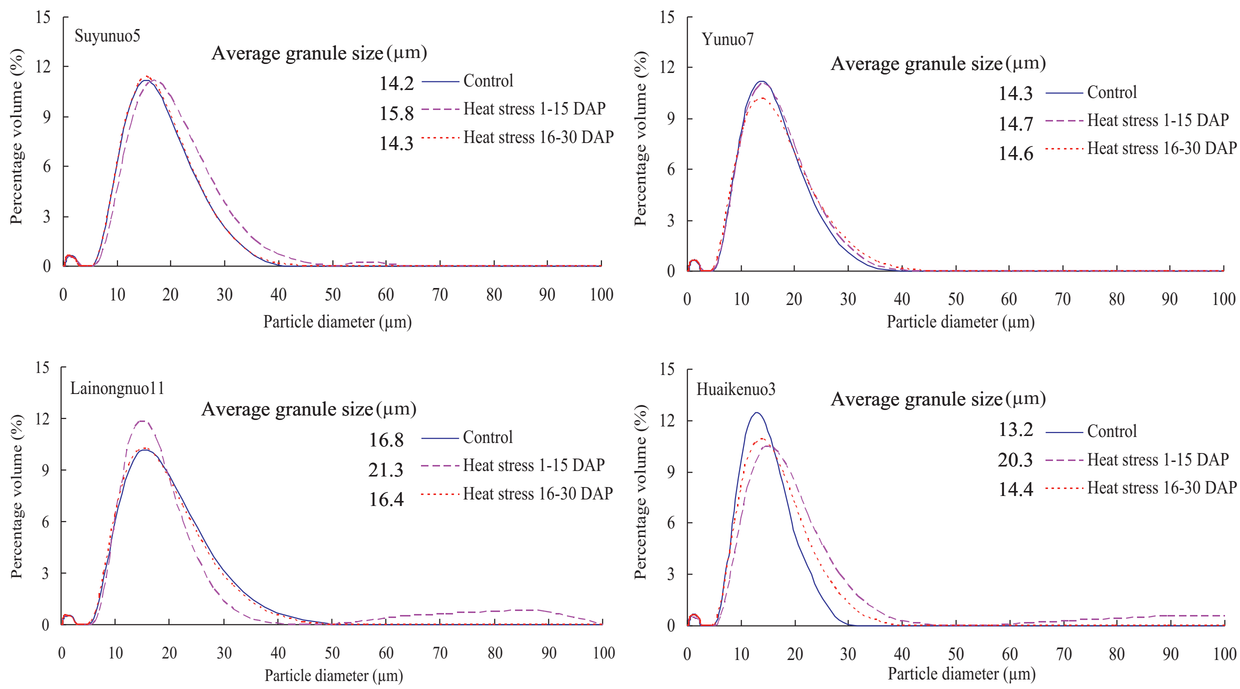

Fig. 4. Effects of heat stress on the starch granule volume distribution of waxy maize.

distribution was affected by heat stress (Fig. 4). Compared with the control, heat stress at the early grain filling stage increased the average granule size in all maize varieties. Heat stress at the late grain filling stage did not affect Suyunuo5 but decreased and increased the average granule size of Lainongnuo11 and both Yunuo7 and Huaikenuo3, respectively. In general, heat stress increased the average granule size, and larger increments were observed during the early grain filling stage than during the late grain filling stage. These results corresponded with the observation by SEM (Fig. 3). Li, Blanco, and Jane (2007) observed that starch granules were increased in the numbers up to 12 DAP and increased in the size from 14 DAP to 30 DAP in the normal maize endosperm. Heat stress decreased both the number and size of starch granules but the effects on large granules were at a lesser extent than on small granules (Lindeboom, Chang, & Tyler, 2004). Heat stress inactivates specific biosynthetic enzymes and restrains the production of small granules (Tester & Karkalas, 2001). In this case, the available substrate is diverted toward pre-existing large granules, allowing them to grow larger and leading to higher ratios of large to small granules by volume (Hurkman et al., 2003).

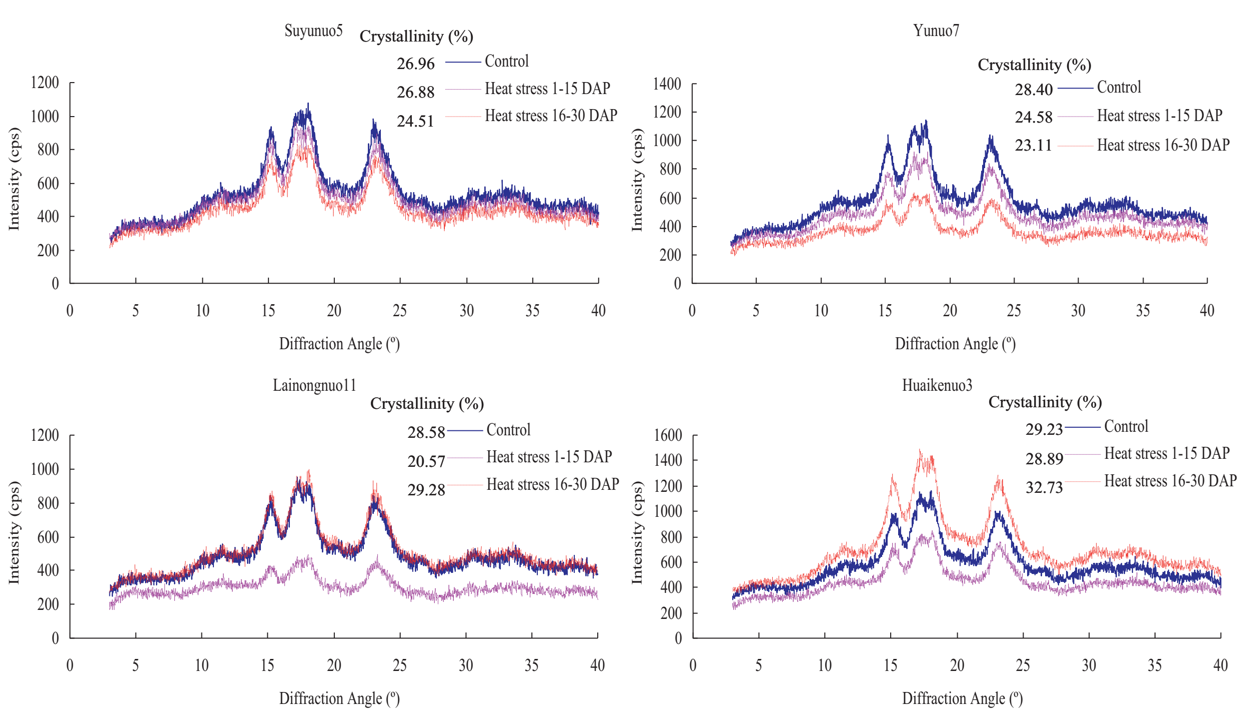

Fig. 5. Effects of heat stress on the X-ray diffraction profiles and crystallinity of waxy maize flour.

Table 1
Effects of heat stress on the iodine binding capacity of waxy maize starch.[c]

Variety	Treatment[a]	Blue value	λ_{max} (nm)	Iodine binding capacity
Suyunuo5	Control	0.076[e]	540.6[c,d]	0.586[d,e,f]
	HS 1-15 DAP	0.079[d,e]	539.6[d,e]	0.603[c]
	HS 16-30DAP	0.086[b,c]	541.7[c]	0.582[e,f]
Yunuo7	Control	0.085[b,c]	538.4[e,f]	0.593[c,d,e]
	HS 1-15 DAP	0.082[c,d]	541.6[c]	0.625[a,b]
	HS 16-30DAP	0.090[a]	538.9[e]	0.598[c]
Lainongnuo11	Control	0.081[d]	536.7[g,h]	0.574[f]
	HS 1-15 DAP	0.075[e]	539.8[d,e]	0.595[c,d]
	HS 16-30DAP	0.078[d,e]	545.3[a]	0.601[c]
Huaikenuo3	Control	0.088[a,b]	535.4[g]	0.561[g]
	HS 1-15 DAP	0.089[a,b]	543.4[b]	0.634[a]
	HS 16-30DAP	0.090[a]	537.4[f,g]	0.620[b]
F-value[b]				
Variety		39.7**	10.8**	16.0**
Heat stress		11.6**	60.2**	93.3**
Variety × heat stress		4.5*	38.9**	19.6**

[a] HS, heat stress; DAP, days after pollination.
[b] *, $p < 0.05$; **, $p < 0.01$.
[c] Mean values in the same column followed by different superscripts (a–g) are significantly different ($p \leq 0.05$).

3.6. Crystalline properties

Environmental factors have significant effects on the crystalline structure of starch (Tester & Karkalas, 2001). All of the samples, regardless of the variety and temperature treatment, displayed an A-type XRD pattern (Fig. 5), similar to our previous results on fresh waxy maize flour (Lu & Lu, 2013). Heat stress at the early grain filling stage decreased the peak intensities at $2\theta = 15°$, 17°, 18°, 20°, and 23° in flours of the four maize varieties, and the decrement observed was more severe for Lainongnuo11 than for the other varieties. The peak intensities of Lainongnuo11 and Huaikenuo3 exposed to high temperature during the late grain filling stage increased whereas those of Suyunuo5 and Yunuo7 decreased. The changes of crystallinities among the treatments were similar to the peak intensities, as those parameters were positively correlated between each other (Lu et al., 2009). Heat stress decreased peak intensities have been observed in maize (Lu et al., 1996), rice (Zhong, Cheng, Wen, Sun, & Zhang, 2005), and wheat (Wang et al., 2008), and the decrement was observed to be more severe from 5 to 7 DAP than from 25 to 27 DAP (Wang et al., 2008), and the crystallinity was decreased by heat stress but no significant difference was observed among different stages that the plants exposed to high temperature.

3.7. Iodine staining

The blue value responses to heat stress during grain filling was variety-dependent. During the early grain filling stage, the blue value decreased by heat stress in Lainongnuo11 but remained similar to the control plants in the other varieties. At the late grain filling stage, however, the blue values increased in Yunuo7 and Suyunuo5, while it remain unaffected for Huaikenuo3 and Lainongnuo11 exposure to high temperature (Table 1). The λ_{max} was increased by heat stress during the late grain filling stage for all varieties, and increased by heat stress during the early grain filling stages for all varieties except for Suyunuo5, which value was similar to control. The iodine binding capacity was increased by heat stress during the early grain filling stage for all varieties, and increased by heat stress during the late grain filling stage for all varieties except for Suyunuo5, which value was similar to control. In general, the iodine binding capacity was increased by heat stress and the increments were larger at the early grain filling stage than at the late grain filling stage. The iodine binding capacity allows estimation of amylose content or the ratio of long chains to short ones in amylopectin (Fiedorowicz & Rebilas, 2002). High iodine binding capacity values under heat stress indicate larger amounts of long chains in the amylopectin. Several studies have observed that heat stress during grain filling increases the amount of long chains of amylopectin in the endosperm (Lu et al., 1996; Matsuki, Yasui, Kohyama, & Sasaki, 2003; Suzuki et al., 2004), which may be due to reductions in starch branching enzyme activities lowering the branching frequency of amylopectin under heat stress (Thitisaksakul et al., 2012).

3.8. Pasting properties

Heat stress during grain filling significantly decreased the pasting quality of rice and wheat flours, and relevant changes were more severe during the early grain development than during the late grain development (Liu et al., 2011; Zhang et al., 2006, 2008). In the present study, heat stress during grain filling decreased the peak, trough, and final viscosities of the waxy maize flours, and the decrements were more severe when stress was applied at 1–15 DAP than at 16–30 DAP. Only Suyunuo5 exhibited an exception to this trend (Table 2), showing peak viscosities similar between the two heat stress treatments and trough and final viscosities were higher at 1–15 DAP than at 16–30 DAP. Pasting temperatures increased by heat stress in Suyunuo5, Yunuo7, and Lainongnuo11; no significant differences were observed between the two grain filling stages. The pasting temperature of Huaikenuo3 also increased by heat stress at the early grain filling stage but it was similar between the control treatment and heat stress at the late grain filling stage. The high pasting temperature under heat stress may be due to the samples have high protein content ($r = 0.75$), high ratio of long chains ($r = 0.61$) and low crystallinity ($r = -0.67$) (Table 4). Heat stress at different grain filling stages showed no significant effects on setback viscosity because the waxy maize starch is composed of 100% amylopectin, which is difficult to recrystallize during cooling (Singh, Sandhu, & Kaur, 2005). The breakdown viscosity was decreased by heat stress and the decrement was more severe during the early grain filling stage in most of the varieties, except Lainongnuo11, which showed similar values between two grain filling stages. The lower peak and breakdown viscosity of maize starch under high temperature stress during early grain development may be due to the bigger granules and higher ratio of long chain in the starch ($r = -0.73$, -0.64, and -0.68, -0.45, respectively), which

Table 2
Effects of heat stress on the pasting properties of waxy maize flour.[c]

Variety	Treatment[a]	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	P_{temp} (°C)
Suyunuo5	Control	1365^{b,c}	974^{a,b,c}	391^{b,c}	1273^{a}	299	70.5^{d,e}
	HS 1-15 DAP	1143^{e}	968^{a,b,c}	175^{g}	1202^{c}	234	72.8^{a}
	HS 16-30DAP	1130^{e,f}	840^{d}	290^{d,e}	1045^{e,f}	206	72.4^{a,b}
Yunuo7	Control	1412^{b}	986^{a,b}	426^{b}	1231^{a,b,c}	246	71.4^{c}
	HS 1-15 DAP	1154^{e}	919^{c}	235^{f}	1122^{d}	203	72.2^{a,b}
	HS 16-30DAP	1313^{c,d}	975^{a,b,c}	338^{c,d}	1209^{c}	235	72.0^{b,c}
Lainongnuo11	Control	1339^{b,c,d}	1015^{a}	325^{d}	1266^{a,b}	252	70.8^{d}
	HS 1-15 DAP	1063^{f}	926^{b,c}	137^{g}	1138^{d}	212	72.7^{a}
	HS 16-30DAP	1154^{e}	990^{a,b}	165^{g}	1221^{b,c}	232	72.3^{a,b}
Huaikenuo3	Control	1509^{a}	917^{c}	592^{a}	1094^{d,e}	178	69.5^{f}
	HS 1-15 DAP	953^{g}	688^{e}	266^{e,f}	852^{g}	165	71.5^{c}
	HS 16-30DAP	1268^{d}	846^{d}	422^{b}	1034^{f}	188	70.0^{e,f}
F-value[b]							
Variety		10.2**	34.5**	79.6**	114.4**	4.9*	44.5**
Heat stress		173.3**	21.6**	170.1**	75.7**	3.1	75.4**
Variety × heat stress		12.5**	9.8**	4.7*	22.7**	1.0	5.0*

[a] HS, heat stress; DAP, days after pollination; PV, peak viscosity; TV, trough viscosity; BD, breakdown viscosity; FV, final viscosity; SB, setback viscosity; P_{temp}, pasting temperature.
[b] *, $p < 0.05$; **, $p < 0.01$.
[c] Mean values in the same column followed by different superscripts (a–g) are significantly different ($p \leq 0.05$).

are hard to break down, in addition to its higher protein content ($r = -0.71$ and -0.71) and lower starch content ($r = 0.69$ and 0.69) (Table 4). This is because high protein content formed a matrix and restrains the starch swell, results its lower peak and breakdown viscosities (Noisuwan, Bronlund, Wilkinson, & Hemar, 2008). Our former study also observed that the peak and breakdown viscosity of waxy maize starch was negatively correlated with large granule size and positively correlated with crystallinity (Lu et al., 2009; Lu, Guo, Dong, & Lu, 2010).

3.9. Thermal properties

Heat stress had significant effects on the thermal properties of waxy maize flour. Heat stress at the late grain filling stage increased the ΔH_{gel} of all varieties. Heat stress at the early grain filling stage also increased the ΔH_{gel} of Lainongnuo11. Under this condition, the ΔH_{gel} of the other three varieties was similar to that of the control treatment (Table 3). Suzuki et al. (2004) observed that ΔH_{gel} increases by heat stress; other studies reported that this response to heat stress is variety-dependent (Lu et al., 1996; Matsuki et al., 2003; Zhong et al., 2005). Heat stress applied during the early grain filling stage increased the T_o of all varieties. Application of heat stress at the late grain filling stage also increased the T_o, except in Huaikenuo3, the T_o of which remained similar to the control plants. Heat stress applied at the late grain filling stage increased the T_p of all varieties, consistent to the pasting temperature observed by RVA. At the early grain filling stage, heat stress also increased T_p, except in Yunuo7, the T_p of which remained similar to that of the control. The T_c was similar between treatments for Suyunuo5 and Yunuo7 but increased by heat stress in Lainongnuo11 and Huaikenuo3. In general, heat stress increased the gelatinization temperatures, especially during the early grain filling stage. This due primarily to high protein content ($r = 0.69$, $p < 0.01$) (Table 4), and enhanced registration of amylopectin double helices and probably enhanced rigidity of amorphous regions, as reviewed by Tester and Karkalas (2001). Similar results have been observed in maize (Lu et al., 1996), wheat (Matsuki et al., 2003), and rice (Suzuki et al., 2004; Zhong et al., 2005).

Table 3
Effects of heat stress on the thermal properties of waxy maize flour.[c]

Variety	Treatment [a]	ΔH_{gel} (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{ret} (J/g)	%*R*
Suyunuo5	Control	9.16^{b,c}	72.3^{b,c}	78.2^{b}	84.2^{b,c,d}	3.72^{b,c,d}	40.6^{c,d}
	HS 1-15 DAP	9.19^{a,b,c}	73.8^{a}	78.6^{a}	84.6^{a,b,c}	4.51^{a}	49.1^{a,b}
	HS 16-30DAP	9.55^{a,b}	73.6^{a}	78.3^{a,b}	84.1^{b,c,d}	3.64^{c,d}	38.1^{d}
Yunuo7	Control	8.46^{d,e,f,g}	71.6^{d}	77.1^{d}	84.1^{b,c,d}	3.60^{c,d}	42.5^{c,d}
	HS 1-15 DAP	8.56^{d,e,f}	72.2^{c}	76.9^{d,e}	83.5^{d,e}	3.63^{c,d}	42.4^{c,d}
	HS 16-30DAP	9.35^{a,b}	72.3^{b,c}	77.6^{c}	84.1^{b,c,d}	4.53^{a}	48.4^{a,b}
Lainongnuo11	Control	8.39^{e,f,g}	71.5^{d}	76.6^{e}	83.0^{e}	2.37^{e}	28.2^{e}
	HS 1-15 DAP	8.87^{c,d}	72.8^{b}	78.0^{b}	84.9^{a,b}	4.52^{a}	51.0^{a}
	HS 16-30DAP	9.62^{a}	72.3^{b,c}	78.2^{b}	85.2^{a}	4.31^{a,b}	44.8^{b,c}
Huaikenuo3	Control	8.03^{g}	70.1^{e}	75.3^{g}	82.6^{e}	3.27^{d}	40.7^{c,d}
	HS 1-15 DAP	8.14^{f,g}	71.4^{d}	77.2^{d}	84.4^{a,b,c,d}	4.21^{a,b,c}	51.7^{a}
	HS 16-30DAP	8.64^{d,e}	70.6^{e}	76.0^{f}	83.6^{c,d,e}	3.45^{d}	39.9^{c,d}
F-value[b]							
Variety		27.8**	95.8**	212.3**	4.1*	1.4	1.7
Heat stress		33.5**	41.3**	76.8**	8.5**	22.2**	31.1**
Variety × heat stress		1.8	1.9	27.4**	4.4*	8.0**	10.8**

[a] HS, heat stress; DAP, days after pollination; ΔH_{gel}, gelatinization enthalpy; T_o, onset temperature; T_p, peak gelatinization temperature; T_c, conclusion temperature; ΔH_{ret}, retrogradation enthalpy; and %*R*, retrogradation percentage.
[b] *, $p < 0.05$; **, $p < 0.01$.
[c] Mean values in the same column followed by different superscripts (a–g) are significantly different ($p \leq 0.05$).

Table 4
Correlation coefficients between various properties of samples from different treatments.

	PV[a]	BD[a]	P_{temp}[a]	ΔH_{gel}[a]	T_p[a]	%R[a]
Protein content	−0.71[c]	−0.71[c]	0.75[c]	0.49	0.69[c]	0.73[c]
Starch content	0.69[c]	0.69[c]	−0.5	−0.16	−0.35	−0.47
λ_{max}	−0.72[c]	−0.68[b]	0.61[b]	0.50	0.64[b]	0.43
Iodine binding capacity	−0.68[b]	−0.45	0.32	−0.05	0.12	0.52
Average granule size	−0.73[c]	−0.64[b]	0.42	−0.13	0.27	0.47
Crystallinity	0.30	0.48	−0.67[b]	−0.36	−0.51	−0.36

[a] PV, peak viscosity; BD, breakdown viscosity; P_{temp}, pasting temperature; ΔH_{gel}, gelatinization enthalpy; T_p, peak temperature; %R, retrogradation percentage.
[b] Correlation is significant ($p < 0.05$).
[c] Correlation is significant ($p < 0.01$).

After the gelatinized samples had been stored at 4 °C for 7 d, retrogradation occurred. Heat stress applied at 1–15 DAP increased the ΔH_{ret} and %*R* of all varieties, except for Yunuo7, which showed results similar to those of the control. Heat stress at 16–30 DAP also increased the ΔH_{ret} and %*R* of Yunuo7 and Lainongnuo11 but did not affect Suyunuo5 and Huaikenuo3. In general, heat stress increased the %*R* of the maize and the increment was larger at the early grain filling stage than at the late grain filling stage. The results of correlation analysis (Table 4) observed that %*R* was positively correlated to protein content ($r = 0.73$, $p < 0.01$), average granule size ($r = 0.47$), iodine binding capacity ($r = 0.52$), and it was negatively correlated to starch content ($r = -0.47$) and crystallinity ($r = -0.36$), indicated the high protein content results in high %*R* as the denatured protein leads to more aggregation (Noisuwan et al., 2008), and high average granule size and the ratio of long chains in amylopectin were also lead to high %*R* (Singh et al., 2005).

4. Conclusion

Heat stress during grain filling decreased the grain weight, restrained starch biosynthesis, and increased the protein content of maize grains. The effects of heat stress on starch physicochemical properties of waxy maize were more severe when plants were exposed to high temperature at the early grain development stage than at the late grain development stage. Heat stress increased average granule size, ratio of long chains to short ones in amylopectin, and amount of abnormal and pitted granules of maize starch, especially at the early filling stage. The XRD patterns of the maize showed no changes and the peak intensities and crystallinities decreased under heat stress during the early development stage in all varieties. By contrast, responses to heat stress during the late development stage were variety-dependent. Heat stress decreased the peak viscosity, breakdown viscosity, and increased their pasting temperature, transition temperature, ΔH_{gel}, and %*R* of the waxy maize flour, resulting in deterioration of starch quality. Differences in physicochemical characteristics were also observed among the four varieties.

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