

## Starch structural and functional properties of waxy maize under different temperature regimes at grain formation stage

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### ABSTRACT

Global warming affects crop productivity, but the influence is uncertain under different temperature regimes. The impact of growth temperatures (T0, 28 °C/20 °C; T1, 32 °C/24 °C; T2, 36 °C/28 °C; T3, 40 °C/32 °C) at grain formation stage on the waxy maize starch physicochemical properties of Suyunuo5 (heat-sensitive hybrid) and Yunuo7 (heat-tolerant hybrid) was studied. Compared with T0, T2 and T3 resulted in a higher number of starch granules with more pitted or uneven surface due to the enhanced enzymatic activities of  $\alpha$ -amylase and  $\beta$ -amylase. Meanwhile, large starch granule size, long amylopectin chain-length, and high relative crystallinity under T2 and T3 resulted in low pasting viscosities and gelatinization enthalpy and high retrogradation percentage, especially under T3. The low coefficient variation of gelatinization temperatures indicated that the differences were meaningless. The influence of T1 on the pasting viscosities were more obvious in Suyunuo5. In conclusion, high temperatures at grain formation stage deteriorated the starch pasting and retrogradation properties.

### Introduction

Climate change characterized by global warming is increasingly threatening crop production and food security. The fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC) pointed out that global temperature will rise by 0.3 °C–0.7 °C in 2016–2035, and global food security is seriously threatened by high temperature stress (HS) (IPCC, 2013). The data from 23 global climate models also showed a high probability (higher than 90 %) that temperatures during the growing season in the tropics and subtropics by the end of the 21st century will exceed the most extreme seasonal temperatures recorded from 1900 to 2006, and in many temperate regions, the hottest seasons on record will represent the future norm (Battisti & Naylor, 2009). Heat stress is a prevalent agricultural problem that can inhibit plant growth and development and reduce agricultural productivity. Without genetic improvement, CO<sub>2</sub> fertilization, and effective adaptation, the yield penalty is 6.0 %, 3.2 %, 7.4 %, and 3.1 % in wheat, rice, maize, and soybean, respectively, with each 1 °C increase in global mean temperature (Zhao et al., 2017).

Maize (*Zea mays* L.) is the most versatile cereal crop and plays a vital

role in ensuring food security. The crop can be used to produce starch, ethanol, syrups, and many other useful non-food products that can be consumed in various forms by both humans and animals. Among different stages, those sensitive to HS are the reproductive development and the post-silking stages, and post-silking HS accelerates the grain-filling rate, shortens the grain-filling period, and restricts the grain component deposition (Lizaso et al., 2018). The enzymatic activities involved in maize starch biosynthesis were downregulated by HS and resulted in less starch content; in vitro, the influence was severe under 35 °C–37 °C (Duke & Doehlert, 1996; Singletary, Banisadr, & Keeling, 1994). Maize grain yield did not decrease in the 36 °C/26 °C during flowering compared with the control (32 °C/22 °C), but reduced by 73.6 % in the 40 °C/30 °C as a result of reduced kernel number rather than kernel weight (Wang et al., 2019).

The influences of HS on the starch structural and functional properties are widely reported in cereal crops. In wheat, the effects of HS applied 6–8 d after pollination (DAP) on starch content and pasting properties were more severe than during other filling periods; severe HS (40 °C) generated a fatal influence, whereas HS at late growth stage (25–27 DAP) decreased the ratio of large granules (Liu et al., 2011). The

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gene expression of the starch synthase was downregulated, and the enzymatic activity of amylase was enhanced; their influence was higher under 40 °C than under 32 °C compared with the control (Kumari et al., 2020). With the severity of HS at flowering stage, the grain yield of canola and stem lodging were aggravated due to poor pod fertility and low success ratio of developed pods (Wu, Duncan, & Ma, 2021). In our previous studies, we observed that post-silking day HS enlarged the starch granule size and increased the amylopectin chain-length, which led to the decrease of pasting viscosity and increase of retrogradation, but the influences depended on hybrids (Lu, Shen, Cai, Yan, Lu, & Shi, 2014), durations (Gu, Huang, Ding, Lu, & Lu, 2018), and stages (Lu, Sun, Yan, Wang, Xu, & Lu, 2013). Information on the effects of intensities of HS at grain formation stage on the structural and functional properties of maize starch is lacking. We hypothesized that different HS degrees may exert different effects on the starch quality of waxy maize. In maize production, the optimal temperature for maize growth was 25 °C–33 °C day/17 °C–23 °C night, and high temperature often set as around 35 °C (Li & Howell, 2021). In China, the day temperature over 35 °C, 37 °C, and 40 °C as taken as high temperatures and announce yellow, orange, and red alert; and the diurnal temperature variation was about 8 °C in summer days (July–August) in southern China (for example, the average day/night temperatures in Yangzhou summer were 32.2 °C/24.4 °C in 2016–2021). Plants of two waxy maize hybrids with different thermostabilities under normal conditions (28 °C/20 °C) and +4, +8, and +12 °C higher at grain formation stage were harvested. The morphology and size of starch granules, amylopectin chain length, crystalline structure, and thermal and pasting properties were analyzed. The results of physicochemical properties under different temperature regimes may improve our knowledge on waxy maize starch under various warmer conditions.

## Materials and methods

### Experimental design

A pot trial was conducted at the Yangzhou University Experimental Farm in 2020 using Suyunuo5 (SYN5, heat-sensitive hybrid) and Yunuo7 (YN7, heat-tolerant hybrid) as materials. The pots (h = 38 cm, d = 43 cm) were loaded with 30 kg of sieved sandy loam soil. The plants per pot (two at seedling stage and one left at the jointing stage) were provided with 10 g compound fertilizer (N/P<sub>2</sub>O<sub>5</sub>/K<sub>2</sub>O = 15 %/15 %/15 %) at transplantation and 6.6 g urea (N = 46 %) at the jointing stage. The plants were grown under field conditions until silking. After manual pollination on the same day, pots were moved to the greenhouse the next day for temperature treatments. The temperatures (day/night) in an intelligent greenhouse were set at 28 °C/20 °C (T0, control), 32 °C/24 °C (T1, mild HS), 36 °C/28 °C (T2, HS), and 40 °C/32 °C (T3, severe HS). The stress duration was 1–15 DAP (grain formation stage). After treatment, all the plants were grown at 28 °C/20 °C until maturity (about 45 DAP). Each treatment included 50 pots.

### Starch isolation

About 100 g grains were steeped in 0.5 L of 1 g/L NaHSO<sub>3</sub> solution at room temperature for 48 h, and starch isolation was conducted following a method we used in a previous report (Lu et al., 2014). Protein, lipid and ash contents in the isolated starch were lower than 4, 2, and 2 mg/g, respectively, indicating that starch purity reached the Chinese National Standard of edible maize starch (GB/T 8885-2017).

### Granule size distribution

Starch granule size was analyzed using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, Worcestershire, England). Instrument accuracy was verified by using Malvern standard glass particles. The instrument, which followed the principle of laser diffraction,

can measure sizes of 0.1 and 2000 μm. Size distribution was expressed in terms of the volume of equivalent spheres.

### Granule morphology

Starch granules were mounted on circular aluminum stubs with double sticky tape, coated with gold, examined by scanning electron microscopy (GeminiSEM 300, Carl Zeiss, Oberkochen, Germany) at an accelerating potential of 5 kV, and then photographed (Yang, Wei, Lu, & Lu, 2021).

### Amylase activity

Waxy maize kernels (middle position of the ears) were stripped from ears at 5, 10, 15, 20, 25, and 30 DAP and stored in a refrigerator maintained at –80 °C. The enzymatic activities of α-amylase and β-amylase were measured by enzyme-linked immunoassay, with three biological replicates. The detection of activity was performed using an MLBIO ELISA kit (catalog numbers ml010786 and ml010787) following the instruction of Shanghai Enzyme-linked Biotechnology Co., Ltd.

### Molecular weight distribution

The starch was debranched with isoamylase (EC 3.2.1.68 Sigma) dissolved in 50 mM of NaAC. The molecular weight distribution was analyzed using a PL-GPC 220 high-temperature chromatograph (Agilent Technologies UK Limited, Shropshire, UK) with three columns (PL110-6100, 6300, and 6525) and a differential refractive index detector (Cai, Cai, Man, Zhou, & Wei, 2014).

### X-ray diffraction pattern

The X-ray diffraction (XRD) patterns of starches were obtained using an X-ray diffractometer (D8 Advance, Bruker-AXS, Karlsruhe, Germany) following a previously described method (Yang et al., 2021). Relative crystallinity (%) was calculated as the percentage of total crystalline peak areas to that of the total diffractogram (total crystalline and amorphous peak areas) by using MDI Jade 6 software.

### Pasting property

Pasting properties of starch (total weight of 28 g; 7 % db, w/w) were estimated by a rapid viscosity analyzer (RVA, Model 3D, Newport Scientific, Warriewood NSW, Australia) according to a method described previously (Yang et al., 2021).

### Thermal property

The thermal characteristics of starch were estimated using differential scanning calorimetry (DSC) (Model 200 F3 Maia, NETZSCH, Germany) according to a method described previously (Yang et al., 2021). Thermal transitions of starch were defined as onset temperature ( $T_o$ ), peak gelatinization temperature ( $T_p$ ), conclusion temperature ( $T_c$ ), and gelatinization enthalpy ( $\Delta H_{gel}$ ). Samples were stored at 4 °C for 7 days after thermal analysis for retrogradation investigations. Retrogradation enthalpy ( $\Delta H_{ret}$ ) was automatically calculated and retrogradation percentage (%R) was computed as  $\%R = 100 \times \Delta H_{ret} / \Delta H_{gel}$ .

### Statistical analysis

In all figures and tables, data were expressed as the average of three repeated observations. Data were subjected to analysis of variance using the Tukey test at the 5 % probability level with the SPSS23.

## Results and discussion

### Granule morphology and activity of amylolytic enzymes

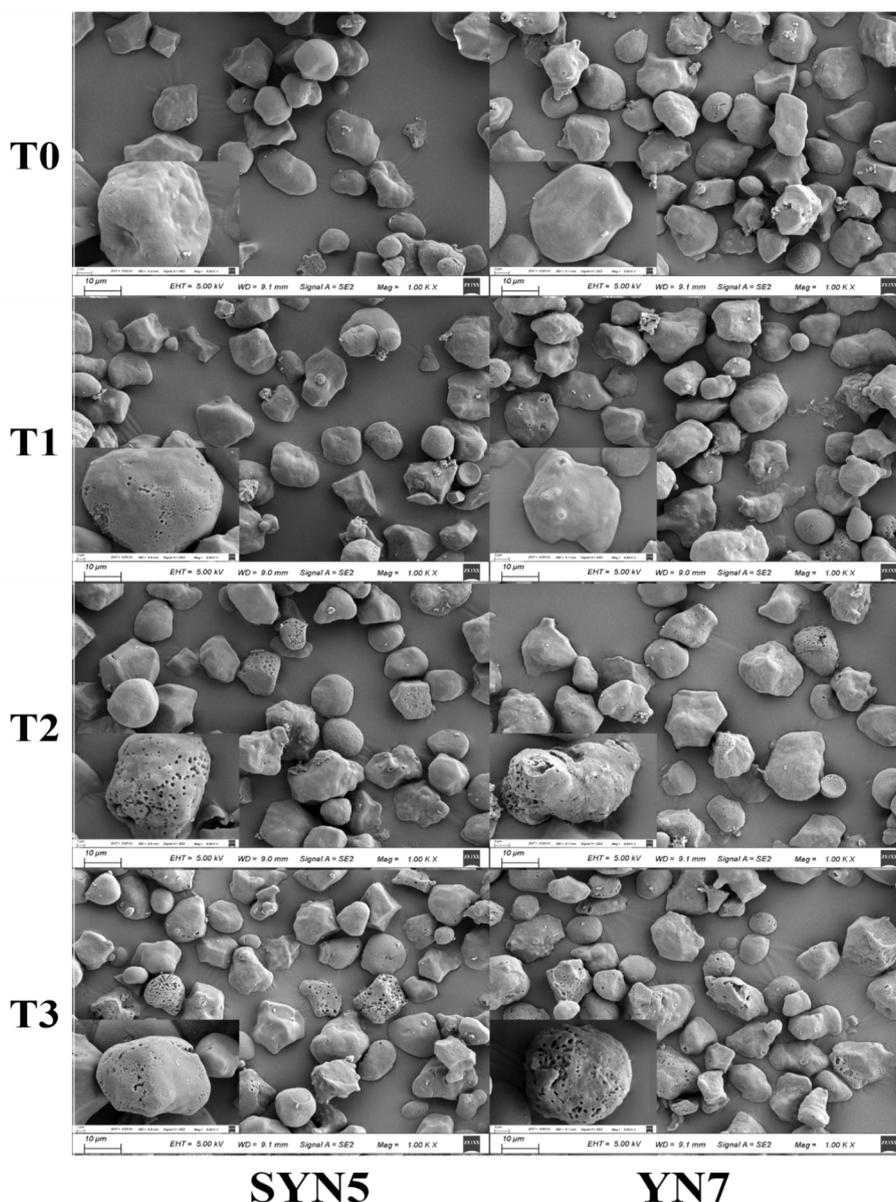
The starch granules under different temperature regimes showed oval, abnormal, and irregular polytopes in both hybrids (Fig. 1). The surface of the starch granules was smooth under T0 (28 °C/20 °C) and T1 (32 °C/24 °C), whereas the proportion of starch granules with pitted, pleated, or uneven surface increased under T2 (36 °C/28 °C) and T3 (40 °C/32 °C) in both hybrids, and more obvious in SYN5. The similar morphology of starch granules under T0 and T1 may be due to the fact that the optimal temperature for maize growth was 25 °C–33 °C day/17 °C–23 °C night (Li & Howell, 2021). Studies on wheat (Li, Zhang, Fu, Li, & Li, 2017; Liu et al., 2011), black gram (Partheeban & Vijayaraghavan, 2020), rice (Lin, Yang, Chen, Yu, Wu, & Xiong, 2020; Yao et al., 2020), normal maize (Commuri & Jones, 1999) and our previous studies (Lu et al., 2014) reported that HS increased the proportion of starch granules with uneven, rough, or pitted surface.

The increased pits or holes on the starch granule surface may be

caused by the enhanced activities of degradative enzymes, which promoted the starch hydrolysis in the endosperm (Li, Zhang, Fu, Li, & Li, 2017). The  $\alpha$ -amylase can make starch produce deeper pores;  $\beta$ -amylase has a weak ability to hydrolyze starch; compound enzymes hydrolysis has a synergistic effect compared with single enzyme (Benavent-Gil & Rosell, 2017). In the present study, the activities of endogenous amylolytic enzymes ( $\alpha$ -amylase and  $\beta$ -amylase) were significantly enhanced at T2 and T3 from 5 to 20 DAP (Fig. S1). The high activities increased the hydrolysis of starch granules and increased the number of pits and holes on the granule surface, which allowed the enzyme to degrade the starch granules more extensively (Kato et al., 2019; Li et al., 2017), and the negative correlation between the  $\alpha$ -amylase activity and small starch granule proportion also partly demonstrated this explanation (Fig. S2).

### Size distribution of starch granules

The size distributions of starch granules in all samples presented dual peaks (Fig. 2). In comparison to YN7, the mean size in SYN5 was higher



**Fig. 1.** Morphology of starch granules under different temperature regimes at grain formation stage in waxy maize. Suyunuo5, SYN5; Yunuo7, YN7; T0, T1, T2, and T3 are 28 °C/24 °C, 32 °C/24 °C, 36 °C/28 °C, 40 °C/32 °C day/night temperatures, respectively.

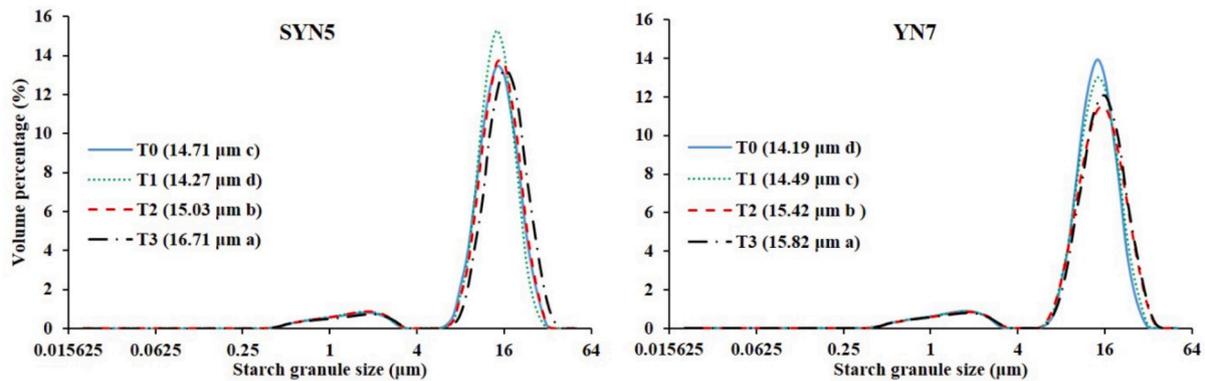


Fig. 2. Size distributions of starch granules under different temperature regimes at grain formation stage in waxy maize. Data in the bracket are average granule size ( $\mu\text{m}$ ). Mean value within each hybrid followed by different letters is significantly different ( $P < 0.05$ ). Suyunuo5, SYN5; Yunuo7, YN7; T0, T1, T2, and T3 are 28 °C/24 °C, 32 °C/24 °C, 36 °C/28 °C, 40 °C/32 °C day/night temperatures, respectively.

at T0 and T3 but was lower at T1 and T2, respectively. The proportion of small granules ( $d < 5 \mu\text{m}$ ) among different treatments ranged from 8.5 % to 9.4 % and was unaffected by the HS. In comparison with T0, the starch granule size was enlarged under T2 and T3, and the largest size was observed under T3 in both hybrids. The size under T1 was reduced in SYN5 and increased in YN7, respectively. The enlarged starch granule size under HS during grain filling were widely reported in waxy maize (Gu et al., 2018; Lu et al., 2013, 2014; Wang, Mao, Huang, Lu, & Lu, 2021), wheat (Liu et al., 2011) and rice (Liu, Zhao, Zhou, Cao, Shi, & Cheng, 2017; Zhao et al., 2019). The size of rice starch granules was decreased by HS under field conditions (Yao et al., 2020), and the stress during the primordial differentiation stage (Lin et al., 2020). In maize, the development of starch granule began with the increase in their number before 14 DAP and enlargement in their size afterward (Li, Blanco, & Jane, 2007). HS for 4–6 d during endosperm cell division disrupted cell division and amyloplast biogenesis in the endosperm cells, resulting in the severe decrease of the number of endosperm cells and starch granules (Commuri & Jones, 1999). In our previous study, we reported that post-silking HS reduced the starch granule number at the early stages (Yang, Shen, Ding, Lu, & Lu, 2017). Therefore, the substrate transferred to existing starch granules, which enlarged starch granule size. The larger granule size in SYN5 under T3 than that of YN7 maybe caused by the lesser starch granule numbers in endosperm cells (Yang, Shen, Ding, Lu, & Lu, 2017). In rice (Chen et al., 2017) and wheat (Liu et al., 2011), the endosperm cells were occupied by loosely packed starch granules under HS conditions, which also demonstrated that the number of starch granules was restricted.

#### Amylopectin structure

The gel permeation chromatography (GPC) profiles of iso-amylase debranched starch presented dual peaks, which differed from the profiles of starch with amylose (Cai et al., 2014; Wu & Gilbert, 2010), thereby indicating the typical waxy character (Fig. 3) (Hsieh, Liu, Whaley, & Shi, 2019). The F1 and F2 fractions in GPC profiles were composed of amylopectin; the F1 fraction contained short starch chains, such as A and short B chains (A + B1 chains), and the F2 fraction consisted of long B chains with higher molecular-weight molecules (Wu & Gilbert, 2010). Between the two hybrids, YN7 has higher F1/F2 ratio under all the temperature regimes, indicated it higher proportion of short amylopectin chains. In comparison with T0, F1/F2 ratio was less affected by T2 and decreased by T1 and T3 in both hybrids, indicating that severe HS at grain formation stage increased the proportion of long chains. This conclusion was widely demonstrated by early studies on different cereal crops (Fan et al., 2019; Hu et al., 2021; Kato et al., 2019; Song, Du, Zhao, & Cui, 2015; Zhao et al., 2019). The low F1/F2 ratio was caused by the large starch granule size (Fig. S2). The higher proportion of F2 may be because the HS decreased the activities of soluble starch synthase and starch branching enzymes, thereby restricting the formation of short amylopectin chains and reducing the branching degree; these phenomena resulted in the higher proportion of longer chains (Fan et al., 2019; Singletary et al., 1994).

#### Crystalline structure

The X-ray diffraction (XRD) of starch provided information on the long-range molecular order, regarded as relative crystallinity (RC),

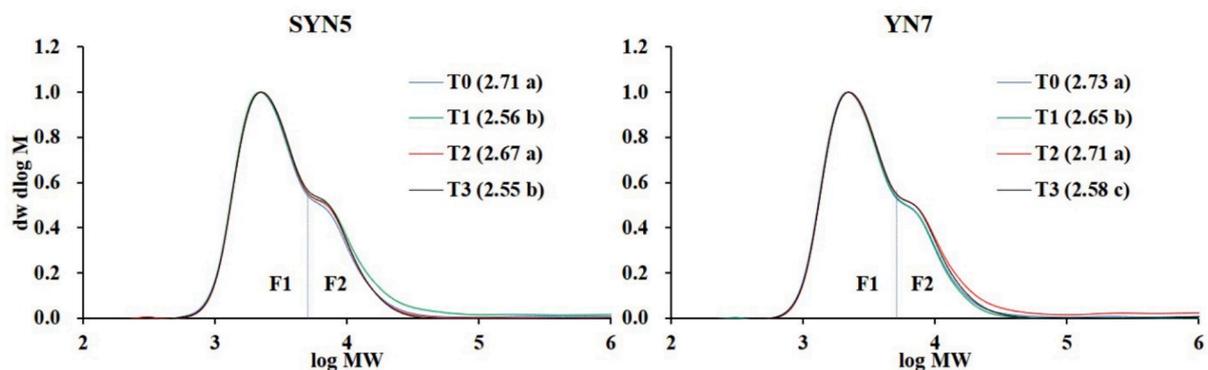


Fig. 3. Gel permeation chromatography profiles of isoamylase debranched starch under different temperature regimes at grain formation stage in waxy maize. Data in the bracket are F1/F2 ratio. Mean value within each hybrid followed by different letters is significantly different ( $P < 0.05$ ). Suyunuo5, SYN5; Yunuo7, YN7; T0, T1, T2, and T3 are 28 °C/24 °C, 32 °C/24 °C, 36 °C/28 °C, 40 °C/32 °C day/night temperatures, respectively.

which was generally associated with ordered arrays of double helices formed by the amylopectin side chains (Chen et al., 2017). All samples presented a typical A-type pattern with strong reflection at 15°, 17°, 18°, and 23°, indicating that HS did not induce the transformation of crystalline polymorphic form (Fig. 4). The RC under T0 and T1 was similar between the two hybrids, whereas it was higher in YN7 at T2 and T3. The lower RC of SYN5 at T2 and T3 may due to more granules were corroded by HS (Foresti et al., 2014). The RC in both hybrids was unaffected by T1 and increased by T2 and T3 compared with T0. Higher RC may due to the starch has large starch granule size and small F1/F2 ratio (Fig. S2). The increased RC under HS was also observed in rice (Hu et al., 2021; Song et al., 2015), but in some studies, the response was dependent on hybrids (Yao et al., 2020), heat stress stage (Lu et al., 2013), and duration (Gu et al., 2018).

### Pasting properties

The pasting profiles of waxy maize starch are shown in Fig. 5 and the pasting characteristics are shown in Table S1. The setback viscosity (SB) ranged from 56 to 126 mPa.s, indicating its typical waxy character. The peak (PV), trough (TV), final (FV), and breakdown (BD) viscosities of SYN5 gradually decreased with increasing temperatures. In comparison with T0, PV, TV, BD, and FV in YN7 were unaffected by T1 and decreased by T2 and T3, and those parameters were similar between T2 and T3. In comparison with T0, the pasting temperature ( $P_{temp}$ ) in SYN5 was reduced by T2 and unaffected by T1 and T3; the value in YN7 was similar among the four temperature regimes. PV, TV, BD, and FV were similar between the two hybrids but SYN5 has higher SB and  $P_{temp}$  in general. The severe decrease of pasting viscosities in SYN5 at T1 and T3 indicated this hybrid more sensitive to HS than YN7. In our previous study, PV, TV, BD, and FV were reduced by post-silking HS, but  $P_{temp}$  response was dependent on hybrids (Wang et al., 2021; Yan, Wang, & Lu, 2020). The decreased pasting viscosities under T2 and T3 may be because the starch was composed of large starch granules with pitting holes and long amylopectin chains and had high relative crystallinity (Fig. S2); the large starch granules with structural integrity were difficult to disintegrate (Wang et al., 2021). Meanwhile, the pasting viscosities decreased after the waxy maize starch was hydrolyzed and degraded (Mendez-Montecalvo, Wang, & Campbell, 2011). The pasting viscosities of wheat starch and flour in response to HS during grain filling depended on the cultivar (Wang, Li, Miao, Zhang, He, & Wang, 2017). The increased PV and BD were observed in delayed sown wheat that was subjected to HS during flowering (Singh et al., 2021). HS during primordial differentiation, pollen filling (Lin et al., 2020), and grain filling (Hu et al., 2021) stages increased the PV, TV, and FV but did not affect the BD of rice starch. Yao et al. (2020) observed that HS during grain filling decreased the SB and TV and increased BD, but PV response

to HS during grain filling depended on cultivar. The discrepancy among different cereals may be due to the HS sensitivity of different samples, heat stress stages, times, and durations, and whether the trials were performed in the field or under pot conditions.

### Thermal property

The gelatinization and retrogradation characteristics of waxy maize starch under different temperature regimes are presented in Table 1 and Fig. S3. In general, SYN5 has similar  $T_p$ , higher  $\Delta H_{gel}$  and  $T_c$ , and lower  $T_o$ ,  $\Delta H_{ret}$  and %R, in comparison to YN7. In comparison to T0, the gelatinization characteristics under T1 was unaffected in YN7 and decreased in SYN5, indicated that SYN5 is more sensitive to the increased temperatures. The  $\Delta H_{gel}$  was lowest under T3 among the different regimes in both hybrids. A study on wheat also observed that the  $\Delta H_{gel}$  was reduced by HS (Wang et al., 2017). In comparison with T0,  $T_o$  in YN7 was unaffected, whereas the value in SYN5 was decreased by T1, T2, and T3, but the decrease was similar.  $T_p$  and  $T_c$  in YN7 were unaffected by temperatures, whereas those two parameters in SYN5 were unaffected by T3 and decreased by T1 and T2 and the decrease were severe under T1. Though the gelatinization temperatures ( $T_o$ ,  $T_p$ , and  $T_c$ ) differed among the four temperature regimes, the low coefficient of variation (1.2, 0.8, and 1.0 for  $T_o$ ,  $T_p$ , and  $T_c$ , respectively) indicated that the difference was meaningless, and this finding was consistent with those obtained in our previous study (Yan, Wang, & Lu, 2020) and the observation on wheat (Wang et al., 2017). In studies on rice starch, researchers observed that the gelatinization characteristics in response to HS during grain filling depended on the cultivar (Hu et al., 2021; Yao et al., 2020), whereas night HS during grain filling increased gelatinization characteristics (Song et al., 2015). The discrepancy may depend on the different cultivars, samples (flour or starch), trials (adjusting sowing date under field conditions or precise temperature control in the greenhouse), and HS stage, severity, duration, and type.

After storing the gelatinized samples at 4 °C for 7 days, the retrogradation occurred. The  $\Delta H_{ret}$  in both hybrids was increased by T1-T3 in comparison with T0, which resulted in the increase of %R. The %R in both hybrids was highest under T3, following by T2 and T1. Similar results were observed in wheat (Wang et al., 2017) and the findings obtained in our previous studies (Gu et al., 2018; Lu et al., 2014; Wang et al., 2021). The increased  $\Delta H_{ret}$  and %R indicated that starches were liable to retrograde after suffered HS during grain filling, which may deteriorate the quality of waxy maize-based food products during storage. The increase in  $\Delta H_{ret}$  and %R under T2 and T3 is due to the starch had large granules, long amylopectin chain length, and high relative crystallinity (Fig. S2) (Gu et al., 2018; Wang et al., 2021). Additionally, the increased hydrolysis may due to the high activity of starch amylolytic enzymes promoted the re-association of amylopectin chains and the

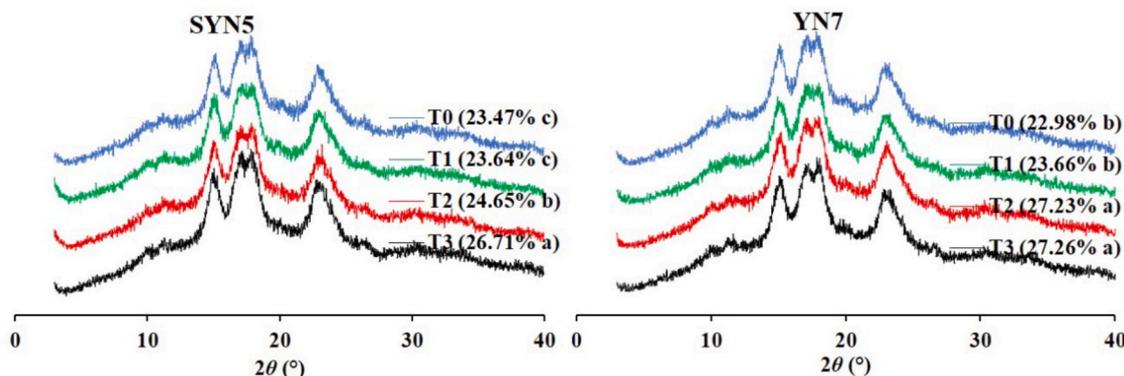


Fig. 4. X-ray diffraction profiles of starch under different temperature regimes at grain formation stage in waxy maize. Data in the bracket are relative crystallinity (%). Mean value within each hybrid followed by different letters is significantly different ( $P < 0.05$ ). Suyunuo5, SYN5; Yunuo7, YN7; T0, T1, T2, and T3 are 28 °C/24 °C, 32 °C/24 °C, 36 °C/28 °C, 40 °C/32 °C day/night temperatures, respectively.

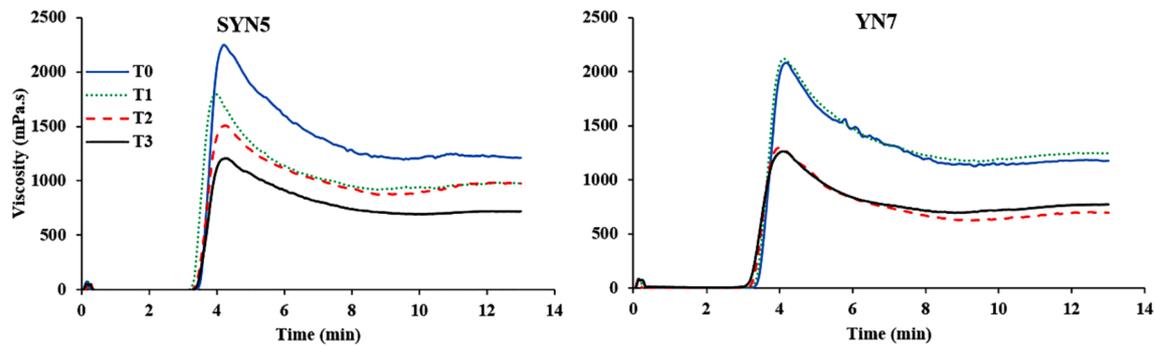


Fig. 5. Pasting profiles of starch under different temperature regimes at grain formation stage in waxy maize. Suyunuo5, SYN5; Yunuo7, YN7; T0, T1, T2, and T3 are 28 °C/24 °C, 32 °C/24 °C, 36 °C/28 °C, 40 °C/32 °C day/night temperatures, respectively.

Table 1

Effects of different temperatures on the thermal properties of waxy maize starch.

Hybrid	Temperature	$\Delta H_{\text{gel}}$ (J/g)	$T_o$ (°C)	$T_p$ (°C)	$T_c$ (°C)	$\Delta H_{\text{ret}}$ (J/g)	%R (%)
Suyunuo5	T0	13.6 a	68.4 a	73.3 ab	81.4 a	5.3 a	38.7 c
	T1	12.8 b	66.0 b	71.5 c	79.2 c	5.2 a	40.9 bc
	T2	12.6 b	66.7 b	72.6 b	80.6 b	5.4 a	42.9 b
	T3	10.0 c	66.9 b	73.7 a	80.7 ab	5.4 a	53.9 a
Yunuo7	T0	12.6 a	67.5 ab	72.7 a	79.5 a	5.0 c	40.1 d
	T1	12.1 a	67.0 b	72.6 a	79.7 a	5.4 b	44.4 c
	T2	12.3 a	68.2 a	72.8 a	79.4 a	5.9 a	48.2 b
	T3	10.4 b	68.3 a	72.5 a	79.1 a	5.7 ab	54.3 a
<i>F</i> -value							
Hybrid		18.0**	13.8**	0.8	72.0**	11.7**	27.8**
Temperature		188.6**	9.6**	14.2**	11.2**	18.2**	158.6**
Hybrid × Temperature		12.3**	8.3**	15.1**	18.4**	9.1**	4.9*

Mean value in the same column within each hybrid followed by different letters is significantly different ( $P < 0.05$ ). \* and \*\* indicate the significant difference at  $P < 0.05$  and  $P < 0.01$  level, respectively.  $\Delta H_{\text{gel}}$ , gelatinization enthalpy;  $T_o$ , onset temperature;  $T_p$ , peak gelatinization temperature;  $T_c$ , conclusion temperature;  $\Delta H_{\text{ret}}$ , retrogradation enthalpy; %R, retrogradation percentage.

consequent formation of crystalline structures after the starch was gelatinized (Mendez-Montevalvo et al., 2011).

## Conclusion

Temperature regimes at grain formation stage affected the starch physicochemical properties of waxy maize. The starch was composed of granules with irregular polytopes under different temperature regimes. The proportion of starch granules with pitted or pleated surface increased with increasing temperatures. This result may due to the increased enzymatic activities of  $\alpha$ -amylase and  $\beta$ -amylase that degraded the starch granules. The starch granule size was enlarged, and the proportion of long amylopectin chains and relative crystallinity increased when plants were subjected to HS (36 °C/28 °C and 40 °C/32 °C) at grain formation stage, thereby resulting in the decrease in pasting viscosity and gelatinization enthalpy and the increase in retrogradation enthalpy and percentage. The temperature increase from 28 °C/20 °C to 32 °C/24 °C did not affect the starch granule size, amylopectin chain length distribution, and relative crystallinity in YN7, indicating its higher thermal tolerance than SYN5. The deteriorated quality under HS was probably useful for waxy maize production. However, the underlying reasons for the involvement of starch biosynthetic enzymes and genes in starch formation, the resulting structure of amylopectin, and the measures that mitigate the influence of heat stress need further investigation.

## CRediT authorship contribution statement

**Xiaotian Gu:** Investigation, Methodology, Data curation, Formal analysis, Writing – original draft. **Xiaoyu Zhang:** Investigation, Visualization, Data curation, Formal analysis, Writing – original draft. **Weiping Lu:** Conceptualization, Supervision. **Dalei Lu:** Conceptualization, Investigation, Methodology, Funding acquisition, Project administration, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100463>.

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