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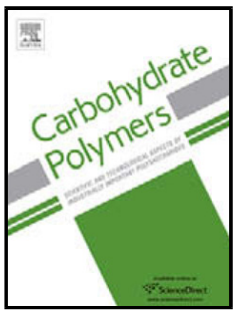

Title: Structural and physicochemical properties of granular starches after treatment with debranching enzyme

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Structural and physicochemical properties of granular starches after treatment with debranching enzyme

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Highlights

- Maize and potato granular starches were successfully debranched at 60ºC.

- Pullulanase was much effective on hydrolyzing potato starch compared to the maize starch.
- Debranched potato starches showed markedly decrease of pasting viscosities and enthalpy changes compared to the debranched maize starches.

Abstract: The present study modified maize and potato granular starches by partial debranching treatment below the gelatinization temperature, and investigated their structural and physicochemical properties. Pullulanase was much effective (more than three times) on hydrolyzing potato starch compared to maize starch as measured from total carbohydrate values in the supernatant. The pullulanase hydrolysis decreased the amount of double helices as observed from DSC measurement. These effects were dependent upon the time of enzyme hydrolysis (24h>8h>1h) as well as type of starch (potato>maize). The pullulanase hydrolysis decreased the peak viscosity of the potato starch paste, whereas the effect was very less pronounced for maize starch. The current results showed that it is possible to achieve the starches with desired physicochemical properties by varying the starch type as well as modification process.

Keywords: maize starch, potato starch, pullulanase, physicochemical properties

1. Introduction

Starch, the second most abundant biomass in nature, is biosynthesized as semi-crystalline granules in higher plants, and generally consists of two polymers, amylose and amylopectin. Amylose is a slightly branched molecule (Takeda，Maruta，& Hizukuri, 1992), whereas amylopectin is a much larger molecule with highly branched structure consisting of ca. 95% α- (1,4) linkages and ca. 5% α- (1,6) linkages (Tester, Karkalas, & Qi, 2004). Starches obtained from different botanical origins vary in granular morphology, crystalline organization and molecular structure, thus their physicochemical and nutritional properties

are origin dependent (Lehmann & Robin, 2007). In order to achieve the desired properties and meet the requirement of food and industrial applications, starches are modified using physical, chemical and enzymic techniques (Chung, Liu, & Hoover, 2009a; Jacobs, Eerlingen, Charwart, & Delcour, 1995; Tester, Karkalas, & Qi, 2004; Zhang, Huang, Luo, Fu, Jiang & Jane, 2011).

To expand the industrial applications of native starches, enzyme modification has been widely used to meet the requirement of the clean labeled food. The enzymatic modification utilizes the ability of enzyme to hydrolyze/synthesize α-(1,4) and/or α-(1,6) linkages of starch molecules. For example, the branch chains of amylopectin and amylose can be selectively cleaved at α- (1,6) linkages by either isoamylase or pullulanase (Cai, Shi, Rong, & Hsiao, 2010; Manners, 1989). The pullulanase debranched starches contained a large number of short branch chains (Liu, Hong, & Gu, 2013), showing a strong retrogradation tendency in a aqueous system (Cai & Shi, 2010). Thus, the combined debranching method with controlled crystallization could be used to alter molecular and supramolecular structure of starches as well as diverse functionality. Furthermore, partially debranched starches had greater capacity to form complex with iodine and fatty acids, and possessed higher solubility but lower viscosity compared with their native counterparts (Klaochanpong, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2015). The debranching hydrolysis could be a novel technique to alter the functionality of native starches without destroying the granular structure. In this study, we compared debranching treatment of A-(maize) and B-(potato) type polymorphic starch granules below the gelatinization temperature, and investigated the structural and physicochemical properties of partially debranched granular starches.

2. Materials and methods

2.1 Materials

Maize and potato starches were obtained from Tiancheng Company (Jilin, China).

Pullulanase (EC 3.2.1.41, 405units/g) was provided by Amano Enzyme Company (Shanghai, China). One unit is defined as the amount of pullulanase that catalyzes the increase of reduction power equivalent to 1 μM of glucose per minute. All other chemicals used in this study were of analytical grade.

2.2 Preparation of debranched granular starches

Starch (30g, dry starch basis, dsb) was mixed with 295 mL of sodium acetate buffer (0.01 M, pH 5.0), and incubated in a water bath at 60°C for 30 min. For enzymatic modification, pullulanase (10 units per dry starch basis) was added and the mixture was kept at 60°C for different time intervals (1, 8, and 24 h) with constant stirring (250 rpm). These starches treated at 60°C without pullulanase hydrolysis are regarded as hydrothermal samples. All hydrothermal and debranched starches were recovered by 3000 *g* centrifugation for 10 min followed by washing with ethanol for three times. The precipitate was oven-dried at 37°C overnight.

2.3 Determination of hydrolysis rate

All hydrothermally treated and debranched starches were recovered by 3000 *g* centrifugation for 10 min. The hydrolysis rate was measured by total carbohydrate values in the supernatant through the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Huang, Fu, He, Luo, Yu, & Li, 2010). The total carbohydrate value was calculated as follows.

$T_C = C \times N \times V$ (Eq.1)

where T_C is the total carbohydrate content of hydrolysate; C is absorbance of diluted hydrolysate according to the regression equation; N is the diluted multiples of sample solution; V is total volume of hydrolysate. The hydrolysis rate was calculated as follows.

$$\text{Hydrolysis rate (\%)} = \frac{T_C \times 0.9}{M_S} \times 100 \quad \text{(Eq.2)}$$

where M_s is the mass of native starch.

2.4 Swelling power

Swelling power (SP) was determined by using 10% starch suspension according to a method reported elsewhere (Singh, Singh, Isono, Noda, & Singh, 2009). The suspension was heated at 60°C with mechanical stirring for 30 min, and centrifuged at 800 *g* for 10 min. The supernatant was discarded and the wet starch residue was weighed. The swelling power was calculated as the weight of starch residue per gram of starch.

$$SP = \frac{M_{\mathrm{r}}}{M_{\mathrm{w}}} \qquad \text{(Eq.3)}$$

where M_{r} is the mass of starch residue after suspension was centrifuged (g), and the M_{w} is the mass of the dry weight (g) of the hydrothermally treated starch.

2.5 Apparent amylose content

Apparent amylose content of starches was determined by measuring iodine affinities of defatted whole starch using a potentiometric autotitrator (888 SM Titrino, Brinkmann Instrument, Westbury, NY, USA) following the method reported elsewhere (Stevenson, Domoto, & Jane, 2006; Takeda, Hizukuri, & Juliano, 1987). Starch samples were dissolved and defatted in 90% dimethyl sulfoxide (DMSO) solution, and followed by alcohol precipitation. An appropriate amount of precipitated sample (100mg) is weighed and transferred to a dry beaker. The water (1 mL) and KOH solution (1M, 5 mL) were added to suspend the sample with occasional stirring. HCl solution (0.5M) was used to neutralize the mixture and then KI (0.5M, 10mL) was added. Sufficient water is added to give a total weight of 100.9g over the weight of the empty beaker. Then the mixture is potentiometrically titrated with iodine at 30ºC with continuous mechanical agitation. Apparent amylose content was calculated by dividing the iodine affinity of the starch by 19.0%, the typical value of iodine affinity for purified maize amylose (Lu, Jane, Keeling & Singletary, 1996).

2.6 Light microscopy

Polarized light microscopy was performed on a BX-51TF microscope (Olympus, Tokyo, Japan). One drop of starch suspension was placed on the microscope slide before covering with a cover slip, and the images were recorded at 500×magnification.

2.7 Scanning electron microscopy (SEM)

Starch granules were mounted on an aluminum stub using double-sided tape, coated with a thin film of gold. The images were examined under scanning electron microscope (TM3000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

2.8 Wide angle X-ray diffraction (XRD)

Starch samples were equilibrated in a chamber with 100% relative humidity at 25°C for 24 h (Jane, Wong, & Mcpherson, 1997). X-ray diffractometer (D8 Advance, Bruker, Germany) was operated at 40 kV and 40 mA with Cu Kα radiation (λ=0.154 nm). The starch powder was packed tightly in a rectangular glass cell, and scanned over the range 5-35 Bragg angles at a rate of 2°/min at room temperature. Relative crystallinity of the starches was calculated using the following equation.

Relative crystallinity (%) = $100 \times A_c / (A_c + A_a)$ (Eq.4)

where A_c is the crystalline area on the X-ray diffractogram, and A_a is the amorphous area.

2.9 Thermal properties

A differential scanning calorimeter (DSC-8000, PerkinElmer, Norwalk, CT, USA) with an intra cooler was used to examine the thermal properties of starch samples. Starch samples (~3mg) were mixed with deionized water (moisture level 70%), and hermetically sealed in high-pressure stainless steel pans (PE No.BO182901) with a gold-plated copper seal (PE No. 042-191758).After equilibrating for 24 h at room temperature, samples were scanned at a heating rate of 5°C /min from 30 to 150°C. The enthalpy change (ΔH), onset (T_o), peak (T_p) and conclusion (T_c) temperatures were calculated by using a Pyris software (Perkin Elmer, Norwalk, CT, USA).

2.10 Pasting properties

The pasting properties of starch samples were measured using a Micro Visco Amylo-Graph (Brabender, Germany). Starch dispersion (6%, w/w, dsb) was directly transferred into a stainless steel canister. The dispersion was heated from 30 to 95°C at a rate of 7.5°C/min, held at 95°C for 5 min, cooled to 50°C at a rate of 7.5°C/min, then held at 50°C for another 5 min. The pasting temperature (T_P), peak viscosity (P_V), breakdown (*BD*) and set back (*SB*) values were recorded

2.11 Statistical analysis

All experiments were performed at least in duplicate, and results are expressed as their means± standard deviation (SD). In the case of XRD, only one measurement was performed. The standard deviations of XRD measurements are typically within 2%. The significance level was set at $p<0.05$. Statistical analysis was performed with SPSS 18.0 statistical software for windows.

3. Results and discussion

3.1 Degree of hydrolysis

The degree of pullulanase hydrolysis of maize and potato starches is shown in Figure 1. Negligible amylose leaching from starches during hydrothermal treatment (HMS-24h, HPS-24h) could be deduced from the total carbohydrate value of the supernatant (0.03%, Figure 1). The degree of hydrolysis for the debranched potato starch (DPS) samples markedly increased as a function of hydrolysis time (up to 34.94% for DPS-24h), whereas the debranched maize starch (DMS) samples showed a slight increase (up to 9.80% for DMS-24h). Potato starch displayed greater susceptibility to pullulanase compared with the maize starch. There can be two possible reasons: i) Hydrothermal treatment below the gelatinization temperature changes the physicochemical properties of starches without destroying the molecular and crystalline structure (Chen, He, & Huang, 2014). Swelling

power indicates the ability of starch to hydrate, and could be used to assess the expansion extent of starch granules as well as enzyme accessibility induced by granule swelling. Potato starch (2.35 g/g) swelled to a larger extent compared with the maize starch (1.88g/g) at the optimum temperature of the pullulanase (60ºC). This could be due to the repulsive force of negatively charged phosphate monoester groups (McPherson & Jane, 1999; Singh, et al., 2009), although potato starch showed a higher amylose content value (37.3%, see Table 1). ii) The branching points of potato starch are mostly located in the amorphous regions, whereas branch linkages of maize starch are more located inside the crystalline region (Jane, et al., 1997). The access to the location of branch points could also be a rate-limiting step which further controls the hydrolysis rate of A-type (maize) and B-type (potato) polymorphic starches.

3.2 Morphological properties

The morphological properties of native and debranched starches are shown in Figure 2. Potato starch granules have smooth and rigid surface structure, whereas maize starch granules show some pinholes on the surface. There is no apparent changes on granular surface after hydrothermal treatment (HMS-24h and HPS-24h), remaining intact and smooth (Fig. 2). Under polarized light microscope, all samples showed characteristic birefringence patterns centered at the hilum, indicating radial orientation of crystallites within the granule (Hibi, Matsumoto, & Hagiwara, 1994). The potato starches were hydrolyzed from surface of the granules, and the more erosion was observed with prolonged debranching time (Fig.2, DPS-24h). The hydrolysis of the maize starch was expected to be related to the surface pores linking from hilum and the surface through interior channels, facilitating ‘inside-out’ digestion (Dhital, Shrestha, & Gidley, 2010). However, it was surprised to find that the surface of DMS was slightly eroded compared with DPS, consistent with the results of hydrolysis degree (Fig.1). This could be attributed to the lower swelling power of maize

starch, preventing the penetration of pullulanase into the starch granules.

3.3 X-ray diffraction

The X-ray diffractogram and relative crystallinity of starches with debranching treatment are shown in Figure 3. Maize starch exhibited the A-type polymorph with main reflections at 15°, 17°, 18°, and 23°2θ, whereas potato starch granules showed B-type crystalline structure with main peaks at 5°, 17°, 22°, and 24° 2θ (Zobel, 1984). Partially debranched maize and potato starches retained their original crystalline type (Fig. 3), and the relative crystallinity of debranched starches slightly decreased compared with their native counterparts, particularly for potato starch.

The crystallinity changes of DPS were lower compared to DMS, which is consistent with the hydrolysis rate (Fig. 1). This could be explained in terms of the difference in location of branching points in maize and potato starches. In general, the branch chains of amylopectin form double helices and contribute to the starch crystallinity (Kainuma & French, 1972). Branch linkages of potato starch are mostly located in amorphous regions compared to the maize starch (Jane, et al., 1997), and it is easier for pullulanase to hydrolyze potato starch and reduce the crystallinity. It was noteworthy that the crystallinity of the potato starch with 24h of debranching treatment (DPS-24h) decreased markedly compared to that of DPS-8h, probably attributed to the reason that more branch chains were debranched with prolonged debranching time.

3.4 Thermal properties

The thermal properties of hydrothermally treated and debranched starches are summarized in Table 1. Starch gelatinization is an endothermic transition corresponding to the dissociation of amylopectin double helices from a semi-crystalline structure to an amorphous conformation. The enthalpy change values primarily reflect loss of double helical

order rather than loss of crystalline register (Cooke & Gidley, 1992). All debranched samples showed higher gelatinization temperatures and lower enthalpy change compared with their native starches (Table 1). The thermal properties are largely affected by the fine structure of the amylopectin, the amylose content, and the phosphate monoester derivatives of starches (Srichuwong & Jane, 2007). In general, starches consisting of amylopectin with longer branch chains show higher gelatinization temperatures, due to the formation of stable double helical crystallites. However, potato starch exhibited relatively lower gelatinization temperature quantified by DSC, resulting from the repulsion effect of phosphate-monoester derivatives between starch molecules and destabilizing the crystalline structure (McPherson, et al., 1999). Higher onset, peak, and conclusion temperatures of debranched starch samples were observed as a function of debranching time, indicating that the loss of branch chains throughout the pullulanase hydrolysis. Higher melting temperature means that considerable more amount of energy is required for starch gelatinization. In addition, the enthalpy change of debranched starches showed a decrease trend with prolonged debranching time (Table 1), particularly for potato starch. These results may be attributed to the loss of double helices of amylopectin during the hydrolysis process, consistent with the XRD data (Fig. 3).

3.5 Pasting properties

The viscosity curves and characteristic values of native and debranched starches are shown in Figure 4 and Table 2, respectively. The viscosity values (i.e., peak viscosity, trough viscosity and final viscosity) of hydrothermally treated and debranched potato pastes

decreased obviously compared with the native potato counterpart. However, there were no markedly changes of the pasting values for the hydrothermally treated and debranched maize samples compared with the native counterpart. More precisely, the debranching treatment slightly decreased the peak viscosity of maize starch from 114 BU (NMS) to 97 BU (DMS 24 h). In comparison, the peak viscosity of all DPS samples decreased markedly, and the peak viscosity of DPS-24h reduced from 433 BU (NPS) to 190 BU (DPS-24h). The peak viscosity is mainly associated with amylose contents, branch chain length distribution of amylopectin, and minor components (Jane, 2006). Amylose inhibits the swelling of starch granules, whereas amylopectin contributes to the swelling of starch granules, especially longer branch chains (Tester & Morrison, 1990). The marked decrease of peak viscosity of DPS may be attributed to the loss of certain amount of branch chains during hydrolysis process, resulting in less swelling of starch granules during heating step. It should be noted that the peak viscosity of HPS-24h was much lower than that of the native starch. It could be attributed to the partial gelatinization of hydrothermally treated samples (HMS-24h, HPS-24), which showed lower enthalpy change (Table 1.) compared with their native counterparts.

All DPS samples showed lower breakdown values compared with the native starch, showing the lower shear resistance. This phenomenon may be attributed to the hydrolysis of branch chains in amylopectin, resulting in the less physical entanglement between amylose and amylopectin upon cooling in starch paste.

4. Conclusions

The enzymatic debranching modification of maize and potato starches was conducted at the sub-gelatinization temperature. We found that pullulanase was much effective on hydrolyzing potato starch and changing physicochemical properties of the partially debranched starches compared to the maize starch. A reduced enthalpy change and increased

gelatinization temperatures of all debranched granular starches were observed from DSC data. The pasting properties showed that the peak viscosities of all debranched potato starches were lower markedly than the native counterpart, whereas the effect was less pronounced for maize starch. These results show that the modification of pullulanase debranching at the sub-gelatinization temperature is dependent on the botanical sources of starches, providing valuable information for designing starches with desired physicochemical properties.

ABBREVIATIONS USED

DMS, maize starch with debranching treatment; DPS, potato starch with debranching treatment; DSC, differential scanning calorimeter; HMS, maize starch with hydrothermal treatment; HPS, potato starch with hydrothermal treatment; NMS, native maize starch; NPS, native potato starch; SEM, scanning electron microscopy; XRD, X-ray diffraction

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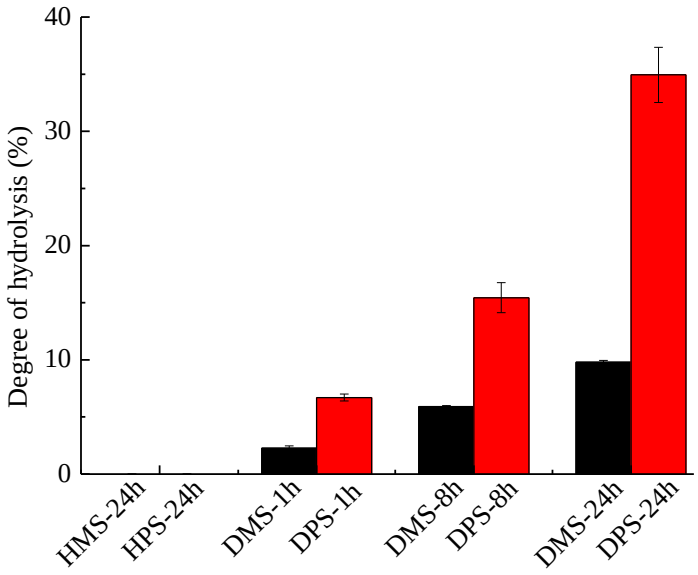

Fig.1 Degree of hydrolysis of debranched maize and potato starches. (HMS-24h maize starch with 24h of hydrothermal treatment; HPS-24h: potato starch with 24 h of hydrothermal treatment; DMS, debranched maize starch; DPS, debranched potato starch; The debranched maize /potato starches with different hydrolysis time (h) were denoted as DMS-1h, DMS-8h, DMS-24h, DPS-1h, DPS-8h, DPS-24h respectively)

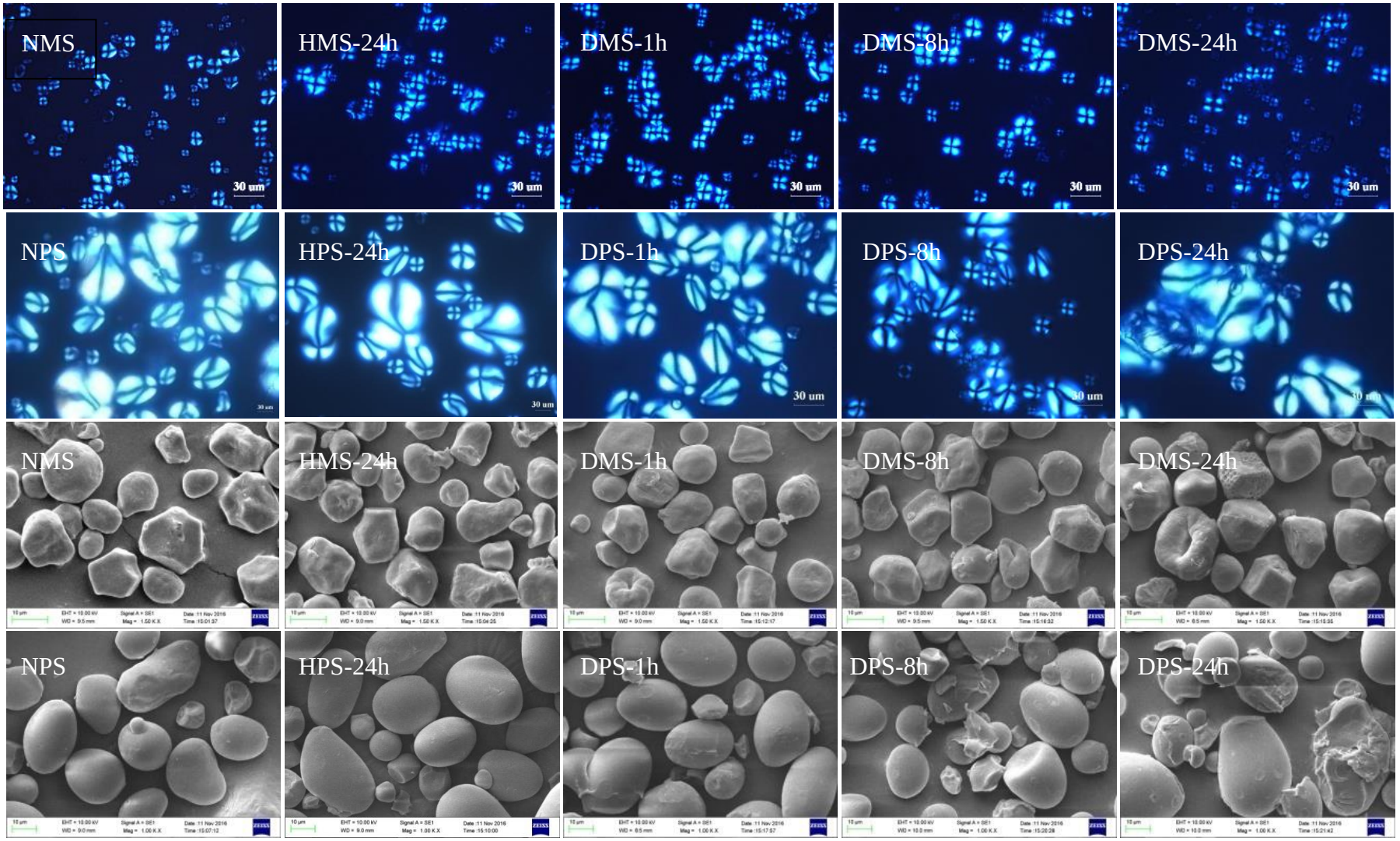

Fig. 2 Morphological properties of debranched maize and potato starches. (NMS, native maize starch; NPS, native potato starch; The debranched maize (DMS)/potato (DPS) starches with different hydrolysis time (h) were denoted as DMS-1h, DMS-8h, DMS-24h, DPS-1h, DPS-8h,

DPS-24h respectively; HMS-24h, maize starch with 24h of hydrothermal treatment; HPS-24h, potato starch with 24h of hydrothermal treatment)

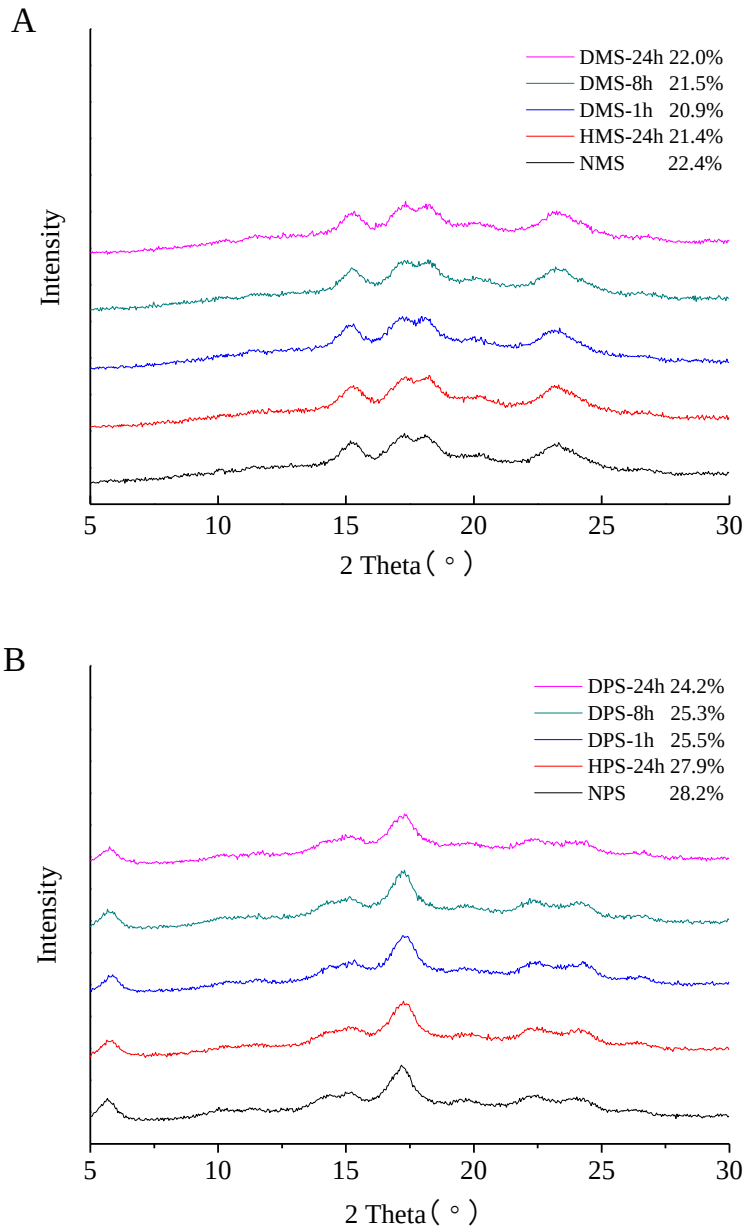

Fig.3 XRD profiles of debranched maize and potato starches. (NMS, native maize starch; NPS, native potato starch; The debranched maize (DMS)/potato (DPS) starches with different hydrolysis time (h) were denoted as DMS-1h, DMS-8h, DMS-24h, DPS-1h, DPS-8h, DPS-24h respectively; HMS-24h, maize starch with 24 h of hydrothermal treatment; HPS-24h, potato starch with 24 h of hydrothermal treatment)

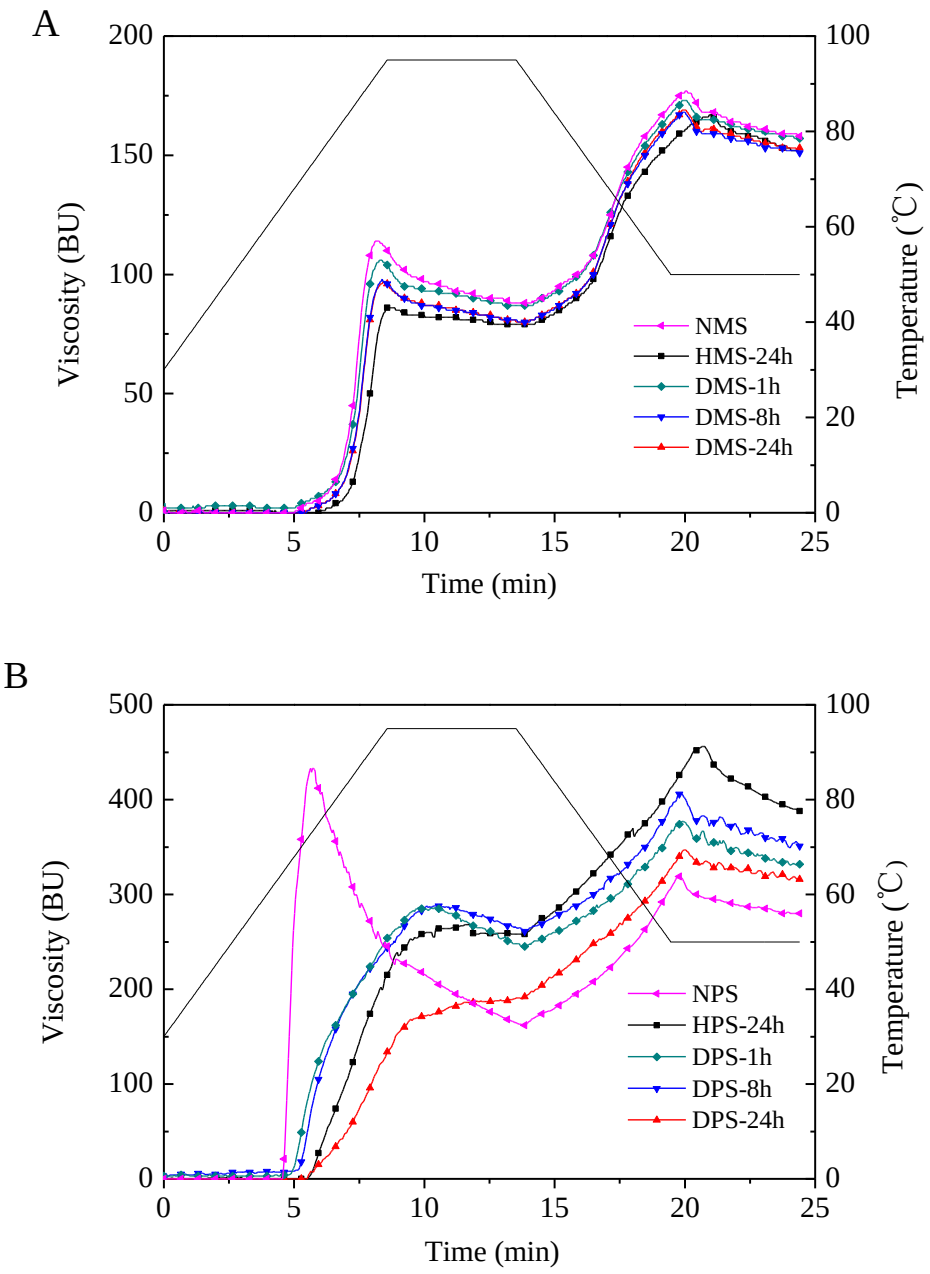

Fig.4 Pasting profiles of debranched maize and potato starches. (NMS, native maize starch; NPS, native potato starch; The debranched maize (DMS)/potato (DPS) starches with different hydrolysis time (h) were denoted as DMS-1h, DMS-8h, DMS-24h, DPS-1h, DPS-8h, DPS-24h respectively; HMS-24h, maize starch with 24h of hydrothermal treatment; HPS-24h, potato starch with 24h of hydrothermal treatment)

Table 1. Thermal properties and apparent amylose content of debranched maize and potato starches[1]

Sample[2]	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	Apparent amylose content (%)[3]
NMS	69.8±0.2^{d}	75.0±0^{b}	81.6±0.7^{c}	14.0±0.2^{a}	34.3±0.3^{d}
HMS-24h	70.7±0.8^{c}	76.7±1.2^{a}	82.9±1.3^{b}	12.7±0.7^{b}	34.4±0.2^{cd}
DMS-1h	73.4±1.5^{b}	78.0±1.5^{a}	83.4± 1.5^{b}	11.7±1.6^{b}	34.5±0.2^{c}
DMS-8h	74.9±0.2^{ab}	79.5±0.3^{a}	88.1 ±0.6^{a}	11.9 ±0.2^{b}	35.0±0.6^{a}
DMS-24h	75.7±1.2^{a}	81.3±0.9^{a}	88.6±0.2^{a}	11.9 ±0.6^{b}	34.5±0.3^{b}
NPS	63.5±0.1^{D}	70.8±0.1^{C}	81.4±0.3^{B}	24.5 ±0.2^{A}	37.3±0.5^{B}
HPS-24h	67.2±1.5^{C}	72.4±1.7^{BC}	83.4±0.9^{B}	20.4±1.8^{A}	37.5±1.5^{B}
DPS-1h	70.2±0.1^{B}	74.0±0.2^{B}	81.7±0.7^{B}	20.1 ±0.1^{B}	38.3±0.4^{A}
DPS-8h	75.7±0.5^{A}	78.8±0.8^{A}	87.8±0.7^{A}	15.7±0.5^{B}	38.5±0.1^{A}
DPS-24h	75.5 ±0.2^{A}	79.1 ±0.5^{A}	88.4±0.6^{A}	13.3±0.4^{C}	40.6±0.3^{A}

[1] All data are averages of triplicate measurements with standard deviation. Means in a column with different letters are significantly different (p<0.05) by the least significant difference (LSD) test.

[2] NMS, native maize starch; NPS, native potato starch; The debranched maize (DMS) / potato (DPS) starches with different hydrolysis time (h) were denoted as DMS-1h, DMS-8h, DMS-24h, DPS-1h, DPS-8h, DPS-24h respectively; HMS-24h, maize starch with 24 h of hydrothermal treatment; HPS-24h, potato starch with 24 h of hydrothermal treatment.

[3] Apparent amylose content was averages of three replicates. Values were calculated from dividing iodine affinity by a factor of 0.199.

Table 2 Pasting characteristics ofdebranchedmaize starch and potato starches[1]

Sample[2]	Pasting temperature (°C)	Peak viscosity (BU)	Trough viscosity (BU)	Final viscosity (BU)	Break down (BU)	Set back (BU)
NMS	78.3±0.2^{d}	114±5^{a}	88±4^{a}	171±4^{a}	26±1^{a}	83±2^{a}
HMS-24h	84.2±0.4^{a}	86±4^{c}	79±4^{a}	155±6^{a}	7±1^{c}	76±3^{a}
DMS-1h	78.8±0.3^{c}	106±1^{ab}	87±3^{a}	167±2^{a}	19±2^{b}	80±5^{a}
DMS-8h	81.4±0.5^{b}	98±6^{bc}	81±5^{a}	163±5^{a}	17±1^{b}	82±3^{a}
DMS-24h	81.3±0.4^{b}	97±5^{bc}	80±6^{a}	164±3^{a}	17±2^{b}	84±3^{a}
NPS	67.5±0.3^{D}	433±15^{A}	165±7^{B}	306±11^{A}	268±8^{A}	141±2^{B}
HPS-24h	73.1±0.9$^{B.}$	268±8^{B}	258±9^{A}	412±10^{A}	10±1^{D}	154±2^{A}
DPS-1h	70.2±0.2^{C}	286±9^{B}	248±11^{A}	364±9^{B}	38±2^{B}	116±4^{D}
DPS-8h	70.9±0.8^{B}	288±6^{B}	265±7^{A}	393±3^{C}	23±5^{C}	128±2^{C}
DPS-24h	76.3±0.2^{A}	190±7^{C}	191±5^{B}	327±3^{C}	0±1^{D}	136±2^{BC}

[1] All data are averages of duplicate measurements with standard deviation. Means in a column with different letters are significantly different (p<0.05) by the least significant difference (LSD) test.

[2] NMS, native maize starch; NPS, native potato starch; The debranched maize (DMS)/potato (DPS) starches with different hydrolysis time (h) were denoted as DMS-1h, DMS-8h, DMS-24h, DPS-1h, DPS-8h, DPS-24h respectively; HMS-24h, maize starch with 24h of hydrothermal treatment; HPS-24h, potato starch with 24h of hydrothermal treatment.