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Characterization of maize amylose-extender (*ae*) mutant starches. Part III: Structures and properties of the Naegeli dextrins

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ABSTRACT

The objective of this study was to understand the crystalline structures of maize *ae*-mutant starches by analyzing the structures and properties of their Naegeli dextrins. After an exhaustive acid hydrolysis of the starch granules with sulfuric acid (15.3%, v/v) at 38 °C for up to 102 days, the maize *ae*-mutant starches produced 18.3–39.5% Naegeli dextrins. The Naegeli dextrins showed the B-type polymorph and displayed similar onset (45.1–51.4 °C), peak (113.9–122.2 °C), and conclusion (148.0–160.0 °C) gelatinization-temperatures and large enthalpy changes (21.8–31.3 J/g) and percentage crystallinity (77.0–79.2%). The Naegeli dextrins showed unimodal molecular-size distributions with the peak molecular-size at degree of polymerization (DP) 16. The molecular-size distributions of the Naegeli dextrins did not significantly change after debranching with isoamylase, indicating predominantly linear molecules. The isoamylase-treated Naegeli dextrins had average chain lengths of DP 23.8–27.5 and large proportions of long chains (DP ≥ 25, 36.7–52.7%), resulting from hydrolysis of amylose double helices.

1. Introduction

Acid hydrolysis of native starch granules has been commonly used to study the crystalline structures of starch granules (Biliaderis, Grant, & Vose, 1981; Gerard, Colonna, Buleon, & Planchot, 2002; Hoover, 2000; Jacobs, Eerlingen, Rouseu, Colonna, & Delcour, 1998; Jane, Wong, & McPherson, 1997; Li, Corke, & Beta, 2007; Robin, Mercier, Charbonniere, & Guilbot, 1974; Robin, Mercier, Duprat, Charbonniere, & Guilbot, 1975; Srichuwong, Isono, Mishima, & Hisamatsu, 2005; Wang & Wang, 2001). Sulfuric acid and hydrochloric acid are used for the purpose, and the remaining starch residues after a prolonged hydrolysis at a moderate temperature are known as the Naegeli dextrin and Lintnerized starch, respectively (Hoover, 2000). Acid hydrolysis of starch granules shows two distinct rates during the course of the hydrolysis. The fast hydrolytic rate in the early stage is attributed to hydrolysis of amorphous starch in the starch granule, whereas the slower hydrolytic rate taking place later is slow erosion of the edge of crystalline starch in the starch granule. After the prolonged acid hydrolysis of starch granules, the remaining starch is considered as starch crystallites present in the starch granules, which resists acid hydrolysis (Biliaderis et al., 1981; Hoover, 2000; Jacobs et al., 1998; Jane et al., 1997; Kainuma & French, 1971; Li et al., 2007; McPherson & Jane, 1999; Perera, Lu, Sell, & Jane, 2001; Planchot, Colonna, & Buleon, 1997; Robin et al., 1974; Robin et al., 1975; Srichuwong et al., 2005; Wang & Wang, 2001).

A public high-amylose maize line registered as GEMS-0067 (PI 643420) was developed using germplasm obtained from USDA-ARS Germplasm Enhancement of Maize (GEM) project (Campbell, Jane, Pollak, Blanco, & O'Brien, 2007). GEMS-0067 is an inbred maize line homozygous for the recessive amylose-extender (*ae*) and high-amylose modifier (HAM) genes (Campbell et al., 2007; Wu, Campbell, Yen, Wicks, & Ibrahim, 2009). Part I of this study reported that GEMS-0067 *ae*-line starches consisted of a significantly larger resistant-starch (RS) contents (39.4–43.2%) than maize *ae* single-mutant starches of H99*ae*, OH43*ae*, B89*ae*, and B84*ae* (11.5–19.1%) (Li, Jiang, Campbell, Blanco, & Jane, 2008). Results of the part II study (Jiang, Campbell, Blanco, & Jane, 2010) demonstrated that long-chain double helices of amylose/intermediate component (IC) retained semi-crystalline structures at 95–100 °C, which were resistant to enzymatic hydrolysis. Microscopic studies showed fusions between adjacent small granules in the amyloplast, likely through amylose interaction, to develop elongated starch granules (Jiang, Horner, et al., 2010). On the basis of these findings, Jiang, Campbell, et al. (2010) proposed that the long-chain double-helical crystallites of amylose/IC molecules were present in the granules of native maize *ae*-mutant starches.

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In the present study, we aimed to understand the starch crystalline structures present in the granules of the GEMS-0067 *ae*-line starches and the existing *ae*-line starches of H99*ae*, OH43*ae*, B89*ae*, and B84*ae*. To achieve this goal, we analyzed the contents and structures of the Naegeli dextrins obtained after prolonged acid hydrolysis of the native starch granules.

2. Materials and methods

2.1. Materials

Maize kernels of three GEMS-0067 *ae*-lines, GUAT209:S13 × (OH43*ae* × H99*ae*) B-B-4-1-2-1-1 (GSOH 1), GUAT209:S13 × (OH43*ae* × H99*ae*) B-B-4-4-2-1-1 (GSOH 2), and GUAT209:S13 × (OH43*ae* × H99*ae*) B-B-4-4-2-1-2 (GSOH 3), and four existing *ae*-lines of H99*ae*, OH43*ae*, B89*ae*, and B84*ae*, were obtained from the USDA-ARS Germplasm Enhancement of Maize (GEM) project at Truman State University (Kirksville, MO). These GEMS-0067 *ae*-lines were F6 generation of GEMS-0067-derived maize *ae*-lines (Campbell et al., 2007). All chemicals were reagent grade and obtained from Sigma–Aldrich Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68) obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan) was used for debranching the Naegeli dextrins.

2.2. Starch isolation

Endosperm starch was isolated from maize kernels using the method as described by Li et al. (2008).

2.3. Acid hydrolysis of starch granules

Acid hydrolysis of starch granules was done following the method described by Jane et al. (1997). Starch (5.0 g, dsb) was suspended in 100 mL of sulfuric acid (15.3%, v/v) and incubated at 38 °C in a water bath. Starch suspensions were gently shaken daily by hand. Starch suspensions (1.5 mL for each) were sampled on 2, 4, 6, 8, 10, 12, 21, 67, and 102 days of acid hydrolysis, and centrifuged at 1000 × *g* for 20 min. The supernatant was analyzed for the total carbohydrate content to determine the degree of starch hydrolysis (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956; Jane et al., 1997). The Naegeli dextrin remaining after 102 days of acid hydrolysis was collected, washed by suspending it in an aqueous ethanol solution (50%, v/v, 40 mL) with gentle shaking for 5 min, and centrifuged at 1000 × *g* for 20 min. The supernatant was discarded. The washing procedure was repeated until the supernatant reached pH ~6.0. The washed Naegeli dextrins were dehydrated by washing with 100% ethanol (40 mL) three times and dried at 37 °C for 24 h.

2.4. Molecular-size distributions of Naegeli dextrins before and after debranching

The prepared Naegeli dextrin (20.0 mg) was wetted with deionized distilled-water (0.2 mL), dispersed in dimethyl sulfoxide (DMSO) (1.8 mL) in a boiling-water bath for 1 h, mechanically stirred at 25 °C for 16 h, precipitated with 20 volumes of ethanol, collected by centrifugation, and then dispersed in boiling water (9.0 mL) with mechanical stirring for 30 min. The dispersed Naegeli dextrin (4.5 mL) was mixed with an acetate buffer solution (0.5 mL, pH 3.5, 100 mM) and debranched using isoamylase (5 units) at 40 °C for 16 h following the method described by Li et al. (2008). The debranched sample was adjusted to pH 7 using a sodium hydroxide solution (0.5 M), heated in a boiling-water bath for 15 min to inactivate the enzyme, and filtered through a membrane filter (1.2 μm pore size). The dispersed Naegeli dextrins and debranched Naegeli dextrins (80 μL for each, 2 mg/mL) were dried at 45 °C using a centrifugal vacuum evaporator for 3 h, labeled with 8-aminopyrene-1,3,6-trisulfonic acid (APTS, Cat. No. 09341, Sigma, St. Louis, MO), and analyzed using a fluorophore-assisted capillary-electrophoresis (FACE) (P/ACE MDQ, Beckman Coulter, Fullerton, CA) following the method of Jiang, Campbell, et al. (2010).

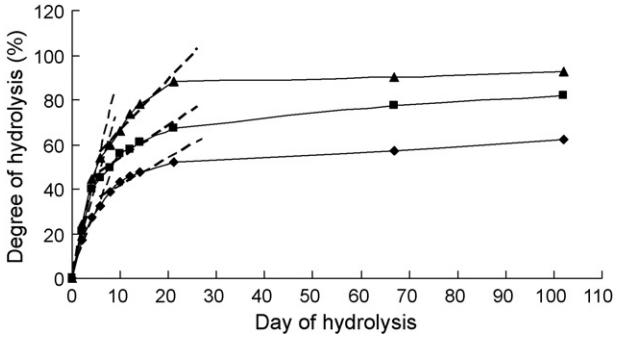

Fig. 1. Acid hydrolysis of starch granules of representative varieties with sulfuric acid (15.3%, v/v) at 38 °C. ♦, GSOH 1; ■, OH43*ae*; ▲, normal maize. Dashed lines are trend lines for the fast and slow hydrolyses of the starch granules. The degree of hydrolysis was determined by measuring the soluble sugars in the supernatant.

2.5. X-ray diffractometry

The Naegeli dextrins obtained after acid hydrolysis of the starch granules for 102 days were equilibrated in a chamber with 100% relative humidity at 25 °C for 24 h. X-ray diffraction patterns and the percentage crystallinity of the Naegeli dextrins were determined using an X-ray diffractometer (D-500, Siemens, Madison, WI) with copper K_α radiation (Ao & Jane, 2007).

2.6. Thermal properties of Naegeli dextrins

Thermal properties of the Naegeli dextrins were analyzed using a differential scanning calorimeter (DSC) (DSC-7, Perkin-Elmer, Norwalk, CT) (Kasemsuwan, Jane, Schnable, Stinard, & Robertson, 1995). The Naegeli dextrin (~10.0 mg, dry basis) was mixed with ~30 μL of deionized distilled-water, sealed in a stainless-steel pan, and equilibrated at 25 °C overnight. The equilibrated sample was heated in the DSC from 10 to 180 °C at a rate of 10 °C/min. A sealed empty stainless-steel pan was used as the reference. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy change (ΔH) of starch gelatinization were obtained using Pyris software (Perkin-Elmer, Norwalk, CT).

3. Results and discussion

3.1. Acid hydrolysis of starch granules

Acid hydrolysis curves of *ae*-mutant- and normal maize starches using sulfuric acid (15.3%, v/v) at 38 °C are shown in Fig. 1. The degrees of hydrolysis and hydrolysis rates of starches are summarized in Table 1. Biphasic patterns of hydrolysis were observed for all the starches, which were in agreement with previous reports (Hoover, 2000; Jacobs et al., 1998; Jane et al., 1997; Srichuwong et al., 2005). As shown in Fig. 1 and Table 1, all the starches were rapidly hydrolyzed during the first 6 days of the acid hydrolysis with rates of 5.4–9.0% per day, which were attributed to hydrolysis of the amorphous starch in the granules. Slower hydrolysis rates (1.1–2.3% per day) observed from the 7th to the 21st day were likely erosion of the edge of crystalline starch in the granules (Biliaderis et al., 1981; Jacobs et al., 1998; Li et al., 2007; Robin et al., 1974; Robin et al., 1975; Srichuwong et al., 2005; Wang & Wang, 2001). The hydrolysis rates either reached plateaus or were very slow after

Table 1
Acid hydrolysis of maize starches.

Sample	Degree of hydrolysis (%)			Hydrolysis rate (% per day)		Naegeli dextrin[a] yield (%)
	6 days	21 days	102 days	0–6 days	7–21 days	
GSOH 1	32.3 ± 0.2	52.4 ± 0.1	62.3 ± 0.6	5.4	1.3	37.7 ± 0.6
GSOH 2	33.0 ± 1.2	49.7 ± 0.4	60.5 ± 0.9	5.5	1.1	39.5 ± 0.9
GSOH 3	34.2 ± 0.2	51.1 ± 0.8	62.6 ± 0.5	5.7	1.1	37.4 ± 0.5
H99*ae*	39.3 ± 2.4	64.0 ± 1.3	73.3 ± 0.1	6.6	1.6	26.7 ± 0.1
OH43*ae*	44.9 ± 0.5	67.4 ± 0.2	81.7 ± 2.5	7.5	1.5	18.3 ± 2.5
B89*ae*	41.7 ± 1.3	65.6 ± 0.5	76.1 ± 0.8	7.0	1.6	23.9 ± 0.8
B84*ae*	42.0 ± 0.1	66.7 ± 0.4	75.1 ± 1.4	7.0	1.6	24.9 ± 1.4
Normal maize	54.1 ± 0.7	88.4 ± 0.0	92.8 ± 1.5	9.0	2.3	7.2 ± 1.5

[a] Naegeli dextrin was the residue remaining after sulfuric acid (15.3%, v/v) hydrolysis of starch granules at 38 °C for 102 days.

21 days. Factors that cause slow down and eventually stop the acid hydrolysis of the crystalline starch in the granules have been proposed by Kainuma and French (1971); and they are (1) the dense packing of the starch molecules in the crystalline region reduces the penetration of hydronium ions; (2) the glucosidic bonds are buried in the interior of the double helix where the hydronium ions cannot readily reach; and (3) the glucose unit in the crystallite is in the $^4C_1$ chair conformation, which requires large activation energy to change to a half-chair conformation in order to be hydrolyzed (Kainuma & French, 1971).

GEMS-0067 *ae*-line starches were hydrolyzed by sulfuric acid at a slower rate than the existing *ae*-line starches and the normal maize starch (Fig. 1 and Table 1). The yields of the Naegeli dextrins were 37.4–39.5%, 18.3–26.7%, and 7.2% for the GEMS-0067 *ae*-line, the existing *ae*-line, and the normal maize starches, respectively (Table 1). The yields of the Naegeli dextrins were significantly correlated ($r = 0.97$, $p < 0.0001$) with the apparent-amylose contents of the starches reported by Jiang, Campbell, et al. (2010). These results indicated that the increase in the Naegeli dextrin yield of GEMS-0067 *ae*-line starch resulted from the large quantity of long-chain double helices of amylose/IC in the GEMS-0067 *ae*-line starch (Jiang, Campbell, et al., 2010). This finding agreed with previous reports showing that starch granules with greater amylose contents were less susceptible to the acid hydrolysis (Jane et al., 1997; Shi, Capitani, Trzasko, & Jeffcoat, 1998). Depending on the source of the starch, native starch granules differ in their susceptibilities to the acid hydrolysis (Srichuwong et al., 2005). The A-type polymorph starch is hydrolyzed faster and to a greater extent than the B-type polymorph starch (Jane et al., 1997; Kainuma & French, 1971; Robin et al., 1974; Robin et al., 1975; Srichuwong et al., 2005).

3.2. Molecular-size distributions of Naegeli dextrins before and after debranching

Molecular-size distributions of the Naegeli dextrins prepared after 102 days of acid hydrolysis are shown in Fig. 2. All the Naegeli dextrins showed unimodal molecular-size distributions with the peak molecular-size at degree of polymerization (DP) 16. The Naegeli dextrins were debranched with isoamylase, and the normalized molecular-size distributions of the debranched Naegeli dextrins in comparison with that of the counterparts without debranching are shown in Fig. 3. The normalized molecular-size distributions of the isoamylase-debranched Naegeli dextrins were similar to that of the Naegeli dextrin counterparts without debranching except a slight increase in chains of DP 21-50 and a complementary decrease in chains of DP 11-20 (Fig. 3). The similarity of the branch-chain length distribution before and after debranching reaction indicated that the Naegeli dextrins consisted of primarily linear molecules. The slight increase in chains of DP 21–50 could be results of debranching of IC that was present in amylose/IC crystallites. This result agreed with previous report showing that the B-type polymorph starch had fewer branch-linkages located in the crystalline region of the starch granule, which were protected from acid hydrolysis (Jane et al., 1997).

The chain length distributions of the debranched Naegeli dextrins, which were all linear molecules and precisely reflected the lengths of the double helices of the Naegeli dextrins, were calculated and are summarized in Table 2. The debranched Naegeli dextrins consisted of chains of DP ≤ 12 (8.6–11.2%), DP 13–24 (38.7–54.1%), DP 25–36 (23.6–30.6%), and DP ≥ 37 (13.1–22.1%). The average chain lengths of the debranched Naegeli dextrins were between DP 23.8 and 27.5 (Table 2), which were substantially larger than that of debranched Naegeli dextrins and Lintnerized starches of the normal and *ae-waxy* starches (DP 13–16) (Jane et al., 1997; Perera et al., 2001; Srichuwong et al., 2005). The short double helices (DP < 25) (Fig. 3) of the Naegeli dextrins were mainly crystalline double-helical branch chains of amylopectin (Jane et al., 1997).

It is known that the Naegeli dextrins of retrograded amylose obtained after a prolonged acid hydrolysis has average DP 31, ranging from DP 25 to 50 (Jane & Robyt, 1984). The debranched Naegeli dextrins of the normal, waxy, and *ae* waxy maize starches have chain lengths mainly distributed between DP 6 and 25 (Jane et al., 1997), which are crystalline double-helical branch chains of the amylopectin (Jane, Xu, Radosavljevic, & Seib, 1992; Kasemsuwan & Jane, 1994). Thus, the long chains of DP ≥ 25 in the debranched Naegeli dextrins of the maize *ae*-mutant starches (Fig. 3) could be attributed to the amylose double helices (Jiang, Campbell, et al., 2010). The presence of amylose double helices in the Naegeli dextrins agreed with the high conclusion gelatinization-temperatures of the native maize *ae*-mutant starches (Li et al., 2008) and their RS residues obtained after digesting the maize *ae*-mutant starches with thermally stable α-amylase at 95–100 °C (Jiang, Campbell, et al., 2010).

3.3. X-ray diffraction patterns of Naegeli dextrins

The X-ray diffraction patterns of the Naegeli dextrins obtained after a prolonged acid hydrolysis of the maize *ae*-mutant starch granules are shown in Fig. 4. All the Naegeli dextrins displayed the B-type polymorphic pattern and had percentages of crystallinity 77.0–79.2%, which were substantially greater than their native starch counterparts (22.8–33.0%) reported by Jiang, Campbell, et al. (2010). The large percentages of crystallinity of the Naegeli dextrins (Fig. 4) suggested that the amorphous starch in the granules was mostly removed and the crystallites of double helices were retained after the prolonged acid hydrolysis. There were no peaks at 8°, 13°, and 20° (Zobel, French, & Hinkle, 1967; Zobel, 1988) observed in the Naegeli dextrins (Fig. 4), indicating no crystalline amylose–lipid complex present in the Naegeli dextrins. This result was consistent with no crystalline amylose–lipid complex found in the native starch counterparts (Jiang, Campbell, et al., 2010).

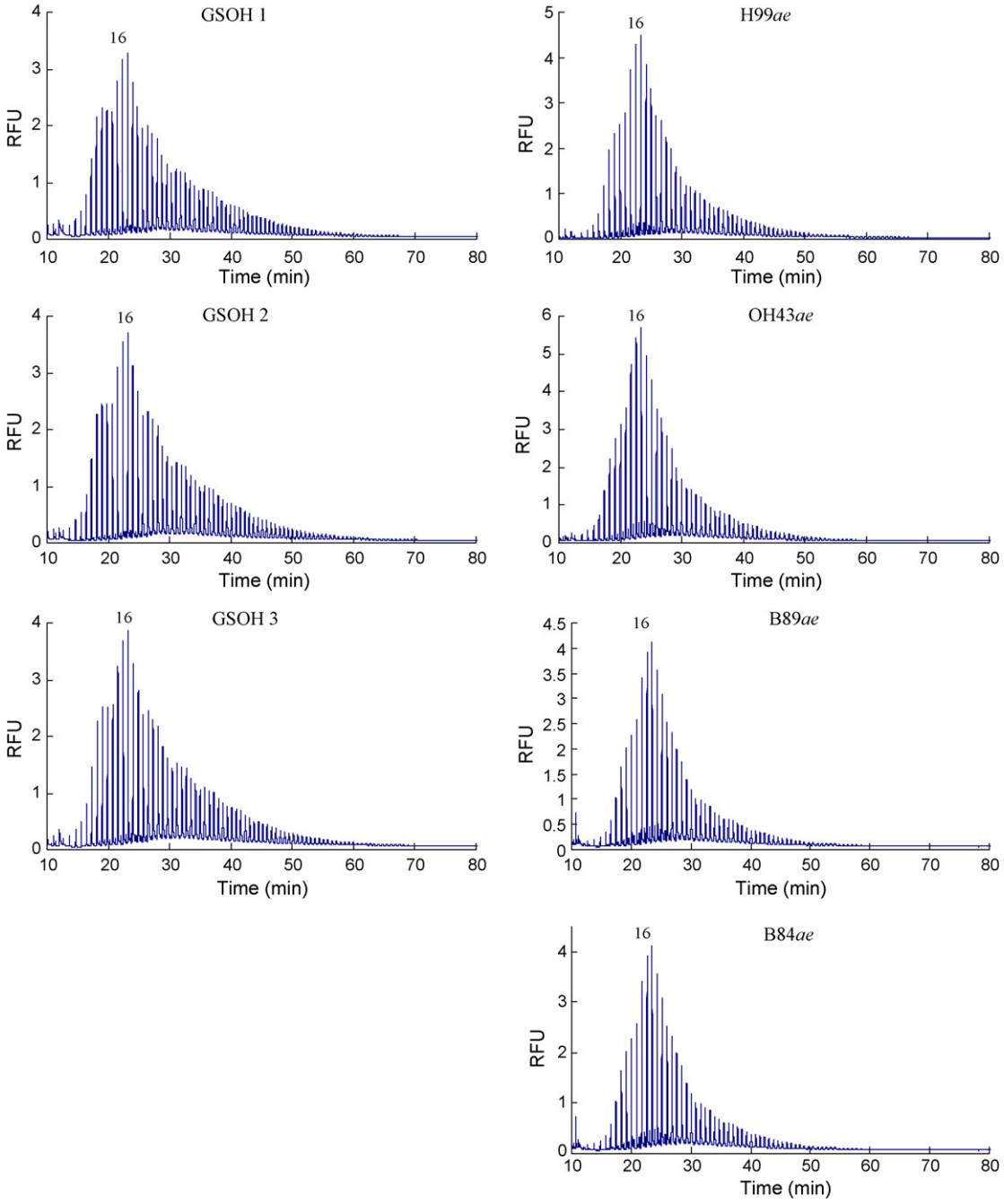

Fig. 2. Molecular-size distributions of Naegeli dextrins determined using a fluorophore-assisted capillary-electrophoresis. The Naegeli dextrins were prepared from sulfuric acid (15.3%, v/v) hydrolysis of maize *ae*-mutant starches at 38 °C for 102 days. RFU, relative fluorescence units. The peak molecular-size of the Naegeli dextrins was at degree of polymerization (DP) 16.

Table 2
Molecular-size distributions of debranched Naegeli dextrins[a,b].

Sample	DP ≤ 12	DP 13–24	DP 25–36	DP ≥ 37	Average DP
GSOH 1	11.2 ± 0.2	42.9 ± 0.1	28.0 ± 0.7	17.9 ± 0.6	25.6 ± 0.3
GSOH 2	8.6 ± 0.2	38.7 ± 0.7	30.6 ± 0.7	22.1 ± 1.5	27.5 ± 0.7
GSOH 3	9.3 ± 0.1	40.0 ± 0.1	28.9 ± 0.2	21.8 ± 0.4	27.3 ± 0.2
H99*ae*	9.5 ± 0.1	52.9 ± 0.8	24.4 ± 0.1	13.2 ± 0.8	23.8 ± 0.2
OH43*ae*	8.7 ± 0.1	49.3 ± 0.1	26.9 ± 0.2	15.1 ± 0.2	24.9 ± 0.1
B89*ae*	9.2 ± 0.1	54.1 ± 0.9	23.6 ± 0.0	13.1 ± 1.0	23.9 ± 0.3
B84*ae*	9.5 ± 0.3	51.8 ± 1.1	25.1 ± 0.0	13.6 ± 0.7	24.1 ± 0.2

DP, degree of polymerization.

[a] Naegeli dextrin was the residue remaining after sulfuric acid (15.3%, v/v) hydrolysis of starch granules at 38 °C for 102 days.

[b] The molecular-size distributions of debranched Naegeli dextrins were analyzed using a fluorophore-assisted capillary-electrophoresis.

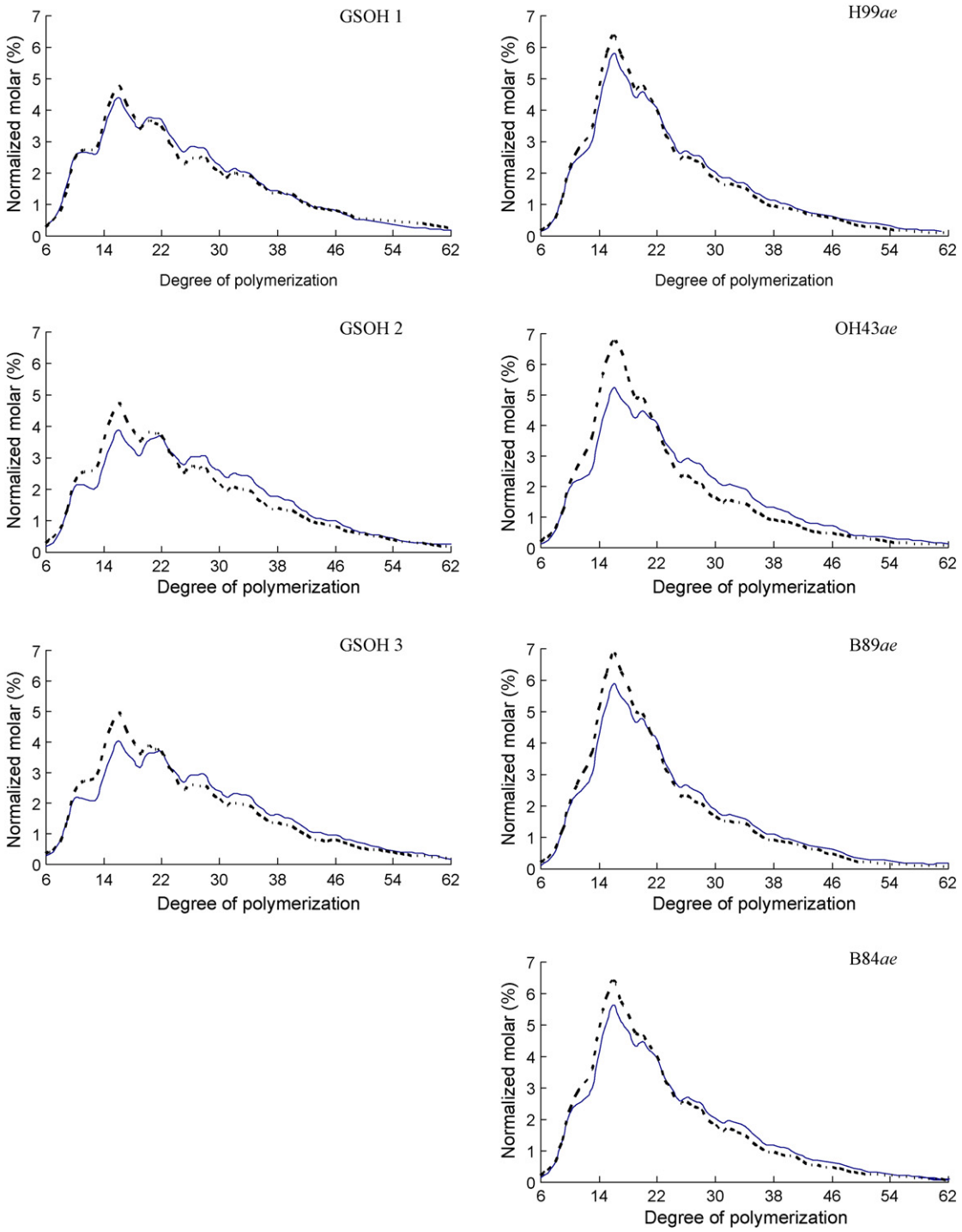

Fig. 3. Normalized molecular-size distributions of isoamylase-debranched Naegeli dextrins (—) and the Naegeli dextrins without debranching (- - - - -) determined using a fluorophore-assisted capillary-electrophoresis. The Naegeli dextrins were prepared from sulfuric acid (15.3%, v/v) hydrolysis of maize *ae*-mutant starches at 38 °C for 102 days.

3.4. Thermal properties of Naegeli dextrins

DSC thermograms of the Naegeli dextrins are shown in Fig. 5. All the Naegeli dextrins displayed similar broad thermal-transitions with onset, peak, and conclusion gelatinization-temperatures of 45.1–51.4, 113.9–122.2, and 148.0–160.0 °C, respectively (Table 3). The thermal-transition temperature ranges of the Naegeli dextrins were similar to those of the Lintnerized high-amylose maize starches as previously reported (Shi et al., 1998). The broad thermal-transition temperature ranges agreed with the broad chain length distributions of the debranched Naegeli dextrins (Fig. 3).

The Naegeli dextrins of the GEMS-0067 *ae*-line starches displayed slightly higher gelatinization-temperatures than that of the existing *ae*-line starches (Table 3). These results were consistent with the longer double helices of the Naegeli dextrins of the GEMS-0067 *ae*-line starches than that of the existing *ae*-line starches (Table 2).

The very high temperatures for the peak (113.9–122.2 °C) and the conclusion gelatinization-temperatures (148.0–160.0 °C) of the

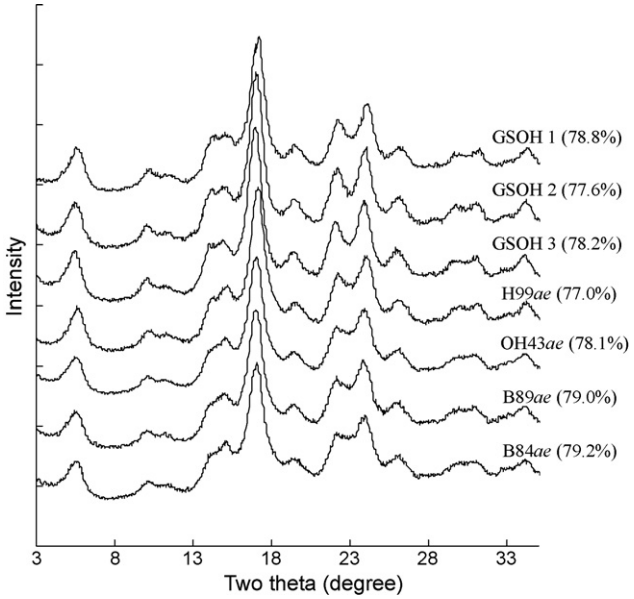

Fig. 4. X-ray diffraction patterns of Naegeli dextrins prepared from sulfuric acid (15.3%, v/v) hydrolysis of maize *ae*-mutant starches at 38 °C for 102 days. The percentage crystallinity is given in parentheses.

Naegeli dextrins (Table 3) were attributed to their long double helices (Table 2 and Fig. 3). It has been reported that the onset, peak, and conclusion temperatures of the retrograded amylose were 131.9, 150.0, and 161.8 °C, respectively (Sievert & Pomeranz, 1989). Thus, the high conclusion gelatinization-temperatures of the Naegeli dextrins agreed with the presence of the amylose double helices.

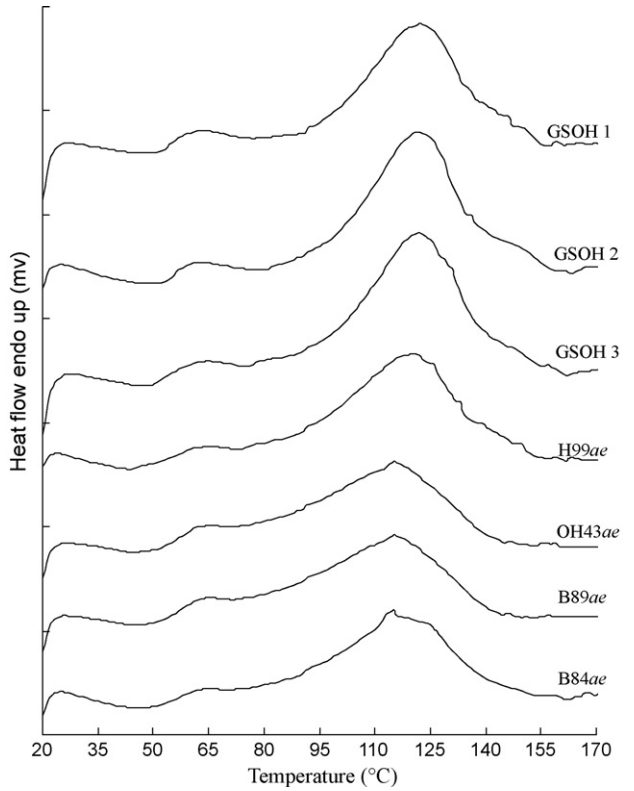

Fig. 5. DSC thermograms of Naegeli dextrins prepared from sulfuric acid (15.3%, v/v) hydrolysis of maize *ae*-mutant starches at 38 °C for 102 days.

Table 3
Thermal properties of Naegeli dextrins[a,b].

Sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
GOSH 1	51.2 ± 0.8	122.2 ± 0.0	158.3 ± 0.3	27.1 ± 1.0
GOSH 2	51.3 ± 0.1	121.9 ± 0.1	158.3 ± 0.7	29.1 ± 0.7
GOSH 3	51.4 ± 0.2	122.0 ± 0.1	160.0 ± 0.0	31.3 ± 1.3
H99*ae*	45.1 ± 0.0	119.0 ± 0.5	156.5 ± 1.5	28.5 ± 1.5
OH43*ae*	50.5 ± 0.5	113.9 ± 0.2	148.3 ± 1.8	21.8 ± 0.6
B89*ae*	51.0 ± 0.0	115.5 ± 0.9	148.0 ± 2.0	22.0 ± 0.6
B84*ae*	50.5 ± 0.5	115.8 ± 0.9	154.1 ± 0.1	22.7 ± 1.6

[a] Naegeli dextrin was the residue remaining after sulfuric acid (15.3%, v/v) hydrolysis of starch granules at 38 °C for 102 days.
[b] Samples (~10.0 mg, dry basis) and deionized distilled-water (~30.0 μL) were used for the analysis; T_o, T_p, T_c and ΔH are onset, peak, and conclusion temperatures, and enthalpy change, respectively.

The enthalpy changes of the Naegeli dextrins ranged from 21.8 to 31.3 J/g (Table 3), which were substantially larger than their native starch counterparts (11.7–17.4 J/g) (Li et al., 2008), indicating that the amorphous starch in the granules was mostly removed after the prolonged acid hydrolysis. Therefore, the Naegeli dextrins obtained after 102 days of acid hydrolysis of the starch granules were highly ordered crystallites of double helices.

4. Conclusions

After a prolonged acid hydrolysis, GEMS-0067 *ae*-line starches produced substantially larger yields of Naegeli dextrins than the existing *ae*-line starches, which were attributed to the greater amylose contents in the GEMS-0067 *ae*-line starches. All the Naegeli dextrins displayed the B-type polymorph and had highly crystalline structures of double helices. All the Naegeli dextrins consisted of essentially linear molecules and showed unimodal molecular-size distributions with the peak molecular-size at DP 16. The average chain lengths of the double helices in the Naegeli dextrins were between DP 23.8 and 27.5. All the Naegeli dextrins displayed similar gelatinization-temperatures of 45.1–51.4, 113.9–122.2, and 148.0–160.0 °C for T_o, T_p, and T_c, respectively. Results of this study supported the presence of amylose double-helical crystallites in the native maize *ae*-mutant starches, which contributed to the resistance of enzymatic hydrolysis at 95–100 °C.

Acknowledgements

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References

Ao, Z., & Jane, J. (2007). Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley. *Carbohydrate Polymers*, *67*, 46–55.
Biliaderis, C. G., Grant, D. R., & Vose, J. R. (1981). Structural characterization of legume starches. II. Studies on acid-treated starches. *Cereal Chemistry*, *58*, 502–507.
Campbell, M. R., Jane, J., Pollak, L., Blanco, M., & O'Brien, A. (2007). Registration of maize germplasm line GEMS-0067. *Journal of Plant Registrations*, *1*, 60–61.
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.
Gerard, C., Colonna, P., Buleon, A., & Planchot, V. (2002). Order in maize mutant starches revealed by mild acid hydrolysis. *Carbohydrate Polymers*, *48*, 131–141.
Hoover, R. (2000). Acid-treated starches. *Food Reviews International*, *16*, 369–392.
Jacobs, H., Eerlingen, R. C., Rouseu, N., Colonna, P., & Delcour, J. A. (1998). Acid hydrolysis of native and annealed wheat, potato and pea starches—DSC melting features and chain length distributions of lintnerized starches. *Carbohydrate Research*, *308*, 359–371.
Jane, J., & Robyt, J. F. (1984). Structure studies of amylose-V complexes and retrograded amylose by action of alpha amylases, and a new method for preparing amylodextrins. *Carbohydrate Research*, *132*, 105–118.

Jane, J., Wong, K.-S., & McPherson, A. E. (1997). Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrins. *Carbohydrate Research*, *300*, 219–227.

Jane, J., Xu, A., Radosavljevic, M., & Seib, P. A. (1992). Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. *Cereal Chemistry*, *69*, 405–409.

Jiang, H., Campbell, M., Blanco, M., & Jane, J. (2010). Characterization of maize amylose-extender (ae) mutant starches. Part II. Structures and properties of starch residues remaining after enzymatic hydrolysis at boiling-water temperature. *Carbohydrate Polymers*, *80*, 1–12.

Jiang, H., Horner, H. T., Pepper, T. M., Blanco, M., Campbell, M., & Jane, J. (2010). Formation of elongated starch granules in high-amylose maize. *Carbohydrate Polymers*, *80*, 534–539.

Kainuma, K., & French, D. (1971). Naegeli amylodextrin and its relation to starch granule structure. I. Preparation and properties of amylodextrins from various starch types. *Biopolymers*, *10*, 1673–1680.

Kasemsuwan, T., & Jane, J. (1994). Location of amylose in normal starch granules. II. Locations of phosphodiester crosslinking revealed by phosphorus-31 nuclear magnetic resonance. *Cereal Chemistry*, *71*, 282–287.

Kasemsuwan, T., Jane, J., Schnable, P., Stinard, P., & Robertson, D. (1995). Characterization of the dominant mutant amylose-extender (Ae1-5180) maize starch. *Cereal Chemistry*, *72*, 457–464.

Li, W., Corke, H., & Beta, T. (2007). Kinetics of hydrolysis and changes in amylose content during preparation of microcrystalline starch from high-amylose maize starches. *Carbohydrate Polymers*, *69*, 398–405.

Li, L., Jiang, H., Campbell, M., Blanco, M., & Jane, J. (2008). Characterization of maize amylose-extender (*ae*) mutant starches. Part I. Relationship between resistant starch contents and molecular structures. *Carbohydrate Polymers*, *74*, 396–404.

McPherson, A. E., & Jane, J. (1999). Comparison of waxy potato with other root and tuber starches. *Carbohydrate Polymers*, *40*, 57–70.

Perera, C., Lu, Z., Sell, J., & Jane, J. (2001). Comparison of physicochemical properties and structures of *sugary-2* cornstarch with normal and waxy cultivars. *Cereal Chemistry*, *78*, 249–256.

Planchot, V., Colonna, P., & Buleon, A. (1997). Enzymatic hydrolysis of alpha-glucan crystallites. *Carbohydrate Research*, *298*, 319–326.

Robin, J. P., Mercier, C., Charbonniere, R., & Guilbot, A. (1974). Lintnerized starches. Gel filtration and enzymic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chemistry*, *51*, 389–406.

Robin, J. P., Mercier, C., Duprat, F., Charbonniere, R., & Guilbot, A. (1975). Lintnerized starches. Chromatographic and enzymic studies of insoluble residues from hydrochloric acid hydrolysis of cereal starches, particularly waxy maize starch. *Starch/Staerke*, *27*, 36–45.

Shi, Y.-C., Capitani, T., Trzasko, P., & Jeffcoat, R. (1998). Molecular structure of a low-amylopectin starch and other high-amylose maize starches. *Journal of Cereal Science*, *27*, 289–299.

Sievert, D., & Pomeranz, Y. (1989). Enzyme-resistant starch. I. Characterization and evaluation by enzymic, thermoanalytical, and microscopic methods. *Cereal Chemistry*, *66*, 342–347.

Srichuwong, S., Isono, N., Mishima, T., & Hisamatsu, M. (2005). Structure of Lintnerized starch is related to X-ray diffraction pattern and susceptibility to acid and enzyme hydrolysis of starch granules. *International Journal of Biological Macromolecules*, *37*, 115–121.

Wang, L., & Wang, Y.-J. (2001). Structures and physicochemical properties of acid-thinned corn, potato and rice starches. *Starch/Staerke*, *53*, 570–576.

Wu, Y., Campbell, M., Yen, Y., Wicks, Z., III, & Ibrahim, A. M. H. (2009). Genetic analysis of high amylose content in maize (*Zea mays* L.) using a triploid endosperm model. *Euphytica*, *166*, 155–164.

Zobel, H. F. (1988). Starch crystal transformations and their industrial importance. *Starch/Staerke*, *40*, 1–7.

Zobel, H. F., French, A. D., & Hinkle, M. E. (1967). X-ray diffraction of oriented amylose fibers. II. Structure of V-amyloses. *Biopolymers*, *5*, 837–845.