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Carbohydrate Research 338 (2003) 2871–2882

CARBOHYDRATE RESEARCH

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Acid hydrolysis of native and annealed starches and branch-structure of their Naegeli dextrins

Yuta Nakazawa, Ya-Jane Wang*

Department of Food Science, University of Arkansas, 2650 N. Young Ave., Fayetteville, AR 72704, USA

Received 26 June 2003; accepted 5 September 2003

Abstract

Eight commercial starches, including common corn, waxy corn, wheat, tapioca, potato, Hylon V, Hylon VII, and mung bean starch, were annealed by a multiple-step process, and their gelatinization characteristics were determined. Annealed starches had higher gelatinization temperatures, reduced gelatinization ranges, and increased gelatinization enthalpies than their native starches. The annealed starches with the highest gelatinization enthalpies were subjected to acid hydrolysis with 15.3% H_2SO_4, and Naegeli dextrins were prepared after 10 days' hydrolysis. Annealing increased the acid susceptibility of native starches in the first (rapid) and the second (slow) phases with potato starch showing the greatest and high amylose starches showing the least changes. Starches with a larger shift in onset gelatinization temperature also displayed a greater percent hydrolysis. The increase in susceptibility to acid hydrolysis was proposed to result from defective and porous structures that resulted after annealing. Although annealing perfected the crystalline structure, it also produced void space, which led to porous structures and possible starch granule defects. The molecular size distribution and chain length distribution of Naegeli dextrins of annealed and native starches were analyzed. The reorganization of the starch molecule during annealing occurred mainly within the crystalline lamellae. Imperfect double helices in the crystalline lamellae improved after annealing, and the branch linkages at the imperfect double helices became protected by the improved crystalline structure. Therefore, more long chains were observed in the Naegeli dextrins of annealed starches than in native starches.

Keywords: Starch; Annealing; Naegeli dextrins; Chain length distribution

1. Introduction

Hydrothermal modification, including annealing and heat-moisture treatment, is a physical modification of the starch granule that involves only heat and water. Annealing is defined as incubation of a starch granule in excess water at a temperature above the glass transition temperature but below the gelatinization temperature of the starch, whereas the treatment carried out at a limited moisture content ($<35\%$, w/w) is described as a heat–moisture treatment. Both processes occur above the glass transition temperature of the starch, but heat–moisture treatment requires an elevated temperature above 100 °C.

Annealing has significant impact on starch physicochemical properties with the most pronounced effects being increased gelatinization temperatures and narrowed gelatinization temperature ranges with increased or unchanged enthalpies.[1,2] The pasting properties[3,4] and the susceptibility to enzymatic hydrolysis[5] of annealed starch are also affected. Although annealing does not change the wide-range X-ray pattern,[2,6] a slightly sharpened pattern and a decreased background have been observed for annealed starches by small-angle X-ray scattering.[7,8] Nonetheless, no change in crystalline and amorphous lamellae repeated distance in wheat and potato starches was reported as a result of the annealing treatment.[8] The exact molecular mechanism of starch annealing is still largely unknown. Possible explanations for the annealing effects on starch properties include improved granule stability[9] and reorganization of granular structure.[10,11] Moreover, it is widely believed that

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* Corresponding author. Tel.: +1-479-575-3871; fax: +1-479-575-6936.

E-mail address: yjwang@uark.edu (Y.-J. Wang).

0008-6215/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2003.09.005

annealing is mainly involved the crystalline and amorphous lamellae perfection such as optimization of crystalline order.[8,12]

The study of starch structure by acid hydrolysis is a means of improving the understanding of the arrangement of amylose and amylopectin within a starch granule. Prolonged, gentle acid hydrolysis is commonly performed with either hydrochloric or sulfuric acid to yield Lintner or Naegeli amylodextrins, respectively. Both amylodextrins still retain the intact granular structure[13] and consist of acid-resistant crystalline regions of amylopectin. Therefore, Lintner and Naegeli amylodextrins have been intensively studied to obtain more information on the nature of the crystalline lamellae in starch granules. Two distinct phases are observed in the course of acid hydrolysis of starch as a function of time. The first phase is attributed to a relatively fast hydrolysis of starch, mainly the amorphous lamellae, while a slow hydrolysis of the crystalline lamellae would occur during the second phase.[14,15] Several factors may contribute to the protection of the crystalline lamellae from acid hydrolysis. The denser packing of starch chains in the crystalline lamellae does not permit acid-ion penetration, and glucosidic bonds are buried in the interior of the double helices where the hydrogen ion cannot reach. Furthermore, in order to be hydrolyzed, the glucose unit must change its conformation from chair to half-chair.[13]

The objective of this study was to better understand the change of the amorphous and crystalline lamellae in the starch granule during annealing. The impact of annealing on the fine structure of Naegeli amylodextrins of annealed starches was also characterized.

2. Experimental

2.1. Materials

Common corn and waxy corn starch were from Cerestar USA, Inc. (Hammond, IN). Wheat starch was from MGP Ingredients, Inc. (Atchison, KS). Potato starch and tapioca starch were from A.E. Staley Manufacturing Company (Decatur, IL). Hylon V and Hylon VII (high-amylose corn starches with 50% and 70% apparent amylose content, respectively) were from National Starch and Chemical Company (Bridgewater, NJ). Mung bean starch was purchased from a local oriental store and subjected to further purification.

2.2. Preparation of annealed starch

Starches were annealed by a multiple-step process. Starch (100 g) and distilled water (300 mL) were placed in a 500-mL beaker, covered with aluminum foil, and incubated at a temperature of incubating the starch slurry that had been previously annealed at each of the lower 5 °C temperature intervals, starting at 40 °C. Starch was annealed at each annealing temperature for 24 h. The annealing process ended when the starch gelatinization enthalpy decreased compared with that of the starch annealed at 5 °C lower temperature. After the annealing treatment, starch samples were filtered through a Whatman No. 4 filter paper and dried at room temperature.

2.3. Gelatinization characteristics

The gelatinization characteristics of each annealed and native starch were measured and recorded by a Perkin–Elmer Pyris-1 Differential Scanning Calorimeter (DSC, Perkin–Elmer, Norwalk, CT). Starch (approximately 4.0 mg, db) was placed in an aluminum pan, and water (8.0 μL) was added with a microsyringe. The starch sample was sealed, equilibrated at room temperature for at least 1 h, and then scanned from 20 to 120 °C at a heating rate of 10 °C/min. The DSC computed the onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy (ΔH) of the gelatinization endotherm. Gelatinization range was calculated as the temperature difference between T_o and T_c. Each starch was measured in triplicate.

2.4. Acid hydrolysis and preparation of Naegeli dextrins

The native and annealed starches that exhibited the largest gelatinization enthalpy were suspended in 15.3% H_2SO_4 (5 g dry starch/100 mL). The beaker was covered with parafilm and placed in a water bath at 38 °C for 2, 4, 6, 8, 10, 20, and 30 days with daily gentle shaking by hand. The supernatant was analyzed for total carbohydrate by the phenol–sulfuric acid method.[16] The dextrin residues were washed with a 3-fold volume of deionized water two times, neutralized with 0.1 M NaOH, washed again with deionized water, centrifuged at 12,000*g* for 10 min, and dried at room temperature.

2.5. Apparent amylose content of annealed, native starches and Naegeli dextrins

The apparent amylose content of native and annealed starches that exhibited the largest gelatinization enthalpy was determined following the method of Juliano.[17] A starch sample (100 g) was placed in a 50-mL Erlenmeyer flask, and 95% EtOH (1 mL) and 1N NaOH (9 mL) were added. The sample was heated in a boiling water bath for 10 min, cooled, transferred into a 100-mL volumetric flask, brought up to volume with deionized water, and well mixed. An aliquot of the starch solution (5 mL) was pipetted into a 100-mL volumetric flask, and 1N AcOH (1 mL) and iodine solution (0.2 g iodine and 2.0 g potassium iodide in 100 mL of aq soln, 2 mL) was added. The solution was allowed to stand at room temperature for 20 min prior to absorbance measurement at 620 nm. Potato amylose from Sigma Chemical Co. (St. Louis, MO) was used as the standard.

2.6. Characterization of Naegeli dextrins of native and annealed starches

The molecular size distribution of Naegeli dextrins of native and annealed starches after acid hydrolysis for 10 days were analyzed by high-performance size-exclusion chromatography (HPSEC) following the method of Kasemsuwan and co-workers[18] as modified by Wang and Wang.[19] The HPSEC system (Waters, Milford, MA) consisted of a 515 HPLC pump with an injector equipped with a 100-μL sample loop, an in-line degasser, a 2410 refractive index detector maintained at 40 °C, and a series of Shodex OHpak columns (KB-802 and KB-804) maintained at 55 °C. The chain-length distribution of Naegeli dextrins was determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) according to the method of Kasemsuwan and co-workers[18] with modifications.[19] The HPAEC–PAD system (Dionex DX 500, Sunnyville, CA) components were a GP50 gradient pump, an LC20-1 chromatography organizer, an ED40 electrochemical detector, a 4 × 50 mm CarboPac PA1 guard column, a 4 × 250 mm CarboPac PA1 analytical column, and an AS40 automated sampler.

3. Results and discussion

3.1. Gelatinization characteristics

The gelatinization characteristics of the native and annealed starches determined by DSC are listed in Table 1 and displayed in Fig. 1. Annealing is temperature dependent, and a multi-step annealing process has been shown to produce higher gelatinization temperatures.[1] Under the conditions in this study, the onset (T_o) and peak (T_p) gelatinization temperatures of annealed starches increased as the annealing temperature increased. However, the gelatinization enthalpy (ΔH) increased and then decreased. Presumably the reduction in ΔH was a result of partial gelatinization at a high annealing temperature. The increase in gelatinization temperature was most pronounced for T_o and least for conclusion temperature (T_c). Similar results were also reported for corn starch by Knutson[1] and for wheat, oat, potato, and lentil starches by Hoover and Vasanthan.[9] The increase in T_o between native and annealed starch with the greatest ΔH followed the order of potato > wheat > Hylon V > tapioca > Hylon VII > waxy corn > mung bean > common corn. The largest ΔH occurred at 50 °C for wheat, tapioca, and mung bean, at 55 °C for common corn, waxy corn and potato, and at 60 °C for Hylon V and Hylon VII. The increase of T_o was very rapid for potato (7.2 °C) and wheat (5.0 °C) starches after annealing at the lowest annealing temperature for 24 h. All annealed starches displayed a narrower gelatinization range compared with their native counterpart, and potato starch exhibited the largest decrease of 7.4 °C.

Hylon V and VII showed a characteristic broad gelatinization endotherm with higher T_o, T_p, and T_c, that were difficult to accurately define. Nevertheless, the annealing effects were also observed in high-amylose corn starches. Their T_o and T_p increased and gelatinization range decreased at higher annealing temperatures, agreeing with the work of Tester and co-workers.[20] Smaller gelatinization ranges for high-amylose corn starches have also been previously reported.[1,10,11] Tester and co-workers[21] suggested that increasing incubation temperature enhanced the order of the amorphous lamellae and subsequently the order of double helices of amylopectin to form more perfect crystalline and helix aggregation. The helix aggregation caused an increase in the length of double helices without increasing hydrogen bonding. The increased double helix length might contribute to improved ordered structure and crystallinity, which consequently increased the gelatinization temperature of annealed starch. Hoover and Vasanthan[9] suggested that an increase in ΔH was due to the interaction between amylose and amylopectin based on their study of amylose leaching. They observed a reduced amount of leached amylose after annealing.

3.2. Hydrolysis as a function of time

The acid hydrolysis rates of starch granules differed with starch type as shown in Fig. 2. Two phases were observed during the acid hydrolysis, the first rapid phase corresponding to hydrolysis of starch amorphous lamellae before 8–10 days,[14] and the second slow phase corresponding to hydrolysis of starch crystalline lamellae between 10 and 30 days. The profiles of rapid hydrolysis and slow hydrolysis could be clearly distinguished between the native and annealed starches in terms of percent hydrolysis. At the end of the 10th day of hydrolysis, native common corn, waxy corn, wheat, tapioca, potato, Hylon V, Hylon VII, and mung bean starches were hydrolyzed to the extent of 31.7, 34.8, 36.7, 40.2, 39.8, 30.8, 30.4, and 37.1%, respectively. The values were generally lower than those reported by Jayakody and Hoover.[22] At the end of the 30th day of hydrolysis, native common corn, waxy corn, wheat, tapioca, potato, Hylon V, Hylon VII, and mung bean starches were hydrolyzed to the extent of 42.2, 45.3, 43.5, 45.6, 49.2, 34.9, 34.0, and 44.0%, respectively. The annealing treatment increased the susceptibility of all starches to acid hydrolysis, ranging from 1.0% for

Table 1
Gelatinization characteristics of native and annealed starches: onset (T_o), peak (T_p) and conclusion (T_c) temperatures, temperature ranges ($T_c - T_o$), and enthalpies (ΔH) (standard deviations in parentheses)

Sample		Native	40°C	45°C	50°C	55°C	60°C	65°C
Common Corn	T_o (°C)	68.1 (0.3)	69.1 (0.3)	70.1 (0.2)	71.3 (0.2)	73.2 (0.3)	74.6 (0.2)	
	T_p (°C)	72.3 (0.3)	73.0 (0.3)	73.7 (0.2)	74.3 (0.1)	76.3 (0.3)	77.3 (0.2)	
	T_c (°C)	77.3 (0.1)	77.7 (0.2)	78.0 (0.3)	78.8 (0.4)	80.2 (0.2)	81.1 (0.3)	
	$T_c - T_o$ (°C)	9.2 (0.4)	8.6 (0.4)	7.9 (0.1)	7.5 (0.0)	7.0 (0.2)	6.5 (0.3)	
	ΔH	12.3 (0.2)	12.8 (0.3)	13.4 (0.2)	14.3 (0.2)	15.6 (0.3)	15.4 (0.3)	
Waxy Corn	T_o (°C)	65.3 (0.2)	67.8 (0.5)	70.2 (0.3)	71.4 (0.1)	72.4 (0.4)	74.5 (0.3)	
	T_p (°C)	72.8 (0.3)	73.3 (0.4)	74.0 (0.2)	74.4 (0.3)	76.3 (0.1)	77.0 (0.3)	
	T_c (°C)	78.2 (0.4)	78.2 (0.6)	78.4 (0.3)	78.8 (0.2)	79.3 (0.1)	81.1 (0.2)	
	$T_c - T_o$ (°C)	12.9 (0.5)	10.4 (0.6)	8.2 (0.4)	7.4 (0.2)	6.9 (0.3)	6.6 (0.3)	
	ΔH	14.9 (0.1)	15.4 (0.2)	16.8 (0.4)	17.5 (0.3)	18.1 (0.2)	17.3 (0.3)	
Wheat	T_o (°C)	58.7 (0.2)	63.7 (0.1)	66.1 (0.3)	69.1 (0.3)	71.8 (0.2)		
	T_p (°C)	65.0 (0.4)	66.8 (0.2)	69.0 (0.3)	71.7 (0.2)	74.0 (0.1)		
	T_c (°C)	68.5 (0.1)	70.7 (0.5)	72.1 (0.4)	75.0 (0.2)	77.2 (0.3)		
	$T_c - T_o$ (°C)	9.8 (0.2)	7.0 (0.3)	6.0 (0.3)	5.9 (0.3)	5.4 (0.3)		
	ΔH	10.0 (0.3)	10.3 (0.2)	11.1 (0.2)	12.4 (0.2)	9.9 (0.2)		
Tapioca	T_o (°C)	61.3 (0.8)	65.0 (0.3)	66.3 (0.2)	69.1 (0.2)	71.7 (0.1)		
	T_p (°C)	70.1 (0.2)	70.3 (0.5)	71.1 (0.1)	73.0 (0.5)	75.6 (0.3)		
	T_c (°C)	76.2 (0.4)	78.6 (0.1)	79.7 (0.1)	79.4 (0.2)	81.3 (0.0)		
	$T_c - T_o$ (°C)	14.9 (0.6)	13.6 (0.2)	13.4 (0.2)	10.3 (0.2)	9.6 (0.1)		
	ΔH	10.6 (0.2)	11.9 (0.2)	12.8 (0.3)	13.6 (0.3)	11.5 (0.3)		
Potato	T_o (°C)	58.4 (0.7)	65.6 (0.2)	67.0 (0.3)	69.9 (0.3)	72.2 (0.3)	74.9 (0.2)	
	T_p (°C)	67.3 (0.3)	68.8 (0.1)	70.0 (0.2)	73.3 (0.2)	77.3 (0.2)	77.3 (0.3)	
	T_c (°C)	73.5 (0.5)	74.1 (0.2)	75.6 (0.3)	78.1 (0.3)	79.9 (0.4)	82.1 (0.3)	
	$T_c - T_o$ (°C)	15.1 (0.6)	8.5 (0.2)	8.6 (0.3)	8.2 (0.3)	7.7 (0.3)	7.2 (0.2)	
	ΔH	16.5 (0.2)	17.1 (0.3)	17.9 (0.2)	18.5 (0.2)	19.6 (0.3)	13.4 (0.4)	
Hylon V	T_o (°C)	68.0 (0.6)	68.8 (0.9)	70.3 (0.2)	71.9 (0.5)	74.3 (0.2)	76.6 (0.9)	78.5 (0.4)
	T_p (°C)	76.2 (0.5)	76.7 (0.7)	77.2 (0.8)	78.5 (0.5)	80.6 (0.4)	83.3 (0.3)	85.4 (1.0)
	T_c (°C)	106.1 (0.9)	107.4 (0.7)	108.0 (0.6)	108.8 (0.3)	108.7 (0.3)	108.9 (0.3)	109.2 (0.2)
	$T_c - T_o$ (°C)	38.1 (0.7)	38.6 (0.5)	37.7 (0.6)	36.9 (0.3)	34.4 (0.3)	32.3 (0.7)	30.7 (0.3)
	ΔH	16.5 (0.5)	17.1 (0.2)	17.9 (0.6)	18.8 (0.7)	19.3 (0.5)	19.4 (0.2)	17.0 (0.3)
Hylon VII	T_o (°C)	68.9 (0.3)	70.0 (0.2)	72.1 (0.8)	73.3 (0.2)	74.4 (0.7)	76.4 (0.9)	76.6 (0.3)
	T_p (°C)	82.0 (0.9)	83.4 (0.6)	84.4 (0.7)	85.1 (0.9)	84.6 (0.6)	86.6 (0.4)	86.6 (0.2)
	T_c (°C)	110.8 (0.9)	113.0 (1.0)	114.8 (0.2)	115.0 (0.4)	115.5 (0.8)	115.4 (1.1)	115.9 (1.0)
	$T_c - T_o$ (°C)	41.9 (0.8)	43.0 (0.6)	42.7 (0.7)	41.7 (0.3)	41.1 (0.7)	39.0 (0.3)	39.3 (0.4)
	ΔH	16.7 (0.6)	18.2 (0.4)	19.4 (0.3)	20.1 (0.5)	20.3 (0.2)	20.3 (0.3)	9.6 (0.3)
Mung Bean	T_o (°C)	64.8 (0.1)	66.3 (0.1)	67.1 (0.0)	71.1 (0.3)	78.1 (0.1)		
	T_p (°C)	70.9 (0.1)	71.8 (0.3)	72.4 (0.2)	75.1 (0.1)	82.0 (0.2)		
	T_c (°C)	78.1 (0.4)	79.8 (0.2)	80.2 (0.3)	80.7 (0.2)	87.9 (0.2)		
	$T_c - T_o$ (°C)	13.3 (0.2)	13.5 (0.2)	13.1 (0,3)	9.6 (0.2)	9.8 (0.3)		
	ΔH	12.3 (0.2)	12.8 (0.4)	13.7 (0.4)	14.4 (0.3)	12.8 (0.3)		

Hylon V to 14.3% for potato. At the end of the 10th day of hydrolysis, annealed common corn, waxy corn, wheat, tapioca, potato, Hylon V, Hylon VII, and mung bean starches were hydrolyzed to the extent 35.5, 43.2, 47.5, 43.3, 54.1, 32.0, 31.4, and 38.8%, respectively. At the end of the 30th day of hydrolysis, annealed common corn, waxy corn, wheat, tapioca, potato, Hylon V, Hylon VII, and mung bean starches were hydrolyzed to the extent of 45.8, 51.7, 52.7, 52.5, 61.3, 37.8, 35.8, and 49.0%, respectively. Potato starch had the highest susceptibility to acid hydrolysis with the most distinct difference between native and annealed starches. For Hylon V and VII, which had the lowest acid hydrolysis susceptibility, the hydrolysis difference between the native and annealed starches was not clear. The high degree of acid hydrolysis for potato starch could possibly be attributed to its B-type less compact organization and its high phosphorous content. Among the four types of corn starches, waxy corn starch showed the highest hydrolysis rate and susceptibility to acid hydrolysis in both annealed and native forms, whereas both native and annealed high-amylose corn starches had a similar low degree of hydrolysis. The difference in the four types of corn starches might be attributed to

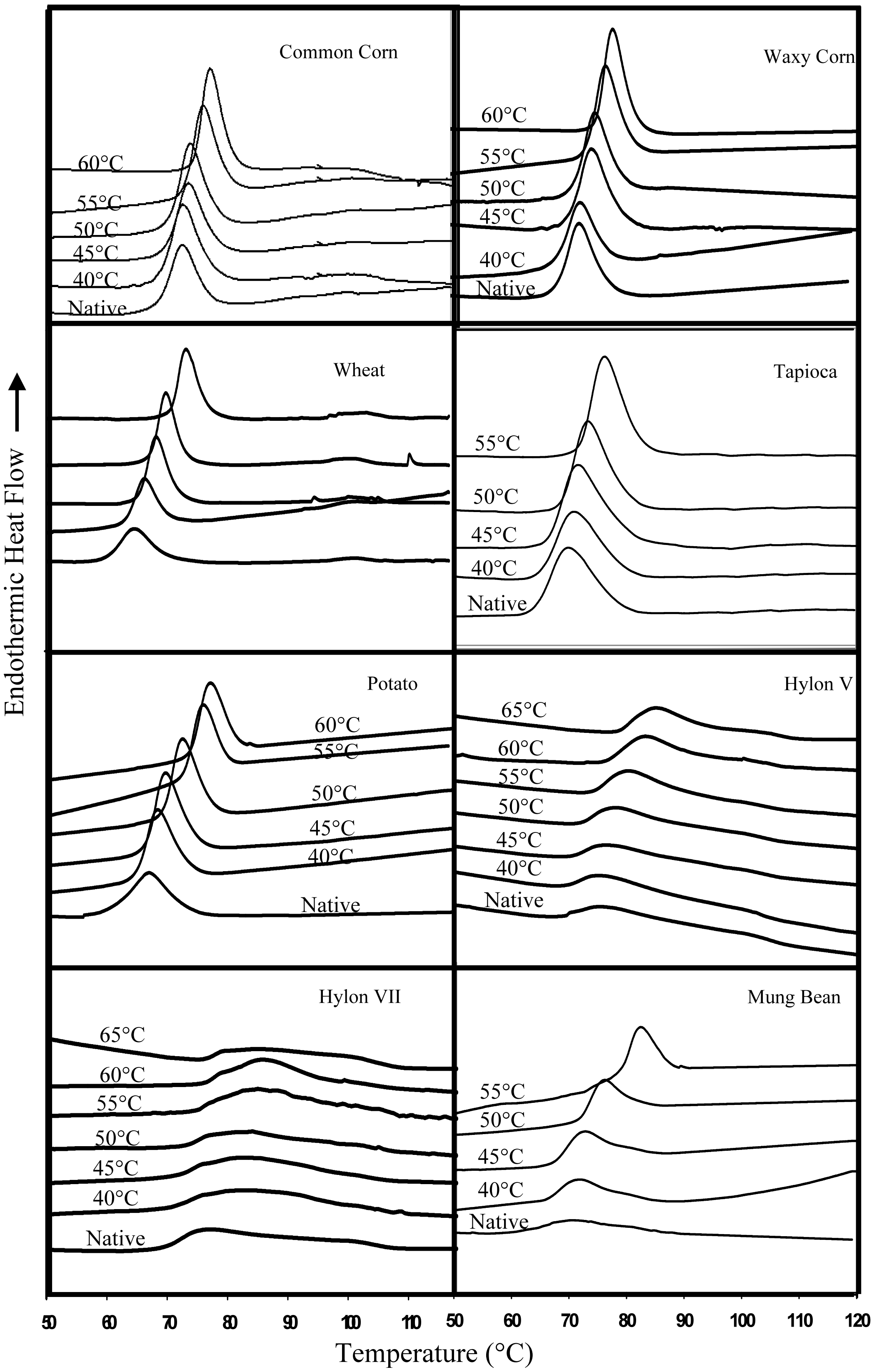

Fig. 1. Gelatinization endotherms of native and annealed starches (labeled temperature denoting the highest annealing temperature).

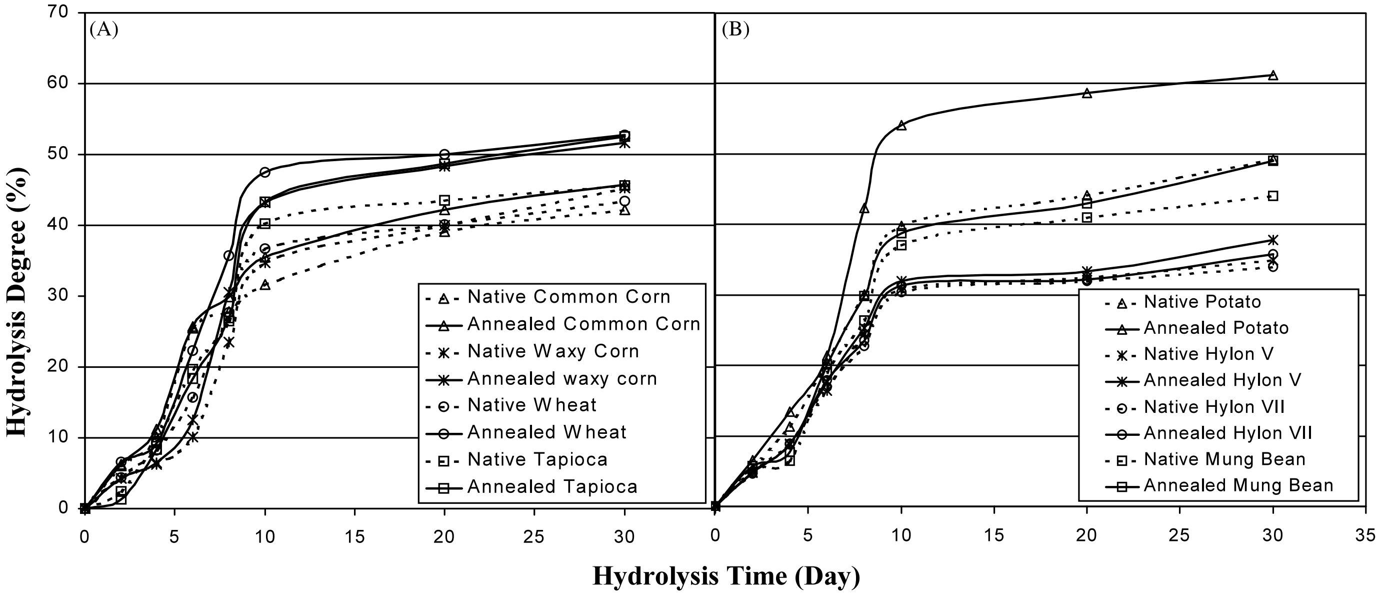

Fig. 2. Acid hydrolysis (15.3% H_2SO_4, v/v) of native and annealed starches at 38 °C for various periods of time: (A) common corn, waxy corn, waxy corn, wheat, and tapioca; (B) potato, Hylon V, Hylon VII, and mung bean.

their difference in amylose content. High amylose corn starches exhibited a restricted swelling compared with waxy corn starch,[20] which might limit the acid penetration into the starch granules and therefore allowed a low percent acid hydrolysis. Furthermore, the smaller proportions of amylopectin in Hylon V and VII would contribute limited reordering during annealing, thus resulting in little change in the percent hydrolysis. Tester and co-workers[23] also observed a more extensive hydrolysis from annealed wheat starch than from native wheat starch during the first phase of acid hydrolysis. However, the extent of hydrolysis for the native and annealed wheat starches were the essentially the same after 7 days of hydrolysis. In contrast to the present results, it was reported that annealing caused a small decrease in susceptibility for potato starch and no significant effect for wheat and pea starches.[24] Hoover and Vasanthan[9] observed a slight decrease in the degree of hydrolysis for annealed both wheat and potato starch. It was suspected that the differences might be due to the procedure of preparing annealed starch. A multiple-step annealing was used in the present study, which could produce annealed starch with more perfect crystallites, whereas others used either one-step or two-step annealing. The different type and concentration acid used might also contribute to the observed discrepancy: 15.3% H_2SO_4 in our study versus 2.2 M HCl in their studies.

The degree of hydrolysis of native starch followed the order of potato > wheat > tapioca, waxy corn > mung bean > common corn > Hylon V > Hylon VII. The difference in percent hydrolysis between native and annealed starches also followed the same order as the percent hydrolysis of native starch. These similar trends implied that starch with a more open, loose structure would rearrange to a greater degree and create void areas that were more susceptible to acid hydrolysis after the annealing treatment. The reorganization of the starch molecule during annealing occurred mainly within the crystalline lamellae as evidenced by much increased hydrolysis during the second slow phase of acid hydrolysis. The numbers of double helices between pre- and post-annealed wheat starch have been studied by Tester and co-workers[23] by ^{13}C-CPMAS-NMR spectroscopy. They discovered that the numbers of double helices between pre- and post-annealed starches were similar and concluded that annealing enhanced the ordering of double helices in the crystalline lamellae rather than facilitating the formation of new double helices. They proposed that hydration and expansion of the amorphous lamellae were involved in the annealing process, which facilitated the optimization of double helical structure in the crystalline lamellae. The amorphous lamellae became more glassy and less mobile, and consequently the glass transition temperature was elevated. Based on the present percent hydrolysis results, the amorphous lamellae seemed to be only slightly affected but the crystalline lamellae became more prone to acid hydrolysis. The increased crystalline lamella perfection might give rise to some void space that allowed for acid penetration and resulted in increased hydrolysis.

When the gelatinization characteristics of native starches were compared with their percentages of hydrolysis, a general trend was noted that starch with a lower T_o and a large decrease in gelatinization range after annealing coincided with a higher percent hydrolysis both before and after annealing. The results demonstrated that annealing treatment caused more significant changes for starches with less organized structures. For starches with less perfect crystalline structures, they were more sensitive toward annealing treatment and would improve their crystalline structure to a greater degree. Nevertheless, the perfection of crystalline lamellae would result in a more open structure for allowing more rapid hydrolysis.

3.3. Apparent amylose content of annealed, native starches and Naegeli dextrins

The apparent amylose content of nonwaxy starches decreased after annealing and the decrease ranged between 2.8% (Hylon VII) and 5.0% (mung bean) (Table 2). Amylose was unavoidably leached out when the annealing temperature approached the gelatinization temperature of the starch. Although high-amylose corn starches have a higher amylose content, they leached out a similar amount of amylose as other starches. The nature of leached amylose molecules in terms of their molecular size, their distribution within the starch granule, and their interaction with amylopectin in the amorphous and crystalline lamellae deserve further investigation.

The apparent amylose contents of native and annealed starches significantly decreased after 10 days' hydrolysis (Table 2). All Naegeli dextrins of native and annealed starches had an amylose content $< 5\%$, except high-amylose corn starches of 10–12%. Since acid first hydrolyzed the amorphous lamellae, the majority of amylose was evidently present in the amorphous lamellae. The annealing treatment seemed to have a slight impact on amylose arrangement with starch granules because the apparent amylose contents of Naegeli dextrin of annealed starches were slightly higher than those of native starches, although the values were not statistically different. It was suspected that annealing also promoted reordering of amylose and/or between amylose and amylopectin, although the reordering was relatively minor. The residual amylose after 10 days' hydrolysis was presumed to be present in the crystalline lamellae and possibly had strong interactions with double helices of amylopectin. Because the apparent amylose content measurement could only measure amylose molecules with a large degree of polymerization ($\mathrm{DP} > 100$), the hydrolyzed amylose molecules with a small DP would not be measured by this method. Therefore, the apparent amylose content of the Naegeli dextrins probably only reflected the portion of amylose that had not been hydrolyzed to a great extent.

Table 2
Apparent amylose content of native and annealed starches [a] (standard deviations in parentheses)

	Starch		Naegeli dextrin [b]	
	Native	Annealed	Native	Annealed
Corn	28.0 (0.2)	24.6 (0.1)	4.4 (0.3)	4.7 (0.4)
Waxy corn	0.6 (0.1)	0.4 (0.1)	0.5 (0.2)	0.3 (0.2)
Wheat	27.1 (0.2)	22.5 (0.1)	3.4 (0.3)	3.4 (0.3)
Tapioca	17.2 (0.1)	13.0 (0.2)	2.2 (0.5)	2.3 (0.6)
Potato	22.5 (0.2)	18.1 (0.1)	1.7 (0.3)	1.9 (0.3)
Hylon V	52.7 (0.2)	48.4 (0.2)	10.2 (0.2)	10.1 (0.5)
Hylon VII	70.3 (0.2)	67.5 (0.2)	11.9 (0.5)	12.4 (0.5)
Mung bean	34.4 (0.1)	29.4 (0.1)	2.4 (0.3)	2.5 (0.3)

[a] Annealed starches with the largest gelatinization enthalpies.

[b] Naegeli dextrin of 10 days' hydrolysis.

3.4. HPSEC of Naegeli dextrins of native and annealed starches

The molecular size distribution of native and annealed starches after acid hydrolysis for 10 days are depicted in Fig. 3. The starches could be classified into two groups based on their chromatograms: (1) A-type starches of common corn, waxy corn, wheat, tapioca, and C-type starch of mung bean with three main populations of chains, and (2) B-type starches of potato, Hylon V, and Hylon VII with two main populations of chains. In general, the chromatogram of the annealed starch was similar to that of the native counterpart, but the relative fraction of large DP chains versus small DP chains changed after annealing for most starches and most obvious for common corn, waxy corn, and tapioca starches.

The A- and C-type starches had three main populations of chains with peak maximal at DP 61–68, 27–34, and 13–16. The higher DP chains ($\mathrm{DP} > 35$) consisted of multiple branched chains, DP 27–30 chains were singly branched, and the DP 13–16 chains were short, linear chains in acid-hydrolyzed starch.[24] After annealing, common, waxy corn, and mung bean starches had increased multiple and single chains, while the fraction of linear chains decreased. On the contrary, tapioca starch had decreased multiple chains but increased linear chains after annealing. The chromatograms of native and annealed wheat starch were similar. The B-type starches had two main populations of chains: one with peak maximal at DP 37 for potato and DP 75 for Hylon V and VII, and one with peak maximal at DP 12–13. The population of singly branched chains (DP around 25) in B-type starches was much smaller than those of multiple-branched and linear chains and did not appear in a separate fraction. High-amylose corn starches were known for their longer branched chains,[25] which consequently led to a longer DP of multiple branched chains with a maximal peak at DP 75.

The changes in HPSEC profiles of Naegeli dextrins after annealing suggested that the distribution of branch

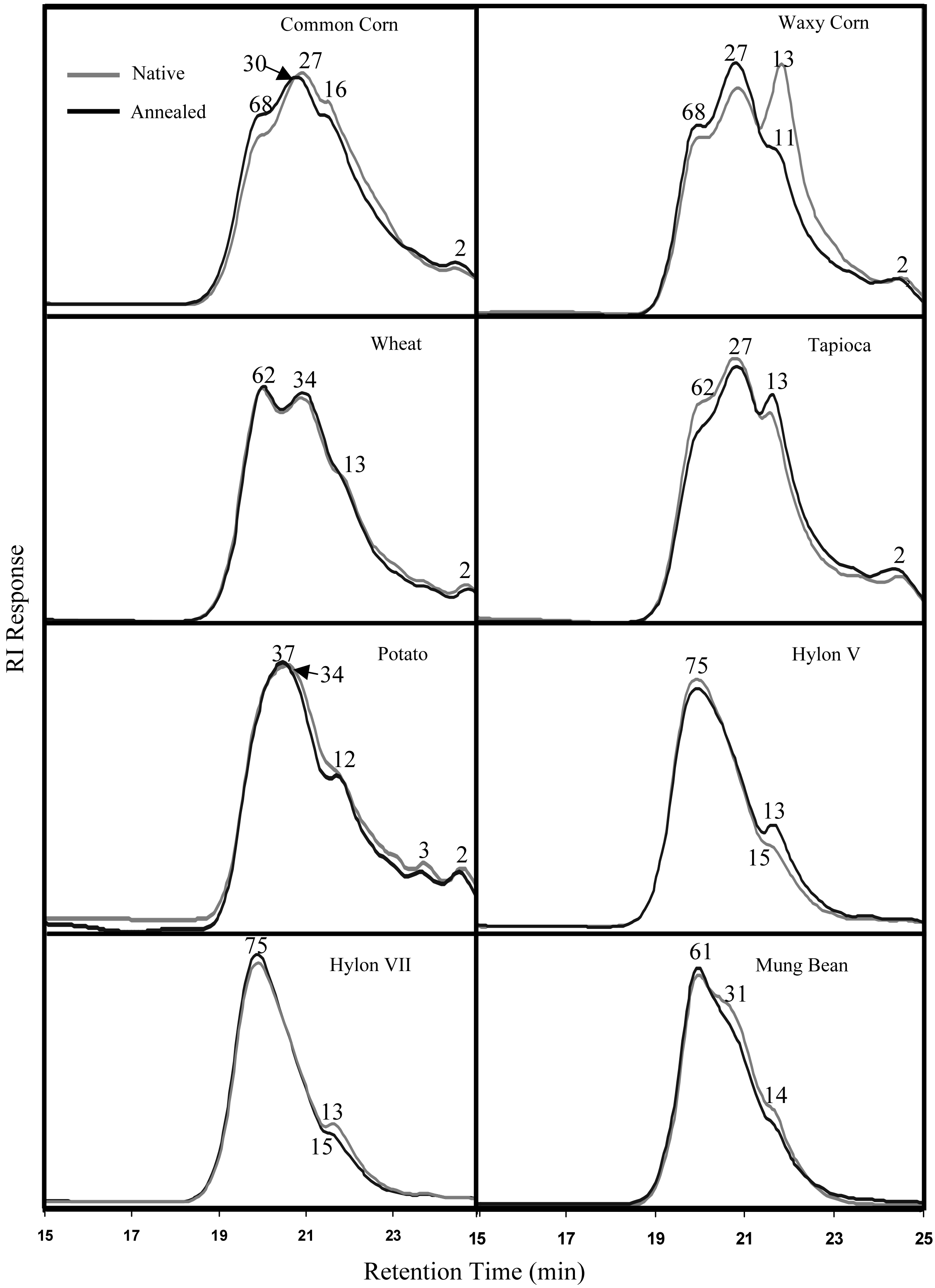

Fig. 3. HPSEC chromatograms of Naegeli dextrins of 10 days' hydrolysis of native and annealed starches with labeled peak degree of polymerization.

linkages of A- and C-type starches was different from that of B-type starches, agreeing with the findings of Jane and co-workers.[26] They studied the branch structure of A- and B-type starches by their Naegeli dextrins and concluded that most of the branch linkages in B-type starches were clustered in the amorphous lamellae compared with more scattered branch linkages in A-type starches. A low concentration of singly branched chains would constitute a more closely clustered branching pattern of the amylopectin. In the current study, the similar HPSEC profiles of native and annealed Naegeli dextrins of B-type starches supported the hypothesis of Jane and co-workers[26] that the branch structures of B-type starches were mostly present in the amorphous lamellae, which was easily hydrolyzed by acid. In contrast, because the branch linkages in A- and C-type starches were largely in the crystalline lamellae, the perfection of the crystalline lamellae changed the HPSEC profiles after annealing and the changes might be determined by their inherent arrangement of branch linkages. The perfection of the crystalline lamellae might produce open space as evidenced by the increased hydrolysis as previously discussed, which consequently modified the HPSEC profiles. Jacobs and co-workers[24] suggested that branch points of amylopectin might become more resistant to acid hydrolysis due to being embedded in a more perfect structure after annealing. The present results indicated that the inherent branch structure of amylopectin influenced how they reorganized during annealing. These results also implied that the helices in the crystalline lamellae of native starches might be loosely organized around the branch linkages, and annealing treatment improved their order.

3.5. HPAEC-PAD of Naegeli dextrins of native and annealed starches

The chain-length distributions of isoamylase-debranched native and annealed starches after acid hydrolysis for 10 days are displayed in Fig. 4. Because the amorphous lamellae in these samples were close to complete hydrolysis, the majority of the chains were DP less than 25. The chains greater than DP 25 were negligible for most starches, except Hylon V and VII. Since native and annealed high-amylose corn starches did not undergo extensive hydrolysis, the majority of B chains were still preserved as noted in their profiles as two main populations with peak maximal at DP around 35 and 50. The presence of chains with $DP < 6$ came from partial hydrolysis of branched chains on the surface of crystallites or at defect structures (weak points) of the crystallites as suggested by Jane and co-workers.[26] The proportions of chains from annealed and native starches were compared, and the results are plotted in Fig. 5. The percent distribution above the line represents the increased percent distribution after annealing, while the distribution below the line represent the decreased percent distribution after annealing. In general, the population of longer chains with $DP > 13$ and short chains DP 1–4 increased, whereas the population of DP 9–13 decreased after annealing. Jacobs and co-workers[24] also observed more longer chains in lintnerized annealed starches (wheat, potato, and pea) compared with their lintnerized native ones, and they proposed such to be a result of the perfection of double helices in the crystalline lamellae. In addition, they also suggested that amylose might become more mobile and aggregated to form double helices during annealing. However, the active role of amylose could not be established according to the present results when comparing waxy corn against common corn or high amylose corn starches. It is more likely that branched chains in amylopectin became more mobile and involved in reorganization. Knutson[1] has also suggested that interaction between amylose and amylopectin in normal starches prior to annealing was relatively minor based on gelatinization behavior of common and waxy corn. The profiles of Naegeli dextrins of wheat starch were different from the rest at $DP < 13$. The presence of amylose–lipid complexes in native wheat starch might exert an effect on the reorganization of the amylopectin branched chain and contribute to its different chain length distribution.

In starch granules, amylopectin is considered to be responsible for the crystalline structure through the ordered arrangement of double helices formed by adjacent branches within the structure.[27] Cooke and Gidley[28] reported two types of double helices in starch granules based on studying of crystalline order by powder X-ray diffraction and of molecular order by ^{13}C-NMR spectroscopy. One type of double helix was large and perfect, the type of double helix involved in crystallites; the other one was a non-ordered helix. Those imperfect double helices might become perfect double helices and form a closer packing of the pre-existing crystalline lamellae within starch granules. In native starches, some branch linkages existed in imperfect double helices, which were hydrolyzed during acid hydrolysis. After annealing, these imperfect double helices might become perfect double helices and part of the crystallites; therefore, they would not be easily hydrolyzed by acid. The decrease in linear chains and increase in single and multiple-branched chains in Naegeli dextrins supported the hypothesis of formation of improved perfect double helices from originally imperfect helices, which protected the branch linkages from acid hydrolysis and resulted in increased longer chains in Naegeli dextrins of annealed starches.

4. Conclusions

Annealing increased the susceptibility of starch to acid due to increased porosity and formation of defects in annealed starches. Although annealing promoted reorganization and perfection of the crystalline lamellae, the improved perfect crystallites might in turn give rise to some void space in the crystalline lamellae, which

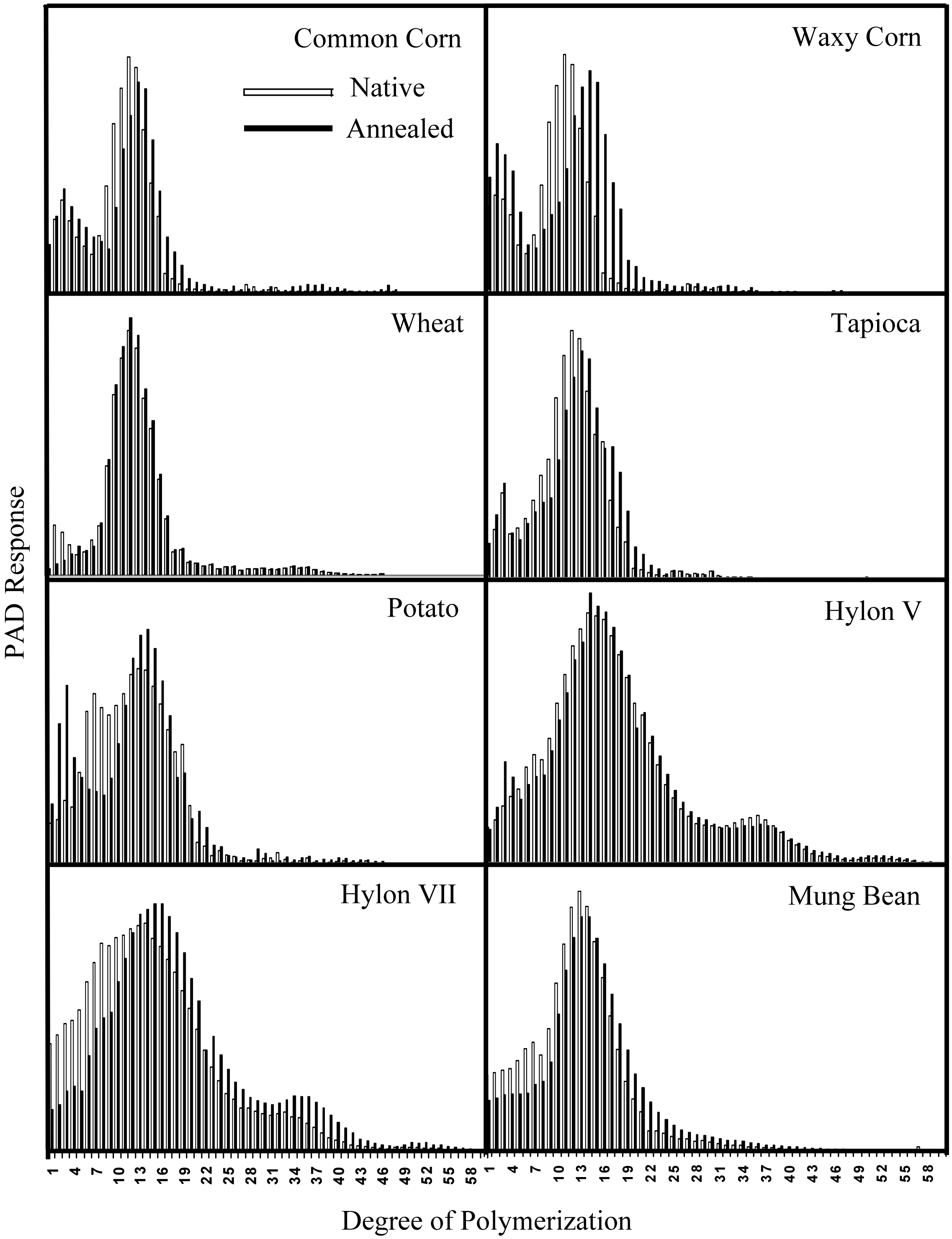

Fig. 4. HPAEC–PAD chromatograms of isoamylase-debranched Naegeli dextrins of 10 days' hydrolysis of native and annealed starches.

consequently allowed rapid hydrolysis. Alternatively, the improved perfect crystallites provide better protection for the embedded branch linkages during hydrolysis. The molecular size distribution of Naegeli dextrins of native and annealed starches indicated a different branch structure of A- and C-type starches from B-type starches. A- and C-type starches had more scattered branch linkages mainly in the crystalline lamellae, whereas the branch linkages were more clustered in the amorphous lamellae for B-type starches. Upon annealing, more changes in the crystalline structure due to reorganization were observed for Naegeli dextrins of A- and C-type starches, but little change was observed for Naegeli dextrins of B-type starches.

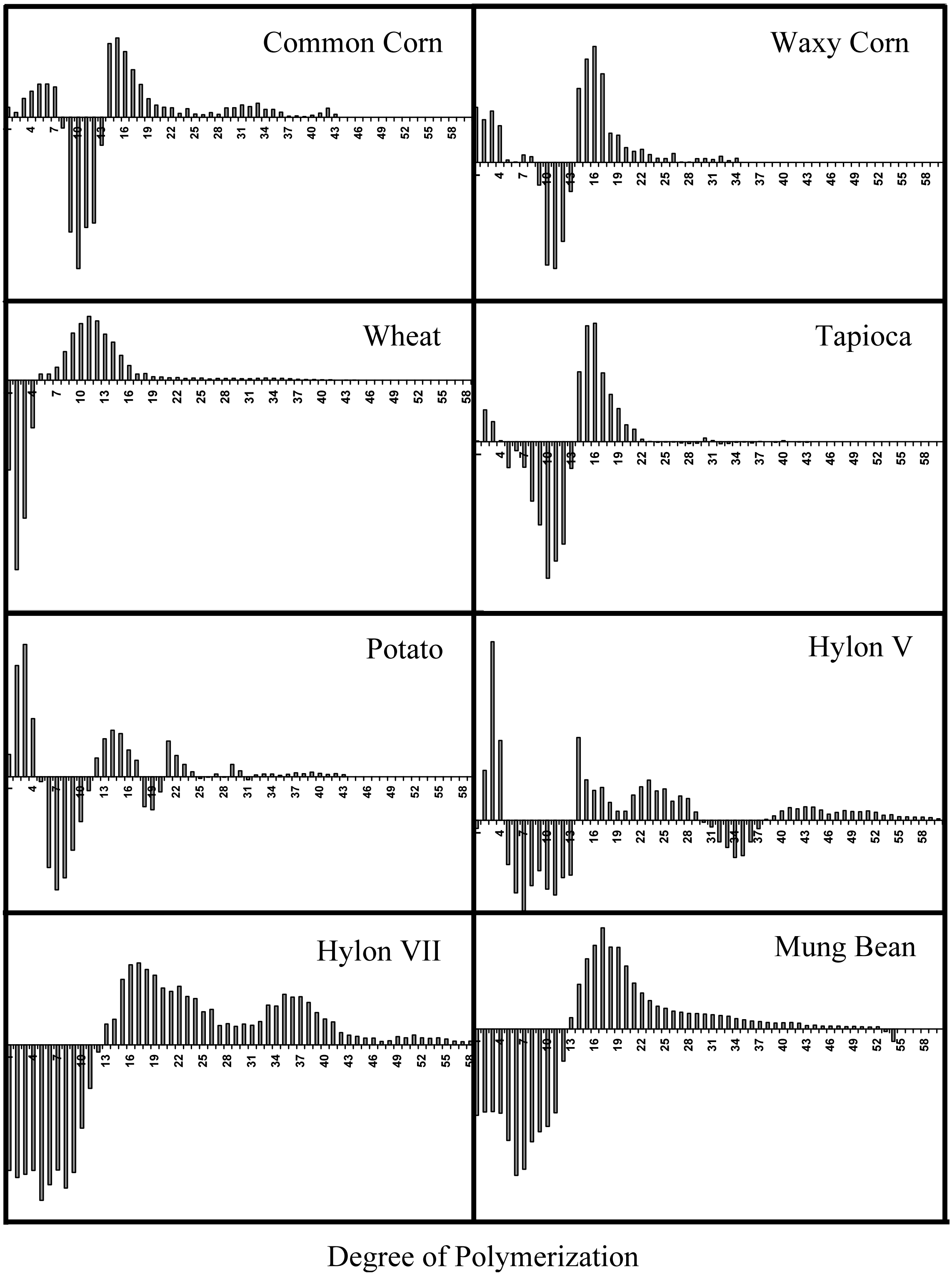

Fig. 5. Differences in percent distribution of HPAEC–PAD chromatograms of isoamylase-debranched Naegeli dextrins of 10 days' hydrolysis of native and annealed starches. The percent distribution above the line represents the increased percent distribution after annealing; the distribution below the line represents the decreased percent distribution after annealing.

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