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Internal structure and physicochemical properties of corn starches as revealed by chemical surface gelatinization

Daris Kuakpetoon[a] and Ya-Jane Wang[b,*]

[a]Department of Food Technology, Chulalongkorn University, Bangkok 10500, Thailand
[b]Department of Food Science, University of Arkansas, 2650 N. Young Avenue, Fayetteville, AR 72704, USA

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Abstract—The organization of amylose and amylopectin within starch granules is still not well elucidated. This study investigates the radial distribution of amylose and amylopectin in different corn starches varying in amylose content (waxy corn starch (WC), common corn starch (CC), and 50% and 70% amylose corn starches (AMC)). Corn starches were surface gelatinized by 13 M LiCl at room temperature to different extents (approximately 10%, 20%, 30%, and 40%). The gelatinized surface starch and remaining granules were characterized for amylose content, amylopectin chain-length distribution, thermal properties, swelling power (SP), and water solubility index (WSI). Except for the outmost 10% layer, the amylose content in CC increased slightly with increasing surface removal. In contrast, amylose was more concentrated at the periphery than at the core for 50% and 70% AMC. The proportion of amylopectin A chains generally decreased while that of B1 chains generally increased with increasing surface removal for all corn starches. The gelatinization enthalpy usually decreased, except for 70% AMC, whereas the retrogradation enthalpy relatively remained unchanged for CC but increased for WC, 50% and 70% AMC with increasing surface removal. The SP and WSI increased with increasing surface removal for all corn starches, with WC showing a significant increase in SP after the removal of the outmost 10% layer. The results of this study indicated that there were similarities and differences in the distribution of amylose and amylopectin chains along the radial location of corn starch granules with varying amylose contents. More amylose–lipid complex and amylopectin long chains were present at the periphery than at the core for amylose-containing corn starches.

Keywords: Amylopectin chain-length distribution; Amylose content; Starch structure; High amylose corn starch; Surface gelatinization; Starch swelling

1. Introduction

Starches from different botanical sources, growing conditions, mutations, or maturity can vary in their chemical structures and physicochemical properties.[1–6] Starch is mainly composed of a mixture of amylose and amylopectin. It was initially believed that most amylose distributed in the amorphous areas with little or no interaction with amylopectin.[7,8] However, more recent studies suggested that some amylose co-crystallized with amylopectin in the crystalline lamellae,[9–11] which supports recent models of amylose biosynthesis.[12,13] These models described that amylose was more likely to be synthesized within the amylopectin matrix. Jenkins and Donald[14] investigated the influence of amylose on starch granule structure by using small-angle X-ray scattering (SAXS) and observed that the combined size of the crystalline and amorphous lamellae was constant at 9 nm for all three corn starches varying in amylose content (0%, 28%, and 70%). However, the size of the amorphous lamellae decreased with increasing amylose content. They suggested that amylose might co-crystallize with amylopectin and pull some amylopectin chains from two adjacent crystalline lamellae closer to each other. Furthermore, amylose and amylopectin may not uniformly distribute in starch. By using a chemical

* Corresponding author. Tel.: +1 479 575 3871; fax: +1 479 575 6936; e-mail: yjwang@uark.edu

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surface gelatinization method, Jane and Shen[15] showed that amylose was more concentrated at the periphery than at the core, and amylopectin had more long-B chains at the core than at the periphery in potato starch. This radial dependence of molecular distribution was also observed in common corn starch.[16]

Although the molecular structures of amylose and amylopectin from various starch sources have been extensively studied, the understanding of amylose and amylopectin distribution in starch granules is still limited. Because of the nature of sample preparation, the current conclusions on starch structure and arrangement are almost exclusively based on the composite results of the whole granule and not from any specific location of the granule. This study employed the chemical surface gelatinization technique on four corn starches with varying amylose contents to better understand the radial distribution of starch composition and structure in starch granules. The relationships of the molecular structure of amylose and amylopectin, the granule organization, and physicochemical properties of starch were also investigated.

2. Results and discussion

2.1. Birefringence and morphology of starch samples

The polarized light micrographs of native waxy corn starch (WC), common corn starch (CC), 50% amylose corn starch (50% AMC), 70% amylose corn starch (70% AMC), and their remaining granules after approximately 40% chemical surface gelatinization are shown in Figure 1. The remaining granules of all corn starches still displayed the Maltese cross arrangement. This suggests that the chemical surface gelatinization did not significantly alter the semicrystalline structure in the remaining starch granules, even after 40% of the surface was removed. In this study, corn starches were not separated into small and large granule sizes as in previous works by Jane and Shen[15] and Pan and Jane[16] because this study intended to obtain composite results from the whole native granule populations to be compared with the results from their hypochlorite-oxidized counterparts.[17] The chemical surface gelatinization seemed to take place on both small and large granules as demonstrated by the presence of both large and small granules even after 40% surface removal (Fig. 1). Therefore, the results from this study could represent the whole granule population.

The scanning electron micrographs of native and remaining granules of WC, CC, 50% AMC, and 70% AMC after approximately 40% chemical surface gelatinization are presented in Figure 2. Rough appearance was observed in all remaining granules, indicating that the granule was peeled from the surface. Furthermore, erosion occurred to both large and small granules, confirming the uniformity of chemical gelatinization. It was noted that the population of rod-shaped granules in 70% AMC decreased while that of small round-shaped granules increased after chemical gelatinization. We speculate that the rod-shaped granules probably were eroded more at the grooves, and eventually the grooves were completely eroded, thus resulting in small round-shaped granules (Fig. 2h). The groove portion of the rod-shaped granules might be highly amorphous and easily gelatinized.

2.2. Distribution of amylose along the radial locations of starch granules

The amylose content of corn starches at different radial locations is summarized in Table 1. The distribution of amylose along the radial location of granules varied among different types of corn starch. The outmost 10% layer of CC granules seemed to be slightly higher in amylose content than the next layer (26.1% vs. 25.5%). After the outmost 10% layer was removed, amylose was slightly more concentrated at the core in CC. This trend was in contrary to the findings of Pan and Jane.[16] This discrepancy might be attributed to the difference in sample preparation. In their study, corn starch was defatted by refluxing in aqueous methanol (85%, v/v) for 24 h prior to chemical surface gelatinization. Vasanthan and Hoover[18] reported that common corn and wheat starches defatted by hot aqueous *n*-propanol (75%, v/v) showed an increase in amylose leaching compared with its undefatted one when heated to 60 °C or higher. It is speculated that some amylose in corn starch could become mobile and gradually move toward the outer surface of the granule during hot alcohol refluxing. Therefore, the defatting process might affect the distribution of amylose within corn starch granules. In contrast, amylose was more concentrated at the periphery in 50% and 70% AMC. The disparity in amylose distribution within corn starch granules seemed to become more pronounced when the amylose content was 50% or greater.

2.3. Amylopectin chain-length distribution along the radial locations of starch granules

The amylopectin branch chains were grouped into four types; namely, A, B1, B2, and B3+ chains, which corresponded to the chain length of the degree of polymerization (DP) 6–12, 13–24, 25–36, and $\geqslant 37$, respectively, according to Hanashiro et al.[19] For WC and CC starch granules, except for the outmost 10% layer, long-B chains (B2 and B3+) were more concentrated at the core than at the periphery (Table 2), agreeing with the findings of Pan and Jane.[16] High AMC granules had more B3+ chains at the periphery but more B1 chains at

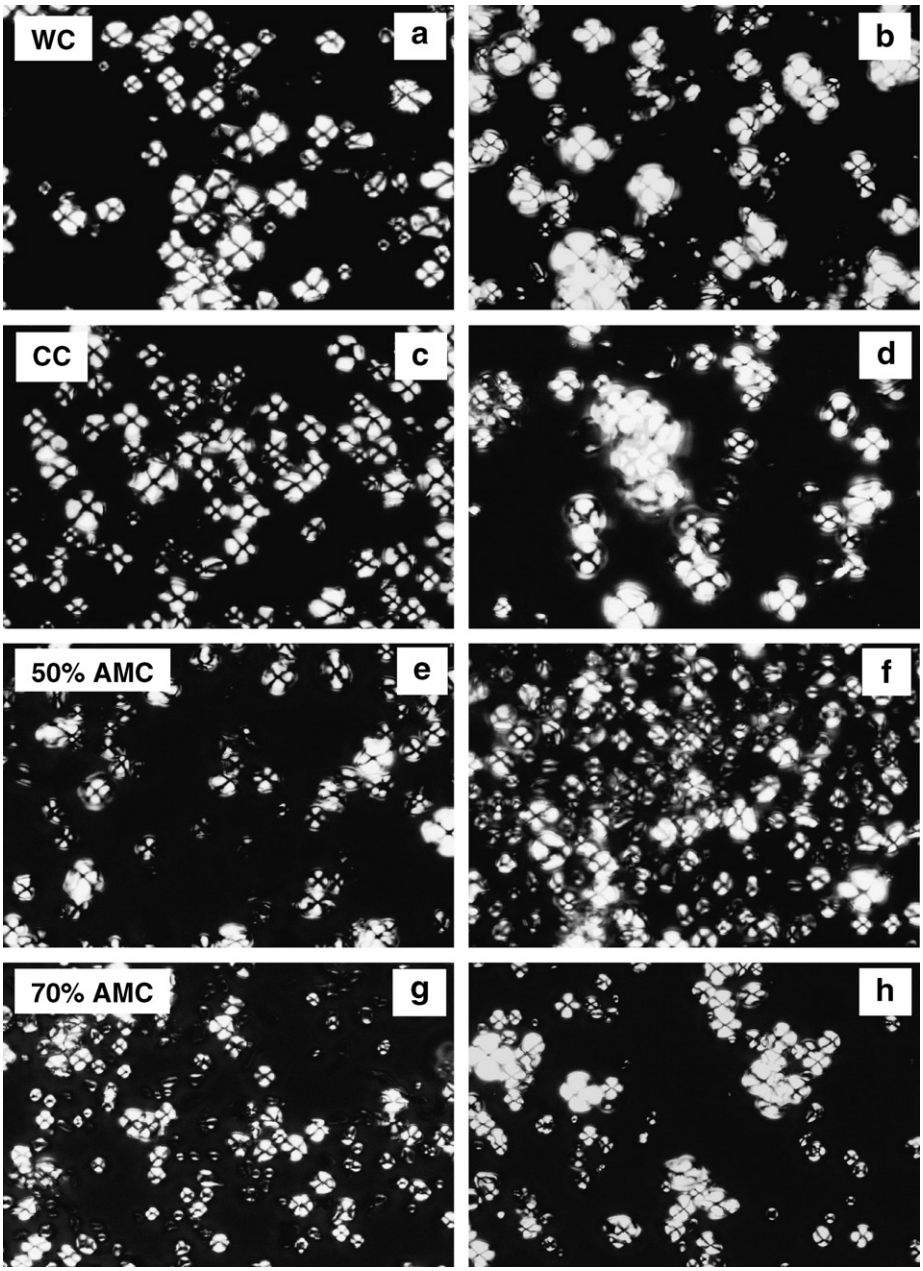

Figure 1. Polarized-light micrographs of native corn starches (a, c, e, g) and their remaining granules after ~40% surface gelatinization (b, d, f, h).

the core. The A chains were more concentrated at the periphery than at the core for all types of corn starches.

Pan and Jane[16] examined the occurrence of structure degradation from mechanical blending by comparing the molecular-weight distribution of the native starch with that of the remaining starch granule, and reported no degradation in amylopectin structure. The proportion of B3+ chains decreased for all corn starches after 10% surface removal. If the degradation did occur, the proportion of B3+ chains should continue to decrease with increasing surface removal. However, the percentage of the B3+ chains increased after 20% surface removal for waxy corn. Therefore, we believe that the decrease in B3+ chains after the 10% surface removal may not be an artifact from mechanical blending but reflected the structural difference.

There were more amylose and amylopectin long chains at the core of CC, whereas 50% and 70% AMC had more amylose and amylopectin long chains at the periphery. Smith et al.[20] proposed that granule bound starch synthase I (GBSSI) was responsible for the production of amylose and for the synthesis of amylopectin long chains. Many studies[21–23] have shown that starch mutants of different origins such as cereals and tubers with missing GBSSI showed lower amylose contents

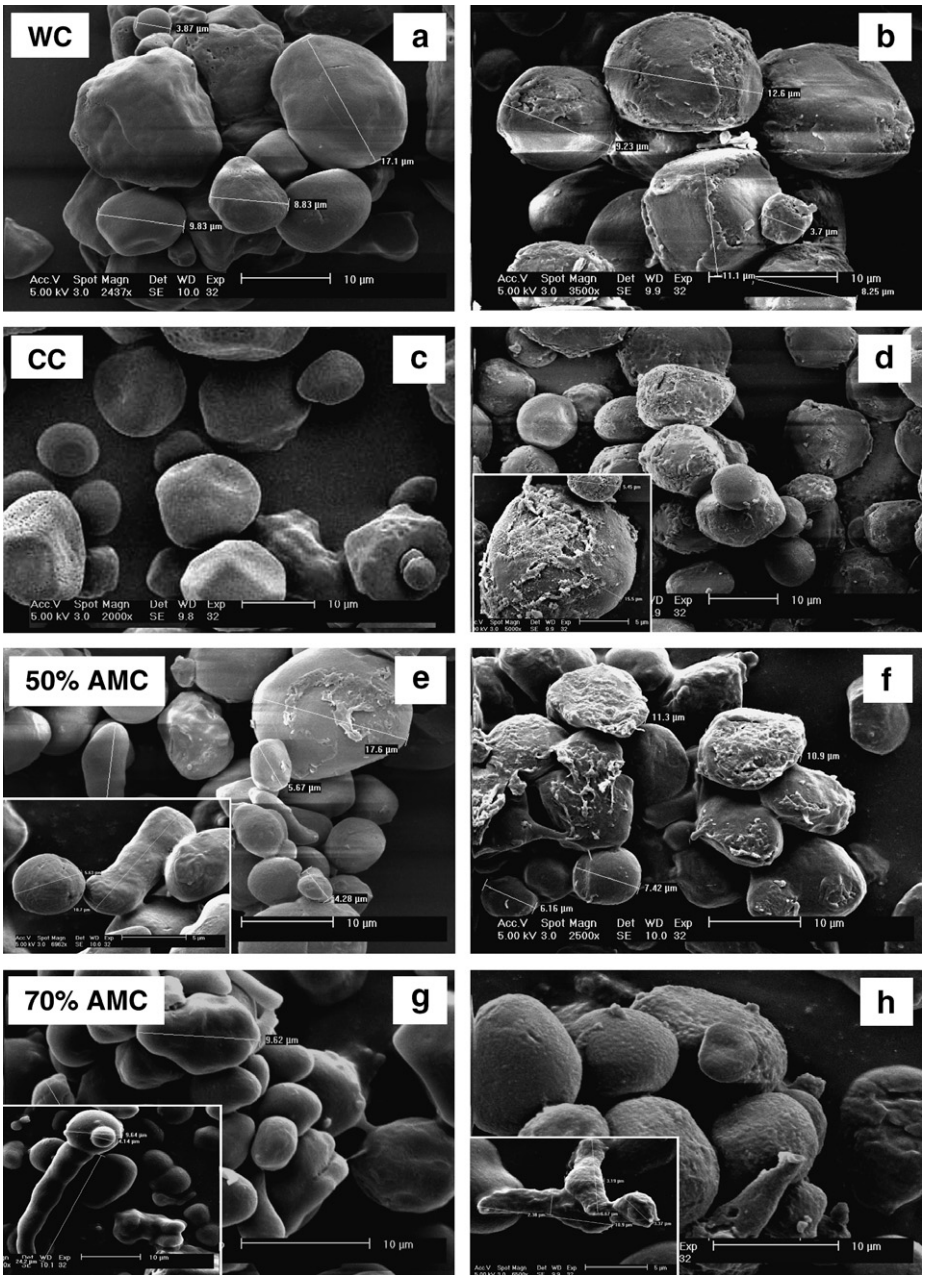

Figure 2. Scanning electron micrographs of native corn starches (a, c, e, g) and their remaining granules after ~40% surface gelatinization (b, d, f, h).

and lower proportions of amylopectin long chains compared with their wild-type counterparts.

2.4. Gelatinization properties

Gelatinization properties of native and remaining granules of corn starches as measured by differential scanning calorimetry (DSC) are summarized in Table 3, and their DSC thermograms are presented in Figure 3. The remaining granules of WC and CC exhibited lower onset gelatinization temperatures (T_0), whereas the T_0 of the 50% and 70% AMC remaining granules was similar or slightly higher when compared with their native counterparts. The gelatinization enthalpies (ΔH_g) of the remaining granules of WC and CC were lower than their native ones and decreased with increasing surface removal, while those of 70% AMC were comparable. For 50% AMC, the trend of change in ΔH_g along the radial locations was not as clear. The ΔH_g of the amylose–lipid complex in CC also decreased with increasing surface removal. It should be noted that the ΔH_g of 50% and 70% AMC included melting of the crystalline structure of amylopectin and the amylose–lipid complex. The DSC thermograms of 50% and 70% AMC (Fig. 3) showed that the endothermic transition from the amylose–lipid complex decreased with increasing surface

Table 1. Amylose content of native corn starches and their remaining granules after different degrees of surface removal[a]

Starch	% Surface removal (approx.)	Amylose content (%)
Common corn	0 (Native)	26.1 ± 0.1
	10	25.5 ± 0.1
	20	26.6 ± 0.0
	30	27.1 ± 0.0
	40	26.8 ± 0.0
50% Amylose corn	0 (Native)	56.2 ± 0.2
	10	53.7 ± 0.1
	20	53.4 ± 0.2
	30	53.5 ± 0.1
	40	50.6 ± 0.2
70% Amylose corn	0 (Native)	71.1 ± 0.0
	10	70.7 ± 0.0
	20	67.9 ± 0.0
	30	65.8 ± 0.3
	40	62.4 ± 0.1

[a] The data are averages of two measurements with standard deviation.

removal, but not the melting of amylopectin. These results support the previous findings that less amylose was present at the core in 50% and 70% AMC granules. The distribution of amylose–lipid complex along the radial location in CC seemed to be contrary to that of amylose content reported in the previous section. Because not all amylose molecules form complexes with lipid, it might be possible that more amylose–lipid complexes were present at the periphery than at the core in CC granules.

The T_0 of starch has been shown to be strongly influenced by the distribution of amylopectin chains. A higher T_0 is generally associated with a higher percentage of B1 chains (DP 13–24) and a lower percentage of A chains (DP 6–12).[4,10,24–27] Both chains are located in a single crystalline cluster,[28] which has a full length approximately equal to DP 18–21 or the length of B1 chains.[29] Therefore, when starch has a larger proportion of B1 chains, it would have a more perfect crystalline structure and consequently a higher T_0.[27] On the other hand, amylopectin long chains also play a role to indirectly affect the T_0 of the crystalline structure. Donovan[30] and Donovan and Mapes[31] suggested that the crystalline lamellae would not melt until the surrounding amorphous regions were first melted. Therefore, if the amorphous regions had a higher glass transition temperature (T_g), the starch would have a higher T_0. Slade and Levine[32] proposed that the high T_g of the amorphous regions in high amylose corn starch was due to a high proportion of amylopectin long chains located in the amorphous regions. These chains form associations among each other and decrease the mobility of the amorphous regions, causing the elevation of the T_g. Shi and Seib[25] also employed this indirect relationship between T_g and T_0 to explain the difference in T_0 among four waxy corn starches.

Besides the amylopectin long chains, amylose that is predominantly located in the amorphous lamellae could also decrease the mobility of the amorphous lamellae by forming associations with the amylopectin long chains,

Table 2. Chain-length distribution of isoamylase-debranched amylopectin from native corn starches and their remaining granules after different levels of surface removal[a]

Starch	% Surface removal (approx.)	Average chain length (DP)	% Chain-length distribution			
			A chain (DP 6–12)	B1 chain (DP 13–24)	B2 chain (DP 25–36)	B3+ chains (DP ⩾37)
Waxy corn	0	21.58 ± 0.17	18.2 ± 0.1	52.3 ± 0.2	18.8 ± 0.2	10.7 ± 0.5
	10	20.84 ± 0.03	19.1 ± 0.0	54.5 ± 0.7	17.4 ± 0.9	9.0 ± 0.0
	20	21.27 ± 0.47	18.3 ± 0.0	54.2 ± 0.6	17.9 ± 0.7	9.7 ± 0.1
	30	21.60 ± 0.04	17.6 ± 0.0	53.6 ± 0.2	18.1 ± 0.2	10.8 ± 0.0
	40	21.94 ± 0.50	16.7 ± 0.6	53.3 ± 0.1	18.8 ± 0.5	11.3 ± 0.2
Common corn	0	21.52 ± 0.04	18.0 ± 0.0	53.5 ± 0.1	17.8 ± 0.0	10.7 ± 0.0
	10	21.23 ± 0.01	18.9 ± 0.0	54.2 ± 0.3	16.6 ± 0.5	10.3 ± 0.2
	20	21.48 ± 0.07	17.7 ± 0.2	54.4 ± 0.0	17.3 ± 0.0	10.6 ± 0.1
	30	21.70 ± 0.05	17.6 ± 0.0	53.6 ± 0.1	17.6 ± 0.0	11.1 ± 0.1
	40	21.47 ± 0.16	17.4 ± 0.0	54.7 ± 0.6	17.5 ± 0.3	10.5 ± 0.5
50% Amylose corn	0	24.77 ± 0.37	16.6 ± 0.3	45.8 ± 0.0	17.9 ± 0.3	19.6 ± 0.0
	10	24.30 ± 0.13	13.1 ± 0.2	50.4 ± 0.8	19.9 ± 0.9	16.6 ± 0.0
	20	24.27 ± 0.06	12.5 ± 0.0	50.9 ± 0.3	19.9 ± 0.3	16.7 ± 0.1
	30	23.93 ± 0.05	13.6 ± 0.0	50.8 ± 0.1	19.6 ± 0.2	15.9 ± 0.2
	40	23.79 ± 0.08	13.0 ± 0.0	51.7 ± 0.2	19.9 ± 0.4	15.4 ± 0.3
70% Amylose corn	0	26.71 ± 0.67	15.3 ± 0.0	40.1 ± 0.4	20.5 ± 0.7	24.1 ± 0.1
	10	24.61 ± 0.23	12.0 ± 0.3	48.9 ± 0.9	22.0 ± 0.1	17.0 ± 0.2
	20	24.77 ± 0.18	12.4 ± 0.0	47.7 ± 0.0	22.3 ± 0.4	17.7 ± 0.5
	30	24.49 ± 0.08	12.4 ± 0.2	48.8 ± 0.2	22.1 ± 0.8	16.8 ± 0.5
	40	24.36 ± 0.04	11.7 ± 0.5	50.7 ± 0.3	21.2 ± 0.2	16.4 ± 0.1

[a] Averages of two measurements with standard deviation.

Table 3. Gelatinization properties of native corn starches and their remaining granules after different degrees of surface removal[a]

Starch	% Surface removal (approx.)	Gelatinization temperature			Gelatinization enthalpy (J/g)
		Onset (°C)	Peak (°C)	End (°C)	
Waxy corn	0 (Native)	71.4 ± 0.3	77.4 ± 0.4	85.0 ± 0.2	16.18 ± 0.46
	10	69.2 ± 0.3	75.9 ± 0.0	83.9 ± 0.0	14.30 ± 0.06
	20	69.2 ± 0.4	75.9 ± 0.0	84.1 ± 0.0	13.88 ± 0.27
	30	69.3 ± 0.2	75.8 ± 0.1	84.0 ± 0.0	13.30 ± 0.20
	40	69.5 ± 0.0	75.4 ± 0.1	83.2 ± 0.1	12.98 ± 0.02
Common corn	0 (Native)	72.2 ± 0.1	76.4 ± 0.1	82.0 ± 0.2	13.77 ± 0.07
		(90.0 ± 0.2)[b]	(102.1 ± 0.6)	(110.0 ± 0.3)	(1.35 ± 0.02)
	10	69.6 ± 0.1	74.1 ± 0.4	79.8 ± 0.4	13.28 ± 0.66
		(91.2 ± 0.5)	(101.7 ± 0.2)	(109.2 ± 0.0)	(1.28 ± 0.07)
	20	69.7 ± 0.2	73.7 ± 0.0	79.4 ± 0.2	11.58 ± 0.31
		(93.8 ± 0.6)	(101.4 ± 0.3)	(110.0 ± 0.5)	(1.31 ± 0.04)
	30	70.1 ± 0.0	74.1 ± 0.0	80.2 ± 0.1	10.69 ± 0.19
		(92.4 ± 0.3)	(101.9 ± 0.2)	(110.3 ± 0.0)	(1.14 ± 0.06)
	40	71.2 ± 0.6	75.1 ± 0.6	81.3 ± 0.6	10.15 ± 0.06
		(92.3 ± 0.1)	(101.9 ± 0.4)	(109.7 ± 0.1)	(0.98 ± 0.08)
50% Amylose corn	0 (Native)	72.4 ± 0.1	80.7 ± 0.5	u.d.[c]	10.11 ± 0.16
	10	72.9 ± 0.2	78.1 ± 0.1	92.2 ± 0.7	12.76 ± 0.06
	20	73.2 ± 0.5	80.0 ± 0.7	92.9 ± 0.5	11.71 ± 0.70
	30	73.5 ± 0.0	80.4 ± 0.1	92.1 ± 0.0	11.33 ± 0.05
	40	73.5 ± 0.3	80.3 ± 0.1	91.0 ± 0.0	9.59 ± 0.64
70% Amylose corn	0 (Native)	71.4 ± 0.2	u.d.	u.d.	10.47 ± 0.10
	10	73.2 ± 0.1	80.5 ± 0.9	u.d.	10.31 ± 0.37
	20	72.7 ± 0.0	84.5 ± 0.2	u.d.	9.90 ± 0.10
	30	72.7 ± 0.2	83.9 ± 0.5	u.d.	10.27 ± 0.02
	40	72.2 ± 0.3	83.5 ± 0.8	u.d.	10.33 ± 0.12

[a] The data are averages of two measurements with standard deviation.
[b] Amylose–lipid complex peak.
[c] Undetectable data by software.

thus resulting in a higher T_g in the amorphous lamellae and consequently a higher T_0 in the crystalline lamellae. The present results suggest the importance of the amorphous regions on T_0 and the important contribution of amylose to the T_g of the amorphous regions. The higher T_0 in native WC and CC than those of their remaining granules could be due to the larger total amount of B2 and B3+ chains in the outermost 10% layer. Furthermore, the higher T_0 of CC could be due to its slightly larger amount of amylose in the outermost 10% layer, which might increase T_g through interacting with amylopectin long chains. The slightly larger amount of amylose–lipid complex in the outermost 10% layer in CC might also elevate the T_g in the amorphous regions.

2.5. Retrogradation properties

Retrogradation properties of native and remaining granules of corn starches measured by differential scanning calorimetry (DSC) are summarized in Table 4, and their DSC thermograms are presented in Figure 4. The retrogradation enthalpies (ΔH_r) of WC and 50% and 70% AMC generally increased with increasing surface removal with the exception of native WC. The ΔH_r of native CC and its remaining granules were similar. These results confirm the conclusion of Shi and Seib[24,25] that the A and B1 chains predominantly controlled AP retrogradation. The greater the proportion of B1 chains and the smaller the proportion of the A chains, the higher the degree of amylopectin retrogradation. Because the full length of a single cluster is equal to the length of the B1 chains,[27] the B1 chains were able to retrograde to the most perfect crystalline structure among other amylopectin chains. On the other hand, the short length of the A chains interfered with the retrogradation of the B1 chains and caused some defects in the crystallites.[32,33] Although the proportion of B1 chains in WC decreased with increasing surface removal, the proportion of A chains decreased to a greater extent. Therefore, retrogradation occurred at a faster rate when the surface of WC was removed to a greater extent. The proportions of A and B1 chains in native and remaining granules of CC were comparable; hence they were similar in ΔH_r.

The much lower ΔH_r in 50% and 70% AMCs cannot be solely explained by their low amylopectin contents. It is suspected that their greater proportions of amylopectin B3+ chains might interfere with the retrogradation of B1 chains.

The ΔH_r of amylose–lipid complex in CC and 70% AMC were higher in their native ones relative to their

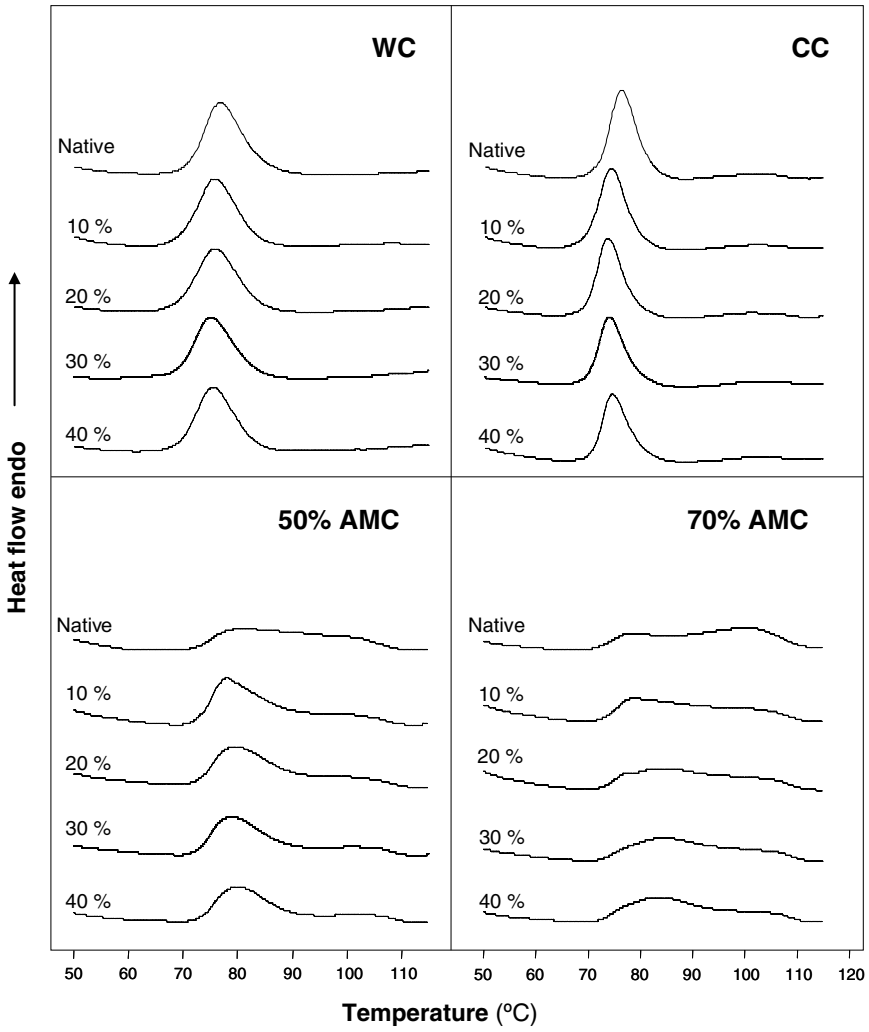

Figure 3. DSC thermograms of native corn starches and their remaining granules after different degrees of surface removal. The number next to each thermogram represents the approximate percentage of surface removal.

remaining granules after 10% surface removal, supporting the previous speculation that amylose was present more in the form of amylose–lipid complex at the periphery. Nevertheless, the ΔH_r of native 50% AMC was not higher than those of its remaining granules, and the reason is not clear. There was little difference in the ΔH_r of the amylose–lipid complex among the remaining granules of CC, 50%, and 70% AMC regardless of the level of surface removal.

2.6. Swelling power and water solubility

Most corn starches exhibited higher SP and WSI with increasing surface removal, and WC showed the most increase after the removal of the outermost 10% surface (Table 5). The increase in SP and WSI suggests that the starch structure at the core of the granule could absorb more water and expand to a greater extent, thus enhancing starch leaching. Tester and Morrison[34] proposed that amylopectin contributes to swelling of starch granules, whereas amylose and lipid restrict the swelling. It is speculated that the higher SP in the remaining granules was a result of their larger proportions of B chains, particularly B1 chains, lower amylose contents, lower amounts of amylose–lipid complex, or combinations thereof. The larger proportion of B chains might contribute to stronger granule integrity during heating by providing stronger intermolecular interaction. This stronger interaction allowed a greater extent of swelling and consequently more water uptake. When amylose was present, the extent of increase in SP reduced, supporting the role of amylose in restricting starch swelling. The lower amounts of amylose and/or amylose–lipid complex toward the core might contribute to the slightly higher SP for all the remaining granules relative to their native ones.

The results of this study indicated that there were similarities and differences in the distribution of amylose

Table 4. Retrogradation properties of native corn starches and their remaining granules after different degrees of surface removal[a]

Starch	% Surface removal (approx.)	Retrogradation temperature			Retrogradation enthalpy (J/g)
		Onset (°C)	Peak (°C)	End (°C)	
Waxy corn	0 (Native)	45.4 ± 0.6	57.0 ± 0.5	70.0 ± 0.0	8.16 ± 0.13
	10	46.4 ± 0.5	57.7 ± 0.3	69.3 ± 0.3	6.90 ± 0.48
	20	45.9 ± 0.2	56.7 ± 0.0	69.7 ± 0.2	7.64 ± 0.17
	30	45.2 ± 0.5	56.9 ± 0.2	69.7 ± 0.0	8.01 ± 0.38
	40	44.8 ± 0.1	56.7 ± 0.3	69.4 ± 0.2	8.39 ± 0.23
Common corn	0 (Native)	46.0 ± 0.4	57.5 ± 0.7	69.7 ± 0.3	6.84 ± 0.08
		(90.8 ± 0.5)[b]	(101.4 ± 0.6)	(109.8 ± 0.4)	(1.44 ± 0.05)
	10	45.9 ± 0.2	56.8 ± 0.1	69.3 ± 0.5	6.30 ± 0.35
		(91.0 ± 0.2)	(101.4 ± 0.1)	(109.2 ± 0.6)	(1.19 ± 0.04)
	20	45.6 ± 0.2	56.6 ± 0.1	68.9 ± 0.3	6.42 ± 0.04
		(93.4 ± 0.1)	(101.8 ± 0.2)	(109.8 ± 0.6)	(1.10 ± 0.03)
	30	45.8 ± 0.3	56.8 ± 0.3	69.0 ± 0.2	6.42 ± 0.39
		(94.6 ± 0.9)	(101.9 ± 0.7)	(108.1 ± 0.7)	(1.05 ± 0.16)
	40	47.4 ± 0.2	57.7 ± 0.2	70.2 ± 0.2	6.47 ± 0.05
		(93.2 ± 0.6)	(101.3 ± 0.1)	(109.6 ± 0.0)	(0.96 ± 0.07)
50% Amylose corn	0 (Native)	50.2 ± 0.7	59.9 ± 0.9	69.8 ± 0.4	0.71 ± 0.04
		(89.9 ± 1.1)	(101.2 ± 0.3)	(109.8 ± 0.2)	(1.81 ± 0.09)
	10	49.3 ± 0.5	58.2 ± 0.2	69.5 ± 0.3	1.39 ± 0.08
		(93.2 ± 0.9)	(101.9 ± 0.1)	(110.0 ± 0.1)	(1.75 ± 0.05)
	20	50.5 ± 0.0	58.9 ± 0.0	70.0 ± 0.2	1.47 ± 0.04
		(95.0 ± 0.4)	(102.3 ± 0.4)	(110.0 ± 0.2)	(1.77 ± 0.06)
	30	49.1 ± 0.7	58.7 ± 0.5	69.7 ± 0.8	1.67 ± 0.08
		(93.4 ± 0.8)	(103.4 ± 0.9)	(110.3 ± 0.1)	(1.89 ± 0.09)
	40	49.7 ± 0.3	59.0 ± 0.3	69.9 ± 0.2	1.61 ± 0.02
		(92.9 ± 0.2)	(102.0 ± 0.5)	(110.2 ± 0.3)	(1.99 ± 0.05)
70% Amylose corn	0 (Native)	47.7 ± 0.2	59.2 ± 0.5	62.8 ± 0.4	0.23 ± 0.04
		(87.6 ± 0.1)	(99.3 ± 0.0)	(109.2 ± 0.6)	(2.41 ± 0.06)
	10	52.6 ± 0.6	59.2 ± 0.0	69.7 ± 0.0	0.49 ± 0.07
		(91.7 ± 0.0)	(101.8 ± 0.1)	(110.5 ± 0.1)	(1.63 ± 0.03)
	20	51.6 ± 0.0	58.3 ± 0.3	69.4 ± 0.4	0.74 ± 0.07
		(92.9 ± 0.3)	(103.9 ± 0.7)	(110.7 ± 0.2)	(1.49 ± 0.17)
	30	49.4 ± 1.0	58.9 ± 0.1	69.7 ± 0.5	0.87 ± 0.16
		(92.4 ± 0.3)	(101.9 ± 0.5)	(111.4 ± 0.2)	(1.66 ± 0.09)
	40	50.9 ± 0.6	59.0 ± 0.4	69.9 ± 0.6	0.96 ± 0.07
		(95.43 ± 0.83)	(101.43 ± 0.59)	(111.06 ± 0.03)	(1.44 ± 0.02)

[a] Averages of two measurements with standard deviation.
[b] Amylose–lipid complex peak.

and amylopectin chains along the radial location of corn starch granules with varying amylose contents. The differences in the thermal properties, swelling power, and water solubility index of the native and remaining granules at different levels of surface removal could be explained based on their difference in amylose content and amylopectin chain-length distribution. It is speculated that more amylose was present in the form of amylose–lipid complex in the outermost 10% layer of starch granules than in the core. The B3+ chains of amylopectin also seemed to be more concentrated in the outermost 10% peripheral region for amylose-containing corn starches. The slightly higher concentration of amylose–lipid complex and amylopectin long chains in the outermost 10% layer could restrict starch swelling and leaching. Once this layer was removed, the remaining granules could swell to a greater degree. For waxy corn starch, it is possible that the extent of swelling was also strongly influenced by amylopectin molecular weight, which will be investigated in the future.

3. Experimental

3.1. Materials

Four commercial corn starches were used in this study. Waxy corn (Cargill Gel 04230), common corn (Cargill Gel 03420), 50% high amylose corn (AmyloGel 03001), and 70% high amylose corn (AmyloGel 03003) starches were provided by Cargill Food and Pharma Specialties North America (Cedar Rapids, IA, USA). Isoamylase (EC3.2.1.68) was purchased from Hayashibara Biochemical Laboratories, Inc. (Kayama, Japan). All chemicals were ACS grade and used without further treatment. Moisture content of starches was determined according to AACC Approved Method 44-15A.[35]

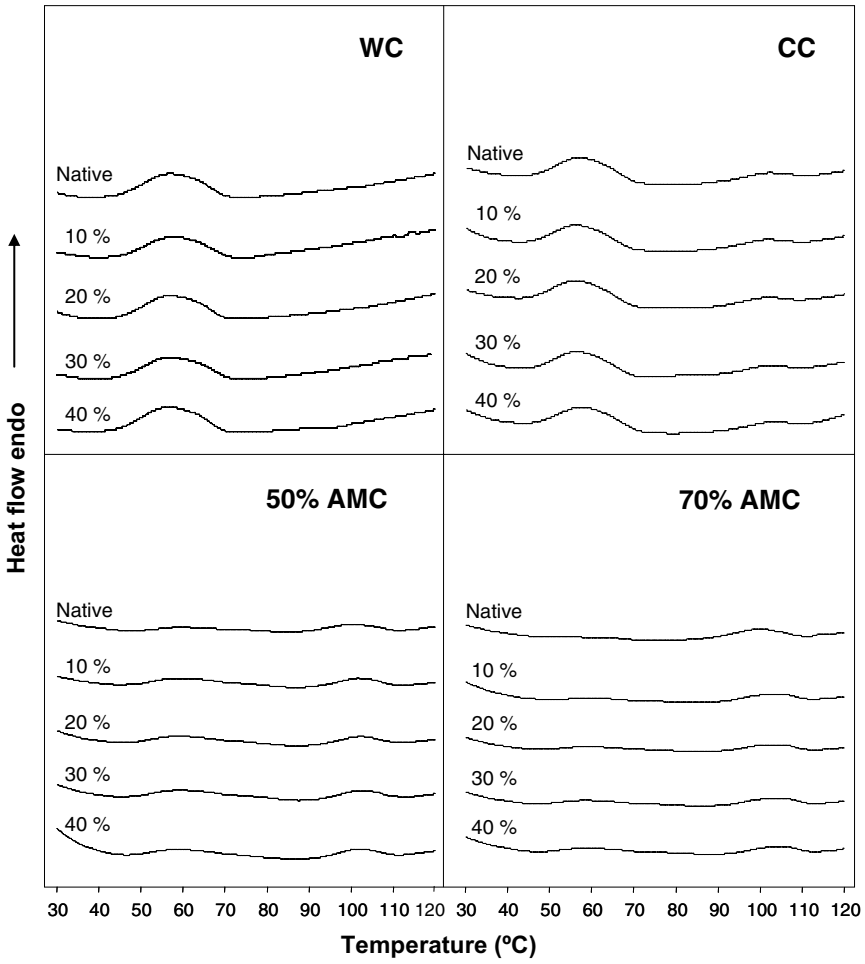

Figure 4. DSC thermograms of starch retrogradation of native corn starches and their remaining granules after different degrees of surface removal. The number next to each thermogram represents the approximate percentage of surface removal.

3.2. Chemical surface gelatinization

The procedure of chemical surface gelatinization was modified after the method of Pan and Jane.[16] Native corn starch (20 g) was suspended in 150 mL of 13 M LiCl with vigorous stirring at room temperature for different periods of time to achieve different levels of surface gelatinization. The gelatinization was stopped by adding 1200 mL of chilled (4 °C) deionized water to the starch suspension with quick mixing. The mixture was then centrifuged at 3840*g* for 15 min, and the supernatant containing LiCl solution was discarded. The surface-gelatinized starch was washed twice with 1500 mL of chilled deionized water.

It should be noted that different starch types required different reaction times to achieve a similar degree of surface gelatinization. Common corn required the longest reaction time, whereas waxy corn required the shortest. For example, the reaction time to achieve approximately 40% surface removal for waxy corn, common corn, 50% high amylose corn, and 70% high amylose corn starches were 30, 90, 45, and 60 min, respectively.

3.3. Separation of gelatinized starch from remaining granules

The surface-gelatinized starch was transferred into a 250-mL Waring stainless steel mini-blender and 120 mL of chilled deionized water was added. The mixture was blended at 22,000 rpm (Waring, 51 BL31, Torrington, CT, USA) for 4 min, and the mixture was centrifuged at 3840*g* for 20 min. The supernatant containing the gelatinized peripheral starch was separated from the remaining granules by decanting, and the remaining granules were re-suspended in 120 mL of chilled deionized water and blended at 22,000 rpm for 4 min. This separation process was repeated three to eight times depending on the extent of gelatinization and the starch type until the supernatant was clear. The supernatant was pooled together and dried in a forced-air oven at 40 °C for 48 h. The purified remaining

Table 5. Swelling power and water solubility index of native corn starches and their remaining granules after different degrees of surface removal at 85 °C[a]

Starch	% Surface removal (approx.)	Swelling power (g/g)	Solubility (%)
Waxy corn	0 (Native)	27.6 ± 0.0	5.4 ± 0.1
	10	34.5 ± 0.4	6.4 ± 0.1
	20	34.0 ± 0.2	8.2 ± 0.0
	30	35.3 ± 0.1	8.4 ± 0.0
	40	35.7 ± 0.0	8.5 ± 0.0
Common corn	0 (Native)	9.0 ± 0.5	6.3 ± 0.0
	10	9.1 ± 0.3	6.6 ± 0.0
	20	9.6 ± 0.2	7.2 ± 0.1
	30	10.3 ± 0.2	8.3 ± 0.2
	40	11.6 ± 0.5	8.8 ± 0.2
50% Amylose corn	0 (Native)	4.4 ± 0.1	3.4 ± 0.4
	10	4.5 ± 0.2	4.2 ± 0.2
	20	5.0 ± 0.3	5.1 ± 0.1
	30	5.1 ± 0.4	5.5 ± 0.1
	40	5.4 ± 0.3	6.4 ± 0.2
70% Amylose corn	0 (Native)	3.7 ± 0.0	2.1 ± 0.1
	10	4.1 ± 0.3	2.8 ± 0.3
	20	4.0 ± 0.1	3.5 ± 0.1
	30	4.0 ± 0.1	3.8 ± 0.1
	40	4.2 ± 0.3	4.2 ± 0.2

[a] Averages of two measurements with standard deviation.

granules were washed twice with 200 mL of abs EtOH and dried at 40 °C for 8 h. The degree of surface gelatinization was calculated as the percentage of surface removal according to the following equation:

$$\begin{aligned}&\text{Percentage of surface removal}\\&= [(\text{Initial starch weight (d.b.)}\\&\quad - \text{Remaining granules weight (d.b.)})\\&\quad \times 100]/\text{Initial starch weight (d.b.)}\end{aligned}$$

3.4. Starch birefringence and morphology

The presence of birefringence in native and surface-gelatinized remaining starch granules was examined with polarized light microscopy. The specimen was prepared from a mixture of 2 mg of starch and 1 mL of 50% glycerol solution and observed by a light microscope (Nikon, Eclipse E400, Tokyo, Japan) using a polarized light filter and equipped with a digital camera (Nikon, Digital Sight DS-L1, Tokyo, Japan) at a magnification of 600×.

The size and surface structure of native and surface-gelatinized remaining starch granules were studied using an ESEM (environmental scanning electron microscope, XL30, FEI Corporation, Eindhoven, Netherlands) at an accelerating voltage of 5 kV. Starch granules were sprinkled onto double-backed cellophane tape attached to a stub before coating with gold–palladium.

3.5. Determination of amylose content

The amylose content of starch samples, including native starch granules and remaining starch granules, was determined by potentiometric titration following the procedure of Schoch.[36] Starch samples were defatted by refluxing with 85% (v/v) MeOH for 24 h. The amylose content was calculated by dividing the iodine affinity of starch samples by 19%, which is the typical iodine affinity value of purified corn amylose.

3.6. Purification of amylopectin from starch samples

Fractionation of amylopectin from all starch samples was done by following the procedure of Takeda et al.[37] with modifications. Approximately 200 mg of defatted starch was heated in 12 mL of 0.2 M NaOH with stirring in a 25-mL capped test tube at 65 °C for 18 h to achieve complete dissolution. The starch solution was then neutralized with 1 M HCl, added with 2.4 mL of BuOH, and stirred at 100 °C for 3 h under nitrogen. The heated dispersion was slowly cooled down to room temperature over 24 h by immersing the sample test tube in a sealed 2-L Dewar flask (Thermo-flask, Lab-line Instruments Inc., Melrose park, IL, USA) filled with hot water to allow the slow formation of the amylose–butanol complex. The cooled dispersion was then placed at 4 °C for 48 h. The amylose–butanol complex was separated by centrifugation at 12,100*g* and 4 °C for 45 min. The supernatant containing mostly amylopectin was further purified for another recrystallization cycle with the addition of 1 mL of BuOH. At the end of the second recrystallization, the purified amylopectin was precipitated with 100 mL of MeOH and kept at room temperature for 24 h. The precipitated amylopectin was collected by centrifugation at 1520*g* for 15 min, washed with 30 mL of MeOH, and dried at 40 °C for 24 h.

3.7. Chain-length distribution of amylopectin

The chain-length distribution of purified amylopectin was characterized by high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) according to the method of Kasemsuwan et al.[38] with modification. The HPAEC-PAD system (DX500, Dionex Co., Sunnyvale, CA, USA) consisted of the following components: GP50 gradient pump, LC20-1 chromatography organizer, ED40 electrochemical detector, 4 × 50-mm CarboPac PA1 guard column, 4 × 250-mm CarboPac PA1 analytical column, and AS40 automated sampler. Purified amylopectin (9 mg) was dissolved in 3.2 mL of deionized water by heating in a boiling water bath with stirring for 1 h. After cooling to room temperature, 30 μL of isoamylase (1770 U) and 0.4 mL of 0.1 M acetate buffer (pH 3.5)

were added to the amylopectin solution, and the mixture was incubated at 40 °C for 48 h. The enzyme was inactivated in a boiling water bath for 20 min, and the mixture was centrifuged at 4500*g* for 5 min. The supernatant was placed into sample vials for injection.

3.8. Thermal properties

Gelatinization and retrogradation characteristics of native and remaining starch granules were assessed by a Pyris-1 differential scanning calorimeter (DSC, Perkin–Elmer, Norwalk, CT, USA). Approximately 12 mg of starch sample was weighed into a stainless steel DSC pan, and then moistened with 24 μL of deionized water using a microsyringe. The pan was sealed, stored at room temperature for 24 h, and scanned from 25 to 140 °C at a rate of 10 °C/min. After scanning, the gelatinized sample was stored at 4 °C for 14 days and rescanned from 5 to 135 °C at a rate of 10 °C/min. Onset, peak, and end temperatures and enthalpy of gelatinization or retrogradation transitions were calculated (Pyris Software for Windows version 3.81, Perkin–Elmer). The instrument was calibrated with indium, and an empty pan was used as the reference.

3.9. Swelling power and water solubility index

Swelling power (SP) and water solubility index (WSI) of native and surface-gelatinized remaining starch granules were determined by heating a 1.7% starch suspension (0.5 g starch in 30 mL water) in a centrifuge tube in a 85 °C water bath for 30 min. Then the suspension was rapidly cooled to room temperature in an ice-water bath for 20 min and centrifuged at 12,100*g* for 20 min. SP was calculated by dividing the sedimented paste weight by the starch dry weight, and WSI by dividing the solid content in the supernatant by the starch dry weight.[39]

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References

1. Boyer, C. D.; Shannon, J. C.; Garwood, D. L.; Creech, R. G. *Cereal Chem.* **1976**, *53*, 327–337.
2. Lineback, D. R. *Bakers Dig.* **1984**, *58*, 16–21.
3. Golachowski, A. *Starch/Stärke* **1985**, *37*, 263–266.
4. Asaoka, M.; Okuno, K.; Fuwa, H. *Agric. Biol. Chem.* **1985**, *49*, 373–379.
5. Morison, W. R.; Gadan, H. J. *Cereal Sci.* **1987**, *5*, 263–275.
6. Kolodziej, Z. *Pol. J. Food Nutr. Sci.* **1993**, *2*, 43–49.
7. Montgomery, E. M.; Sexson, K. R.; Senti, F. R. *J. Polym. Sci.* **1958**, *28*, 1–9.
8. Nikuni, Z. *Sci. Cookery* **1969**, *2*, 6 (in Japanese).
9. Kasemsuwan, T. In Location of amylose in normal corn starch revealed by phosphodiester cross-linking and phosphorus-31 NMR; Master Thesis; Iowa State Univ. Ames: Iowa, 1991.
10. Jane, J.-L.; Xu, A.; Radosavljevic, M.; Seib, P. A. *Cereal Chem.* **1992**, *69*, 405–409.
11. Kasemsuwan, T.; Jane, J.-L. *Cereal Chem.* **1994**, *71*, 282–287.
12. Ball, S. G.; van de Wal, M. H. B. J.; Visser, R. G. F. *Trends Plant Sci.* **1998**, *3*, 462–467.
13. Zeeman, S. C.; Smith, S. M.; Smith, A. M. *Plant Physiol.* **2002**, *128*, 1069–1076.
14. Jenkins, P. J.; Donald, A. M. *Int. J. Biol. Macromol.* **1995**, *17*, 315–321.
15. Jane, J.-L.; Shen, J. *J. Carbohydr. Res.* **1993**, *247*, 279–290.
16. Pan, D. D.; Jane, J.-L. *Biomacromolecules* **2000**, *1*, 126–132.
17. Kuakpetoon, D.; Wang, Y.-J. *Carbohydr. Res.*, in press.
18. Vasanthan, T.; Hoover, R. *Food Chem.* **1992**, *45*, 337–347.
19. Hanashiro, I.; Abe, J.-I.; Hizukuri, S. *Carbohydr. Res.* **1996**, *283*, 151–159.
20. Smith, A. M.; Denyer, K.; Martin, C. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 67–87.
21. Delrue, B.; Fontaine, T.; Routier, F.; Decq, A.; Weiruszeski, J.-M. *J. Bacteriol.* **1992**, *174*, 3612–3620.
22. Flipse, E.; Keetels, C. J. A. M.; Jacobsen, E.; Visser, R. G. F. *Theor. Appl. Genet.* **1996**, *92*, 121–127.
23. Maddelein, M.-L.; Libessart, N.; Bellanger, F.; Delrue, B.; D'Hulst, C. *J. Biol. Chem.* **1994**, *269*, 25150–25157.
24. Shi, Y. C.; Seib, P. A. *Carbohydr. Res.* **1992**, *227*, 131–145.
25. Shi, Y. C.; Seib, P. A. *Carbohydr. Polym.* **1995**, *26*, 141–147.
26. Yuan, R. C.; Thompson, D. B.; Boyer, C. D. *Cereal Chem.* **1993**, *70*, 81–89.
27. Jane, J.; Chen, Y. Y.; Lee, L. F.; McPherson, A. E.; Wong, K. S.; Radosavljevic, M.; Kasemsuwan, T. *Cereal Chem.* **1999**, *76*, 629–637.
28. Hizukuri, S. *Carbohydr. Res.* **1986**, *147*, 342–347.
29. Cameron, R. E.; Donald, A. M. *Polymer* **1992**, *33*, 2628–2635.
30. Donovan, J. W. *Biopolymers* **1979**, *18*, 263–275.
31. Donovan, J. W.; Mapes, C. J. *Starch/Starke* **1980**, *32*, 190–193.
32. Slade, L.; Levine, H. *Carbohydr. Polym.* **1988**, *8*, 183–208.
33. Krusi, H.; Neukom, H. *Starch/Stärke* **1984**, *36*, 300–305.
34. Tester, R. F.; Morrison, W. R. *Cereal Chem.* **1990**, *67*, 551–557.
35. American Association of Cereal Chemists. Method 44-15A, 46-13, 61-02; In *Approved Methods of AACC*, 9th ed, 1995.
36. Schoch, T. J. *Methods Carbohydr. Chem.* **1964**, *4*, 157–160.
37. Takeda, Y.; Hizukuri, S.; Juliano, B. O. *Carbohydr. Res.* **1986**, *148*, 299–308.
38. Kasemsuwan, T.; Jane, J.-L.; Schnable, P.; Stinard, P.; Robertson, D. *Cereal Chem.* **1995**, *72*, 457–464.
39. Holm, J.; Björck, L.; Asp, N. G.; Sjöberg, L. B.; Lundquist, I. J. *Cereal Sci.* **1985**, *3*, 193–206.