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Locations of hypochlorite oxidation in corn starches varying in amylose content

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Abstract—The general oxidation mechanism by hypochlorite on starch has been well studied, but the information on the distribution of the oxidation sites within starch granules is limited. This study investigated the locations where the oxidation occurred within corn starch granules varying in amylose content, including waxy corn starch (WC), common corn starch (CC), and 50% and 70% high-amylose corn starch (AMC). Oxidized corn starches were surface gelatinized by 13 M LiCl at room temperature to different extents (approximately 10%, 20%, 30%, and 40%). The surface-gelatinized remaining granules were separated and studied for structural characteristics including carboxyl content, amylose content, amylopectin chain-length distribution, thermal properties, and swelling properties. Oxidation occurred mostly at the amorphous lamellae. More carboxyl groups were found at the periphery than at the core of starch granules, which was more pronounced in oxidized 70% AMC. More amylose depolymerization from oxidation occurred at the periphery of CC. For WC and CC, amylopectin long chains (>DP 36) were more prone to depolymerization by oxidation. The gelatinization properties as measured by differential scanning calorimetry also supported the changes in amylopectin fine structure from oxidation. Oxidized starches swelled to a greater extent than their unmodified counterparts at all levels of surface removal. This study demonstrates that the locations of oxidation and physicochemical properties of oxidized starches are affected by the molecular arrangement within starch granules.

Keywords: Oxidized starch; Hypochlorite; Carboxyl; Carbonyl; Amylopectin chain-length distribution; Amylose content; Starch structure; High-amylose corn starch; Surface gelatinization

1. Introduction

Oxidized starch is commonly produced by reacting starch with a specified amount of NaOCl under controlled temperature and pH.[1] There are two main reactions involved in oxidation. Firstly, hydroxyl groups in starch molecules are oxidized to carbonyl groups and then to carboxyl groups, which primarily takes place at C-2, C-3, and C-6.[1] Secondly, oxidation also causes depolymerization of starch molecules by cleaving α-(1→4)-glucosidic linkages.[1] Therefore, carboxyl and carbonyl content and/or extent of depolymerization in oxidized starches could indicate oxidation efficiency.

Oxidation efficiency can be affected by many factors, including pH, temperature, hypochlorite concentration, starch molecular structure, and starch organization.[1–8] Our previous study[8] showed that potato starch was much more prone to oxidation than were corn and rice starches under the same oxidation conditions. The loose arrangement of B-type crystalline structure[9,10] in potato starch may provide more accessible sites for oxidation.

Hypochlorite could be consumed during starch oxidation by three possible mechanisms: namely, lipid oxidation, depolymerization of amylose and amylopectin, and formation of carboxyl and carbonyl groups.[11] Significant differences in oxidation efficiency were observed among corn starches with varying amylose content when they were oxidized under the same conditions.[11] Hypochlorite was suggested to first react with lipid,

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presumably from an amylose–lipid complex, and then either to depolymerize the starch molecule or form carboxyl and carbonyl groups. More hypochlorite was consumed for depolymerization of starches containing more amylose and amylopectin long chains such as in high-amylose corn starch. In contrast, more carboxyl formation was observed for starches low in amylose as in waxy corn starch. Amylopectin was more prone to carboxyl formation than was amylose.

Previous work[12,13] has shown non-uniformly distribution of amylose and amylopectin along the radial location within starch granules. Amylose was found to be more concentrated at the periphery, and more amylopectin long-B chains were at the core in common corn starch.[12] This radial dependence of molecular distribution was also recently confirmed and suggested to be influenced by starch composition.[14] The objectives of this study were to investigate the distribution of oxidation sites along the radial locations within corn starch granules varying in amylose content by using chemical surface gelatinization, and to characterize the structure and physicochemical properties of the remaining oxidized corn starches after different degrees of surface removal.

2. Results and discussion

2.1. Birefringence and morphology of starch samples

The polarized light micrographs of hypochlorite-oxidized waxy corn starch (WC), common corn starch (CC), 50% amylose corn starch (50% AMC), 70% amylose starch corn (70% AMC), and their remaining

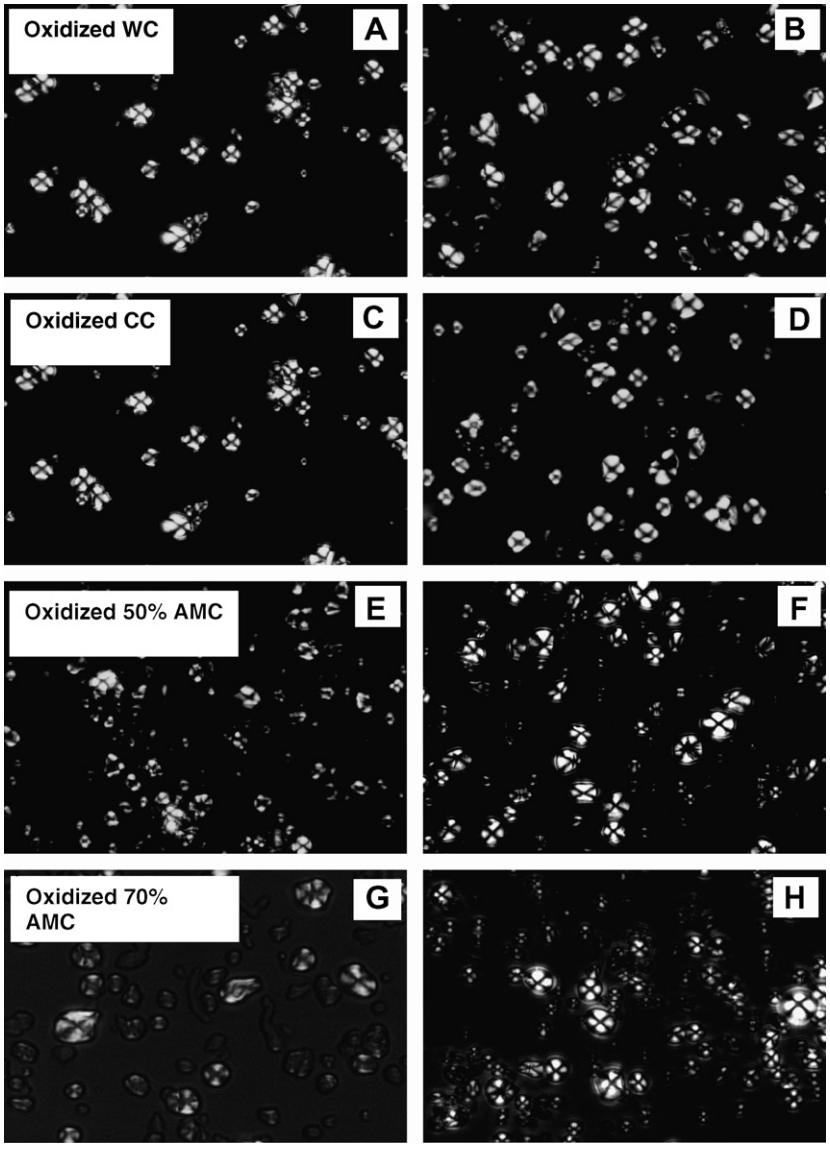

Figure 1. Polarized light micrographs of oxidized starches (A, C, E, G) and their remaining granules after ~40% surface gelatinization (B, D, F, H).

granules after approximately 40% chemical surface gelatinization are shown in Figure 1. For all oxidized corn starches, their remaining granules still displayed the Maltease cross-pattern. This suggests that the chemical surface gelatinization did not significantly alter the crystalline structure within starch granules, even at the highest percentage (~40%) of surface gelatinization. In this study, corn starches were not separated into small and large granules as in other previous work[12,13] because this study intended to obtain representative results of oxidation from all sizes of granules present in the same starch type.

The scanning electron micrographs of the remaining granules of oxidized starches showed a rougher surface than their native counterparts (Fig. 2). Surface removal was observed on both large and small granules, confirming the uniformity of chemical gelatinization. Oxidation seemed to produce holes in some WC and CC granules, presumably a result of extensive oxidation.

2.2. Carboxyl content along the radial location

The carboxyl content at different radial locations of oxidized corn starch granules are presented in Table 1. The carboxyl content decreased with increasing amylose content for the four corn starches. For the same starch type, the carboxyl content was higher at the periphery than at the core. The significantly lower carboxyl content in 50% and 70% AMCs suggests the negative impact of high-amylose content. It is proposed that the lipid present in the amylose–lipid complex may preferentially react with NaOCl, and thus less NaOCl became available to produce carboxyl groups. Lipids, particularly esters of fatty acids, are readily oxidized under

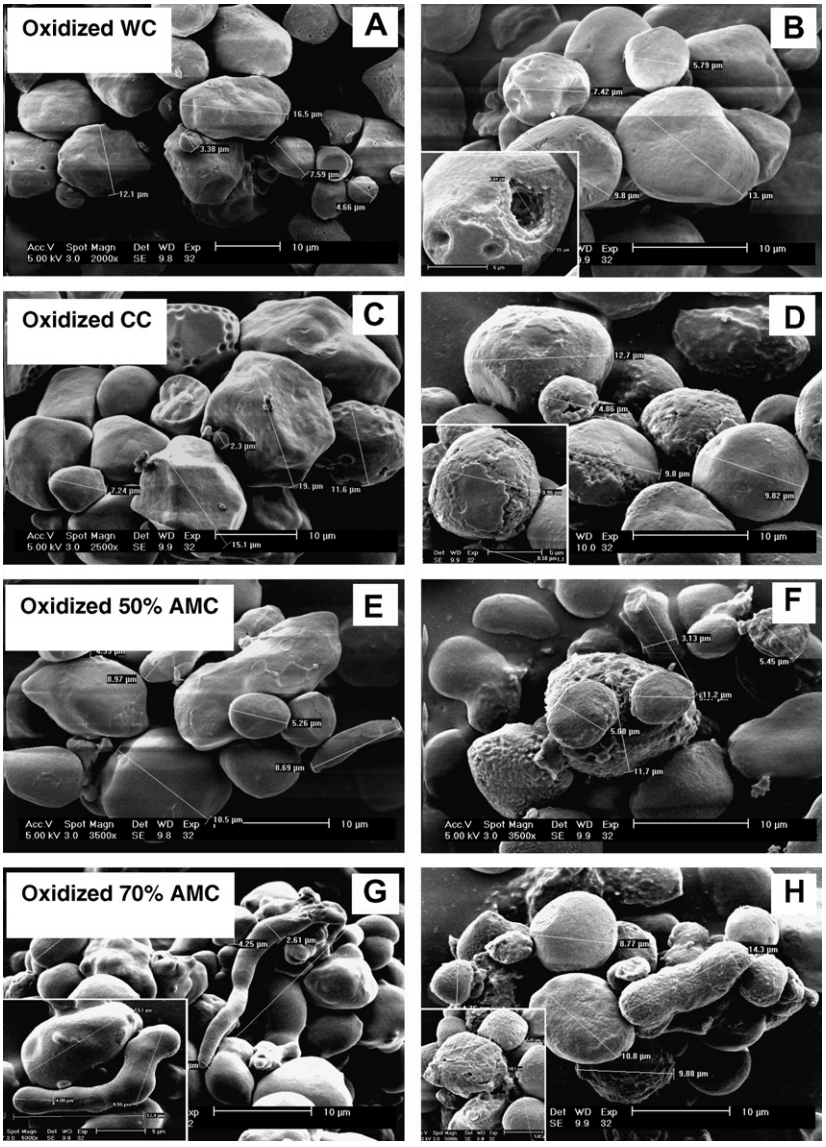

Figure 2. Scanning electron micrographs of oxidized starches (A, C, E, G) and their remaining granules after ~40% surface gelatinization (B, D, F, H).

alkaline conditions (P. Tomasik, personal communication). Additionally, our previous work[11] demonstrated that amylose and long-chain amylopectin were more prone to depolymerization than carboxyl formation during oxidation. Therefore, the extent of oxidation could be affected by starch composition and structure.

The presence of carboxyl groups in different layers of starches demonstrates that oxidation occurred throughout starch granules. The decrease in the number of carboxyl groups from the surface to the center for all types of granules could be due to their difference in amylose and amylopectin content along the radial location within granules as shown by our previous study.[14] According to the oxidation of amylopectin,[11] oxidative depolymerization preferentially took place on the B2 and B3+ chains, while carboxyl formation was likely to occur near the branching point of the A and B1 chains. Because amylopectin consists of more A chains, the number of branching points in amylopectin is also high. Consequently, there will be more available sites for carboxyl formation. This rationale seems to be in accord with the radial distribution of carboxyl groups in this study. For all four types of corn starches, more A chains were present at the periphery than at the core;[14] therefore, there should be more branching points at the periphery in amylopectin. Consequently, this A-chain distribution pattern supported the decrease in carboxyl content from the surface to the center of the granule for all corn starches.

Table 1. Carboxyl content of hypochlorite-oxidized corn starches and remaining granules after different degrees of surface removal[a]

Oxidized starch	% Surface removal (approx.)	Carboxyl content (%)
Waxy corn	0	0.162
	10	0.160
	20	0.159
	30	0.156
	40	0.152
Common corn	0	0.149
	10	0.151
	20	0.147
	30	0.141
	40	0.136
50% Amylose corn	0	0.096
	10	0.088
	20	0.086
	30	0.086
	40	0.086
70% Amylose corn	0	0.076
	10	0.075
	20	0.080
	30	0.060
	40	0.046

[a] Averages of duplicate measurements with all standard deviations 0.000.

2.3. Amylose content along the radial location

The amylose content of unmodified and oxidized corn starches at different radial locations are summarized in Table 2. The amylose content as measured by potentiometric titration slightly decreased for oxidized CC, but significantly increased for oxidized 50% and 70% AMCs when compared with their unmodified counterparts. The increase in amylose content for both AMCs is proposed to originate from degraded amylose molecules from oxidation. Oxidation would cleave starch molecules in addition to producing carboxyl groups. The cleavage of high-molecular-weight amylose molecules would yield some amylose that still could be detected in the

Table 2. Amylose content of unmodified and hypochlorite-oxidized starch and their remaining granules after different degrees of surface removal[a]

Starch	% Surface removal (approx.)	Amylose content (%)		
		Unmodified[b]	Oxidized	Difference
Common corn	0	26.1 ± 0.1	24.5 ± 0.0	−1.6
	10	25.5 ± 0.1	24.6 ± 0.3	−0.9
	20	26.6 ± 0.0	26.0 ± 0.1	−0.6
	30	27.1 ± 0.0	25.7 ± 0.1	−1.4
	40	26.8 ± 0.0	25.5 ± 0.2	−1.3
50% Amylose corn	0	56.2 ± 0.2	64.0 ± 0.1	+7.9
	10	53.7 ± 0.1	61.9 ± 0.1	+8.7
	20	53.4 ± 0.2	59.6 ± 0.1	+6.2
	30	53.5 ± 0.1	59.4 ± 0.3	+5.9
	40	50.6 ± 0.2	56.3 ± 0.3	+5.7
70% Amylose corn	0	71.1 ± 0.0	76.0 ± 0.1	+4.9
	10	70.7 ± 0.0	75.1 ± 0.0	+4.4
	20	68.0 ± 0.0	72.4 ± 0.2	+4.5
	30	65.8 ± 0.3	70.6 ± 0.0	+4.8
	40	62.4 ± 0.1	68.8 ± 1.0	+6.4

[a] Averages of two measurements with standard deviations.
[b] Results from Kuakpetoon and Wang.[14]

measurement. Nevertheless, some degraded amylose in CC was probably too small to complex with iodine.

The amylose at the surface of oxidized CC and 70% AMC degraded slightly, and the extent of amylose depolymerization generally increased through the core of the granule after the outermost 10% layer. The increased degradation of amylose at the core might be due to the fact that more amylose was possibly present in the free form.[14] Nevertheless, this trend was not observed in 50% AMC, possibly due to the slightly greater amount of amylose–lipid complex at the core of the granule.[14]

2.4. Amylopectin chain-length distribution at different radial locations

The chain-length distributions of amylopectin of oxidized corn starches and their remaining granules after different degrees of surface removal are summarized in Table 3. AP chains were grouped into four types, namely, A, B1, B2, and B3+ chains corresponding to their chain length in degree of polymerization (DP) according to Hanashiro et al.[15] When compared with their unmodified counterparts,[14] the proportions of B2 and B3+ chains in oxidized WC decreased, indicating their degradation into short chains. Furthermore, the reduction in proportions of B2 and B3+ chains increased with increasing surface removal. More NaOCl was probably consumed for the depolymerization of amylopectin chains at the core of oxidized WC, which explains the lower carboxyl content at its core. Because A and B1 chains are located within a single cluster, and B2 and B3+ chains are long enough to span two or more clusters,[16] the degradation of each chain type from oxidation could indicate the oxidation site. The greater depolymerization of B2 and B3+ chains in oxidized WC and its remaining granules supports our previous results[11] that oxidation mainly took place in the amorphous lamellae throughout the whole granule.

The degradation of amylopectin chains in CC seemed to follow the same trend as in WC in that most oxidation took place in the amorphous lamellae, and the degradation of the B2 and B3+ chains occurred more at the core than at the periphery. Slightly more degradation of amylopectin B2 and B3+ chains was found at the 10–20% surface layer, which might be due to the slightly lower amylose content in this layer. Consequently, more NaOCl could react with amylopectin long chains via oxidative degradation.

When compared with their unmodified counterparts,[14] the degradation of amylopectin chains in 50% AMC was not evident. It was likely that B2 and B3+ chains were also degraded as in WC and CC. However, because of the occurrence of amylose degradation, their proportions remained relatively unchanged. The degradation of B2 and B3+ chains seemed to be random along the radial location. The decrease in the proportion of A chains suggests that the A chains were also degraded, particularly at the core of the granule, possibly due to the lower amylose content at the core of 50% AMC. The full length of a crystallite is approximately equal to DP 18–21 or the length of B1 chain.[17]

Table 3. Chain-length distribution of amylopectins of isoamylase-debranched hypochlorite-oxidized corn starch and remaining granules after different degrees of surface removal[a]

Oxidized 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	21.2 ± 0.3	19.3 ± 0.2	53.6 ± 0.2	17.2 ± 0.4	9.9 ± 0.1
	10	20.9 ± 0.2	20.0 ± 0.3	53.3 ± 0.1	17.2 ± 0.2	9.6 ± 0.3
	20	21.0 ± 0.4	18.7 ± 0.1	54.6 ± 0.3	17.1 ± 0.1	9.7 ± 0.4
	30	21.1 ± 0.1	18.9 ± 0.1	54.2 ± 0.4	17.4 ± 0.1	9.6 ± 0.2
	40	20.8 ± 0.2	19.5 ± 0.2	54.2 ± 0.3	17.2 ± 0.2	9.1 ± 0.3
Common corn	0	21.1 ± 0.3	19.0 ± 0.3	54.2 ± 0.2	17.0 ± 0.3	9.8 ± 0.2
	10	19.7 ± 0.4	24.2 ± 0.4	53.2 ± 0.3	14.7 ± 0.5	8.0 ± 0.4
	20	20.9 ± 0.1	18.5 ± 0.2	55.5 ± 0.4	16.4 ± 0.2	9.6 ± 0.2
	30	20.8 ± 0.1	18.7 ± 0.6	55.6 ± 0.1	16.6 ± 0.3	9.2 ± 0.3
	40	20.6 ± 0.2	18.7 ± 0.3	56.5 ± 0.1	16.3 ± 0.1	8.5 ± 0.5
50% Amylose corn	0	24.7 ± 0.3	16.5 ± 0.4	45.8 ± 0.3	18.7 ± 0.2	19.0 ± 0.2
	10	23.9 ± 0.2	13.5 ± 0.1	49.8 ± 0.4	20.4 ± 0.3	16.2 ± 0.1
	20	24.1 ± 0.4	12.6 ± 0.3	51.4 ± 0.2	19.2 ± 0.5	16.8 ± 0.3
	30	23.9 ± 0.2	12.6 ± 0.1	51.2 ± 0.3	20.5 ± 0.3	15.8 ± 0.1
	40	24.0 ± 0.2	12.6 ± 0.2	51.8 ± 0.2	19.1 ± 0.1	16.5 ± 0.1
70% Amylose corn	0	25.2 ± 0.1	16.7 ± 0.3	41.2 ± 0.1	21.0 ± 0.3	21.1 ± 0.1
	10	25.5 ± 0.1	14.2 ± 0.1	43.5 ± 0.3	21.3 ± 0.2	20.9 ± 0.1
	20	25.3 ± 0.2	14.1 ± 0.2	44.4 ± 0.3	21.9 ± 0.2	19.7 ± 0.2
	30	24.4 ± 0.2	12.6 ± 0.1	48.7 ± 0.4	21.6 ± 0.4	17.1 ± 0.1
	40	23.9 ± 0.3	13.5 ± 0.4	50.6 ± 0.4	19.4 ± 0.3	16.6 ± 0.3

[a] Averages of two measurements with standard deviations.

Therefore, B1 chains would form a more perfect crystalline structure with stronger double helical association than A chains. When amylopectin was the predominant constituent as in WC and CC, the less-ordered structure formed by A chains might be protected from oxidation by other well organized chains. However, when amylopectin became the minor constituent as in 50% and 70% AMC, this protection might diminish.

For 70% AMC, amylopectin B3+ chains were significantly degraded at the outermost 10% layer, whereas the proportions of other chains slightly increased compared to their unmodified counterparts.[14] This could be attributed to the larger proportion of B3+ chains at the periphery relative to other internal layers. The decrease in proportions of B1 and B2 chains in all remaining granules suggests that oxidative degradation also occurred to the crystalline lamellae, which was not as obvious in other oxidized starches. This phenomenon could be possibly ascribed to the interrupted crystalline structure due to a greater percentage of amylose in high AMC as proposed by Jenkins and Donald.[18] They investigated the influence of amylose on corn starch granule structure using small-angle X-ray scattering (SAXS). They reported 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%), but the size of the amorphous lamellae decreased with increasing amylose content. They suggested that amylose might disrupt the packing of amylopectin double helices by co-crystallizing with amylopectin chains and pulling some amylopectin chains from two adjacent crystalline lamellae closer to each other. Therefore, some B1 chains might not be closely aligned with each other and become exposed, thus resulting in increased susceptibility to oxidation. A chains were also possibly degraded, and the increase in the proportion of A chains was assumed to arise from degradation of B chains. Nevertheless, the degradation of B1 chains was less pronounced at the core, probably due to its slightly lower amylose content (Table 2). The interruption to the crystalline structure from amylose was more pronounced in 70% AMC than in 50% AMC. Additionally, the crystallites formed by amylopectin chains might be individually located and not associated with each other in 70% AMC because amylose is the predominant component, which creates an open area around the crystallites and allows for more degradation to B1 chains by NaOCl.

2.5. Gelatinization properties

Gelatinization properties as measured by differential scanning calorimetry (DSC) of oxidized corn starches at different degree of surface removal are summarized

Table 4. Gelatinization properties of hypochlorite-oxidized corn starches and remaining granules after different degrees of surface removal[a]

Oxidized starch	% Surface removal (approx.)	Gelatinization temperature			Gelatinization enthalpy (J/g)
		Onset (°C)	Peak (°C)	End (°C)	
Waxy corn	0	69.8 ± 0.5	76.0 ± 0.3	84.0 ± 0.7	15.93 ± 0.36
	10	69.4 ± 0.5	75.9 ± 0.0	84.3 ± 0.2	15.61 ± 0.18
	20	69.5 ± 0.5	75.8 ± 0.6	84.4 ± 0.9	15.14 ± 0.11
	30	68.7 ± 0.4	73.4 ± 0.2	84.2 ± 0.4	15.00 ± 0.25
	40	69.4 ± 0.1	75.8 ± 0.1	84.7 ± 0.6	14.39 ± 0.11
Common corn	0	70.5 ± 0.1	75.7 ± 0.3	82.0 ± 0.6	14.40 ± 0.22
		(94.2 ± 0.5)[b]	(105.5 ± 0.4)	(110.5 ± 0.4)	(1.03 ± 0.01)
	10	70.2 ± 0.2	75.5 ± 0.4	82.2 ± 0.6	12.56 ± 0.35
		(96.0 ± 0.2)	(105.9 ± 0.5)	(110.2 ± 0.4)	(0.60 ± 0.10)
	20	70.9 ± 0.4	75.6 ± 0.6	82.2 ± 0.9	12.98 ± 0.29
		(93.6 ± 0.2)	(104.4 ± 0.5)	(110.5 ± 0.4)	(1.04 ± 0.09)
	30	70.7 ± 0.4	75.1 ± 0.4	81.4 ± 0.4	12.14 ± 0.13
		(93.0 ± 0.1)	(105.3 ± 0.1)	(110.2 ± 0.1)	(0.95 ± 0.00)
	40	71.1 ± 0.4	75.6 ± 0.4	81.9 ± 0.5	12.10 ± 0.25
		(93.8 ± 0.6)	(103.1 ± 0.3)	(110.6 ± 0.6)	(0.87 ± 0.05)
50% Amylose corn	0	70.9 ± 0.3	76.9 ± 0.5	100.0 ± 0.2	7.97 ± 0.46
	10	70.9 ± 0.4	77.4 ± 0.1	94.5 ± 0.4	8.09 ± 0.34
	20	70.5 ± 0.2	77.9 ± 0.6	92.4 ± 0.6	8.59 ± 0.26
	30	71.6 ± 0.7	79.3 ± 0.5	94.6 ± 0.4	8.71 ± 0.24
	40	70.7 ± 0.1	78.0 ± 0.1	91.3± 0.3	9.38 ± 0.14
70% Amylose corn	0	71.8 ± 0.0	101.6 ± 0.1	110.3 ± 0.0	7.08 ± 0.16
	10	71.1 ± 0.6	76.6 ± 0.3	96.7 ± 0.6	7.09 ± 0.17
	20	71.4 ± 0.7	76.7 ± 0.4	110.3 ± 0.8	7.39 ± 0.39
	30	71.0 ± 0.1	76.6 ± 0.3	110.0 ± 0.0	7.31 ± 0.16
	40	71.4 ± 0.3	76.9 ± 0.2	109.9 ± 0.4	7.21 ± 0.04

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

in Table 4, and their DSC thermograms are presented in Figure 3. The onset temperature (T_o) of oxidized WC was significantly lower than that of the unmodified counterpart (71.4 °C), which was reported in our previous study.[14] However, the T_o of the oxidized remaining granules was comparable to its unmodified one at different levels of surface removal. The gelatinization enthalpy (ΔH_g) of oxidized WC was similar to that of unmodified WC at the surface (16.18 J/g), but the ΔH_g of the oxidized remaining granules for all levels of surface removal was significantly higher. The differences between the gelatinization properties of oxidized and unmodified WC granules along the radial locations seemed to relate to the degradation pattern of amylopectin chains previously discussed. At the surface of oxidized WC granules, the degradation took place mostly on the B2 and B3+ chains in the amorphous lamellae (Table 3), which could increase mobility and decrease the glass transition of amorphous lamellae. Consequently, the crystalline lamellae would start to melt at a lower T_o. The higher ΔH_g in oxidized WC remaining granules relative to their unmodified counterparts could possibly result from the hemiacetal or hemiketal pseudo-cross-linking formation among carboxyl groups on amylopectin chains, particularly on the A and B1 chains.[19,20] Therefore, more energy is required to break down these extra intermolecular bonds.

A similar trend was observed in CC as in WC. Oxidized CC generally displayed a lower T_o and a higher ΔH_g compared with the unmodified counterpart for different degrees of surface removal. The melting temperature of the amylose–lipid complex in the remaining granules was significantly higher, but ΔH_g was significantly smaller for all levels of surface removal. It is speculated that the remaining amylose–lipid complex might be arranged in a more ordered form than those preferentially attacked, or it may be located in the crystalline lamellae that protected them from oxidation.

In contrast to WC and CC, the T_o and ΔH_g of the remaining granule in oxidized 50% and 70% AMC at all levels of surface removal were lower than their unmodified counterparts (Table 4). The pronounced decrease in T_o and ΔH_g could be attributed to two structural changes from oxidation. Firstly, the degradation of amylose into smaller DPs might lower the glass transition temperature of the amorphous lamellae, and subsequently lower T_o. Secondly, the degradation of the crystalline lamellae as previously discussed could lower the ΔH_g. The crystalline lamellae were more easily degraded in high AMC, probably a result of the interruption from amylose molecules and less association with each other due to a smaller amount of amylopectin. The pseudo-cross-linking effect on ΔH_g was not observed in oxidized high AMC, presumably because

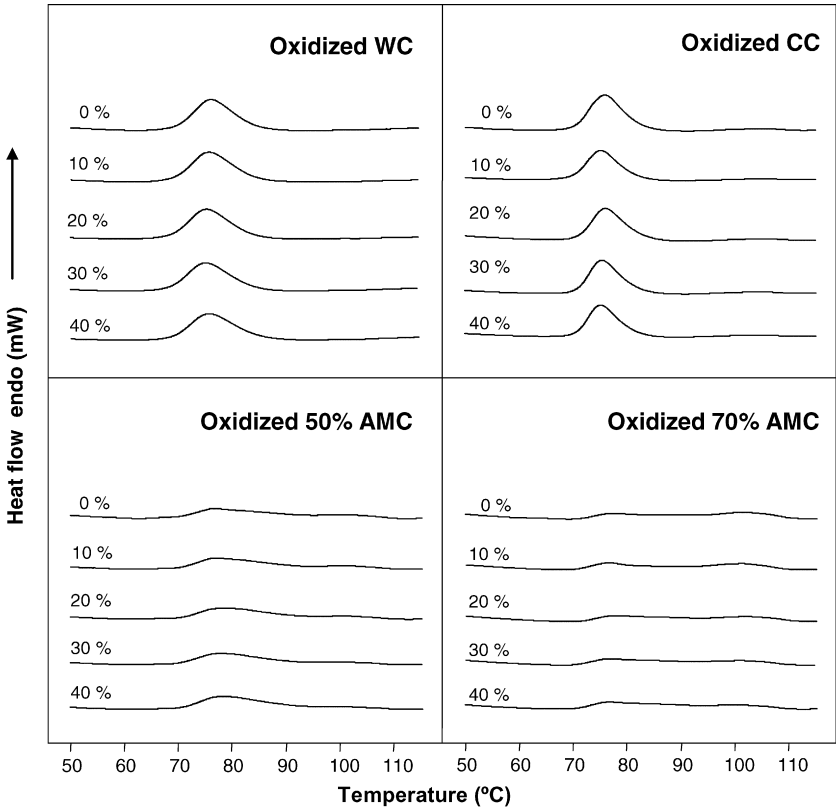

Figure 3. DSC thermograms of oxidized corn starches at different degrees of surface removal. The number next to each thermogram represents the approximate percentage of surface removal.

amylopectin molecules were not located close to each other to form the pseudo-cross-links.

2.6. Swelling power and water solubility index

All oxidized corn starches exhibited a higher swelling power (SP) and water solubility index (WSI) than their unmodified counterparts at all levels of surface removal (Table 5), which was attributed to the hemiacetal or hemiketal cross-linking among amylopectin molecules and between amylose and amylopectin.[19,20] At a low level of oxidation such as that from 2% NaOCl, these cross-links could stabilize the swelling of granules and overcome minor depolymerization.[8,20,21] However, this cross-linking effect was less pronounced in high AMC, probably because of the insufficient amylopectin to form the cross-link network. In general, the remaining granules showed higher SP and WSI for all oxidized starches, which was similar to the results of unmodified ones.[14] The lower SP and WSI in native starches were ascribed to a large amount of amylose–lipid complex at the periphery of the granules.[14] When the periphery was removed, the SP and WSI increased with the increasing degree of surface removal. This trend was not as pronounced in the oxidized corn starches, probably due to the extensive degradation of amylopectin from oxidation.

The differences in starch composition, structure, and organization along the radial location clearly influenced the efficiency of hypochlorite oxidation. The number of carboxyl groups was positively correlated with the number of A chains, which were more concentrated at the periphery than at the core for all four types of corn starch granules. Therefore, there were more carboxyl groups at the periphery than at the core of oxidized corn starches. Depolymerization took place mainly in the amorphous lamellae and more at the core than at the periphery. The crystalline lamellae were more loosely packed in high-amylose corn starches, possibly because of the disruption from a greater amount of amylose and insufficient amylopectin. Consequently, depolymerization also took place in the crystalline lamellae of AMCs. Depolymerization of starch molecules by oxidation seemed to preferentially occur in amylose, followed by amylopectin long chains, amylopectin short chains in the amorphous lamellae, and lastly the short chains in the crystalline lamellae.

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

Oxidized starch	% Surface removal (approx.)	Swelling power (g/g)	Water solubility index (%)
Waxy corn	0	48.8 ± 0.1	33.1 ± 0.1
	10	55.2 ± 0.2	32.2 ± 0.4
	20	52.6 ± 0.1	47.0 ± 0.1
	30	54.4 ± 0.0	40.4 ± 0.1
	40	52.7 ± 0.0	48.9 ± 0.1
Common corn	0	30.4 ± 1.5	23.6 ± 0.4
	10	25.9 ± 0.1	21.2 ± 0.1
	20	28.5 ± 0.1	24.6 ± 0.5
	30	22.1 ± 0.2	18.7 ± 0.2
	40	29.2 ± 0.7	23.0 ± 0.2
50% Amylose corn	0	4.6 ± 0.2	9.1 ± 0.1
	10	5.5 ± 0.6	9.8 ± 0.1
	20	5.4 ± 0.4	10.1 ± 0.3
	30	6.2 ± 0.6	11.5 ± 0.1
	40	6.5 ± 0.4	11.2 ± 0.3
70% Amylose corn	0	4.1 ± 0.2	4.5 ± 0.3
	10	4.1 ± 0.2	4.8 ± 0.1
	20	4.0 ± 0.6	5.4 ± 0.1
	30	4.1 ± 0.2	6.2 ± 0.0
	40	4.3 ± 0.1	7.0 ± 0.3

[a] Averages of two measurements with standard deviations.

3. Experimental

3.1. Materials

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 gifts from Cargill Food & Pharma Specialties North-America (Cedar Rapids, IA, USA). *Pseudomonas* isoamylase (EC 3.2.1.68) was purchased from Hayashibara Biochemical Laboratories, Inc. (Kayama, Japan). NaOCl containing 6% active chlorine was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). All other chemicals were ACS grade. The moisture content of the starches was determined according to AACC Method 44-15A.[22]

3.2. Starch oxidation

The oxidation procedure followed the method of Autio et al.[23] with modifications. A 40% (w/w) starch slurry was prepared by adding deionized water to starch (450 g, dry basis) to a final weight of 1125 g in a 2-L reaction vessel equipped with a heating mantle. The starch slurry was maintained at 35 °C by occasionally turning off the mantle heating power, and the pH was adjusted to 9.5 with 2 M NaOH. NaOCl (150 g) (2 g Cl/100 g starch, 2% w/w) was slowly added into the starch slurry within 30 min while maintaining the pH at 9.5 with 1 M H_2SO_4. After the addition of NaOCl, the pH of the slurry was maintained at 9.5 with 2 M NaOH for an additional 50 min. The slurry was then neutralized to pH 7.0 with 1 M H_2SO_4, filtered through suction (Whatman filter #4), washed with deionized water, and dried in an oven (40 °C, 48 h).

3.3. Chemical surface gelatinization

The procedure of chemical surface gelatinization followed the method of Pan and Jane[12] with modifications. Oxidized corn starch (20 g) was quickly added into 13 M LiCl (150 mL) with vigorous mixing by a magnetic stirring bar at room temperature, and the extent of surface gelatinization depended on the length of mixing. The chemical gelatinization was terminated by the addition of chilled (4 °C) deionized water (1200 mL) to the starch mixture. The cooled starch slurry was then centrifuged (3840*g*, 15 min), and the supernatant containing LiCl solution was decanted and discarded. The residue was washed two more times each with 1500 mL of chilled deionized water and then centrifuged (3840*g*, 15 min).

It was observed that both starch type and oxidation affected the reaction time in conducting chemical surface gelatinization. Oxidized starches required a shorter reaction time than did native starches to achieve the same degree of surface gelatinization. Native waxy, common, 50% amylose, and 70% amylose corn starches required 10, 20, 15, and 5 min, respectively, to achieve about 10% surface gelatinization; their oxidized counterparts required 7.5, 16, 5, and 1.5 min, respectively.

3.4. Separation of the gelatinized starch from the remaining granules

The surface-gelatinized starch was blended (22,000 rpm, 4 min) with chilled deionized water (120 mL) in a 250-mL Waring stainless-steel mini-blender (Waring, 51 BL31, Torrington, CT, USA). The mixture was then centrifuged (3840*g*, 20 min). The supernatant containing the gelatinized peripheral starch was separated from the remaining granules by decanting, and the remaining granules were again re-blended (22,000 rpm, 4 min) with chilled deionized water (120 mL). This separation process was repeated three to eight times depending on the degree of gelatinization and the starch type until the supernatant was clear. The supernatants from each blending cycle were pooled into a plastic tray (10 cm × 10 cm) and dried in a forced-air oven (40 °C, 48 h). The separated remaining granules were washed twice with abs EtOH (200 mL) and dried (40 °C, 8 h). The degree of surface gelatinization was calculated as the percentage of surface removal as follows:

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

3.5. Carboxyl group content

The carboxyl group content of oxidized starches and oxidized remaining starch granules was determined according to the modified procedure of Chattopadhyay et al.[24] Approximately 2 g of starch was mixed with 0.1 M HCl (25 mL), and the slurry was stirred occasionally for 30 min with a magnetic stirrer. The slurry was then vacuum filtered through a 150-mL medium-porosity fritted glass funnel and washed with deionized water (400 mL). The starch cake was then carefully transferred to a 500-mL beaker, and the volume was adjusted to approximately 300 mL with deionized water. The starch slurry was heated in a boiling water bath with continuous stirring for 15 min to achieve complete gelatinization. The hot starch dispersion was then adjusted to approximately 450 mL with boiling deionized water and immediately titrated to pH 8.3 with standardized 0.01 M NaOH. The amount of 0.01 M NaOH used in mL was recorded. Unmodified starches were used as the blanks for oxidized starches, and the native remaining starch granules were used as the blanks for their oxidized remaining starch granules at the same degree of surface removal. Instead of 0.1 M HCl, 2 g of unmodified starch was stirred with 25 mL of deionized water.

Carboxyl group content was calculated as follows:

$$\begin{aligned}&\text{Milliequivalents of acidity/100 g starch}\\&= [(\text{Sample-Blank) mL} \times \text{Molarity of NaOH} \times 100]\\&\quad /\text{Sample weight (dry basis) in g}\\&\text{Percentage of carboxyl content}\\&= [\text{milliequivalents of acidity/100 g starch}] \times 0.045\end{aligned}$$

3.6. Polarized light microscopy

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

3.7. Scanning electron microscopy (SEM)

The morphology of starch granules was examined with 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, and excess starch granules on the tape were removed by compressed air. The starch samples were

coated with gold–palladium in a vacuum evaporator for 2 min.

3.8. Determination of amylose content

The amylose content of starch samples was determined by potentiometric titration following the procedure of Schoch.[25] Starch samples were defatted by refluxing with 85% (v/v) MeOH for 24 h. The amylose content data were calculated by dividing the iodine affinity of starch samples by 19%, which is the theoretical iodine affinity value of purified amylose from corn starch.

3.9. Purification of amylopectin from starch samples

Amylopectin of all starch samples was purified according to the method of Takeda et al.[26] with modifications. Defatted starch (200 mg) was dissolved in 0.2 M NaOH (12 mL) by heating (65 °C, 18 h) with stirring in a 25-mL capped test tube. The starch solution was then neutralized with 1 M HCl, added with 1-butanol (2.4 mL), flushed with nitrogen, capped, and heated (100 °C, 3 h) in an oil bath with stirring. The heated dispersion was gradually cooled to room temperature over 24 h by immersing the 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 dispersion was then stored at 4 °C for 48 h. The amylose–butanol complex was removed by centrifugation (12,100*g*, 45 min, 4 °C). The supernatant containing mostly amylopectin was purified for another recrystallization cycle with the addition of 1-butanol (1 mL). At the end of the second recrystallization cycle, the purified amylopectin in the supernatant was added with MeOH (100 mL), and the resulting precipitate was formed over 24 h at room temperature. The precipitated amylopectin was then recovered by centrifugation (1520*g*, 15 min), washed with MeOH (30 mL), and dried (40 °C, 24 h).

3.10. 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.[27] with modification. The HPAEC-PAD (DX500, Dionex Co., Sunnyvale, CA, USA) system 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 deionized water (3.2 mL) by heating in a boiling water bath with stirring (1 h). After cooling to room temperature, *Pseudomonas* isoamylase (1770 U) and acetate buffer (0.4 mL, 0.1 M, pH 3.5) were added to the amylopectin solution, and the mixture was incubated in a shaker water bath (40 °C, 48 h) to allow for the enzymatic debranching. The enzyme was inactivated by heating in a boiling water bath (20 min), and the mixture was centrifuged (4500*g*, 5 min). The supernatant was placed into sample vials for analysis.

3.11. Thermal properties

The gelatinization transition of starch samples was investigated using a Pyris-1 differential scanning calorimeter (DSC) (Perkin–Elmer, Norwalk, CT, USA). The starch sample (12 mg) was weighed into a stainless-steel DSC pan, and deionized water (24 μL) was added to obtain a starch-to-water ratio of approximately 1:2. The pan was hermetically sealed, equilibrated at room temperature for 24 h, and scanned from 25 to 140 °C at a rate of 10 °C/min.

3.12. Swelling power and water solubility index

Swelling power (SP) and water solubility index (WSI) were determined by following the method of Holm et al.[28] Starch (0.5 g) was suspended in deionized water (30 mL) in a pre-weighed centrifuge tube, and the tube was heated at 85 °C for 30 min. Thereafter, the suspension was cooled rapidly by immersing in an ice-water bath (20 min) and centrifuged (12,100*g*, 20 min). The supernatant containing the leached starch was carefully poured into a 100-mL pre-weighed beaker, and the beaker was dried (105 °C, 24 h). Then the beaker was cooled to room temperature and weighed. The centrifuge tube containing the starch paste was also weighed. The SP and WSI were calculated as follows:

$$\mathrm{SP} = \text{Starch gel weight}/(\text{Initial starch weight (d.b.)} - \text{Dried supernatant weight})$$

$$\mathrm{WSI} = \text{Dried supernatant weight} \times 100/\text{Initial starch weight (d.b.)}$$

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