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Physicochemical properties of common and waxy corn starches oxidized by different levels of sodium hypochlorite

Ya-Jane Wang*, Linfeng Wang

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

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Abstract

Structure and physicochemical properties of oxidized common and waxy corn starches by different levels of sodium hypochlorite (0.25–3.0% active chlorine based on dry starch weight) were studied. The oxidized common corn starch generally had a higher carboxyl content but a similar carbonyl content compared with the oxidized waxy corn starch. The introduction of carboxyl and carbonyl groups promoted starch hydration and swelling. The gelatinization temperature of the oxidized starches increased but the enthalpy remained unchanged. Oxidation at low concentrations of chlorine ($\leq 1.0\%$) produced starch with significantly higher peak and final viscosities and less breakdown as measured by Micro Viscoamylography. The carboxyl groups formed hemiacetal crosslinks that strengthened the starch integrity. However, as more amylose and amylopectin were degraded at higher oxidation levels, the depolymerization of starch molecules overrode the effects of crosslinking. Both amylose and amylopectin were oxidized and degraded during oxidation but amylose was more susceptible to oxidation.

Keywords: Oxidation; Sodium hypochlorite; Common corn starch; Waxy corn starch; Crosslinking

1. Introduction

Oxidized starch is widely used in food and industrial applications to provide surface sizing and coating property (Scallet & Sowell, 1967). Oxidized starch is commonly prepared by reacting starch with a specified amount of oxidant under controlled temperature and pH. Although many oxidants have been used, commercial production of oxidized starch generally employs sodium hypochlorite as the oxidizing reagent. During oxidation reaction, hydroxyl groups on starch molecules are first oxidized to carbonyl groups and then to carboxyl groups. Therefore, the numbers of carboxyl and carbonyl groups on oxidized starch indicate the extent of oxidation, which takes place primarily at the hydroxyl groups of C-2, C-3, and C-6 positions (Wurzburg, 1986). The reaction of starch oxidation is also always accompanied by an extensive hydrolysis of glucosidic linkages leading to starch depolymerization (Autio, Suortti, Hamunen, & Poutanen, 1992; Boruch, 1985; Forssell, Hamunen, Autio, Suortti, & Poutanen, 1995; Parovuori, Hamunen, Forssell, Autio, & Poutanen, 1995; Zhu & Bertoft, 1997). Hence, oxidized starches exhibit a low viscosity at a high solid concentration from depolymerization and improved clarity and stability from the introduction of functional groups.

Starch is composed of two types of macromolecules, amylose, which is essentially linear and mostly distributed in the amorphous growth ring with small amounts associated with the semi-crystalline growth ring (Montgomery & Senti, 1958), and amylopectin, which is highly branched and constitutes the crystalline lamellae. Whistler and Schweiger (1959) observed that amylopectin was rapidly depolymerized in the initial oxidation catalyzed by hydrogen peroxide. Torneport, Salomonsson, and Theander (1990), however, found that amylose was more easily degraded to small fragments by the bromine oxidant than was amylopectin for potato starch and the unit chains of amylopectin were not noticeably degraded at a low degree of oxidation. The mean molecular weight of the starch and the viscosity of corresponding samples decreased as the concentration of bromine oxidant increased.

Most oxidation in the literature was conducted using high levels of oxidant and little work has been done to

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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).

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study the changes of amylose and amylopectin by low levels of oxidant. In this study, both common and waxy corn starches were oxidized by different levels of hypochlorite to investigate their changes in structure and physicochemical properties. Because of the results from our previous work (Kuakpetoon & Wang, 2001) showed that the slightly oxidized common corn starch exhibited characteristics of crosslinked starches with increased peak viscosity and less breakdown as measured by Brabender Viscoamylography, this study also aimed at understanding how oxidation modified starch swelling.

2. Materials and methods

Both unmodified common corn starch and waxy corn starch were obtained from Cerestar, USA (Hammond, IN). Sodium hypochlorite containing 5.1% active chlorine was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ).

2.1. Preparation of oxidized starches

A 35% starch slurry was prepared by adding deionized water to 200 g starch (dry basis) to a final weight of 571 g in a 2-L reaction vessel and 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 2N NaOH. Sodium hypochlorite, 10 g (0.5 g active chlorine/200 g starch, 0.25% active chlorine w/w), was slowly added into the starch slurry in 30 min while maintaining the pH at 9.5 with 1N H_2SO_4. After the addition of NaOCl, the pH of the slurry was maintained at 9.5 with 1N NaOH for an additional 50 min. The slurry was then adjusted to pH 7.0 with 1N H_2SO_4, filtered by suction with a Buchner filter funnel (Whatman filter #4), washed with twofold volume deionized water and dried in a convection oven at 40 °C for 48 h. The same procedure was applied to both common and waxy corn starch and with different active chlorine concentrations (0.25, 0.50, 0.75, 1.0, 1.25, 1.5, 2.0, and 3.0% w/w).

2.2. Carbonyl content

The carbonyl content was determined by following the titrimetric method of Smith (1967). Four grams of starch sample was suspended in 100 ml distilled water in a 500 ml flask. The suspension was gelatinized in a boiling water bath for 20 min, cooled to 40 °C, adjusted to pH 3.2 with 0.1N HCl, and added with 15 ml of hydroxylamine reagent. The flask was stoppered and placed in a 40 °C water bath for 4 h with slow stirring. The excess hydroxylamine was determined by rapidly titrating the reaction mixture to pH 3.2 with standardized 0.1N HCl. A blank determination with only hydroxylamine reagent was performed in the same manner. The hydroxylamine reagent was prepared by first dissolving 25 g hydroxylamine hydrochloride in 100 ml of 0.5N NaOH before the final volume was adjusted to 500 ml with distilled water. Carbonyl content was calculated as follows:

Percentage of carbonyl content = [(Blank − Sample) ml × Acid normality × 0.028 × 100]/Sample weight (dry basis) in g

2.3. Carboxyl content

The carboxyl content of oxidized starch was determined according to the modified procedure of Chattopadhyay, Singhal, and Kulkarni (1997). About 2 g of starch sample was mixed with 25 ml of 0.1N HCl, 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 400 ml of distilled water. The starch cake was then carefully transferred into a 500 ml beaker, and the volume was adjusted to 300 ml with distilled water. The starch slurry was heated in a boiling water bath with continuous stirring for 15 min to ensure complete gelatinization. The hot starch dispersion was then adjusted to 450 ml with distilled water and titrated to pH 8.3 with standardized 0.01N NaOH. A blank test was performed with unmodified starch. Carboxyl content was calculated as follows:

milliequivalents of acidity/100 g starch = [(Sample − Blank) ml × Normality of NaOH × 100]/Sample weight (dry basis) in g

Percentage of carboxyl content = [milliequivalents of acidity/100 g starch] × 0.045

2.4. Physicochemical properties

The β-amylolysis limit was determined by hydrolyzing the starch samples (9.0 mg) with β-amylase (150 U) at 30 °C in 50 mM acetate buffer (pH 4.8) for 180 min. Maltose produced was determined by the methods of Nelson (1944) and Somogyi (1952).

The thermal properties, including onset and peak gelatinization temperatures and enthalpy, were assessed by a Perkin–Elmer Pyris-1 Differential Scanning Calorimeter (DSC, Perkin–Elmer Co., Norwalk, CT) following the method of Wang, White, and Pollak (1992).

The pasting properties of native and oxidized starches (10%, w/w) were determined using a Micro Viscoamylograph (C.W. Brabender Instruments, Inc., South Hackensack, NJ) equipped with a 300 cmg cartridge and operated at a speed of 250 rpm. Starch slurry was heated from 30 to 95 °C at a rate of 7 °C/min, held at 95 °C for 5 min, cooled down to 50 °C at a rate of 7 °C/min and held at 50 °C for 3 min.

The swelling power (SP) and water solubility index (WSI) were measured by suspending a 1.7% starch suspension (0.5 g dry starch in 30 ml water) into a centrifuge tube with cap and heating the tube from 55 to

Table 1
Carbonyl and carboxyl contents of oxidized starches[1]

	Active chlorine concentration (%)							
	0.25	0.5	0.75	1.0	1.25	1.5	2.0	3.0
Carbonyl (%)								
Common	0.016^{b}	0.022^{b}	0.024^{a}	0.030^{a}	0.036^{a}	0.041^{a}	0.044^{a}	0.061^{a}
Waxy	0.006^{a}	0.014^{a}	0.020^{a}	0.027^{a}	0.035^{a}	0.040^{a}	0.048^{a}	0.061^{a}
Carboxyl (%)								
Common	0.023^{b}	0.036^{c}	0.052^{b}	0.062^{c}	0.102^{c}	0.114^{c}	0.177^{c}	0.269^{b}
Waxy	0.017^{b}	0.017^{a}	0.022^{a}	0.042^{b}	0.050^{b}	0.056^{b}	0.121^{b}	0.297^{c}

[1] Mean values of three measurements in the same column with different letters are significantly different ($p < 0.05$).

95 °C at 10 °C intervals and the sample was kept at that temperature for 30 min. The heated sample was rapidly cooled to room temperature in an ice-water bath and centrifuged at 7700 × *g* for 20 min. SP was determined by measuring the sedimented paste weight and WSI by the solid content of the supernatant (Holm, Björck, Asp, Sjöberg, & Lundquist, 1985).

2.5. Structural characterization

The carbohydrate profiles of native and isoamylase-debranched unmodified and oxidized starches were obtained by high-performance size-exclusion chromatography (HPSEC) (Waters Corporate, Milford, MA) according to the method of Kasemsuwan, Jane, Schnable, Stinard, and Robertson (1995) with modification (Wang & Wang, 2000).

The chain-length distribution of amylopectin was characterized by a high-performance anion-exchange chromatograph equipped with a pulsed amperometric detector (HPAEC-PAD) according to the method of Kasemsuwan et al. (1995) with minor modifications. The HPAEC-PAD (Dionex DX500) 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. Six-mg defatted starch was added with 3.2 ml deionized water and gelatinized in a boiling water bath for 1 h. After cooling to room temperature, 30 μl of isoamylase (3100 enzyme units, Hayashibara Biochemical Laboratories Inc. Okayama, Japan) and 0.4 ml of acetate buffer (pH 3.5) were added to the starch sample, 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 filtered through a 0.45 μm filter prior to injection.

The oxidized starches (0.5 g dry starch in 30 ml deionized water) were also heated at 90 °C for 30 min, cooled in ice water for 20 min, and centrifuged at 7700 × *g* for 20 min. The supernatants were filtered through a 0.45 um filter and analyzed for carbohydrate profile by HPSEC without further treatment.

2.6. Statistical analysis

All analyses were done in duplicate unless otherwise indicated. Experimental data were analyzed by using the General Linear Models Procedure (SAS Software Institute, Inc. Cary, NC 1999) to identify differences among data. All differences were reported at the 95% significant level.

3. Results and discussion

3.1. Carboxyl and carbonyl contents

The carboxyl and carbonyl contents of oxidized starches are listed in Table 1. Both carboxyl and carbonyl content of oxidized starches increased as active chlorine concentration increased. The increase was much greater for the carboxyl content than for the carbonyl content because oxidation conducted in an alkaline condition, pH 9.5, and by hypochlorite promoted the production of carboxyl groups

Table 2
β-Amylolysis limits of oxidized starches[1]

	Active chlorine concentration (%)								
	0	0.25	0.5	0.75	1.0	1.25	1.5	2.0	3.0
Common	58.4(0.0)	56.1(0.7)	55.1(1.1)	52.7(0.6)	50.9(0.4)	49.6(0.3)	44.9(1.4)	45.7(0.6)	47.9(0.2)
Waxy	54.6(1.7)	52.2(0.1)	48.6(1.1)	47.8(0.4)	45.5(2.2)	44.2(1.1)	44.0(1.9)	47.7(0.7)	48.5(0.3)

[1] Mean value (standard deviation) of two measurements.

Table 3
Thermal properties of oxidized common and waxy corn starches by differential scanning calorimetry [1]

		Active chlorine concentration (%)								
		0	0.25	0.5	0.75	1.0	1.25	1.5	2.0	3.0
Common	T_o (°C)	68.1^c	68.8^{bc}	68.8^{bc}	69.3^b	69.3^b	70.4^a	69.5^b	69.2^b	69.3^b
	T_p (°C)	72.2^c	72.8^b	72.8^b	73.1^b	73.2^b	74.3^a	73.9^a	73.1^b	73.2^b
	ΔH (J/g)	13.1^a	13.2^a	12.7^a	13.4^a	12.6^a	12.8^a	13.0^a	12.4^a	12.7^a
Waxy	T_o (°C)	67.6^c	68.2^{bc}	68.1^{bc}	68.7^{ab}	69.1^a	68.7^{ab}	68.4^b	68.5^b	66.9^c
	T_p (°C)	72.6^{cd}	72.7^{cd}	73.0^{bcd}	73.4^{ab}	73.9^a	73.4^{ab}	73.4^{ab}	73.3^{abc}	72.5^d
	ΔH (J/g)	15.6^a	15.3^a	15.9^a	15.3^a	16.2^a	16.0^a	15.2^a	15.1^a	15.7^a

[1] Mean values of three measurements in the same row with different letters are significantly different ($p < 0.05$), T_o: onset temperature; T_p: peak temperature; ΔH: enthalpy.

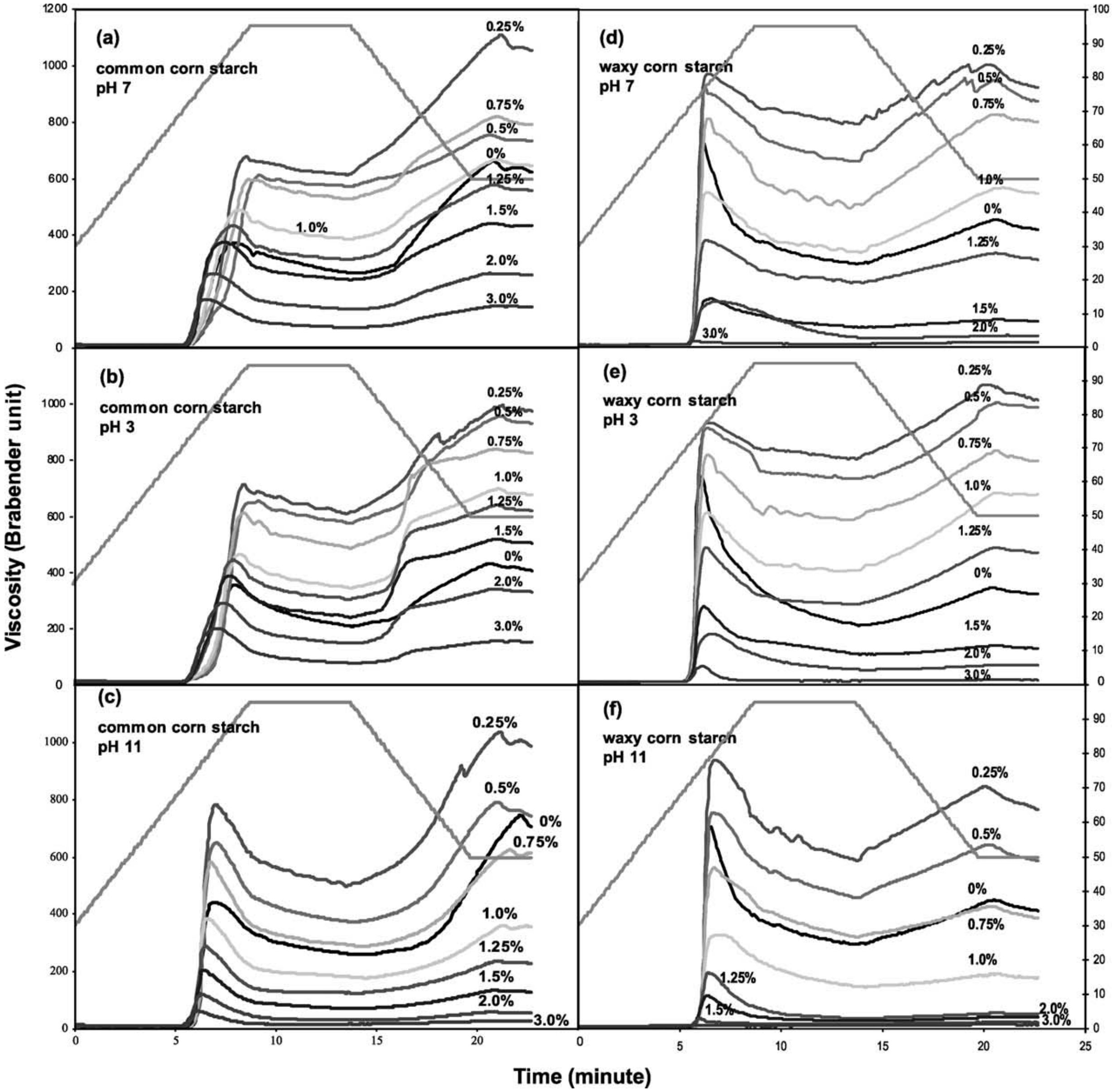

Fig. 1. Pasting curves of oxidized starches by Micro Viscoamylography. (a) common corn starch at pH 7; (b) common corn starch at pH 3; (c) common corn starch at pH 11; (d) waxy corn starch at pH 7; (e) waxy corn starch at pH 3; (f) waxy corn starch at pH 11.

in comparison with the oxidation conducted in an acidic condition and by hydrogen peroxide (Boruch, 1985; Parovuori et al., 1995). There was no significant difference in carbonyl content between common and waxy starches except at very low chlorine concentrations (0.25 and 0.5%). In contrast, the carboxyl content in oxidized common corn starch was consistently higher than in oxidized waxy corn starch, except at 3.0% active chlorine, suggesting that common corn starch was more susceptible to oxidation. Both common and waxy corn starches are cereal starches with a similar A-type X-ray diffraction pattern, granule size and shape, but differ in their amylose contents. Previous work (Farley & Hixon, 1942; Kuakpetoon & Wang, 2001; Schmorak & Lewin, 1963; Schmorak, Mejzler, & Lewin, 1962) has shown that the occurrence of oxidation was mainly in the amorphous lamellae of the semi-crystalline growth rings in starch granules. The amorphous lamella mainly consists of amylose and the regions around the branches in amylopectin. The present results implied that amylose was more susceptible to oxidation than was amylopectin, which may be due to the more accessible nature and the linear structure of amylose. Nevertheless, the carboxyl content was highest for waxy corn starch oxidized by 3% active chlorine. Amylopectin might become readily oxidized once some structural barriers were overcome.

3.2. Physicochemical properties

The β-amylolysis limits of both common and waxy corn starches gradually decreased as the extent of oxidation increased but increased slightly at 2 and 3% chlorine levels (Table 2). The decrease in β-amylolysis limits was presumed to come from the introduction of carbonyl and carboxyl groups near the non-reducing ends in the amylopectin component (Manelius, Buleon, Nurmi, & Bertoft, 2000), whereas the increase at higher hypochlorite concentrations might result from greater depolymerization of amylopectin molecules which created more available non-reducing ends. The oxidized waxy corn starch had a higher β-amylolysis limit than the oxidized common corn starch at 2 and 3% active chlorine, which agreed with the carboxyl results that more amylopectin became oxidized and degraded at high hypochlorite concentrations.

The thermal properties of unmodified and oxidized starches are summarized in Table 3. In general, oxidized starches had higher onset and peak temperatures but shared a similar enthalpy compared with the unmodified counterparts. The increased gelatinization temperature was attributed to the hydrolysis of the amorphous lamella, which functions to destabilize the crystalline lamella by increasing the hydration and swelling of the crystallites (Donovan, 1979). Therefore, when the amorphous lamella was degraded, the gelatinization transition shifted to a higher temperature up to 1.25% active chlorine. When the active chlorine level exceeded 1.25%, the onset and peak temperatures decreased, possibly from degradation of crystalline lamellae. The enthalpy, nevertheless, remained unchanged possibly because the amount of crystallites was not affected by oxidation. Manelius et al. (2000) observed a lower melting temperature for oxidized potato starch,

Table 4
Swelling power and water solubility index of oxidized starches[1]

		Active chlorine concentration (%)								
		0	0.25	0.5	0.75	1.0	1.25	1.5	2.0	3.0
Swelling power										
Common corn starch	65 °C	5.3	5.4	5.0	3.3	5.0	4.6	4.3	4.7	5.1
	75 °C	7.6	7.7	8.1	8.6	9.0	9.4	9.4	10.0	11.5
	85 °C	10.1	9.5	10.1	11.7	14.1	13.9	14.8	16.5	13.8
	95 °C	15.9	14.0	11.8	12.6	16.2	14.0	13.6	12.0	8.1
Waxy corn starch	65 °C	5.5	6.6	6.3	5.7	5.0	4.9	3.8	4.3	3.4
	75 °C	15.2	11.7	13.1	12.6	12.1	13.4	11.5	12.4	13.6
	85 °C	19.9	16.2	14.8	16.0	17.6	20.0	11.5	15.1	0
	95 °C	27.0	17.1	16.5	18.0	20.0	18.8	0[a]	0	0
Water solubility index										
Common corn starch	65 °C	1.1	0.9	1.4	1.2	1.9	1.2	1.3	2.4	6.0
	75 °C	2.0	2.1	1.4	2.8	3.8	2.7	4.6	8.5	12.8
	85 °C	4.6	4.5	5.1	6.9	7.9	11.1	12.6	16.7	34.4
	95 °C	7.9	10.7	11.8	14.6	17.0	22.7	46.2	49.4	66.3
Waxy corn starch	65 °C	2.1	0.8	1.7	2.6	3.9	3.6	3.9	5.1	13.1
	75 °C	2.2	1.5	4.0	4.9	9.8	15.3	21.8	20.6	34.2
	85 °C	13.4	4.4	3.5	6.0	10.2	22.0	48.2	48.0	100
	95 °C	33.9	4.9	5.1	7.6	34.1	35.8	100	100	100

[1] Mean values of two measurements with standard deviation <10%

[a] No paste formation.

presumably because of the high concentration of active hypochlorite (20% of dry starch weight) used in their study.

The pasting properties of oxidized common and waxy corn starches as measured by Micro Viscoamylography at pH 7 are presented in Fig. 1(a) and (d), respectively. The pasting temperatures of the oxidized common corn starches were decreased by oxidation but those of the oxidized waxy corn starches remained unchanged. The most notable differences for slightly oxidized starches (≤1.0% chlorine for common corn starch and ≤0.75% chlorine for waxy corn starch) compared with the unmodified counterparts (0% chlorine) were their increased peak and final viscosities, which are the characteristics of slightly crosslinked starches because of the improved starch integrity from chemical crosslinking. However, unlike the conventional chemically crosslinked starch, which usually exhibits a higher pasting temperature, these slightly oxidized starches had lower pasting temperatures. Farley and Hixon (1942) rationalized that slightly oxidized starch granules can swell to a higher degree than their unmodified starch granules because of their higher hydration capacity from the introduction of carboxyl groups, resulting in a higher peak viscosity. Seib (1997) further suggested that the aldehyde groups generated at first by the low levels of oxidant formed hemiacetal cross-links, which stabilized the swelling of granules and overrode the effects of minor depolymerization. The hemiacetal cross-links would be one structure in a dynamic equilibrium with three possible structural forms, the free carbonyl form, its hydrated form with water, and its hemiacetal form with one of its hydroxyl group in the hydrated form being replaced by OR where OR is a starch side chain of –C(OH, OR) (Seib, P. personal communication). The formation of internal hemiacetal or hemiketal bonds is more energetically favored over the free aldehydo or keto form of the sugar.

In order to understand the nature of the ‘cross-links’ by slight oxidation, the pasting properties of the oxidized starches were also measured at pH 3 and 11 (Fig. 1(b) and

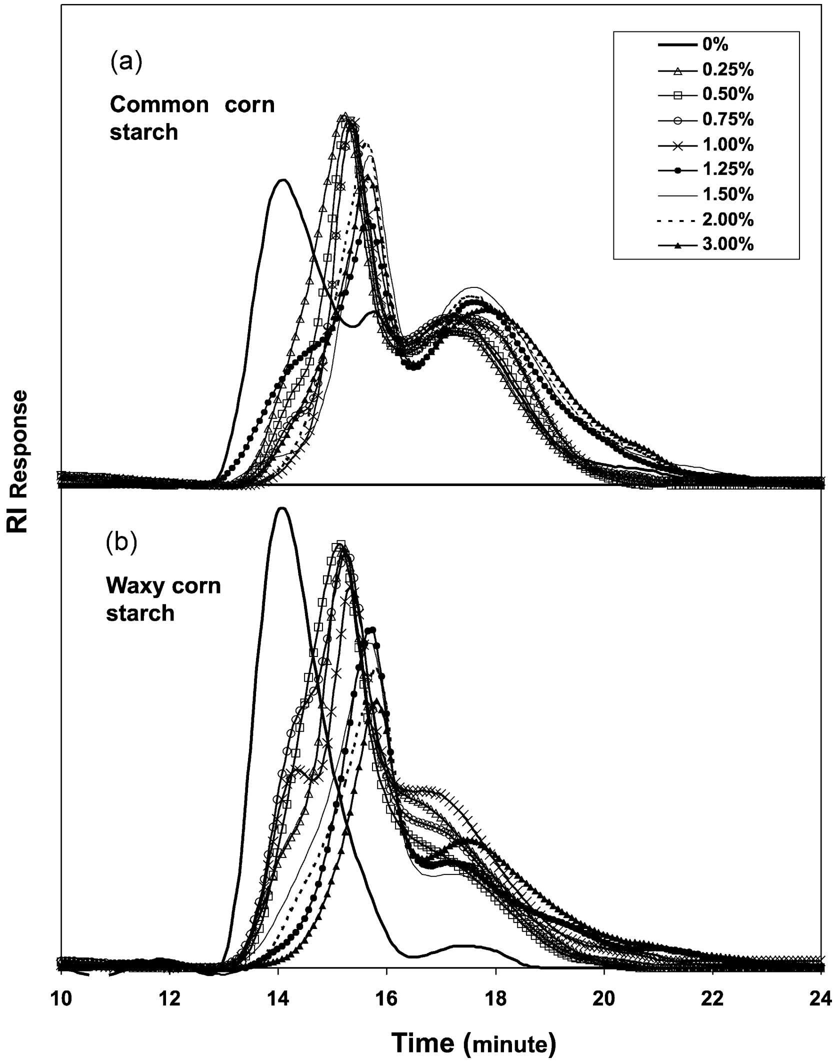

Fig. 2. Carbohydrate distributions of oxidized starches. (a) common corn starch; (b) waxy corn starch.

(c) for common corn starch and Fig. 1(e) and (f) for waxy corn starch, respectively). When the pH was adjusted to 3, the pasting profiles of both common and waxy corn starches were similar to those at pH 7. However, when the pH was adjusted to 11, both types of starches generally showed increased breakdown and decreased final viscosities. The results demonstrated that carboxyl groups played an important role in forming the cross-links and ionized carboxyl groups were not as effective as protonated carboxyl groups in maintaining the starch integrity, possibly due to their negative charge repulsion. Although the carboxyl contents in oxidized starches were only 0.023% for common corn starch and 0.017% for waxy corn starch at 0.25% chlorine, the hemiacetal bonds were strong enough to augment the starch integrity similar to crosslinked starches by bi-functional reagents, such as phosphoryl chloride.

The SP and WSI of the oxidized starches were also studied and results are presented in Table 4. The SP of the oxidized common corn starch was similar to or higher than the unmodified counterpart at 75 and 85 °C but lower at 95 °C. In contrast, the SP of the oxidized waxy corn starch was lower than the unmodified counterpart at all incubation temperatures, except some at 65 °C, and no paste was obtained for the highly oxidized starches at 85 or 95 °C. When amylose was preferentially hydrolyzed in the initial stage of oxidation, the SP increased at 75 and 85 °C incubation temperatures, suggesting the depolymerization of amylose assisted in starch swelling. However, when amylopectin was hydrolyzed, the starch lost the ability of holding the absorbed water, particularly at 95 °C. Therefore, these results evidently supported that starch swelling is controlled by both amylose and amylopectin, supporting Tester and Morrison (1990) that amylopectin contributes to swelling and pasting of starch granules, whereas amylose and lipids inhibit the swelling. The present results also support the hypothesis proposed by Jenkins and Donald (1995) that amylose disrupts the crystalline lamella packing by co-crystallizing with amylopectin. The randomly distributed, isolated amylose would not have exerted such a significant impact on starch swelling. Because of its long linear structure, amylose extends into several crystalline lamellae and would be able to reinforce the starch integrity only when

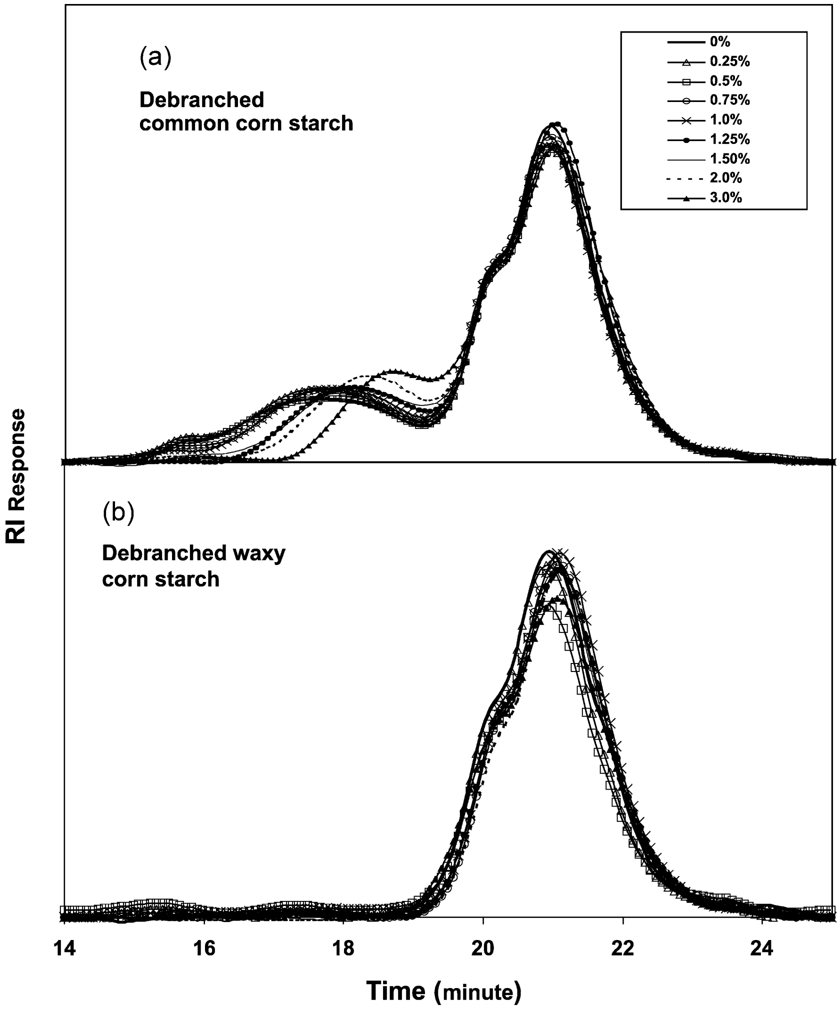

Fig. 3. Carbohydrate distributions of isoamylase-debranched oxidized starches. (a) common corn starch; (b) waxy corn starch.

it is complexed with amylopectin. It has also been suggested that amylose chain would intertwine with branch chains of amylopectin to hold the integrity of starch granules during heating (Jane et al., 1999). Although the slightly oxidized starches had higher peak viscosities than the unmodified ones as shown in Fig. 1, their SPs at 95 °C were lower, particularly for waxy corn starch. It is proposed that the oxidized starch had a spongy, porous structure that was able to imbibe water during heating but could not retain the absorbed water under centrifugation, whereas the unmodified starch held the absorbed water much stronger. The amorphous lamella was suggested to be responsible for holding the absorbed water in addition to the crystalline lamella.

WSI represents the amount of solubilized starch molecules at a certain temperature. In general, the WSI increased as the extent of oxidation progressed and as the incubation temperature increased, agreeing with previous findings (Autio et al., 1992; Parovuori et al., 1995) Although 65 °C was below the onset gelatinization temperature as measured by DSC (67–70 °C), a small amount of starch molecules were capable of leaching out of granules at 65 °C, presumably amylose molecules. The WSI of the more highly oxidized starches had higher WSIs than the unmodified one at 65 °C, probably because of the improved hydration from the introduced functional groups. However, the waxy corn starch oxidized by 0.25, 0.5, or 0.75% chlorine showed very low WSIs at 85 and 95 °C, supporting the presence of cross-links that prevented the amylopectin molecules from leaching out. In comparison, oxidized common corn starch at the same low levels of chlorine had higher WSIs, which may be from depolymerized amylose molecules. At 1.5% chlorine or greater, the WSIs of both oxidized common and waxy corn starch drastically increased, assuming from depolymerization of both amylose and amylopectin. These data again agreed with the previous results that the cross-links were present in oxidized starches and amylose was more prone to breaking glucosidic linkages in oxidation.

3.3. Structural characterization

The HPSEC of unmodified and oxidized corn starches are displayed in Fig. 2. The starches oxidized at 0.25–1.0% chlorine could not be completely dissolved in 90% dimethyl sulfoxide like other samples and required four times of autoclaving (121 °C and 19 psi for 95 min) before complete dissolution. The first fraction of the common corn starch (Fig. 2(a)) with a shorter retention time was the amylopectin with a large molecular size, and the second fraction was the amylose with a small molecular size or the degraded amylopectin. The amylopectin degraded to a great extent even at low levels of oxidation for both common and waxy corn starches and the proportion of low molecular weight fraction increased as the extent of oxidation increased. However, it was noted that the change in carbohydrate profile did not follow the extent of oxidation. For example, the oxidized waxy corn starches

Table 5
Amylopectin average chain length and branch chain distribution of oxidized starches[1]

		Average chain length (glucose unit)	Branch chain distribution (%)			
			A chains (DP 5–12)	B1 chains (DP 13–24)	B2 chains (DP 25–36)	B3 chains (DP 37–63)
Common corn starch						
Active chlorine	0%	19.7	35.2	43.3	12.2	9.3
	0.25%	19.9	33.9	43.5	13.2	9.3
	0.5%	19.8	34.1	43.7	13.0	9.2
	0.75%	19.8	33.8	44.3	13.1	8.8
	1.0%	19.7	34.5	43.7	12.9	8.9
	1.25%	19.5	34.7	44.4	12.7	8.2
	1.5%	19.4	34.9	44.2	12.9	8.0
	2%	19.0	36.3	44.2	12.5	7.0
	3%	18.5	37.8	43.9	11.7	6.6
Waxy corn starch						
Active chlorine	0%	20.5	33.0	41.8	14.6	10.6
	0.25%	20.2	33.9	42.0	13.9	10.2
	0.5%	20.5	33.4	43.0	13.4	10.2
	0.75%	20.6	32.3	42.4	14.9	10.4
	1.0%	20.5	32.5	42.3	14.9	10.2
	1.25%	20.4	32.8	42.4	14.9	10.0
	1.5%	20.2	33.0	42.8	14.8	9.5
	2%	20.3	32.9	42.5	15.2	9.5
	3%	19.6	34.1	43.3	14.9	7.7

[1] Average of duplicate measurements.

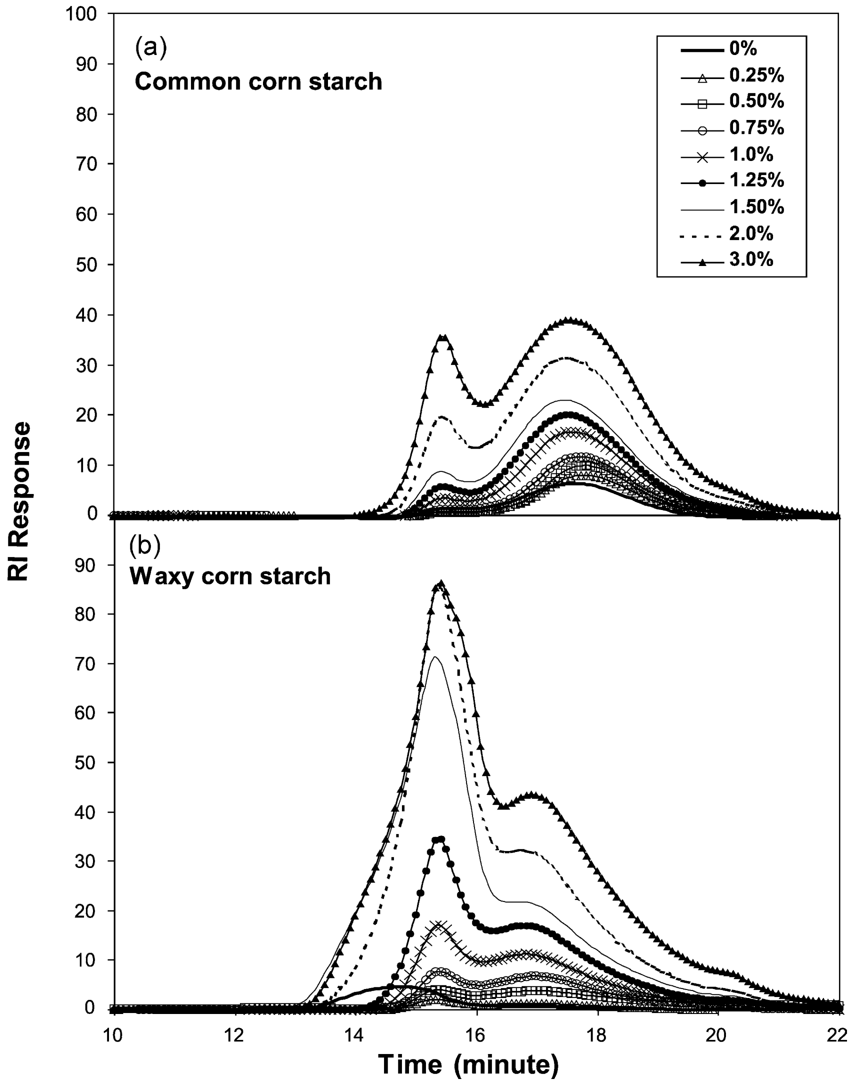

Fig. 4. Carbohydrate distributions of supernatant of oxidized starches heated at 90 °C for 30 min. (a) common corn starch; (b) waxy corn starch.

at 0.75 and 1.0% active chlorine showed a shoulder at the shorter retention time, which had a larger molecular size than the starch oxidized at either 0.25 or 0.5% chlorine. It is speculated that the inter-molecular cross-links resulted in the increase in molecular size, thus shifting the molecule to a shorter retention time. The different trends in common corn starch versus in waxy corn starch might be due to their difference in amylose content and the susceptibility of amylose versus amylopectin to oxidation. Amylose has been suggested to form complexes with amylopectin within the crystalline lamella (Blanshard, 1987; Jenkins & Donald, 1995; Kasemsuwan & Jane, 1994). When amylose and amylopectin were oxidized, the carboxyl groups in both amylose and amylopectin would be capable of forming hemiacetal bonds with nearby molecules, which suggested both amylose and amylopectin could be involved in the formation of cross-links. If amylopectin chains formed cross-links with each other, the molecular size would increase drastically, resulting in the appearance of the shoulder in Fig. 2(b). The influence of the cross-links from amylose on molecular size change was not as significant and evident as from amylopectin.

The normalized HPSEC chromatograms of isoamylase-debranched unmodified and oxidized starches are shown in Fig. 3. The results show that amylose was also degraded to lower MW molecules and the change in carbohydrate profile followed the trend of the extent of oxidation. The molecular weight change of amylopectin branch chains after debranching was not as significant as compared with the native starches (Fig. 2); nevertheless, the peak retention time of the amylopectin fraction shifted to a slightly longer retention time, indicating some degradation occurred to amylopectin branch chains.

The chain-length distributions of amylopectin from isoamylase-debranched unmodified and oxidized starches by HPAEC-PAD are summarized in Table 5. The amylopectin fractions are grouped into four chain types and corresponding DP according to Hanashiro, Abe, and Hizukuri (1996). The average chain-length and the proportion of B1 and B2 chains remained mostly unchanged, whereas the percentage of A chains increased and B3 chains decreased at high oxidation levels, which agreed with our previous work (Kuakpetoon & Wang, 2001). The constant B1 chains suggested the occurrence of oxidation in the amorphous region because the crystalline regions is mainly constituted of A and B1 chains and the decrease in B3 chains indicated the involvement of amylopectin long chains in oxidation (Hizukuri, 1985; Hizukuri, Kaneko, & Takeda, 1983). Because the chains in fraction B3 extend into 3 clusters (Hizukuri, 1986), the portion of the B3 chain in the amorphous lamella had a greater chance to be oxidized or hydrolyzed in oxidation as the amylose fraction.

The carbohydrate profiles of supernatants from oxidized starches heated at 90 °C for 30 min are depicted in Fig. 4. At low levels of oxidation, the amylose peak became larger and shifted to a longer retention time while almost no soluble amylopectin was detected in the slightly oxidized common corn starches (Fig. 4(a)), which again confirmed that amylose was preferentially oxidized in the beginning of oxidation to smaller molecular size molecules. As oxidation progressed, more amylose and amylopectin became solubilized, possibly from improved hydration and depolymerization. For waxy corn starch (Fig. 4(b)), oxidation clearly degraded amylopectin into two molecular size groups similar to the carbohydrate profiles of oxidized common corn starches, but the second peak of the waxy corn starch had a shorter retention time than the amylose peak because it was from the degraded amylopectin. The results also demonstrated that more solubles leached out from oxidized waxy corn starch than from oxidized common corn starch since the refractive index (RI) response represented the concentration of the solubles.

4. Conclusions

The present study supported the swelling mechanism proposed by Mat Hashim et al. (1992) that the breakage of some linkages particularly in amylopectin contributes to swelling and subsequent disintegration of the granule at high temperatures, which will facilitate the loss of this material from the swollen granule. The crystalline lamella played a pivotal role in maintaining the integrity of starch granules. Both amylose and amylopectin were degraded but amylose was more susceptible to oxidation. The cross-links from hemiacetal bonds of carboxyl groups in oxidized starches permitted the greater extent of swelling. Both the amorphous and crystalline lamellae in starch granules were important in absorbing and retaining water.

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