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Specific oxidation pattern of soluble starch with TEMPO-NaBr-NaClO system

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ABSTRACT

Oxidized starch, one of the most important starch derivatives, has many different properties and applications. Currently, there are two ways to produce oxidized starch, through specific and nonspecific oxidation. Specific oxidation using the stable nitroxyl radical, 2,2,6,6-tetramethyl preparidinloxy (TEMPO), with NaBr and NaClO can produce oxidized starches with different properties under good quality control. In the current study, we examine the products of specifically oxidized starch. As the amount of oxidant and the temperature, two critical factors impacting the oxidation of starch were thoroughly investigated. Analysis of the molecular weight (MW), degree of oxidization (DO) and the detailed structures of corresponding products was accomplished using gel permeation chromatography with multi-angle laser light scattering (GPC-MALLS), infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and quadrapole time-of-flight mass spectrometry (Q/TOF-MS). According to the analytical results, the oxidation patterns of starch treated with specific oxidant TEMPO-NaBr-NaClO were established. When high amounts of oxidant was applied, more glucose residues within starch were oxidized to glucuronic acids (higher DO) and substantial degradation to starch oligosaccharides was observed. By selecting a reaction temperature of 25 °C a high DO could be obtained for a given amount of oxidant. The reducing end sugar residue within oxidized starch was itself oxidized and ring opened in all TEMPO-NaBr-NaClO reactions. Furthermore, extra oxidant generated additional novel structures in the reducing end residues of some products, particularly in low temperature reactions.

1. Introduction

Starch is an α-glucan polysaccharide, widely occurring in seeds and tubers of plants (Tharanathan, 2005). Starch consists of an α (1 → 4) linked-glucose (Glc) main chain (backbone) having a number of α (1 → 6) branches (Lindeboom, Chang, & Tyler, 2004; Serrero et al., 2010; Tavares et al., 2004). The higher the degree of branching, the lower the solubility of starch (Lin et al., 2016). Starch can be modified physically, chemically, and enzymatically to produce starch derivatives with varying properties, including solubility, viscosity, stability, gelatination, etc. (Zhang, Wang, Zhao, & Wang, 2012; Zhang et al., 2007). These starch derivatives are widely used in the food, cosmetic, medical and pharmaceutical industries (Komulainen, Verlackt, Pursiainen, & Lajunen, 2013; Zhang, Wang, Zhang, Yang, & Wang, 2010).

Oxidized starch, one of the most important starch derivatives, has improved solubility, high transparency, high adhesive force, and low viscosity (Kuakpetoon & Wang, 2006). Oxidized starch has important applications as raw material for papermaking and spinning, food additives, and excipients, (Komulainen et al., 2013; Kuakpetoon & Wang, 2006; Haar et al., 2010). Currently, there are two ways to produce oxidized starch, through specific and nonspecific oxidation. The better the structural characterization of an oxidized starch the more widely it can be applied in different applications. Periodate, hypocholoride and bromate are generally used for the nonspecific oxidation of starch. In these reactions both the primary hydroxyl groups, at position 6, and the secondary hydroxyl groups at other positions with glucose can be oxidized (Serrero et al., 2010; Kuakpetoon & Wang, 2006; Komulainen et al., 2013). The structures of these nonspecifically oxidized products are always complicated. The specific oxidation of starch with 2,2,6,6-tetramethylpiperidine-1-oxyl/sodium bromide/sodium hypochlorite (TEMPO-NaBr-NaClO) system has been recently reported. In this specific oxidation, the primary hydroxyl

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groups at position 6 are selectively oxidized to afford glucuronic acid residues (Ding et al., 2008; Haar et al., 2010; Watanabe, Habu, & Isogai, 2013). This specific oxidation process provides the opportunity to produce oxidized starches having different properties under good quality control. However, it has been reported that the degree of oxidation (DO) by this method is relatively low and that oxidation can result in degradation (Cao & Yang, 2011; Komulainen et al., 2013). The detailed structural characteristics of TEMPO-NaBr-NaClO oxidation product has yet to be completely determined.

In this work, we carefully examine the products of TEMPO-NaBr-NaClO oxidized starch. The amount of oxidizing agent and the temperature, two critical factors that impact the oxidation, were thoroughly investigated. The molecular weight (MW), degree of oxidization (DO) and the detailed structures of corresponding products were determined using gel permeation chromatography with multi-angle laser light scattering (GPC-MALLS), infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and quadrapole time-of-flight mass spectrometry (Q/TOF-MS). The specific oxidation pattern of starch afforded using TEMPO-NaBr-NaClO was determined.

2. Experimental

2.1. Materials

Soluble starch, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), sodium bromide (NaBr) and sodium hypochlorite (NaClO, 5% active chlorine) solution were obtained from Aladdin Industrial Co. (Shanghai, China). Concentrated hydrochloric acid (HCl) and sodium hydroxide (NaOH) were from Chinasun Specialty Products CO., LTD. (Changshu, China). Ammonium acetate (NH_4OAc) was purchased from Sigma-Aldrich (Shanghai, China). Methanol (HPLC grade) was obtained from Merck Chemicals (Darmstadt, Germany). Deuteroxide (D_2O, atm.%D $\geq$ 99.9%) was from Energy Chemicals (Shanghai, China). High-purity water (resistivity $\geq$18.2 M$\Omega \times$ cm, 25 °C) was used throughout the study. All other chemicals were of analytical reagents.

2.2. Methods

2.2.1. Preparation of oxidized starch samples

All reactions in this work were carried out at a scale of 1 g starting material (soluble starch) in 50 mL water. In each reaction, soluble starch (1 g) was suspended in 50 mL water and heated at 80 °C to afford a transparent solution. Each solution was cooled down to room temperature before 32 mg TEMPO and 320 mg NaBr were added. Each reaction system was then adjusted to pH 10 by adding 20% NaOH solution. The reaction temperatures were maintained at either 4, 25 or 50 °C, and different amounts of oxidant (4, 8 or 12 mL of NaClO solution, 5% active chlorine) were applied to each reaction at each temperature. The pH of each reaction solution was kept at ~10 by the drop wise addition of 20% NaOH solution throughout the reaction. A pH meter was used to monitor the pH in this process. After 4 h, 1 mL of ethanol was added to terminate the reaction. The mixture was then brought to pH 6 by adding 4 M HCl solution and dialyzed against distilled water in a 500 Da molecular weight cut-off dialysis bag to remove TEMPO and other salts. The products retained in the dialysis bags were evaporated under vacuum at 45 °C and then lyophilized to obtain oxidized starch powder.

2.2.2. Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR spectra of oxidized starch samples were acquired on a FT-IR spectrometer (Bruker, Germany). The scanning range was set at 4000–600 cm^{-1}. The resolution of the instrument was 4 cm^{-1} and the scanning number was 16. All samples were dried at 50 °C for 24 h before measurement. The data was processed with OPUS software.

2.2.3. NMR analysis

Each oxidized starch sample (50 mg) was dissolved in 0.7 mL D_2O for NMR analysis. A 600 MHz NMR spectrometer (Agilent, CA, USA) operated at 600 MHz and 150 MHz, and the scan numbers were set at 16 and 10240 for ^1H and ^{13}C data, respectively. The data was processed with MestReNova software of version 6.1.1.

2.2.4. Measurement of the molecular weight distribution by GPC-MALLS

The weight-average MW of each oxidized starch sample was determined by GPC-MALLS. In this experiment, the analysis performed on an Agilent 1260HPLC system (CA, USA) coupled with an 18-angles MALLS (Wyatt, USA) tandem a refractive index (RI) detector (Agilent, USA). The dn/dc value was set at 0.138 mL/g. The separation was performed on an ACQUITY UPLC@BEH125 SEC column (1.7 μm, 4.6 × 300 mm, Waters, USA) at 0.1 mL/min. The mobile phase was 80 mM ammonium acetate aqueous solution. The injection volume was 20 μL and the column temperature was set at 25 °C. The data were processed with ASTRA software of version 6.1.

2.2.5. Q/TOF MS analysis

Each sample solution (5 mg/mL, 5 μL) was injected on an Agilent 6540 electrospray ionization (ESI) Q/TOF-MS system (CA, USA). The MS spectra were recorded in negative mode with a MS scan range between 100–3000 *m/z*. This range covers the most ions including the oligosaccharide with degree of polymerization (dp) of up to 15 in singly charged form as well as molecular ions of oligosaccharides having higher dp as multiply charged species. Nitrogen gas was used in the nebulizer at a pressure of 30 psi. The spray voltage was 3.5 kV and a flow of nitrogen gas of 8 L/min at 350 °C assisted in the drying process. Fragment voltage was set to 120 V. MassHunter software was used for the data analysis.

3. Results

3.1. The analysis of the degree of oxidization using IR spectroscopy

The IR spectra of native starch and the oxidized starches, prepared with different amounts of oxidant (4, 8 and 12 mL of NaClO solution) at 25 °C, were obtained (Fig. 1A–D). The broad absorption band at ~3200 cm^{-1} was assigned as the signal of hydroxyl groups of the sugar (Zhang et al., 2012; Pavlovic & Brandao, 2003). All of the spectra in this Figure were normalized against the intensity of this peak. The strong and narrow absorption band at ~1600 cm^{-1} was assigned to the carbonyl signal of carboxyl group (Komulainen et al., 2013; Williams & Fleming, 1987). The absence of this signal in the spectrum of native starch and the increasing of intensities of this signal in the spectra of oxidized starches from second top to bottom demonstrates that larger amounts of oxidant results in the formation of more carboxyl groups. The absorption bands at 1500–1200 and at 1200–1000 cm^{-1} were assigned as the specific signals of sugar ring and the signals of C—OH on the sugar ring (Komulainen et al., 2013; Serrero et al., 2010), respectively, demonstrating the retention of the sugar ring in these oxidation reactions.

The IR spectra of native starch and oxidized starches prepared using identical amounts of oxidant (12 mL NaClO solution) but at different temperatures (4, 25 and 50 °C) are shown in Supplementary Fig. 1. These spectra were also normalized against the absorption band at ~3200 cm^{-1}. The absorption bands at ~1600 cm^{-1} assigned to the carbonyl of the carboxyl groups was present in all the spectra of oxidized starches. The carboxyl group

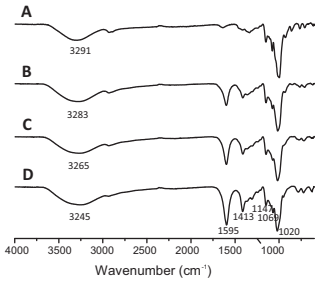

Fig. 1. FT-IR spectra of native starch and the oxidized starches. (A) native starch; (B) oxidized starch carried out with 4 mL NaClO solution; (C) oxidized starch carried out with 8 mL NaClO solution; (D) oxidized starch carried out with 12 mL NaClO solution.

content was lowest in the starch oxidized at 4 °C and was higher and similar in starches oxidized at 25 and 50 °C. Based on these intensities the DO of starch oxidized at low temperature (4 °C) was lower than those oxidized at higher temperature but increasing the temperature from 25 to 50 °C did not result in higher DO.

3.2. The analysis of the degree of oxidization using NMR spectroscopy

^{1}H and ^{13}C NMR spectroscopy was applied to provide more structural information of these oxidized starches for a deeper insight into the oxidation pattern of starch treated with TEMPO-NaBr-NaClO.

The ^{1}H NMR spectra of native starch and oxidized starches prepared using different amounts of oxidant (4, 8 and 12 mL of NaClO solution) at 25 °C are presented in Fig. 2A–D. In the ^{1}H NMR spectrum of native starch, the chemical shift at 5.42 ppm was assigned to the anomeric hydrogen of the α – linked glucose residue (Glc, H 1). The chemical shifts at 3.99, 3.87 and 3.63 ppm were assigned as the other hydrogens on the sugar ring (H 3 and 5, H 6 a and b, and H 2 and 4, respectively) (Kato, Matsuo, & Isogai, 2003). On treatment with a low concentration of oxidant, the intensity of signal at 5.42 ppm decreased and two new signals at 5.61 and 5.48 ppm appeared (4 mL $NaClO_4$, Fig. 2B). These were assigned to the anomeric hydrogens of glucuronic acid (GlcA) linked to GlcA and the GlcA linked to Glc, respectively (Kato et al., 2003; Komulainen et al., 2013). On treatment with a higher concentration of oxidant, the intensity of signal at 5.42 ppm further decreased, the intensity of signal at 5.61 ppm increased and the signal at 5.48 ppm became the dominant anomeric hydrogen in the spectrum (8 mL NaClO solution, Fig. 2C). In the treatment with the highest amount of oxidant, the signal at 5.61 ppm corresponding to GlcA linked to GlcA was the only anomeric hydrogen signal observed in the spectrum (12 mL NaClO solution, Fig. 2 D). The other protons on sugar ring in the fully oxidized starch were assigned at 4.11, 4.01, 3.75 and 3.57 corresponding to the signals of H5, H3, H4 and H2, respectively (Kato et al., 2003). Thus, as oxidation proceeds, the anomeric hydrogen signal corresponding to Glc linked to Glc, at 5.42 ppm, decreases, the intensity of the anomeric hydrogen signal corresponding to GlcA linked to Glc, at 5.48 ppm increases and then decreases, and the intensity of the anomeric hydrogen signal corresponding to of GlcA linked to GlcA, at 5.61 ppm, increases. This is consistent with increased oxidant generating increased GlcA until most of Glc is selectively converted to GlcA when the reaction is carried out with 12 mL NaClO solution and at 25 °C.

The ^{13}C NMR spectra of native starch and oxidized starches were next examined (Fig. 2E–H). The signals at 99.5, 76.6, 73.3, 71.5, 71.1 and 60.4 ppm in the spectrum of native starch α–linked glucose residues were assigned to the anomeric carbon C 1, and other carbons on the sugar ring (C 4, C 2, C 3, C 5 and C6), respectively (Kato et al., 2003; Serrero et al., 2010; Zhang, Zhang, Wang, & Wang, 2009). The intensity of the signals corresponding to the carbon in primary hydroxyl group, positon 6 of the Glc residue, decreased as the oxidation of starch moved towards completion and completely disappeared in the sample treated with the highest amount of oxidant (12 mL NaClO solution, Fig. 2H). New signals, at 175–180 ppm, corresponding to carboxyl groups, appeared and increased as more oxidant was applied, consistent with the generation of GlcA as Glc residues were selectively oxidized (Fig. 2F–H). These signals were not observed in the native starch (Fig. 2E).

The impact of temperature on the oxidation was next investigated using ^{1}H- and ^{13}C NMR spectroscopy. The ^{1}H- and ^{13}C NMR spectra of native starch and the oxidized starches carried out with same amount of oxidant (12 mL NaClO solution) but different temperatures (4, 25 and 50 °C) are presented in Supplementary Fig. 2. Only a small portion of the Glc residues were oxidized to GlcA in low temperature (4 °C). Interestingly, the oxidation of starch, carried out at 25 °C, afforded the highest DO, just slightly higher than that produced at 50 °C. The results from NMR analysis were consistent with those obtained using IR spectroscopy.

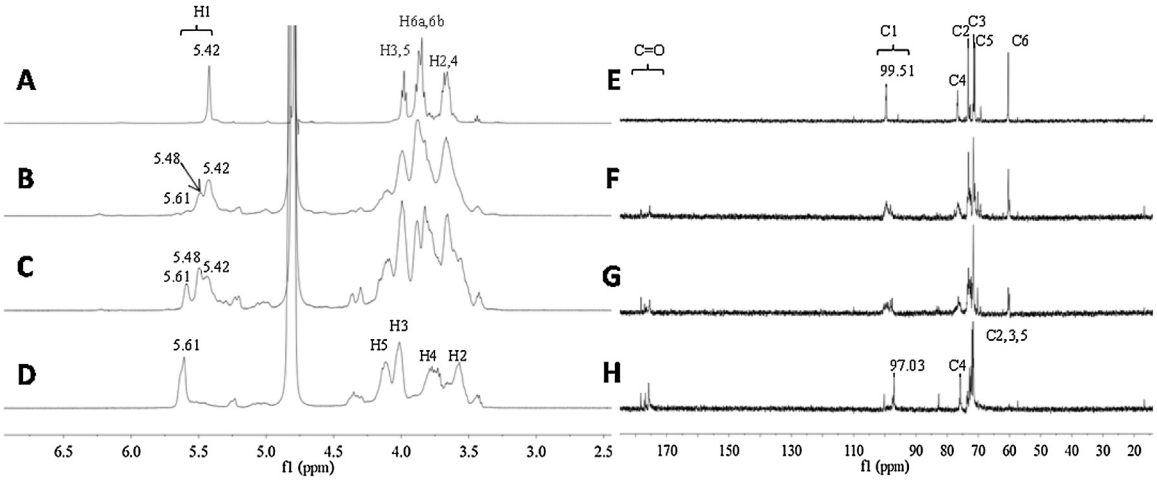

Fig. 2. NMR spectra of native and oxidized starches. (A) ^{1}H NMR spectrum of native starch; (B) ^{1}H NMR spectrum of oxidized starch (4 mL NaClO solution); (C) ^{1}H NMR spectrum of oxidized starch (8 mL NaClO solution); (D) ^{1}H NMR spectrum of oxidized starch (12 mL NaClO solution); (E) ^{13}C NMR spectrum of native starch; (F) ^{13}C NMR spectrum of oxidized starch (4 mL NaClO solution); (G) ^{13}C NMR spectrum of oxidized starch (8 mL NaClO solution); (H) ^{13}C NMR spectrum of oxidized starch (12 mL NaClO solution).

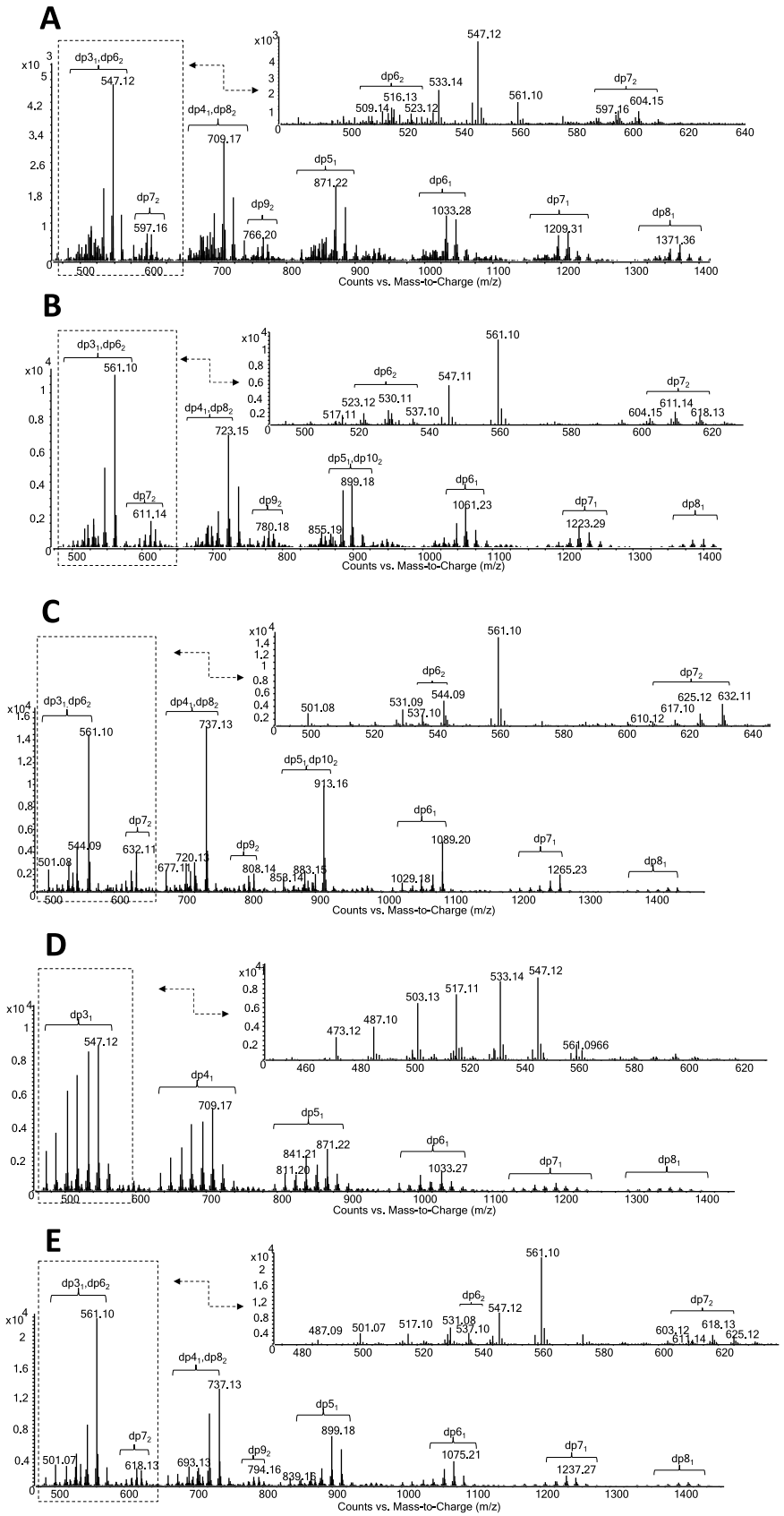

Fig. 3. Mass spectra of oxidized starch oligosaccharides. (A) oligosaccharides carried out with low amount of oxidant (4 mL NaClO solution) at 25 °C; (B) oligosaccharides carried out with relatively high amount of oxidant (8 mL NaClO solution) at 25 °C (C). oligosaccharides carried out with high amount of oxidant (12 mL NaClO solution) at 25 °C; (D) oligosaccharides carried out with high amount of oxidant (12 mL NaClO solution) at 4 °C; (E) oligosaccharides carried out with high amount of oxidant (12 mL NaClO solution) at 50 °C.

3.3. MW analysis using GPC-MALLS

The weight-averaged molecular weights (Mws), number-averaged molecular weights (Mns) and the polydispersities of native starch and oxidized starches were next determined with GPC-MALLS (Table 1). According to the results, the polysaccharide starch was degraded to oligosaccharides in all reactions. The smallest oligosaccharides (Mw, 1.7 kD; Mn, 1.0 kD) resulted from treatment with high concentration of oxidant (12 mL NaClO) at 4 °C while the largest oligosaccharides (Mw, 4.3 kD; Mn, 2.5 kD) resulted from treatment with low concentration of oxidant (4 mL NaClO) at 25 °C. Significant degradation occurred with all TEMPO-NaBr-NaClO oxidations as long as the amount of oxidant was ≥4 mL NaClO (~5% active chlorine). The application of higher amounts of oxidant at each temperature resulted in a lower molecular weight of oxidized products. In this study, relatively harsh oxidation conditions were required to obtain smaller oligosaccharides from which detailed structural information and oxidation pattern could be easier obtained. It might be possible to use a native starch having a higher molecular weight to obtain larger oligosaccharide products. Alternatively, milder oxidation conditions might be used to obtain oxidized starches having higher molecular weights.

3.4. Structural analysis with Q/TOF-MS

Based on the results from molecular weight analysis, a large proportion of the oxidized products were converted to oligosaccharides that are amenable to MS analysis. While the mass spectral signals corresponding to oligosaccharides with a high dp are present at too low intensity to be analyzed, the smaller oligosaccharides that can be analyzed are representative of the structural features of the product mixture. The MS spectra of the oxidized starch oligosaccharides, prepared using different amount of oxidant (4, 8 and 12 mL NaClO solution) at 25 °C, are presented in Fig. 3A–C.

In the spectra of oxidized starch oligosaccharides prepared using a low amount of oxidant (4 mL NaClO solution), several peaks were observed corresponding to each oligosaccharide degree of polymerization (dp) (Fig. 3A–C). The molecular ions, corresponding to the oligosaccharides with lower dp, showed a singly charged ion and is labeled with a subscript "1" next to the dp number. The molecular ions of larger oligosaccharides showed doubly charged ions and are labeled with a subscript "2" next to the dp number. Molecular ion peaks at *m*/*z* 533, 547 and 561 were observed as singly charged ions and were assigned as different oxidized trisaccharides (Fig. 3A insert). The assignments are listed in Table 2, and their structures are shown in Fig. 4A. The hemiacetal group at the reducing end of these oligosaccharides are exposed to oxidant in the reaction and the reducing end sugar ring of these oligosaccharides were all oxidized to carboxyl group at the position 1 and ring opened. (Structure 1, ox1 form) Molecular ions at *m*/*z* 533, 547 and 561 are reducing terminal sugar ring opened trisaccharides with one Glc oxidized to GlcA $(Glc_2GlcA)_{ox1}$, two Glc oxidized to GlcA $(GlcGlcA_2)_{ox1}$ and all three Glc oxidized to GlcA $(GlcA_3)_{ox1}$, respectively. The peak at *m*/*z* 547 was the dominant signal in the trisaccharides. In general, two to three Glc residues were oxidized to GlcA in the major component of every oligosaccharide dp produced under these conditions (Fig. 3A and Table 2). This indicates that the products were partially oxidized and that the larger the oligosaccharide was, the lower its DO was under these conditions.

The molecular ion peaks at *m*/*z* 533, 547 and 561 were also observed in the oxidized trisaccharides (Fig. 3 B insert and Table 2) derived using higher amounts of oxidant (8 mL NaClO solution). The ion peak at *m*/*z* 561, corresponding to fully oxidized trisacchairde $(GlcA_3)_{ox1}$, dominated the spectrum. In general, three to four Glc residues were oxidized to GlcA residues in the major components of oligosaccharides produced under these conditions (Fig. 3B and Table 2). The DOs of these oligosaccharides, derived with higher amount of oxidant, were higher than those observed in the oligosaccharides derived with a low amount of oxidant.

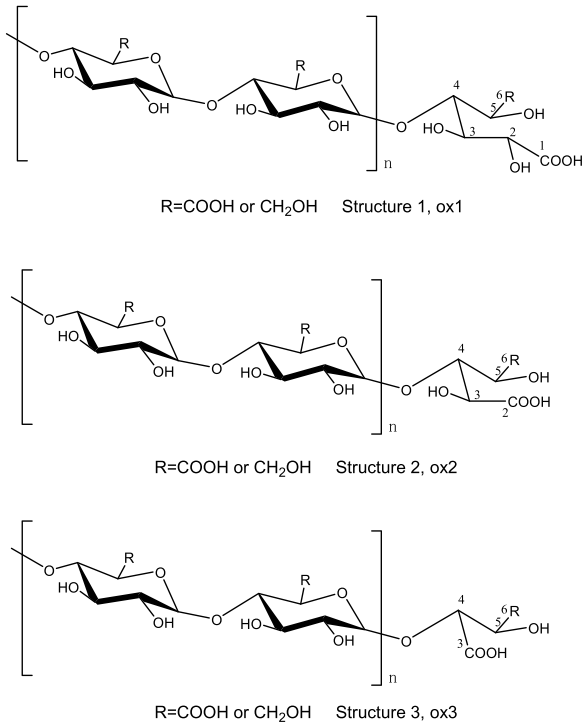

Fig. 4. Structures of different oxidation forms.

The major components of the starch oligosaccharides derived with the highest amount of oxidant (12 mL NaClO solution) at room temperature are about fully oxidized (Fig. 3C), and few partially oxidized products were observed in small oligosaccharides (dp3-dp6). Their molecular ions were assigned at *m*/*z* 561, 737, 913 and 1089, respectively. The magnified spectrum corresponding to oligosaccharide of dp3 was shown in insert of Fig. 3C, and the assignments are provided in Table 2. Thus, higher oxidant produces oxidized starch with higher DO and the starch can be fully oxidized using 12 mL NaClO solution at 25 °C, consistent with the results obtained from IR and NMR spectroscopy.

The spectrum of the oxidized starch oligosaccharides derived with 12 mL NaClO solution at high temperature (50 °C) is shown in Fig. 3E. Some peaks with low intensity corresponding to partially oxidized oligosaccharides were also observed coupled with dominant peaks for fully oxidized oligosaccharides. The MS assignments (Fig. 3E and Table 2) of these oligosaccharides confirmed that their DO was slightly lower than that of fully oxidized products obtained at room temperature, which is consistent with the results from NMR spectroscopy.

Low DOs were observed in the oligosaccharides derived at lower temperature according to their assignments in the MS spectrum (Fig. 3D and Table 2), in which the ion peak of fully oxidized oligosaccharide, for example trisaccharide at *m*/*z* 561, had very low intensity and the ion peaks of partially oxidized oligosaccharides, for example trisaccharides $(GlcGlcA_2)_{ox1}$ and $(Glc_2GlcA)_{ox1}$ at *m*/*z* 547 and 533, were observed as major signals. In addition, more ion peaks were present in each oligosaccharide dp compared to those observed in other products. For instance, four more ion peaks were observed at *m*/*z* 473, 487, 503 and 517 in the spectrum of trisaccharides of this product (Fig. 3E insert). The ions at *m*/*z* 473 and 503 were assigned as the structures of $(Glc_2GlcA)_{ox3}$ and $(Glc_2GlcA)_{ox2}$, (Structure 2 and 3, Fig. 4B and C), which were derived

Table 1
MWs of native starch and oxidized starches.

Samples		Mw (kDa)	Mn (kDa)	Polydispersity
Temperature (°C)	Volume of oxidant (mL)			
–	–	417.3 ± 4.7%	50.9 ± 4.8%	8.2
4	4	3.1 ± 3.7%	1.6 ± 6.1%	1.7
4	8	1.9 ± 3.8%	1.1 ± 6.9%	1.7
4	12	1.7 ± 4.0%	1.0 ± 7.4%	1.7
25	4	4.3 ± 3.7%	2.5 ± 4.9%	1.7
25	8	3.7 ± 5.4%	2.2 ± 3.6%	1.7
25	12	3.6 ± 4.2%	2.1 ± 5.3%	1.7
50	4	3.4 ± 4.0%	2.0 ± 5.6%	1.7
50	8	2.3 ± 4.6%	1.4 ± 6.5%	1.6
50	12	2.2 ± 4.5%	1.3 ± 7.0%	1.6

Table 2
MS Assignments of the oligosaccharides (dp3-6).

			Intensities in MS spectra				
	Structures	*m/z*	4 mL/25 °C	8 mL/25 °C	12 mL/25 °C	12 mL/50 °C	12 mL/4 °C
dp3	$(Glc_2GlcA)_{ox3}$	473	–	–	–	–	Low
	$(Glc_2GlcA)_{ox2}$	503	–	–	–	–	High
	$(Glc_2GlcA)_{ox1}$	533	Low	–	–	–	High
	$(GlcGlcA_2)_{ox3}$	487	–	–	–	Low	Middle
	$(GlcGlcA_2)_{ox2}$	517	–	–	–	Low	High
	$(GlcGlcA_2)_{ox1}$	547	High	Middle	–	Middle	High
	$(GlcA_3)_{ox3}$	501	–	–	Low	Low	–
	$(GlcA_3)_{ox2}$	531	–	–	Low	Low	–
	$(GlcA_3)_{ox1}$	561	Low	High	High	High	Low
dp4	$(Glc_3GlcA)_{ox3}$	635	–	–	–	–	Low
	$(Glc_3GlcA)_{ox2}$	665	–	–	–	–	Middle
	$(Glc_3GlcA)_{ox1}$	695	Low	–	–	–	High
	$(Glc_2GlcA_2)_{ox3}$	749	–	–	–	–	Middle
	$(Glc_2GlcA_2)_{ox2}$	679	–	–	–	–	High
	$(Glc_2GlcA_2)_{ox1}$	709	High	Low	–	–	High
	$(GlcGlcA_3)_{ox3}$	663	–	–	–	Low	–
	$(GlcGlcA_3)_{ox2}$	693	–	–	–	Low	–
	$(GlcGlcA_3)_{ox1}$	723	Middle	High	–	High	Low
	$(GlcA_4)_{ox3}$	677	–	–	Low	Low	–
	$(GlcA_4)_{ox2}$	707	–	–	Low	Low	–
	$(GlcA_4)_{ox1}$	737	–	Middle	High	High	–
dp5	$(Glc_4GlcA)_{ox3}$	797	–	–	–	–	Low
	$(Glc_4GlcA)_{ox2}$	827	–	–	–	–	Low
	$(Glc_4GlcA)_{ox1}$	857	Low	–	–	–	Middle
	$(Glc_3GlcA_2)_{ox3}$	811	–	–	–	–	Low
	$(Glc_3GlcA_2)_{ox2}$	841	–	–	–	–	High
	$(Glc_3GlcA_2)_{ox1}$	871	High	–	–	–	High
	$(Glc_2GlcA_3)_{ox3}$	825	–	–	–	Low	–
	$(Glc_2GlcA_3)_{ox2}$	855	–	–	–	Low	–
	$(Glc_2GlcA_3)_{ox1}$	885	Middle	Middle	–	Low	Low
	$(GlcGlcA_4)_{ox3}$	839	–	–	–	Low	–
	$(GlcGlcA_4)_{ox2}$	869	–	–	–	Low	–
	$(GlcGlcA_4)_{ox1}$	899	–	High	–	High	–
	$(GlcA_5)_{ox3}$	853	–	–	Low	–	–
	$(GlcA_5)_{ox2}$	883	–	–	Low	–	–
	$(GlcA_5)_{ox1}$	913	–	Low	High	High	–
dp6	$(Glc_5GlcA)_{ox3}$	959	–	–	–	–	Low-Low
	$(Glc_5GlcA)_{ox2}$	989	–	–	–	–	Low-Low
	$(Glc_5GlcA)_{ox1}$	1019	–	–	–	–	Low
	$(Glc_4GlcA_2)_{ox3}$	973	–	–	–	–	High-low
	$(Glc_4GlcA_2)_{ox2}$	1003	–	–	–	–	High-High
	$(Glc_4GlcA_2)_{ox1}$	1033	High	–	–	–	High
	$(Glc_3GlcA_3)_{ox3}$	987	–	–	–	–	–
	$(Glc_3GlcA_3)_{ox2}$	1017	–		–	–	–
	$(Glc_3GlcA_3)_{ox1}$	1047	Middle	Middle	–	–	Low
	$(Glc_2GlcA_4)_{ox3}$	1001	–	–	–	Low	–
	$(Glc_2GlcA_4)_{ox2}$	1031	–	–	–	Low	–
	$(Glc_2GlcA_4)_{ox1}$	1061	Low	High	–	Middle	–
	$(GlcGlcA_5)_{ox3}$	1015	–	–	–	Low	–
	$(GlcGlcA_5)_{ox2}$	1045	–	–	–	Low	–
	$(GlcGlcA_5)_{ox1}$	1075	–	Low	–	High	–
	$(GlcA_6)_{ox3}$	1029	–	–	Low	–	–
	$(GlcA_6)_{ox2}$	1059	–	–	Low	–	–
	$(GlcA_6)_{ox1}$	1089	–	–	High	Low	–

from the structure of $(Glc_2GlcA)_{ox1}$. The ions at m/z 487 and 517 were assigned as the structures of $(GlcGlcA_2)_{ox3}$ and $(GlcGlcA_2)_{ox2}$, (Structure 2 and 3, Fig. 4B and C), which were derived from the structure of $(GlcGlcA_2)_{ox1}$. It appears that the oxidation did not stop after the reducing end of oligosaccharides were oxidized to carboxyl group (C1) at lower temperature. Subsequently, with extra oxidant the bond linking C1 and C2 in the reducing end residue was cleaved, and position 2 was oxidized to carboxyl group to form the Structure 2. Furthermore, the bond linked C2 and C3 in the reducing end residue was cleaved and position 3 was oxidized to carboxyl group to form the Structure 3.

4. Discussion and conclusion

In this work, the specific oxidation pattern of soluble starch with TEMPO-NaBr-NaClO system was investigated. According to the results from IR, NMR and MS, higher amount of oxidant (NaClO) is applied, more Glc is oxidized to GlcA at room temperature, and the oxidation is specific. The starch was nearly fully oxidized with 12 mL NaClO solution (~5% active chlorine) at room temperature. Higher temperature did not improve the specific oxidation, but low temperature showed reduced specific oxidation. The activity of TEMPO is low at low temperature, which decreased the DO of oxidized starch, and the extra oxidant then oxidized hemiacetal group directly (ox1 form) and furthermore produced additional structures at the reducing end residues (ox2 and ox3 forms). The NaClO oxidant easily decomposes at high temperatures. Some of oxidant might decompose before it catalytically oxidizes the primary hydroxyl groups on sugar residues at 50 °C. This results in the DO of these products being slightly lower than the products obtained at room temperature.

It is hard to avoid the degradation of oxidized starch in this system. Most polysaccharides in starch were converted to oligosaccharides in the reactions carried out in this work. Much less oxidant could be applied to avoid the significantly degradation, but the corresponding DO would be also very low based on the oxidation trends demonstrated with different amounts of oxidant. In addition, the highest MW was observed with the oxidized product carried out at room temperature, the lowest MW was observed with the oxidized products carried out at 4 °C. The different DO could be one of the reasons as the glycosidic bonds between Glc and Glc was reported weaker than those between GlcA and GlcA (Haar et al., 2010). The lower the DO is, more Glc-Glc bonds are present and these have more chances to be subsequently degraded in the reaction. Finally, this study shows that oxidized starches with different properties can be produced by selection of the conditions used in the TEMPO-NaBr-NaClO reaction system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2016.03.040.

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