Carbohydrate Polymers 93 (2013) 73–80

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Oxidation and degradation of native wheat starch by acidic bromate in water at room temperature

Sanna Komulainen[a], Christof Verlackt[b], Jouni Pursiainen[a], Marja Lajunen[a,*]

[a] Department of Chemistry, University of Oulu, P.O. Box 3000, FI-90014 University of Oulu, Finland
[b] Department of Chemistry, University of Antwerp, Groenenborgerlaan, 171-2020 Antwerp, Belgium

ARTICLE INFO

Article history:
Received 31 October 2011
Received in revised form 28 March 2012
Accepted 1 June 2012
Available online 21 June 2012

Keywords:
Oxidation
Bromate
Synthetic method
Water-soluble oxidized starch

ABSTRACT

Native wheat starch was oxidized by benign acidic bromate in water at room temperature. HPLC-ELSD study indicated that starch degraded in the course of oxidation but it still had a polymeric structure characterized by ^{1}H, ^{13}C, HSQC and HMBC NMR measurements. Products were generally water-soluble fragments but the use of a short reaction time and dilute reaction mixture yielded water-insoluble products. Titration of the products showed, that the increase of the starch content and reaction time increased the content of carbonyl and carboxyl groups in the range of 0.5–2.5% and 1.7–17.2%, respectively, in the product fragments. A mechanism for the oxidation reaction was proposed.

1. Introduction

Starch is a natural carbohydrate polymer obtained from numerous botanical sources like cereals, roots and tubers (Tharanathan, 2005). It is biodegradable, abundantly available and contains various possibilities for modification (Serrero et al., 2010; Zhang, Zhang, Wang, & Wang, 2009). Starch has a granular structure, which consists of a practically linear amylose whose glucose units are linked by $\alpha(1\rightarrow4)$ bonds and a highly branched amylopectin with a $\alpha(1\rightarrow4)$ backbone containing $\alpha(1\rightarrow6)$ branches (Izydorczyk, 2005; Tavares et al., 2004). Starch and its derivatives are widely used in many fields including plastics, food and medical industry (Zhang, Wang, Zhang, Yang, & Wang, 2010), cosmetics and pharmaceuticals (Rinaudo, 2008).

Oxidized starch (Kato, Matsuo, & Isogai, 2003; Wing & Willett, 1997; Zhu, Sjöholm, Nurmi, & Bertoft, 1998) is one of most studied starch derivatives and dialdehyde starch (DAS) is most valuable one (Du et al., 2008). Oxidized starch is useful for its low viscosity, high stability, film forming and binding properties (Kuakpetoon & Wang, 2006) and therefore widely used in paper (Teleman, Kruus, Ämmälahti, Buchert, & Nurmi, 1999) and textile (Kuakpetoon & Wang, 2006) industry for coating and surface sizing purposes. It also interests food industry as a coating and sealing agent in confectionary, as a conditioner of bread as well as a binding agent in batter applications (Kuakpetoon & Wang, 2006).

Dialdehyde starch is usually prepared by oxidation like with sodium periodate (Serrero et al., 2010; Tharanathan, 2005) or sodium hypochlorite (Kuakpetoon & Wang, 2006; Tharanathan, 2005; Wang & Wang, 2003) and it has been further oxidized to a dicarboxylic acid derivative (Teleman et al., 1999). Primary hydroxyl groups of starch glucose units can be selectively oxidized with TEMPO–NaOCl–NaBr system carboxyl groups (Kato et al., 2003).

Bromate is a benign oxidant which has been used for oxidation of secondary alcohols (Natarajan & Venkatasubramanian, 1969; Gupta, Banerjee, & Chatterjee, 1992) together with a metal catalyst (Tomioka, Oshima, & Nozaki, 1982; Yamamoto, Suzuki, & Morooka, 1985) or in plain water with bromide (Pääkkönen, Pursiainen, & Lajunen, 2010, 2012). Bromate cleaves and oxidates C—C bond to ketones (Gupta, Kumar, Sen, Banerjee, & Chatterjee, 1991) or dicarboxylic acids (Bierenstiel, D'Hondt, & Schlaf, 2005). Besides bromate has been used to oxidate primary C-6 hydroxyl groups of a polysaccharide to carboxyl groups in strongly acidic (80–85% H_3PO_4) medium (Pagliaro, 1998; Varela, 2003).

Under neutral conditions molecular bromine has oxidated C-2—C-3 carbon bond of glucose unit via a ketone intermediate (Salomonsson, Andersson, Torneport, & Theander, 1991; Torneport, Salomonsson, & Theander, 1990). The increase of the bromine amount in the oxidation mixture has lowered the molecular weight of starch (Muhrbeck, Eliasson, & Salomonsson, 1990). Optimum conditions for a hypobromite (BrOH) oxidation are alkaline (pH 9) (Hollingsworth & El-Gewely, 1996). Bromine based oxidants are much less studied in starch oxidations than other corresponding halogen compounds even

* Corresponding author. Fax: +358 8 5531629.
E-mail address: Marja.Lajunen@oulu.fi (M. Lajunen).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.06.001

thought they are stronger oxidants than chloro compounds (Hollingsworth & El-Gewely, 1996) and less expensive than iodo compounds. Bromate has been used as a modifier for flour (http://www.inchem.org/documents/jecfa/jecmono/v024je03.htm; http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/ucm191033.htm) but information about products or oxidation mechanism was slight.

We have shown that bromate with bromide in plain acidic water is a rapid and sensitive method for oxidation of secondary alcohols yielding only a negligible amount of side products (Pääkkönen et al., 2012). In this study we have examined the bromate oxidation of native wheat starch in acidic water at room temperature in order to produce water-soluble oxidized starch for a metal complexation studies (Komulainen, Pursiainen, Perämäki, & Lajunen, under review). Besides the oxidation of primary hydroxyl groups, the cleavage of glucose ring between C-2 and C-3 occurred. Products were generally water-soluble, degraded oxidized fragments. A short reaction time with a dilute reaction mixture yielded products insoluble to water. Oxidation products were characterized by HPLC, NMR and titration methods, and a mechanism for the oxidation was proposed.

2. Experimental

2.1. General

All commercially available reagents (Sigma–Aldrich, FF Chemicals, Baker, Ciba, Fluka) were reagent grade and used as received without further purification. Oxidation reactions were performed at room temperature. The oxidized starch precipitated by ethanol was dried at 35 °C for 2 days. NMR spectrum of samples was measured by a Bruker DPX 400 spectrometer at room temperature and the spectrum was reported in ppm the solvent residue with ^{1}H (D_2O, $\delta_H = 4.80$ ppm, when DMSO, $\delta_H = 0$) as the internal standard and ^{13}C (DMSO, $\delta_C = 39.51$ ppm and EtOH as a secondary standard, $\delta_{CH_3} = 18.01$) as the internal standard. The HPLC-ELSD instrument (Shimadzu) consisted of three isocratic pumps (LC-10AD) equipped with a control unit (SCL-10A), a degasser (DGU-14A), an automatic sampler (SIL-10AD), an evaporative light scattering detector (Polymer Laboratories PL-ELS 2100), a guard column (Phenomenex PolySep-GFC-P) and an analytical column (Phenomenex Poly Sep-GFC-Linear 300 mm × 7.8 mm).

2.2. General oxidation procedure and isolation of product

The native wheat starch (3.0 g) was weight out into a flask, 25 mL or 10 mL of distilled water (oxidations A or B, respectively) and 1.05 equiv. of $NaBrO_3$ (2.927 g) per glucose unit was added to each. H_2SO_4 (5.5 mL, 1.77 M) was manually added by dropwise within 60 min. The reaction was stopped by neutralizing the mixture with NaOH (1 M) until pH 9. Addition of ethanol (100 or 250 mL) precipitated the oxidation product, which was filtered under vacuum through sintered glass funnel #4 (porosity 10–16 μm), washed three times with a water–ethanol mixture (30 mL of distilled H_2O and 300 mL of cold ethanol). Ethanol used in the precipitation was evaporated from the filtrate and reused in the next isolation.

2.2.1. Determination of carboxyl group content of oxidized starch

The carboxyl content of the oxidized, water-soluble starch was determined by a titration typical for weak acids (Wing & Willett, 1997). Starch solution (0.5 g of dry, oxidized starch in 300 mL of distilled water) was adjusted to pH 2.5 with 0.1 M HCl at room temperature, stirred for 15 min and titrated to pH 8.3 with standardized 0.1 M NaOH. Native wheat starch was used as blank sample of the determination. The known contents of acetic and citric acid (0.025 and 0.012 M, respectively) were used as standards of the procedure.

Carboxyl content of the sample was calculated as follows (Kuakpetoon & Wang, 2006):

$$\text{Percentage of carboxyl content} = \frac{[(\text{Sample} - \text{Blank})\text{mL} \times \text{molarity of NaOH} \times 100 \times 0.045]}{\text{Sample weight in grams}}$$

2.2.2. Determination of carbonyl group content of oxidized starch

The carbonyl content of the oxidized starches was determined by the following titrimetric method (Kuakpetoon & Wang, 2006): a dry sample of oxidized starch (0.5 g) was suspended into 100 mL of distilled water in a flask and the solution was adjusted to pH 3.2 with 0.1 M HCl. Hydroxylamine reagent (20 mL) (prepared by dissolving 25 g of hydroxylamine hydrochloride in 100 mL of 0.5 M NaOH in a volumetric flask (500 mL) and filled with distilled water to the mark) was added. The reaction mixture was stirred for 4 h. The unreacted hydroxylamine was determined by a rapid titration to pH 3.2 with standardized 0.1 M HCl. A blank experiment with native wheat starch and hydroxylamine reagent was performed similarly.

Carbonyl content of the sample was calculated as follows (Kuakpetoon & Wang, 2006):

$$\text{Percentage of carboxyl content} = \frac{[(\text{Blank} - \text{Sample})\text{mL} \times \text{molarity of HCl} \times 100 \times 0.028]}{\text{Sample weight in grams}}$$

2.3. NMR spectroscopy

For a 1D NMR measurement a sample (ca. 0.3 mL) from the neutralized oxidized mixture A or B was taken, evaporated to dry and dissolved into D_2O. 2D NMR measurements were performed with isolated, oven dried oxidized starch (20 mg in 0.5 mL). All the measurements were performed at room temperature.

2.4. Determination of water-solubility of oxidized starch

Water-solubility of oxidized starches was determined by dissolving a sample of 20 mg into 1 mL of distillated water. Suspension was mixed vigorously and centrifuged with 13 400 g for 2 min. Supernatant was removed and the amount of dried precipitate was measured.

2.5. HPLC-ELSD study of oxidized starch

An oven dried samples (5 mg) from the oxidation A (10 mass% of starch) or from the oxidation B (20 mass% of starch) were dissolved into 1 ml of distilled water. Products from the oxidation B were fully soluble as well as the 24 h product from the reaction A. In the oxidation A the products of the 2 or 5.5 h reaction were not equally soluble. A sample from each solution (10 or 30 μL) was injected to the HPLC equipment. The temperature of the injector and columns was kept at 40 °C by using a column heather (Shimadzu, CTO-10AS). The ELS detector was optimized for the analytes and following parameters were used: the evaporative temperature 80 °C, the temperature of the nebulizer 50 °C and the nitrogen gas flow 0.90 ml/min. The mobile phase was distilled deionized water passed through in-line membrane filters (0.45 μm Millipore, Bedford, MA) at the flow rate of 0.4 ml/min. The system was controlled and data was handled by using LC solution program from the same source.

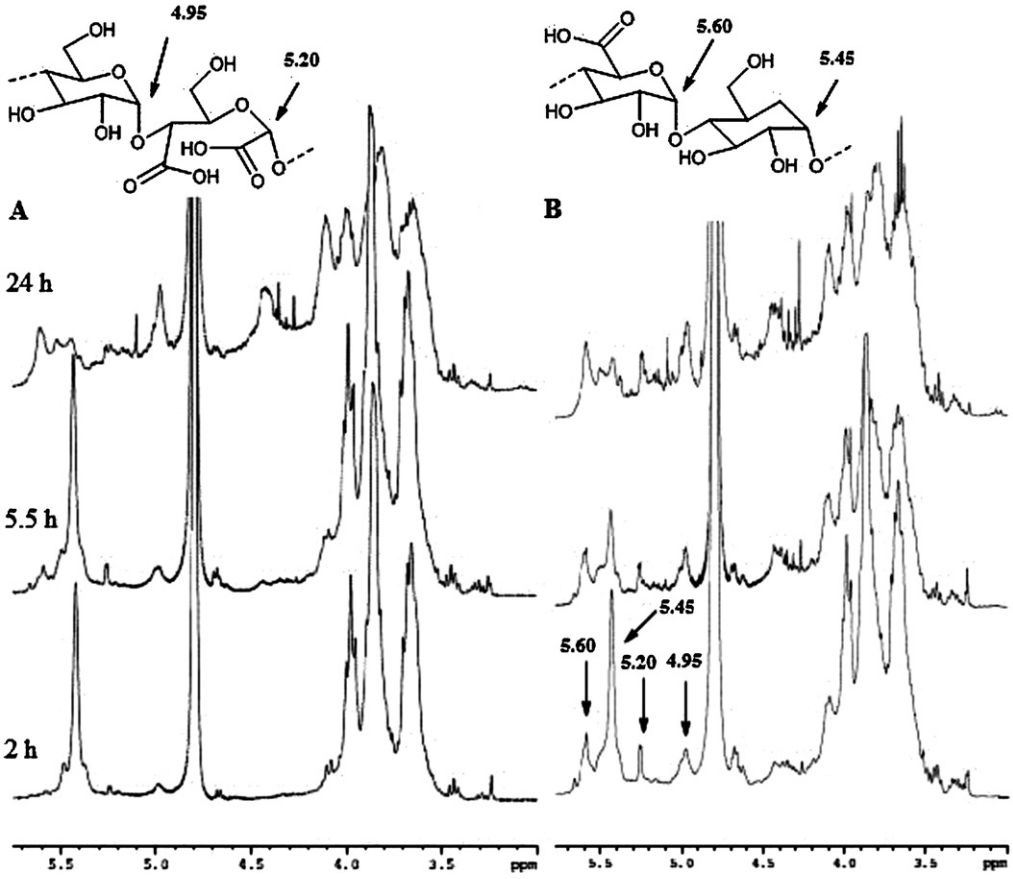


Fig. 1. Progress of starch oxidations by using starch contents of 10 or 20 mass% (A) and (B), respectively, followed by ^{1}H NMR after the reaction times of 2, 5.5 and 24 h.

2.6. FT-IR measurements

FT-IR spectra were measured with a Bruker IFS 66 FTIR spectrometer using KBr pellets. All samples were dried in oven at 35 °C for 2 days and measured by standard KBr pellet method. Spectra were collected with 32 scans at 4 cm^{-1} resolution.

3. Results and discussion

3.1. Oxidation of starch and characterization of products with ^{1}H NMR and HPLC

Small granular native wheat starch was used in this study. It was suspended in water at room temperature. $NaBrO_3$ (1.05 equiv. per glucose unit) was added to the suspension. H_2SO_4 (1.77 M, 0.525 equiv.) was slowly dropped into the stirred reaction mixture within 60 min. The progress of the oxidation was followed by ^{1}H NMR (Fig. 1) and HPLC-ELSD (Kärkkäinen, Lappalainen, Joensuu, & Lajunen, 2011) (Fig. 2). The oxidation degraded starch to water-soluble fragments. The progress of the oxidation was compared between two water contents: 10 and 25 mL (oxidation A and B, respectively).

In the oxidation A (10 mass% of starch) the reaction occurred slowly and the product mixture became water-soluble after 24 h reaction time. The increase of the starch content to 20 mass% (oxidation B) clearly accelerated the oxidation and yielded water-soluble products in 2 h. The amount of H_2SO_4 had a significant effect on the oxidation. As it was reduced from 0.525 to 0.125 equiv. the oxidation as well as the degradation of starch was essentially slower. Starch suspension did not become water-soluble even after 26 h reaction time.

A sample for ^{1}H NMR study was taken from each oxidation mixture after the reaction times of 2, 5.5 and 24 h. The sample was neutralized by 1.0 M NaOH, evaporated to dryness and analyzed. The ^{1}H spectra of the samples from the oxidations A (Fig. 1a) and B (Fig. 1b) show characteristic signals of oxidized starch.

In Fig. 1 the signal at 5.45 ppm was characterized to the anomeric proton of the anhydroglucose unit (AGU). Its intensity decreased along the oxidation, which indicated the degradation of the product and progress of the oxidation (Nilsson, Gorton, Bergquist, & Nilsson, 1996). Fig. 1 shows that various anomeric protons start to appear at the region of 4.95–5.56 ppm along the increasing reaction time. Signals of ring protons started to shift downfield from the region of 3.5–4.05 to 4.1–4.6 ppm indicating an appearance of electronegative groups at vicinal positions (De Graaf, Lammers, Janssen, & Beenackers, 1995). The signal at 4.95 ppm was characterized to the anomeric proton of the glucose unit next to the unit containing a dicarboxyl group and the signal at 5.20 ppm was H-1 of the dicarboxyl unit (Teleman et al., 1999). The signal at 5.56 ppm was characterized to the anomeric proton in glucuronic acid unit (Kato et al., 2003). Along the increasing reaction time the intensity of these characteristic signals increased indicating the progress of the oxidation.

The size of the starch degradation products was roughly estimated by HPLC-ELSD studies (Fig. 2) (Kärkkäinen et al., 2011). Glucose, triose and dextran of 1000 Da (Fig. 2a) and dextrans with increasing size 5000, 25 000, 50 000 and 150 000 Da (Fig. 2b) were used as standards.

Comparison of the chromatograms 2c and 2d of the reaction mixtures A and B, respectively, with standards 2a and 2b (Fig. 2) shows that the acidic reaction conditions degraded starch to shorter

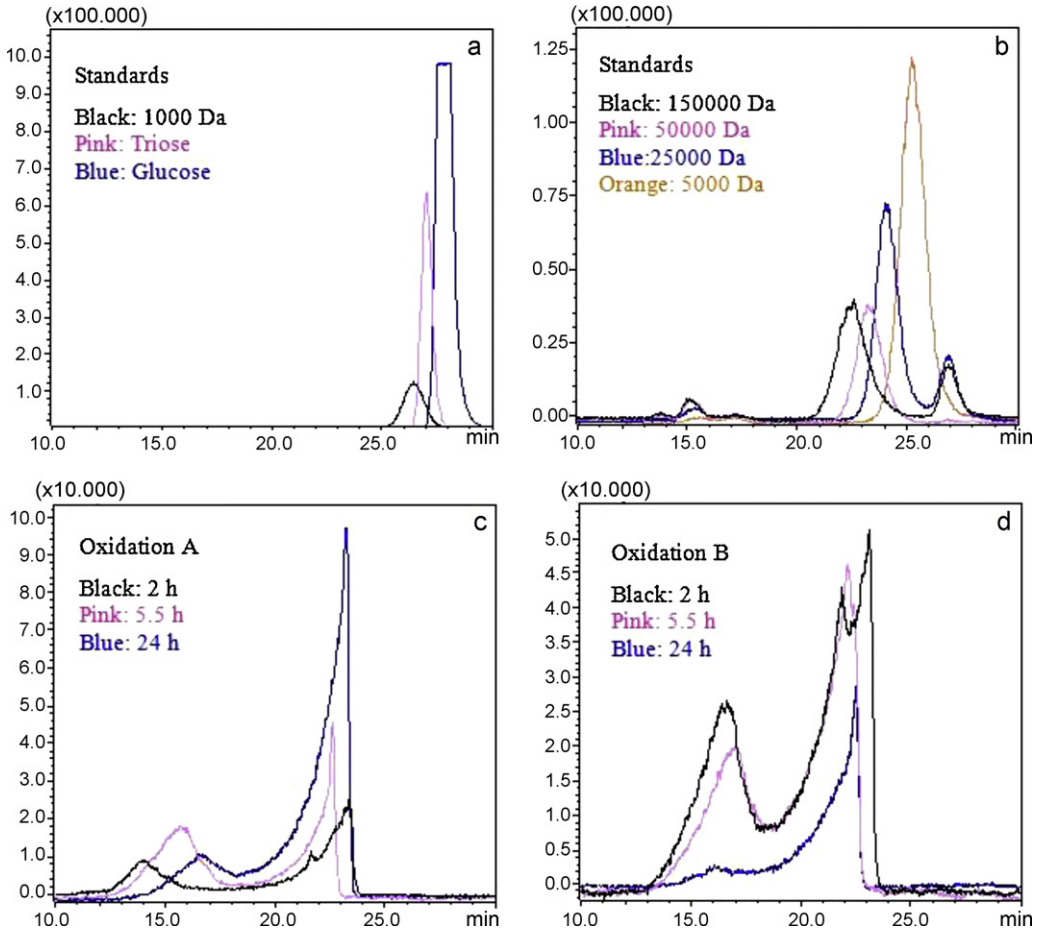


Fig. 2. HPLC-ELSD studies of the standards and samples from the oxidation mixtures A and B. Chromatogram (a) shows the retention times of glucose, triose and 1000 Da sized dextran, (b) the retention times of larger dextrans (5000, 25 000, 50 000 and 150 000 Da), (c) the chromatogram of a sample (5 mg/mL in distillated water) from the oxidation mixture A after variable reaction times and (d) the chromatograms of the corresponding samples from the oxidation mixture B.

fragments but that the product still had the polymeric structure. The size of oxidation products decreased as the reaction time increased. After 2 h reaction time the oxidation mixture A (Fig. 2c) contained mainly fragments of two lengths: long chains (>150 000 Da) at the retention time of 13.9 min and short chains (25 000–150 000 Da) at the retention time of 23.3 min. After the reaction times of 5.5 and 24 h the amount of the long chains had decreased and correspondingly the amount of short chains had increased as seen at the retention times of 15.6, 16.6 and 23.3 min, respectively. The degraded, oxidized product at the retention time of 23.3 min was the main product of the reaction after 24 h reaction time.

In the oxidation B (Fig. 2d) a similar trend was seen. After 2 h reaction time starch had degraded more than in the oxidation A and long and short product fragments were seen at the retention times of 16.6 min and 22.8 min, respectively. Correspondingly, along the reaction the amount of the long fragments at the retention time of 16.6 min decreased and the short fragments at 22.8 min increased indicating that the degradation had happened. After 24 h reaction time starch was nearly completely degraded to short fragments (Fig. 2d).

Based on the HPLC retention times of the product fragments and standards, the size of the long fragments was estimated to be higher than 150 000 Da and the size of the short fragments varied in the range of 5000–150 000 Da.

3.2. Structure evaluation of oxidized starch

A sample from the isolated product of the oxidation B after 24 h reaction time was chosen for 2D NMR measurements. The product was a highly water-soluble mixture of fragments whose size varied mainly in the range of 25 000–150 000 Da. Heteronuclear Single Quantum Coherence (HSQC) spectrum shows a connection over a single bond. HSQC spectrum of the sample (Fig. 3) showed several C—H cross-peaks, which gave a detailed information about the structure. Cross-peaks and supposed corresponding monosaccharide units of the oxidized starch connected to them have been designated with letters **a**–**f**.

Anomeric cross-peaks are shown at higher shifts in the spectrum. Cross-peaks α- and β-H-1α at 5.22 ppm is connected to C-1α at 93.73 ppm, and H-1β at 4.63 ppm connected to C-1β at 97.57 ppm, respectively, belong to the anomeric unit **a** indicating a degradation of starch (Falk, Stanek, & Wutka, 1997; Mora-Gutierrez & Baianu, 1991). The region of H-1 at 5.35–5.45 ppm correlates with C-1 at region 100.5–102 ppm. The signals are characteristic to units b and d (anhydroglucose unit without neighboring oxidized glucose units) (Kato et al., 2003; Teleman et al., 1999). H-1 at 5.56 ppm is connected to the C-1 at 99.18 ppm and is identified to the anomeric position of glucuronic acid unit **c** (Kato et al., 2003). The correlation of H-1 at 5.20 ppm with C-1 at 101.68 ppm is characteristic to the dicarboxyl unit **e** (Salomonsson et al., 1991; Teleman et al., 1999).

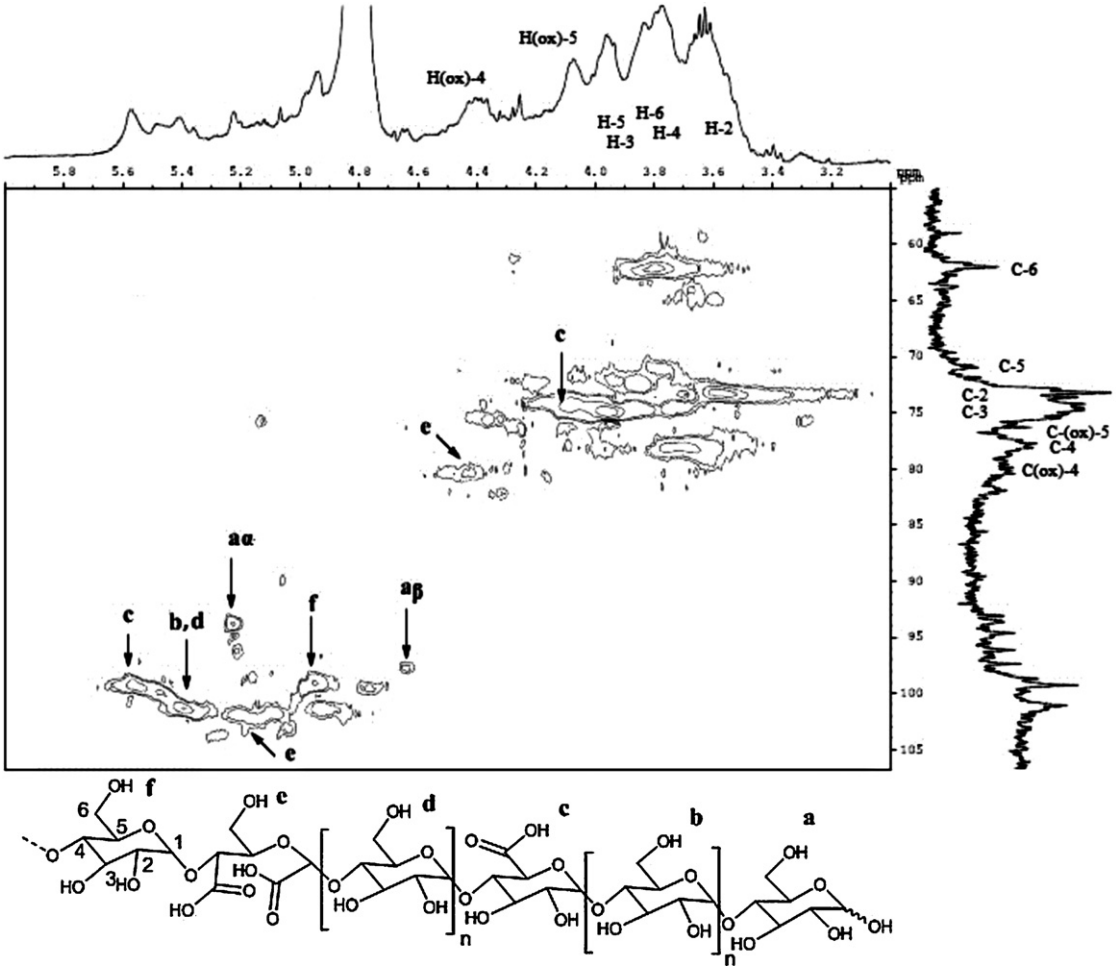


Fig. 3. HSQC spectrum of the oxidized starch sample (60 mg/mL in D_2O) from the oxidation B after 24 h reaction time.

H-1 at 4.95 ppm connected to a C-1 at 98.84 ppm is characteristic to the glucose unit **f** next to dicarboxyl unit **e** (Teleman et al., 1999).

The signals in HSQC spectrum are broad because of the overlap of the adjacent signals. Signal broadening is also affected by hemiacetal structure of the glucose unit (Kato et al., 2003). In Fig. 3 the signal at 4.95 ppm in the proton spectrum has divided into two signals in HSQC spectrum. The other HSQC signal could be a consequence from a unit containing three carboxylic groups. That is supported by the signal H-1 at 5.48 ppm connected to C-1 at 99.90 ppm because carboxylic acid group (unit **c**) shifts the anomeric proton downfield from 5.38 ppm to 5.55 ppm and the anomeric carbon from 102 ppm to 100 ppm. Dicarboxylic acid unit **e** shifts proton from 5.37 to 5.26 ppm and carbon signal downfield from 100.61 to 102.93 ppm. Here proton is shifted downfield and carbon slightly upfield as a combination of these.

Besides anomeric correlations, other identifications can be detected from the HSQC spectra as well. Ring protons of AGU exist at the region of 3.5–4.05 ppm. Ring protons of the oxidized units were shifted downfield and can be characterized from the spectrum. H-4 at 4.5 ppm correlates with C-4 at 79 ppm and is characteristic to the dicarboxyl unit **e** (Teleman et al., 1999). H-5 near 4.1 ppm is connected to C-5 at 71 ppm and was identified to glucuronic unit **c** (Kato et al., 2003). Consequently it was concluded that the oxidation product contained carboxylic groups at the positions C-2 and C-3 besides the primary C-6.

Several minor signals existed at the higher shifts in the HSQC spectra. The proton at 8.44 ppm (Serrero et al., 2010) was connected to carbon at 173.03 ppm (Zhang et al., 2009) and the proton at 8.15 ppm connected to carbon at 143.84 ppm (Kato et al., 2003), which were interpreted to aldehyde protons. The proton at 7.08 ppm (Serrero et al., 2010) was connected to carbon at 116.02 ppm, which is typical for a hemiacetal formation in water (Serrero et al., 2010).

^{13}C NMR spectrum contained several signals at the region of 175–182 ppm, which are characteristic to carbonyl groups. In the HSQC spectrum these signals did not have connections to ring protons.

The heteronuclear multiple bond correlation (HMBC) spectrum shows connections over several bonds (Fig. 4). It revealed that the carbonyl groups were mainly connected to ring protons of higher shifts at 4.1–4.4 ppm region. The carboxyl signals close to 180 ppm were identified to carboxylic groups at C-6 and the signals around 175 ppm to carboxylic groups at C-2 and C-3 (Kato et al., 2003; Teleman et al., 1999).

3.3. FT-IR measurements

FT-IR spectra of the native wheat starch and its oxidation products from the oxidation A are shown in Fig. 5. In the spectrum of starch, the bands can be divided in specific regions. The bands in regions between 3500–3300 cm^{-1} and 3000–2800 cm^{-1} are related to OH and CH stretching, respectively (Pavlovic & Brandao, 2003). The bands in the range of 1500–1200 cm^{-1} are assigned to the coupled modes of vibration that involves C—H bending (Serrero et al., 2010). In the region of 1200–1000 cm^{-1} the bands are due to the stretching of CH—OH and CH_2—OH groups (Serrero et al., 2010). The

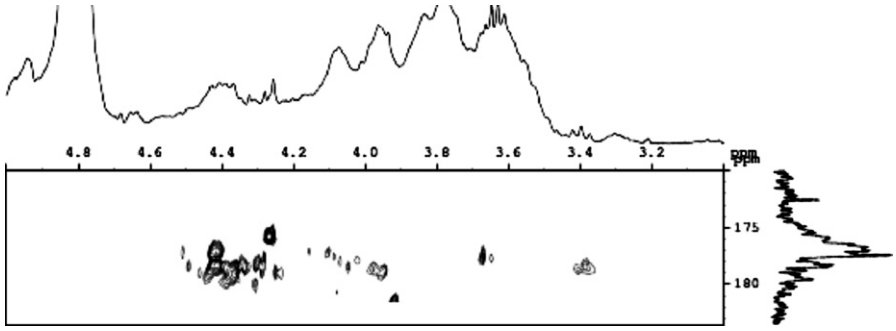


Fig. 4. HMBC spectrum of the oxidized starch sample (60 mg/mL in D_2O) from the reaction B after 24 h reaction time.

region between 1000–700 cm^{-1} shows bands due to the atomic group involved in the anomeric form (Pavlovic & Brandao, 2003).

In the spectrum of the oxidized starches (Fig. 5) the same interpretations can be made. The strong band of the carboxylate group is observed at 1611 cm^{-1} (Williams & Fleming, 1987) which indicates the oxidation of native starch. In the spectrum of 2 h oxidation reaction the bromate signals at 801 and 445 cm^{-1} are observed and refer that with less water-soluble products the isolation process requires longer filtration time.

3.4. Determination of carbonyl and carboxyl group contents of the oxidized starch

The content of carbonyl and carboxyl groups of the oxidized starch was determined by using general titration methods for this purpose (Kuakpetoon & Wang, 2006; Wing & Willett, 1997). A sample was taken from the oxidations A and B after 2, 5.5 and 24 h reaction times and was titrated. Calculated carbonyl and carboxyl contents are presented in Table 1.

After 2 h reaction time the product of the oxidation A had slightly oxidized (Table 1, entry 1). As the oxidation progressed, the content of the carbonyl and carboxyl groups increased (Table 1, entries 2 and 3). The content of both oxidized groups increased in the oxidation B (Table 1, entries 4–6) due to the increased reaction rate as a result of the increased contents of starch and bromate. The carbonyl content started to decrease after 5.5 h reaction time, indicating the decreased rate of the aldehyde formation. In both oxidations the carbonyl content was low compared to the carboxyl content. This indicated that the carbonyl groups oxidized further. Table 1 shows that, the oxidation of the aldehyde to the corresponding acid was faster in more concentrated reaction mixture.

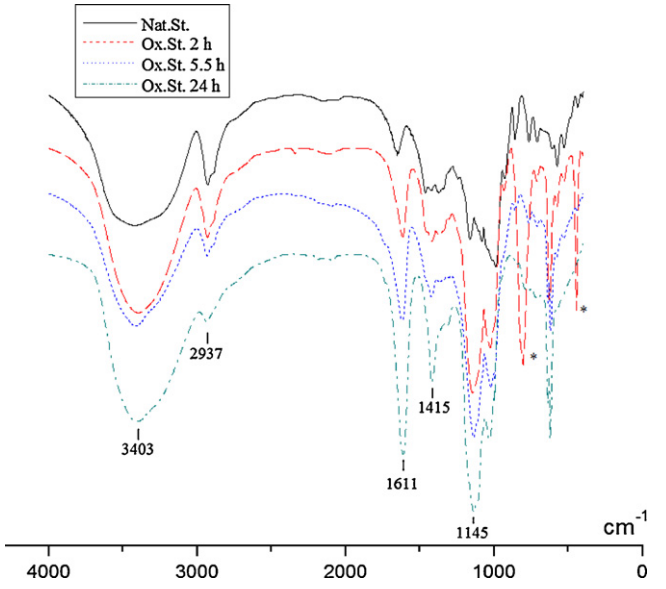


Fig. 5. FT-IR spectra of native wheat starch and its oxidation products from the oxidation A after the reaction time of 2, 5.5 and 24 h. Band of bromated impurities are marked with * in spectrum of 2 h oxidation product.

Table 1
Carbonyl and carboxyl group contents of oxidized starch after variable reaction times.

Entry	Reaction time (h)	Content of starch (mass%)	CO (%)	COOH (%)
1	2	10	0.5	1.7
2	5.5	10	1.6	7.7
3	24	10	2.4	11.5
4	2	20	1.7	6.8
5	5.5	20	2.5	11.6
6	24	20	1.9	17.2

3.5. Water-solubility of oxidized starch

The water-solubility of oxidation products from oxidation A and B was determined and results are presented in Table 2.

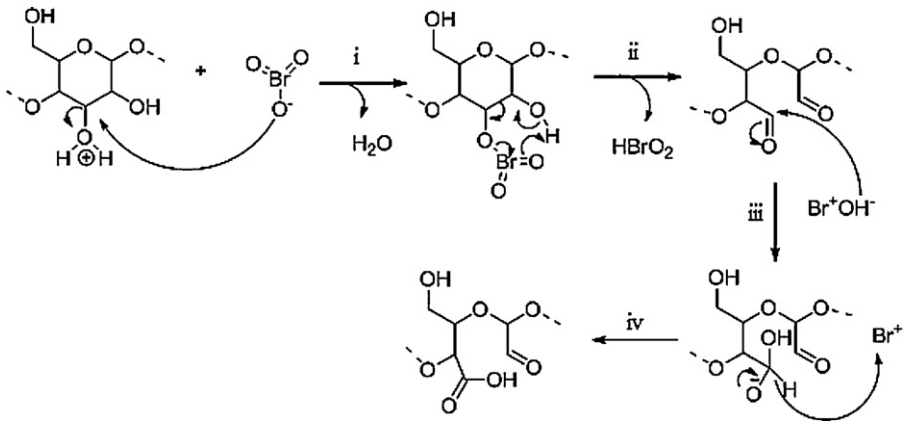
Table 2 indicates that the water-solubility of oxidized starch increased according to the increasing reaction time in both oxidations. Products from the oxidation A (10 mass%) were less soluble than from the oxidation B (20 mass%) but after the reaction time of 24 h products from both oxidations were fully soluble.

3.6. Mechanism of oxidation

In the oxidations of starch by bromine (Salomonsson et al., 1991; Torneport et al., 1990), hypobromite (Hollingsworth & El-Gewely, 1996) or hypochlorite (Kuakpetoon & Wang, 2006) in neutral or alkaline conditions the cleavage of C-2—C-3 bond has been reported to proceed via a ketone intermediate, which was supported by the ^{13}C NMR signal at 206.4 ppm typical for a ketone (Salomonsson et al., 1991). In this study no signal existed at that region. Presumably the mechanism of acidic bromate oxidation differs from that of bromine or hypobromite oxidation. The cleavage of C—C bond between vicinal diols is suggested to occur through a cyclic mechanism (Gupta et al., 1991). The supposed mechanism in the oxidation

Table 2
Water solubility oxidized starch after variable reaction times with two starch contents.

Entry	Reaction time (h)	Content of starch (mass%)	Water solubility (%)
1	2	10	75
2	5.5	10	90
3	24	10	100
4	2	20	99
5	5.5	20	99
6	24	20	100



Scheme 1. Mechanism supposed for the ring cleavage of starch in acidic bromate oxidation (Gupta et al., 1991; Farkas et al., 1949a, 1949b; Kajigaeshi et al., 1986; Metsger & Bittner, 2000).

of starch with acidic bromate is presented in Scheme 1. Under acidic conditions the protonated hydroxyl group leaves as water and the bromate ion attacks to the anhydroglucose unit (i). Bromate ester is formed and reacts through a cyclic mechanism to dialdehyde starch (ii). Dialdehyde starch reacts subsequently with Br_2 or BrOH present in the reaction mixture to the carboxylic acid (iii and iv) (Farkas, Perlmutter, & Schachter, 1949a, 1949b; Kajigaeshi, Nakagawa, Nagasaki, Yamasaki, & Fujisaki, 1986; Metsger & Bittner, 2000).

The oxidation of the primary C-6 hydroxyl produces the glucuronic acid unit as reported before (Pagliaro, 1998) or the hydroxyl group oxidizes with Br_2/BrOH system (Farkas et al., 1949a, 1949b; Pääkkönen et al., 2012).

4. Conclusions

In this contribution we have demonstrated that the acidic bromate oxidation of native wheat starch in water at room temperature produced water-soluble oxidized starch. ^{1}H NMR and HPLC-ELSD studies showed that some degradation of starch happened during oxidation but it still preserved its polymeric structure. Depending on the reaction time and concentration of the oxidation mixture it was possible to oxidize starch with a different oxidation degree and length of the chain. According to NMR studies glucose rings of oxidized starch partly opened and contained carboxyl groups in C-2, C-3 or C-6 positions. The ring opening happened between C-2 and C-3 of the monosaccharide unit. The content of the oxidized groups (carbonyl and carboxyl) in starch was determined by titration methods. Titration of the products showed, that the increase of the starch content and reaction time increased the content of carbonyl and carboxyl groups in the range of 0.5–2.5% and 1.7–17.2%, respectively, in the product fragments. Based on the NMR and titration results, the oxidized starch mainly contained carboxylic groups but aldehyde and hemiacetal groups were detected as well. No signals of keto groups were detected.

The oxidation mixture was strongly acidic and it was supposed that under these conditions the ring cleavage proceeded through the cyclic mechanism as concluded from the fact that no ketone intermediate was detected during NMR measurements.

Comparison of the oxidation products with dextran standards of variable sizes by using HPLC-ELSD verified the degradation of starch under oxidation. It also showed that the product maintained its polymeric nature, although it was strongly degraded. The increase of starch content from 10 to 20 mass% accelerated the oxidation. The oxidation products from the more concentrated oxidation B were completely water-soluble within 2 h reaction time at room temperature. With the lower starch content 24 h reaction time was required to produce water-soluble products. Generally, the oxidation procedure was simple to perform and proceeded at room temperature rapidly. In this work we have showed that bromate salt is an effective oxidant for starch and does not need any additional catalyst or co-oxidants.

Acknowledgements

This work was financially supported by the Inorganic Materials Chemistry Graduate Program and Emil Aaltonen Foundation. The authors thank Mrs. Päivi Joensuu and Ph.D. Johanna Kärkkäinen for their assistance with the HPLC measurements. The group of Ph.D. Sampo Mattila helped kindly with NMR measurements.

References

Bierenstiel, M., D'Hondt, P. J., & Schlaf, M. (2005). Investigations into the selective oxidation of vicinal diols to α±-hydroxy ketones with the $NaBrO_3$/$NaHSO_3$ reagent: pH dependence, stoichiometry, substrates and origin of selectivity. *Tetrahedron*, *61*(21), 4911–4917.

De Graaf, R. A., Lammers, G., Janssen, L. P. B. M., & Beenackers, A. A. C. M. (1995). Quantitative analysis of chemically modified starches by ^{1}H-NMR spectroscopy. *Starch – Stärke*, *47*(12), 469–475.

Du, Y.-L., Cao, Y., Lu, F., Li, F., Cao, Y., Wang, X.-L., et al. (2008). Biodegradation behaviors of thermoplastic starch (TPS) and thermoplastic dialdehyde starch (TPDAS) under controlled composting conditions. *Polymer Testing*, *27*(8), 924–930.

Falk, H., Stanek, M., & Wutka, R. (1997). Structural aspects of partially acetylated degraded amylopectins – A ^{13}C NMR study. *Starch – Stärke*, *49*(12), 488–491.

Farkas, L., Perlmutter, B., & Schachter, O. (1949a). The oxidation of ethyl alcohol in the presence of bromate and bromine. *Journal of the American Chemical Society*, *71*(8), 2833–2835.

Farkas, L., Perlmutter, B., & Schachter, O. (1949b). The reaction between ethyl alcohol and bromine. *Journal of the American Chemical Society*, *71*(8), 2829–2833.

Gupta, K. K. S., Kumar, S. C., Sen, P. K., Banerjee, A., & Chatterjee, H. (1991). Relative reactivities of some mono-ols and vicinal diols towards bromate ion in acid medium. *Journal of Chemical Research, Synopses*, *5*, 118–119.

Hollingsworth, R., & El-Gewely, M. R. (1996). The chemical degradation of starch: Old reactions and new frontiers. In *Biotechnology annual review*. Elsevier. (pp. 281–291)

Izydorczyk, M. (2005). Understanding the chemistry of food carbohydrates. In S. W. Cui (Ed.), *Food carbohydrates* (p. 52). Boca Raton: CRC Press/Taylor & Francis Group.

Kajigaeshi, S., Nakagawa, T., Nagasaki, N., Yamasaki, H., & Fujisaki, S. (1986). Oxidation of alcohols and ethers using sodium bromate–hydrobromic acid system. *Bulletin of the Chemical Society of Japan*, *59*(3), 747–750.

Kato, Y., Matsuo, R., & Isogai, A. (2003). Oxidation process of water-soluble starch in TEMPO-mediated system. *Carbohydrate Polymers*, *51*(1), 69–75.

Komulainen, A., Pursiainen, J., Perämäki, P., & Lajunen, M. Complexation of iron(III) with water-soluble oxidized starch, under review.

Kuakpetoon, D., & Wang, Y.-J. (2006). Structural characteristics and physicochemical properties of oxidized corn starches varying in amylose content. *Carbohydrate Research*, *341*(11), 1896–1915.

Kärkkäinen, J., Lappalainen, K., Joensuu, P., & Lajunen, M. (2011). HPLC-ELSD analysis of six starch species heat-dispersed in [BMIM]Cl ionic liquid. *Carbohydrate Polymers*, *84*(1), 509–516.

Metsger, L., & Bittner, S. (2000). Autocatalytic oxidation of ethers with sodium bromate. *Tetrahedron*, *56*(13), 1905–1910.
Mora-Gutierrez, A., & Baianu, I. C. (1991). Carbon-13 nuclear magnetic resonance studies of chemically modified waxy maize starch, corn syrups, and maltodextrins. Comparisons with potato starch and potato maltodextrins. *Journal of Agricultural and Food Chemistry*, *39*(6), 1057–1062.
Muhrbeck, P., Eliasson, A. C., & Salomonsson, A. C. (1990). Physical characterization of bromine oxidised potato starch. *Starch – Stärke*, *42*(11), 418–420.
Natarajan, R., & Venkatasubramanian, W. (1969). The kinetics of oxidation of secondary alcohols by potassium bromate. *Tetrahedron Letters*, *10*(57), 5021–5024.
Nilsson, G. S., Gorton, L., Bergquist, K.-E., & Nilsson, U. (1996). Determination of the degree of branching in normal and amylopectin type potato starch with ^{1}H-NMR spectroscopy improved resolution and two-dimensional spectroscopy. *Starch – Stärke*, *48*(10), 352–357.
Pagliaro, M. (1998). Autocatalytic oxidations of primary hydroxyl groups of cellulose in phosphoric acid with halogen oxides. *Carbohydrate Research*, *308*(3–4), 311–317.
Pääkkönen, S., Pursiainen, J., & Lajunen, M. (2010). Fast oxidation of secondary alcohols by the bromate–bromide system using cyclic microwave heating in acidic water. *Tetrahedron Letters*, *51*(51), 6695–6699.
Pääkkönen, S., Pursiainen, J., & Lajunen, M. (2012). Room-temperature oxidation of secondary alcohols by bromate–bromide coupling in acidic water. *Synthetic Communications*, *42*(4), 534–540.
Pavlovic, S., & Brandao, P. R. G. (2003). Adsorption of starch, amylose, amylopectin and glucose monomer and their effect on the flotation of hematite and quartz. *Minerals Engineering*, *16*(11), 1117–1122.
Rinaudo, M. (2008). Main properties and current applications of some polysaccharides as biomaterials. *Polymer International*, *57*(3), 397–430.
Salomonsson, A.-C. B., Andersson, R. E., Torneport, L. J., & Theander, O. (1991). A 1H- and 13C-N.M.R. study of bromine-oxidised potato starch. *Carbohydrate Research*, *217*, 221–225.
Sen Gupta, K. K., Banerjee, A., & Chatterjee, H. (1992). Oxidative behaviours and relative reactivities of some Î±-hydroxy acids towards bromate ion in hydrochloric acid medium. *Tetrahedron*, *48*(25), 5323–5330.
Serrero, A., Trombotto, S., Cassagnau, P., Bayon, Y., Gravagna, P., Montanari, S., et al. (2010). Polysaccharide gels based on chitosan and modified starch: Structural characterization and linear viscoelastic behavior. *Biomacromolecules*, *11*(6), 1534–1543.
Tavares, M. I. B., Bathista, A. L. B. S., Silva, E. O., Costa, P. M., Filho, N. P., & Nogueira, J. S. (2004). 13C-NMR study of dipteryx alata vogel starch. *Journal of Applied Polymer Science*, *92*(4), 2151–2154.
Teleman, A., Kruus, K., Ämmälahti, E., Buchert, J., & Nurmi, K. (1999). Structure of dicarboxyl malto-oligomers isolated from hypochlorite-oxidised potato starch studied by 1H and 13C NMR spectroscopy. *Carbohydrate Research*, *315*(3–4), 286–292.
Tharanathan, R. N. (2005). Starch—Value addition by modification. *Critical Reviews in Food Science and Nutrition*, *45*(5), 371–384.
Tomioka, H., Oshima, K., & Nozaki, H. (1982). Cerium catalyzed selective oxidation of secondary alcohols in the presence of primary ones. *Tetrahedron Letters*, *23*(5), 539–542.
Torneport, L. J., Salomonsson, B. A. C., & Theander, O. (1990). Chemical characterization of bromine oxidized potato starch. *Starch – Stärke*, *42*(11), 413–417.
Wang, Y.-J., & Wang, L. (2003). Physicochemical properties of common and waxy corn starches oxidized by different levels of sodium hypochlorite. *Carbohydrate Polymers*, *52*(3), 207–217.
Varela, O. (2003). Oxidative reactions and degradations of sugars and polysaccharides. In *Advances in carbohydrate chemistry and biochemistry*. Academic Press. (pp. 307–369).
Williams, D. H., & Fleming, I. (1987). *Spectroscopic methods in organic chemistry*. London: McGrawhill Book Company. (p. 38).
Wing, R. E., & Willett, J. L. (1997). Water soluble oxidized starches by peroxide reactive extrusion. *Industrial Crops and Products*, *7*(1), 45–52.
Yamamoto, Y., Suzuki, H., & Moro-oka, Y. (1985). Ruthenium-catalyzed oxidation of alcohols with sodium bromate. *Tetrahedron Letters*, *26*(17), 2107–2108.
Zhang, S.-D., Wang, X.-L., Zhang, Y.-R., Yang, K.-K., & Wang, Y.-Z. (2010). Preparation of a new dialdehyde starch derivative and investigation of its thermoplastic properties. *Journal of Polymer Research*, *17*(3), 439–446.
Zhang, S.-D., Zhang, Y.-R., Wang, X.-L., & Wang, Y.-Z. (2009). High carbonyl content oxidized starch prepared by hydrogen peroxide and its thermoplastic application. *Starch – Stärke*, *61*(11), 646–655.
Zhu, Q., Sjöholm, R., Nurmi, K., & Bertoft, E. (1998). Structural characterization of oxidized potato starch. *Carbohydrate Research*, *309*(2), 213–218.

Further reading

Potassium bromide in flour. http://www.inchem.org/documents/jecfa/jecmono/v024je03.htm. Accessed November 2011. International Programme on Chemical Safety. 30.4.2011.

Listing of food additive status. Part I: http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/ucm191033.htm. Accessed November 2011. U.S. Food and Drug Administration (FDA). 30.4.2011.