



Position of modifying groups on starch chains of octenylsuccinic anhydride-modified waxy maize starch



Yanjie Bai^a, Rhett C. Kaufman^b, Jeff D. Wilson^b, Yong-Cheng Shi^{a,*}

^a Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA

^b USDA-ARS, Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan, KS 66502, USA

ARTICLE INFO

Article history:

Received 20 July 2013

Received in revised form 26 November 2013

Accepted 3 December 2013

Available online 9 December 2013

Keywords:

Substitution distribution
Octenylsuccinic anhydride
Modified starch
Waxy maize starch
Starch structure

ABSTRACT

Octenylsuccinic anhydride (OSA)-modified starches with a low (0.018) and high (0.092) degree of substitution (DS) were prepared from granular native waxy maize starch in aqueous slurry. The position of OS substituents along the starch chains was investigated by enzyme hydrolysis followed by chromatographic analysis. Native starch and two OS starches with a low and high DS had β -limit values of 55.9%, 52.8%, and 34.4%, respectively. The weight-average molecular weight of the β -limit dextrin from the OS starch with a low DS was close to that of the β -limit dextrin from native starch but lower than that of the β -limit dextrin from the OS starch with a high DS. Debranching of OS starches was incomplete compared with native starch. OS groups in the OS starch with a low DS were located on the repeat units near the branching points, whereas the OS substituents in the OS starch with a high DS occurred both near the branching points and the non-reducing ends.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Starch is often modified to enhance its functional properties (Wurzburg, 1986). The three general approaches to starch modification are physical, chemical, and enzymatic (Huber & BeMiller, 2009). After modification, the physical and chemical properties of native starch are altered via molecular scission, molecular rearrangement, oxidation, and introduction of substituent chemical groups (Wurzburg, 1986). Substituted starch is of great industrial and academic interest due to significant improvements in selected starch properties.

The structure of substituted starch is often characterised at three levels: universal, granular, and molecular (Huber & BeMiller, 2009). At the universal level, substituted starch is characterised by the degree of substitution (DS) and molar substitution (MS), which reflects the overall extent of modification. At the granular level, substituted starch is characterised by whether substituents occur at the surface or interior of granules, and whether they occur in

amorphous or crystalline regions. At the molecular level, starch is characterised by the substitution position on repeating anhydroglucose units (AGUs) and along the starch chain (Richardson & Gorton, 2003).

Octenylsuccinic anhydride (OSA)-modified starch is one of the chemically modified starches obtained by substitution modification (Sweedman, Tizzotti, Schäfer, & Gilbert, 2013). Food-grade octenylsuccinated (OS) starch is limited legally to treatment with up to 3 wt% OSA reagent, and is commonly prepared by reacting granular starch with OSA in an aqueous system (Sui, Huber, & BeMiller, 2013; Trubiano, 1986). The functional properties of OS starch depend on its DS and distribution of OS groups as well as molecular structure of starch.

The structure of OS starch has been investigated at three levels. First, the overall extent of substitution, or DS, has been determined by titrimetric and nuclear magnetic resonance (NMR) methods (Bai & Shi, 2011; Bai, Shi, Herrera, & Prakash, 2011; Tizzotti, Sweedman, Tang, Schaefer, & Gilbert, 2011). Second, in regards to substitution at the granular level, most of the starch granules are accessible and react with OSA (Bai, Shi, & Wetzel, 2009; Shogren, Viswanathan, Felker, & Gross, 2000). OS substitution occurred primarily in the amorphous region (Bai & Shi, 2011; He, Song, Ruan, & Chen, 2006; Shogren et al., 2000) and X-ray diffraction pattern of a starch is generally not changed after reacting with 3% OSA. However, the distribution of the OS groups is not uniform among individual starch granules at DS 0.05, as determined by FT-IR microspectroscopy (Bai et al., 2009). Backscattered electron imaging of

Abbreviations: OSA, octenylsuccinic anhydride; OS, octenylsuccinate; DS, degree of substitution; OS-S-L, octenylsuccinate starch of DS 0.018; OS-S-H, octenylsuccinate starch of DS 0.092; GPC, gel permeation chromatography; SEC-MALS, size exclusion chromatography with multi-angle light scattering; HPAEC, high-performance anion exchange chromatography.

* Corresponding author. Address: 201 Shellenberger Hall, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA. Tel.: +1 785 532 6771; fax: +1 785 532 7010.

E-mail address: ycshi@ksu.edu (Y.-C. Shi).

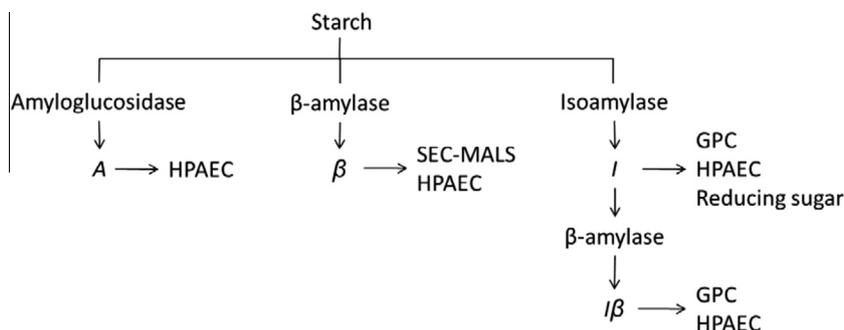


Fig. 1. Enzymatic and analytical methods used to study the structure of octenylsuccinate starch. A = amyloglucosidase hydrolysates; β = β -amylase hydrolysates; I = isoamylase hydrolysates; I β = isoamylase and successive β -amylase hydrolysates; HPAEC = high-performance anion-exchange chromatography; GPC = gel permeation chromatography; SEC-MALS = size exclusion chromatography with multi-angle light scattering.

osmium-stained OS waxy maize starch granules revealed that OS groups were located throughout the starch granules, but X-ray photoelectron spectroscopy suggested that the surface concentration of OS was approximately 3–4 times that of the bulk (Shogren et al., 2000). Also using X-ray photoelectron spectroscopy, Huang et al. (2010) reported that surface concentration of OS groups in modified normal maize starch granule was 2 times that of the bulk. Using confocal FT-IR microscopy (Wetzel, Shi, & Reffner, 2010) and confocal Raman spectroscopy (Wetzel, Shi, & Schmidt, 2010) we found that OS groups were located primarily on the external surface of waxy maize starch granules. In studying OSA modification of high-amylose maize starch, Zhang et al. (2011) reported that the OS groups were distributed throughout granules, but were in higher concentration at the granule surface as examined by confocal laser scanning microscopy. Third, at the molecular level, OS substitution positions on AGUs varied for the modified starches produced by reaction of different physical forms. For modified granular starch, OS substitution occurred primarily at OH-2 and OH-3 for DS up to 0.056 (Bai & Shi, 2011; Bai et al., 2011), whereas for the modified maltodextrin, OS groups were substituted at OH-2, OH-3, OH-6, and at reducing ends (Bai & Shi, 2011). In studying OS waxy maize starch of DS 0.03–0.11, Shogren et al. (2000) suggested that OS substitution was not uniform at the branch level based on the analysis of the debranched chains even though OS groups were distributed throughout the granule.

Based on the literature review, one question that is still unknown is how the OS groups are distributed along starch chains. Substitution distribution along the starch chain has been studied for various chemically modified starches and is often obtained by analyzing the limit dextrans of a modified starch after treatment by a single enzyme or a combination of enzymes. Limit dextrans are oligomeric or polymeric saccharides that remain after exhaustive treatment of starch with a hydrolytic enzyme. Enzymes including α -amylase, β -amylase, amyloglucosidase, isoamylase, and pullulanase are most commonly used for starch structure characterisation (Hizukuri, Abe, & Hanashiro, 2006; Richardson & Gorton, 2003). It has been suggested that the action of starch-degrading enzymes is stopped by the presence of a substituent on a glucose residue or on an adjacent glucose residue (Mischnick & Momcilovic, 2010; Richardson & Gorton, 2003). Therefore, the substitution distribution can be inferred by comparing the structures of enzyme hydrolysates of a native starch with that of its modified components and this has been a subject of two reviews (Mischnick & Momcilovic, 2010; Richardson & Gorton, 2003).

In this study, OSA-modified starches with a low and high DS were prepared from granular waxy maize starch in an aqueous system. Waxy maize starch contains only amylopectin, highly branched polymers, and is the source of most commercial OS starch products (Sweedman et al., 2013). The recovered modified starches were solubilized and hydrolysed exhaustively by various

enzymes including amyloglucosidase, isoamylase, and β -amylase followed by chromatographic analysis as shown in Fig. 1. Using light scattering to analyse the limit dextrans after exhaustive hydrolysis by exo-acting enzymes, we were able to determine and compare the MW of the limit dextrans of the modified starches with that of the limit dextrin from the native starch. The distribution of OS groups along starch chains were deduced from the comprehensive analyses of the enzyme hydrolysates.

2. Materials and methods

2.1. Materials

Waxy maize starch was obtained from National Starch LLC (Bridgewater, NJ). Amyloglucosidase from *Rhizopus* sp. (A-7255) were purchased from Sigma–Aldrich (St. Louis, MO). Based on the information from Sigma–Aldrich, enzyme activity of amyloglucosidase was over 5000 units/g solid and one unit liberated 1.0 mg of glucose from soluble starch in 3 min at pH 4.5 at 55 °C. β -Amylase (Diazyme BB) was obtained from Danisco (Madison, WI). The enzyme activity was expressed as degrees Diastatic Power (DP°) and was 1320 ± 90 DP°/g as indicated by the product brochure. One DP° unit is defined as the amount of enzyme that produces sufficient reducing sugars from a standard starch substrate to reduce 5 ml of Fehling's solution. The starch substrate is hydrolysed at pH 4.6 and 20 °C for 30 min. The reducing ends produced during the hydrolysis are measured by a titrimetric procedure using alkaline ferricyanide. Isoamylase (EC 3.2.1.68) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The enzyme activity was 1.41×10^6 isoamylase activity units (IAU)/g, where 1 unit is an increase in absorbance of 0.008 at 610 nm when incubating the enzyme with soluble waxy maize starch in the presence of iodine for 30 min at pH 3.5 and 40 °C (Joint FAO/WHO Expert Committee on Food Additives., 2007). Glucose, maltose, and a series of oligosaccharides from maltotriose to maltoheptaose were purchased from Sigma–Aldrich (St. Louis, MO). Dextran standards were purchased from American Polymer Standards Corp. (Mentor, OH). Other chemicals were analytical grade.

2.2. OSA modification

The OSA reaction was performed as previously described (Bai & Shi, 2011). Briefly, a starch slurry (250 g) of 40% solid content was adjusted to pH 7.5 by 3 wt% sodium hydroxide. OSA (3% or 15% based on the weight of starch) was added to the starch suspension and the reaction was maintained at pH 7.5 by 3 wt% sodium hydroxide. After the pH remained stable for 30 min, the reaction was stopped by adjusting to pH 6 by adding 1 M hydrochloric acid. OS starch was recovered by filtration, washed with methanol

(300 mL), and the product dried in an oven at 45 °C overnight. The DS of OS starches was determined by NMR as previously described (Bai & Shi, 2011).

2.3. Amyloglucosidase hydrolysis

Waxy maize starch or OS starches (0.1 g) was dispersed in 5 mL acetate buffer (0.05 M, pH 4.5) and the slurry was heated with agitation in a boiling water bath at 100 °C for 1 h. After cooling to 55 °C, amyloglucosidase (1% based on the weight of starch or 5 units of activity) was added, and the digest was incubated at 55 °C for 24 h. Another 1% (based on the weight of starch) amyloglucosidase was added to the first starch digest and the mixture was incubated for another 24 h. The resulting solution was diluted and its glucose content was determined by high-performance anion exchange chromatography (HPAEC). The percentage amyloglucosidase hydrolysis was calculated as:

$$\frac{\text{Weight of glucose in the hydrolysate} \times 0.9}{\text{Weight of starch}} \times 100\%$$

2.4. β -Amylase hydrolysis

Waxy maize starch or OS starch (0.5 g) was dispersed in 20 mL acetate buffer (0.01 M, pH 5.5) and the mixture was heated in a boiling water bath at 100 °C for 1 h. After cooling to 55 °C, β -amylase (2% wt% based on the weight of starch) was added, and the digest was incubated at 55 °C for 24 h followed by heating in a boiling water bath for 30 min which denatured the enzymes. Starch hydrolysates were diluted and the solution was assayed by HPAEC and size-exclusion chromatography with multi-angle light scattering (SEC-MALS). The amount of maltose liberated from a starch was used to calculate its β -limit value as follows:

$$\frac{\text{Weight of maltose in the hydrolysate} \times 0.95}{\text{Weight of starch}} \times 100\%$$

2.5. Isoamylase hydrolysis and successive β -amylolysis

Waxy maize starch or OS starch (0.5 g) was dispersed in 25 mL acetate buffer (0.05 M, pH 3.5) and heated in a boiling water bath at 100 °C for 1 h. After cooling to 50 °C, isoamylase (1% wt%) was added and incubated at 50 °C for 24 h. A 15 mL portion of the reaction mixture was freeze dried and the debranched starch was assayed for reducing sugar (dextrose equivalents) and chain-length distribution by GPC and HPAEC. The remainder of the isoamylase hydrolysate (10 mL) was successively hydrolyzed by β -amylase as follows. The starch solution after debranching was cooled to 40 °C and adjusted to pH 4.8 by adding 0.05 M sodium acetate. β -amylase (2 mL) was added, and the starch solution was incubated at 40 °C for 24 h. The solution was freeze-dried and the amount of maltose liberated by β -amylase was determined by HPAEC. The percentage β -amylase hydrolysis (β -limit) was calculated as stated above.

2.6. Gel permeation chromatography (GPC)

GPC analysis was performed as previously described (Cai, Shi, Rong, & Hsiao, 2010). Starch hydrolysates (4–16 mg) were dissolved in DMSO (4 mL) by stirring at room temperature for 12 h. The solutions were filtered through a 2 μ m filter (Millex-AP, Millipore, Billerica, MA) and injected by an autosampler into a PL-GPC 220 system (Polymer Laboratories Inc., Amherst, MA, USA) with three Phenogel columns (00H-0642-K0; 00H-0644-K0; 00H-0646-K0; Phenomenex Inc., Torrance, CA, USA), one guard

column (03B-0290-K0, Phenomenex Inc., Torrance, CA, USA), and a differential refractive index detector. The eluting solvent was DMSO containing with 0.5 mM NaNO₃, and the flow rate was 0.8 mL/min. The column oven temperature was controlled at 80 °C. Standard dextrans (American Polymer Standards Co., Mentor, OH, USA) with different MWs were used for MW calibration. GPC results were analysed using Cirrus™ GPC Software Version 3.0 (Agilent Technologies, Santa Clara, CA).

2.7. SEC-MALS

The SEC-MALS system consisted of a chromatograph (1200 HPLC, Agilent, Palo Alto, CA.), a multi-angle light scattering (MALS) detector (DAWN® HELEOS® II, Wyatt Technology, Santa Barbara, CA), a Shodex OHPak SB-806 M HQ column in series with a Shodex OHPak SB-805 HQ column (Showa Denko America, New York, NY) and a Shodex OHPak SB-G guard column. A refractive index detector was used to determine mass flow rate ($dn/dc = 0.147$). The MALS detector was calibrated with toluene and normalised with bovine serum albumin (BSA). SEC-MALS experiments were performed at a column temperature of 55 °C with 0.1 M NaNO₃ as eluent and a flow rate of 0.5 mL/min. Starch solution (10 mg/mL) was filtered through a 1 μ m filter before injecting. The injection volume was 100 μ L. Data was analysed using Astra 6 software.

2.8. HPAEC

HPAEC was done on a Dionex ICS-3000 chromatograph (Dionex Corp., Sunnyvale, CA) equipped with a pulsed amperometric detector, a guard column, a CarboPac™ PA1 analytical column, and an AS-DV autosampler. Eluent A was 150 mM NaOH, and eluent B was 150 mM NaOH containing 500 mM sodium acetate. The gradient program for debranched starch was: 85% of eluent A at 0 min, 30% at 20 min, 25% at 30 min, 0% at 35 min, and 85% at 41 min. The gradient program for hydrolysates from amyloglucosidase and β -amylase was: 85% of eluent A at 0 min, 45% at 15 min, 40% at 20 min, 0% at 21 min, and 85% at 26 min. The separations were carried out at 25 °C with a flow rate of 1 mL/min. Peak assignments were done with reference to standard samples of glucose, maltose, and a series of multi oligosaccharides of DP 3–6.

2.9. Reducing sugar analysis

Dextrose equivalent (DE) of debranched starches was determined by Nelson-Somogyi reagent (Somogyi, 1952). DP was calculated as 100/DE. The mole percentage of resistant branches was calculated as:

$$\frac{\text{mole of dextrin from native starch} - \text{mole of dextrin from OS starch}}{\text{mole of dextrin from native starch}} \times 100\%$$

2.10. Statistical analysis

Each sample was measured in triplicates and means and standard deviations were reported. Means were compared with Student's *t* test and least significant differences were computed at $p < 0.05$.

3. Results and discussion

3.1. Amyloglucosidase hydrolysis

Native starch was 96.2% converted to glucose by amyloglucosidase (Table 1), which agreed with the results of other workers (Hood & Mercier, 1978; Richardson et al., 2003). In contrast, OS

Table 1

Structural characteristics of native waxy maize starch and octenylsuccinated starches with degrees of substitution of 0.018 (OS-S-L) and 0.092 (OS-S-H).

Starch	β			I			$I\beta$
	% Hydrolysis	β -Limit value (%)	β -Limit dextrin Mw ($\times 10^6$)	DE	DP	Resistant branches (%)	% Hydrolysis
Native starch	96.2 \pm 1.3c	55.9	1.08 \pm 0.07a	4.5 \pm 0.0c	22 \pm 0a	–	100.0 \pm 0.3c
OS-S-L	84.7 \pm 2.3b	52.8	1.26 \pm 0.02b	3.9 \pm 0.1b	26 \pm 1b	13.3 \pm 0.3a	91.1 \pm 0.1b
OS-S-H	58.0 \pm 1.8a	34.4	7.04 \pm 0.10c	3.3 \pm 0.1a	31 \pm 1c	26.7 \pm 0.3b	70.6 \pm 1.5a

A = amyloglucosidase hydrolysates; β = β -amylase hydrolysates; I = isoamylase hydrolysates; $I\beta$ = isoamylase and successive β -amylase hydrolysates; DE = dextrose equivalent; DP = number average degree of polymerisation.

Numbers in the same column followed by a letter in common are not significantly different at $p < 0.05$.

starch of DS 0.018 (OS-S-L) and 0.092 (OS-S-H) gave 84.7% and 58.0% hydrolysis, respectively (Table 1). Amyloglucosidase is an exo-acting enzyme that releases glucose by hydrolyzing α -1,4 and α -1,6 linkages from the non-reducing end of a starch chain. If the enzyme encounters an OS-substituted glucose unit, the action of amyloglucosidase would stop at the modifying group because the substitution groups interfere with the binding between enzyme and starch substrates (Hood & Mercier, 1978; Richardson et al., 2003). The glucose units released by amyloglucosidase would contain no OS substituents, whereas the residual dextrans would contain all the OS groups. Based on the amount of glucose released by glucoamylase (Table 1), and invoking the cluster model of amylopectin (Hizukuri et al., 2006), the OS starch with a low DS (OS-S-L) contained almost no OS groups near the non-reducing ends as opposed to the product with a high DS (OS-S-H) which did.

3.2. β -Amylase hydrolysis

Native starch and the two OS starches were hydrolyzed exhaustively by β -amylase, and the limiting β -amylolysis value was 55.9%, 52.8% and 34.4%, respectively (Table 1). The β -limit value for the native starch is in agreement with that previously reported (Manners, 1989). For the two modified starches with a low and high DS, the β -limit value was reduced 3.1% and 21.5% from that of the native starch, respectively, indicating the action of β -amylase was inhibited by OS substituents on starch chains. Inhibition on β -amylolysis has been reported for many chemically modified starches (Hood & Mercier, 1978; Kavitha & BeMiller, 1998; Richardson et al., 2003). β -Amylase is an exo-acting hydrolase that removes unsubstituted maltosyl units from the non-reducing ends of amylopectin, leaving the inner part (β -limit dextrin) intact (Robyt, 2009). In agreement with our results on glucoamylase hydrolysis, β -amylolysis of the OS-S-L product gave a β -limit value that was close (-3.1%) to that of the native starch, whereas that of OS-S-H product was considerably reduced (-21.5%), which was reflected by its much lower β -limit value.

The β -limit dextrin generated from waxy maize starch had a weight-average molecular weight (Mw) of 1.08×10^6 (Table 1). The Mw of the β -limit dextrin from OS-S-L was 1.26×10^6 , which was only slightly higher than that of the native starch (Table 1), indicating that OS substitutions were close to the outer branching points of the starch molecules in the OS starch with a low DS. In contrast, the β -limit dextrin of OS-S-H had an Mw of 7.04×10^6 which was 7 times that of native starch and OS-S-L (Table 1 and Fig. 2). However, based on the β -limit value of the unmodified starch (amylopectin), which was 55.9% (Table 1), the original amylopectin molecule would have ca. double size of the Mw of β -limit dextrin, not 7 times that size. The unusual high Mw value of the β -limit dextrin with a high DS compared with that of the β -limit dextrin from unmodified starch suggest that starch molecules may be associated in water due to hydrophobic interaction. Other solvent (e.g. DMSO) and columns need to be used to confirm this in the future study.

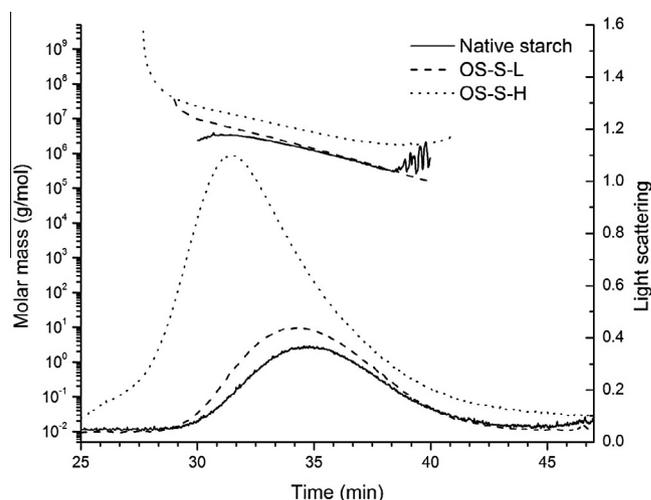


Fig. 2. Molecular weight distributions (bottom) and molar masses (top) of β -limit dextrans of native waxy maize starch and octenylsuccinated starches with degrees of substitution of 0.018 (OS-S-L) and 0.092 (OS-S-H), all determined by SEC-MALS.

3.3. Isoamylase hydrolysis and successive β -amylolysis

3.3.1. Isoamylase hydrolysis

Isoamylase is also known as a debranching enzyme for starch because it hydrolyzes the α -1,6 linkages located in the interior of amylopectin molecules. The product of isoamylase debranched unmodified starch is a mixture of maltooligosaccharides varying in DP, mostly below 80 anhydroglucose units (Robyt, 2009). The average DP of the debranched waxy maize starch and the two OS starch products were 22, 26, and 31, respectively (Table 1). The greater DP values of debranched OS-modified starches, as indicated by their low dextrose equivalent (DE) value, verify that the action of isoamylase was inhibited by OS groups. Considering that native starch was 100% debranched by isoamylase, branches resistant to isoamylase were calculated to be 13.3% for the OS starch with a low DS (OS-S-L) and 26.7% for the OS starch with a high DS (OS-S-H) (Table 1).

Inhibition of isoamylase action by OS groups also was reflected in the molecular-size distribution of the debranched native starch and OS starches as determined by GPC (Fig. 3 and Table 2). Debranched waxy maize starch gave a bimodal distribution as observed by previous work (Bertoft, 2004; Biliaderis, 1982). Approximately 22% of molecules were eluted from 26 to 29 min (Fraction 1), which are thought to be long B2 and B3 chains in the cluster model. The remaining molecules of $\sim 78\%$ were eluted from 29 to 34 min (Fraction 2) and those are believed to be short A and B1 chains. The high-molecular size fraction (Fraction 1) increased to ca. 32% and 68%, respectively, in the debranched OS-S-L and OS-S-H products (Table 2). Moreover, the average DP of Fraction 1 was much larger for debranched OS-S-H compared

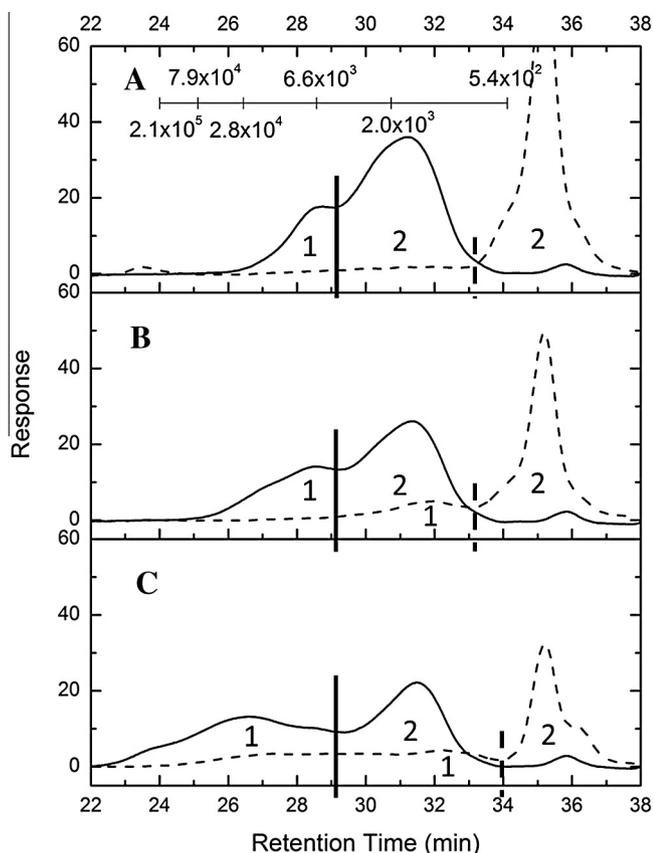


Fig. 3. Molecular size distributions of debranched starch before (—) and after successive β -amylolysis (-----). A = native waxy maize starch, B and C = octenylsuccinate starches with degrees of substitution, respectively, of 0.018 and 0.092. The large and small molecular weight fractions were assigned as fraction 1 and 2, respectively. The cut-off points for debranched starch before and after β -amylolysis were approximately 29 and 34 min, respectively.

Table 2

Large (Fraction 1) and small (Fraction 2) molecular size fractions of debranched native starch and octenylsuccinate (OS) starch before (*I*) and after successive β -amylolysis (*I* β) as determined by GPC.

Treatment	Sample	Fraction 1		Fraction 2	
		DP	% Area	DP	% Area
<i>I</i>	Retention time (min)	<29		>29	
	Native starch	68	21.7	12	78.3
	OS starch of DS 0.018	105	32.1	12	67.9
	OS starch of DS 0.092	500	67.9	105	32.1
<i>I</i> β	Retention time (min)	<34		>34	
	Native starch	ND	ND	2	100.0
	OS starch of DS 0.018	10	15.9	2	84.1
	OS starch of DS 0.092	89	36.9	2	63.1

DP = number average degree of polymerisation; DS = degree of substitution; ND = not detected.

to debranched OS-S-L, which reinforces the postulate that more OS was substituted near the branching points in OS-S-H.

Unit-chain length profiles of debranched starches were determined by HPAEC. Debranched starches showed a range of DP's between 6 and 64 (Fig. 4A). The distribution pattern found for the waxy maize starch was similar to previous reports (Bertoft, 2004), where short chains of DP 6 to DP 32 were assigned to A and B1 chains. The distribution pattern also showed long chains of DP 33 to 64, and these chains were assigned to B2 and B3 chains in the cluster model. HPAEC chromatograms of the starches appeared to be different compared with the corresponding GPC

chromatograms especially in the long chain region. This is due to the decrease in detector response with the increasing degree of polymerisation (Henshall, 1999). It has been suggested that the peak area from amperometric detection is not directly proportional to the molar concentrations of the maltooligosaccharides of different lengths (Koizumi, Fukuda, & Hizukuri, 1991; Shi & Seib, 1992). However, when the total amount of injected dextrans is the same for each sample, as was the case in this study, then a comparison can be made for each DP oligomer between samples. OS starches had reduced areas compared to the native starch over the entire unit-chain length profile (Fig. 4A). Peak area differences were calculated by subtracting the area of each unit chain length of the native starch from the corresponding area of the OS starches. The difference represents the relative number of chains that were not released by debranching. The more negative the value, the fewer of the chains with that length was released. The OS starch with a high DS (OS-S-H) had significantly fewer starch chains released than the OS starch with a low DS (OS-S-L) (Fig. 4B). Moreover, chains between DP 15 and 32 seemed to be most inhibited by the debranching enzyme and could not be cleaved, suggesting that OS groups were located mostly on or near those chains.

3.3.2. β -Amylase hydrolysis

To further elucidate the structure of the OS starches, we used β -amylase to hydrolyze debranched native and OS starches. β -Amylase was able to completely convert debranched waxy maize starch to 100% maltose equivalents (Table 1). HPAEC of the β -amylase converted debranched starch (*I* β hydrolysate) showed predominantly maltose with little glucose, confirming that the molecules of debranched waxy maize starch were linear. For debranched OS-S-L, ca. 91% was converted to the theoretical yield of maltose by β -amylase (Table 1). These results further suggest that OS groups in OS starch with a low DS were located mostly near the branching points of amylopectin. In contrast, the β -amylolysis of the debranched OS-S-H gave only ca. 70% maltose equivalents (Table 1), and that *I* β hydrolysate contained a large molecular fraction with an average DP of ca. 89, compared to 10 for OS-S-L (Table 2). These results suggest the presence of OS groups near the non-reducing end of the starch chains in the OS starch with a high DS.

3.4. A model of substitution distribution in OSA-modified starches

Chemical substitution of starch granules has been suggested to occur preferentially in the amorphous regions of the partially crystalline granules (van der Burgt et al., 1999) although the exact location of modifying groups on starch molecules is not always clear. For example, substitution occurred near the branching points as well as at the non-reducing ends for acetylated (DS 0.06) distarch phosphate made from smooth pea starch and for hydroxypropylated (DS 0.09) distarch phosphate made from waxy maize starch, but the distribution of acetyl and hydroxypropyl groups within the starch granule was different (Biliaderis, 1982). Hydroxypropylation was more uniform throughout the starch granule, whereas acetylation occurred in certain parts of the granule. In this study, the substitution groups in OS starches of low DS were found close to the branch points of starch molecules; however, at a DS of 0.092, OS starch contained modifying groups near their non-reducing ends (model in Fig. 5). In the initial stage of the reaction of OSA with starch granules, the OS groups are substituted near the branching points of the starch. Those initial substituents may cause some limited swelling of granules in the slightly alkaline (pH 8.5) reaction medium, which exposes the non-reducing ends of starch chains to reaction with OSA.

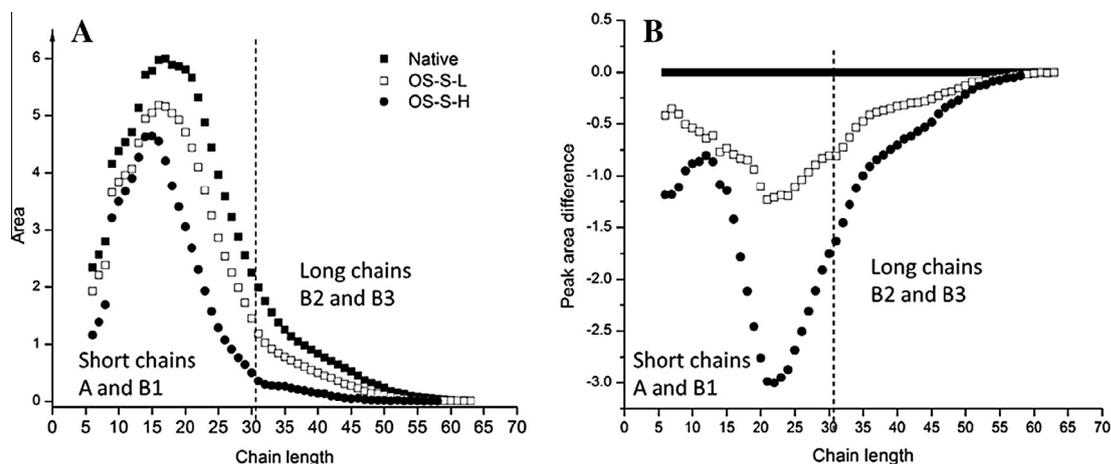


Fig. 4. Chain length distributions (A) and peak area differences (B) of debranched waxy maize starch and octenylsuccinated starches with degrees of substitution (DS) of 0.018 (OS-S-L) and 0.092 (OS-S-H). Peak area differences were the area of each peak from an OS-substituted starch minus that of waxy maize starch.

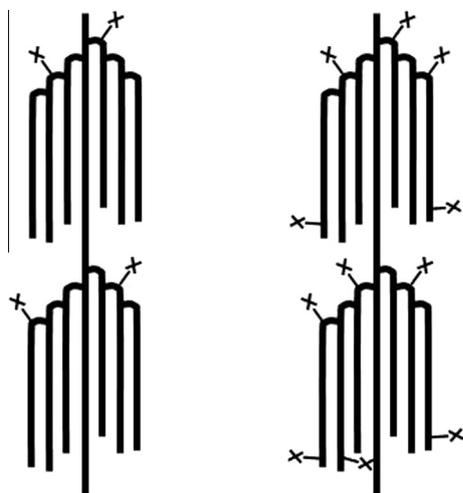


Fig. 5. Proposed model structures for octenylsuccinated starches with degrees of substitution of 0.018 (left) and 0.092 (right).

4. Conclusions

The distribution of OS groups along starch chains in OS starches was revealed by enzyme hydrolysis followed by structural analysis of reaction products. At a low DS of 0.018, most OS groups were located near the branching points of the amylopectin and the MW of its β -limit dextrin was close to that of the β -limit dextrin of the unmodified starch. As DS increased to 0.092, modifying groups were located near the branching points and on or close to non-reducing ends.

Acknowledgements

This is contribution number 13-212-J from the Kansas Agricultural Experiment Station. We thank Dr. Paul Seib for reviewing the manuscript and helpful discussions.

References

Bai, Y., & Shi, Y.-C. (2011). Structure and preparation of octenyl succinic esters of granular starch, microporous starch and soluble maltodextrin. *Carbohydrate Polymers*, 83, 520–527.

Bai, Y., Shi, Y.-C., Herrera, A., & Prakash, O. (2011). Study of octenyl succinic anhydride-modified waxy maize starch by nuclear magnetic resonance spectroscopy. *Carbohydrate Polymers*, 83, 407–413.

Bai, Y., Shi, Y.-C., & Wetzel, D. L. (2009). Fourier transform infrared (FT-IR) microspectroscopic census of single starch granules for octenyl succinate ester modification. *Journal of Agricultural and Food Chemistry*, 57, 6443–6448.

Bertoft, E. (2004). Analysing starch structure. In A. C. Eliasson (Ed.), *Starch in food. Structure, function and applications* (pp. 57–96). Boca Raton, FL: CRC Press LLC.

Biliaderis, C. G. (1982). Physical characteristics, enzymic digestibility and structure of chemically modified smooth pea and waxy maize starches. *Journal of Agricultural and Food Chemistry*, 30, 925–930.

Cai, L., Shi, Y.-C., Rong, L. X., & Hsiao, B. S. (2010). Debranching and crystallization of waxy maize starch in relation to enzyme digestibility. *Carbohydrate Polymers*, 81, 385–393.

He, G. Q., Song, X. Y., Ruan, H., & Chen, F. (2006). Octenyl succinic anhydride modified early indica rice starches differing in amylose content. *Journal of Agricultural and Food Chemistry*, 54, 2775–2779.

Henshall, A. (1999). High performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD). In L. Prosky, S. S. Cho, & M. Dreher (Eds.), *Complex carbohydrates in foods* (pp. 267–289). Boca Raton, FL: CRC Press.

Hizukuri, S., Abe, J.-I., & Hanashiro, I. (2006). Starch: Analytical aspects. In A.-C. Eliasson (Ed.), *Carbohydrates in food* (2nd ed., pp. 305–390). Boca Raton, FL: CRC Press.

Hood, L. F., & Mercier, C. (1978). Molecular structure of unmodified and chemically modified manioc starches. *Carbohydrate Research*, 61, 53–66.

Huber, K. C., & BeMiller, J. N. (2009). Modified starch: Chemistry and properties. In A. Bertolini (Ed.), *Starches: Characterization, properties and applications* (pp. 145–203). Boca Raton, FL: CRC Press.

Huang, Q., Fu, X., He, X. W., Luo, F. X., Yu, S. J., & Li, L. (2010). The effect of enzymatic pretreatments on subsequent octenyl succinic anhydride modifications of cornstarch. *Food Hydrocolloids*, 24, 60–65.

Kavitha, R., & BeMiller, J. N. (1998). Characterization of hydroxypropylated potato starch. *Carbohydrate Polymers*, 37, 115–121.

Koizumi, K., Fukuda, M., & Hizukuri, S. (1991). Estimation of the distributions of chain length of amylopectins by high-performance liquid chromatography with pulsed amperometric detection. *Journal of Chromatography A*, 585, 233–238.

Manners, D. J. (1989). Recent developments in our understanding of amylopectin structure. *Carbohydrate Polymers*, 11, 87–112.

Mischnick, P., & Momcilovic, D. (2010). Chemical structure analysis of starch and cellulose derivatives. *Advances in Carbohydrate Chemistry and Biochemistry*, 64, 117–210.

Richardson, S., & Gorton, L. (2003). Characterisation of the substituent distribution in starch and cellulose derivatives. *Analytica Chimica Acta*, 497, 27–65.

Richardson, S., Nilsson, G., Cohen, A., Momcilovic, D., Brinkmalm, G., & Gorton, L. (2003). Enzyme-aided investigation of the substituent distribution in cationic potato amylopectin starch. *Analytical Chemistry*, 75, 6499–6508.

Robyt, J. F. (2009). Enzymes and their action on starch. In J. BeMiller & R. Whistler (Eds.), *Starch: Chemistry and technology* (3rd ed., pp. 237–292). New York, NY: Elsevier Inc.

Shi, Y. C., & Seib, P. A. (1992). The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydrate Research*, 227, 131–145.

Shogren, R. L., Viswanathan, A., Felker, F., & Gross, R. A. (2000). Distribution of octenyl succinate groups in octenyl succinic anhydride modified waxy maize starch. *Starch – Stärke*, 52, 196–204.

Somogyi, M. (1952). Notes on sugar determination. *Journal of Biological Chemistry*, 195, 19–23.

Sui, Z. Q., Huber, K. C., & BeMiller, J. N. (2013). Effects of the order of addition of reagents and catalyst on modification of maize starches. *Carbohydrate Polymers*, 96, 118–130.

Sweedman, M. C., Tizzotti, M. J., Schäfer, C., & Gilbert, R. G. (2013). Structure and physicochemical properties of octenyl succinic anhydride modified starches: A review. *Carbohydrate Polymers*, 92, 905–920.

- Tizzotti, M. J., Sweedman, M. C., Tang, D., Schaefer, C., & Gilbert, R. G. (2011). New ^1H NMR procedure for the characterization of native and modified food-grade starches. *Journal of Agricultural and Food Chemistry*, 59, 6913–6919.
- Trubiano, P. C. (1986). Succinate and substituted succinic derivatives of starch. In O. B. Wurzburg (Ed.), *Modified starches: Properties and uses* (pp. 131–147). Boca Raton, FL: CRC Press.
- van der Burgt, Y. E. M., Bergsma, J., Bleeker, I. P., Mijland, P. J. H. C., van der Kerk-van Hoof, A., Kamerling, J. P., et al. (1999). Distribution of methyl substituents over crystalline and amorphous domains in methylated starches. *Carbohydrate Research*, 320, 100–107.
- Wetzel, D. L., Shi, Y.-C., & Reffner, J. A. (2010). Synchrotron infrared confocal microspectroscopic detection of heterogeneity within chemically modified single starch granules. *Applied Spectroscopy*, 64, 282–285.
- Wetzel, D. L., Shi, Y.-C., & Schmidt, U. (2010). Confocal Raman and AFM imaging of individual granules of octenyl succinate modified and natural waxy maize starch. *Vibrational Spectroscopy*, 53, 173–177.
- Wurzburg, O. B. (1986). *Modified starches: Properties and uses*. Boca Raton, FL: CRC Press.
- Zhang, B., Huang, Q. A., Luo, F. X., Fu, X. O., Jiang, H. X., & Jane, J. L. (2011). Effects of octenylsuccinylation on the structure and properties of high-amylose maize starch. *Carbohydrate Polymers*, 84, 1276–1281.