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Study of octenyl succinic anhydride-modified waxy maize starch by nuclear magnetic resonance spectroscopy

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ABSTRACT

Granular waxy maize starch was reacted with two levels (3 and 15%, based on the weight of starch) of octenyl succinic anhydride (OSA). Structure of the OSA and modified starches was studied by one-dimensional (1D) ^{1}H and ^{13}C and two-dimensional (2D) homonuclear correlation and heteronuclear correlation nuclear magnetic resonance (NMR) spectroscopy. The modified starches were converted to α-limit dextrins prior to NMR analysis. By applying the 1D and 2D NMR techniques, complete assignments of ^{1}H and ^{13}C NMR spectra of the OSA reagent were achieved, and the position of the double bond and ratio of *trans* to *cis* isomers were determined. As level of OSA substitution increased, the peak (≈5.38 ppm) for the anomeric proton of internal α-1,4-D-glucosyl units became broader in ^{1}H NMR spectra, suggesting that substitution occurred at the O-2 position. Compared with the ^{13}C NMR spectrum of the native starch, the modified starches gave additional signals at the C-4 peak region and broadening of the C-1, C-2, C-3, and C-4 resonances, but not of the C-6 signal. Those results further suggest that the OS groups were substituted at the O-2 and O-3 positions, but not at the O-6 position.

1. Introduction

Native starches are used in food and industrial applications (Daniel, Whistler, Roper, & Elvers, 2007), but shortcomings of the unmodified starches limit their use in many commercial applications (Wurzburg, 1986). These shortcomings, among others, include insolubility or failure of the starch granules to develop viscosity in cold water; the cohesive texture of the cooked starch, particularly from waxy maize, potato, and tapioca starch; the loss of viscosity by acids or mechanical shear; lack of clarity and the tendency to retrograde during storage; and the lack of emulsification properties (Trubiano, 1986; Wurzburg, 1986).

Modified starches have been developed to overcome one or more of the shortcomings. Caldwell and Wurzburg (1953) disclosed the reaction between starch and octenyl succinic anhydride (OSA). Native starch molecules are hydrophilic, but with the incorporation of hydrophobic groups from OSA, the OSA-modified starch becomes lipophilic and finds applications in beverage emulsion; salad dressings; oil- and petroleum-based cosmetics or pharmaceutical pastes; alcohol-based lotions and body deodorant sprays; encapsulation of flavors, fragrances, vitamins, clouds, and oils (Rutenberg & Solarek, 1984; Trubiano, 1986; Wurzburg, 2006, Chapter 3). In addition, it has been used in biodegradable plastics (Jane et al., 1991) and emulsified foods as fat replacers (Cho, Lim, Park, Hwang, & Lim, 1999; Kim, Sandhu, Lee, Lim, & Lim, 2010). OSA modification also makes a starch more resistant to enzyme digestion and increases the levels of slowly digestible and resistant starch (Han & BeMiller, 2007; He, Liu, & Zhang, 2008; Viswanathan, 1999; Wolf et al., 2001). The properties of OSA-modified starch depend on the level of bound OS or the degree of substitution (DS). Titration (Bao, Xing, Phillips, & Corke, 2003; Bhosale & Singhal, 2006; He, Song, Ruan, & Chen, 2006; He et al., 2008; Hui, Chen, Fu, Xu, & He, 2009; Jeon, Viswanathan, & Gross, 1999; Liu et al., 2008; Shogren, Viswanathan, Felker, & Gross, 2000) and nuclear magnetic resonance (NMR) (Čížová, Koschella, Heinze, Ebringerová, & Sroková, 2007; Choi, Girek, Shin, & Lim, 2002; Jeon et al., 1999; Shih & Daigle, 2003) methods have been used to determine the DS of OSA-modified starch.

Despite the extensive investigation of the reaction of OSA with starch reported in the literature, a number of questions remain. For instance, the OSA reagent contains a double bond. Are there *trans* and *cis* isomers (Fig. 1a and b) in the reagent? If so, what is the percentage of each form? Configuration of the OSA may influence properties of the modified starch. Furthermore, the position of the double bond is not clear from published information on OSA. Several Web sites show the structure as 1-octenyl succinic anhydride (IUPAC name: 3-[(E)-oct-1-enyl]oxolane-2,5-dione) (Fig. 1c and d). Some researchers stated that they used 1-octenyl succinic anhydride (Scheffler et al., 2010) and 1-octenyl succinic

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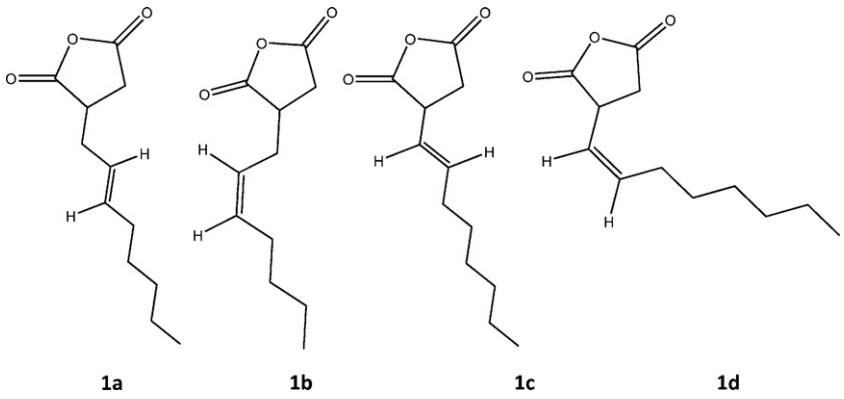


Fig. 1. Four possible structures of octenyl succinic anhydride (OSA) reagent: 3-(E-oct-2-enyl)dihydrofuran-2,5-dione (1a); 3-(Z-oct-2-enyl)dihydrofuran-2,5-dione (1b); 3-(E-oct-1-enyl)dihydrofuran-2,5-dione (1c); and 3-(Z-oct-1-enyl)dihydrofuran-2,5-dione (1d).

anhydride-modified starch (Wolf et al., 2001) in their studies. Others reported using 2-octenyl succinic anhydride to make OSA-modified starches (Bao et al., 2003; Bhosale & Singhal, 2006; He et al., 2006; Hui et al., 2009; Kim et al., 2010; Liu et al., 2008). However, to our knowledge, no study has been reported on the detailed structure of the OSA reagent, and no method has been reported on how to differentiate 1- and 2-octenyl succinic anhydride and their *cis* and *trans* isomer. Also, the internal glucosyl repeat units in starch have three hydroxyl groups available for substitution, but no study has reported the distribution of the OS substituents. The goal of this work was to answer these questions by studying the structure of OSA and OSA-modified starch using high-resolution one-dimensional (1D) and two-dimensional (2D) NMR techniques. 1D ^{1}H and ^{13}C NMR and 2D ^{1}H-^{1}H homonuclear correlation and ^{1}H-^{13}C heteronuclear correlation NMR experiments were used to examine the OSA reagent and OSA-modified waxy maize starches having different levels of substitution, completely assign the resonances of the protons and carbons in the NMR spectra of the OSA reagent, and elucidate the structures of OSA and OSA-modified starch. The information gained in this study is needed to relate the structures of OS starches to different reaction conditions and to their functional properties and enzyme digestibility.

2. Material and methods

The OSA and waxy maize starch (Amoica TF) were obtained from National Starch LLC (Bridgewater, NJ). Alpha-amylase (Liquozyme SC DS) was provided by Novozymes (Franklinton, NC). Other chemicals used were analytical grade.

2.1. Preparation of OSA-modified starches

Waxy maize starch (100 g, dry weight) was suspended in water at 40% solid content, and the slurry was adjusted to pH 7.5 by adding 3% (wt%) NaOH. The starch slurry was continuously mixed by an overhead stirrer, and OSA (3 or 15% based on the weight of starch) was added dropwise from a burette while the pH was maintained at 7.5 by adding 3% (wt%) NaOH using a pH controller (Model 501-3400, Barnant Co., IL). After the addition of OSA, the pH was stable in 30 min and the reaction mixture was adjusted to pH 6.0 by 1.0N HCl. The modified starch was collected by filtration, and then washed with 300 mL of water and dried in an oven at 35 °C for 48 h to about 10% moisture.

2.2. NMR spectroscopy

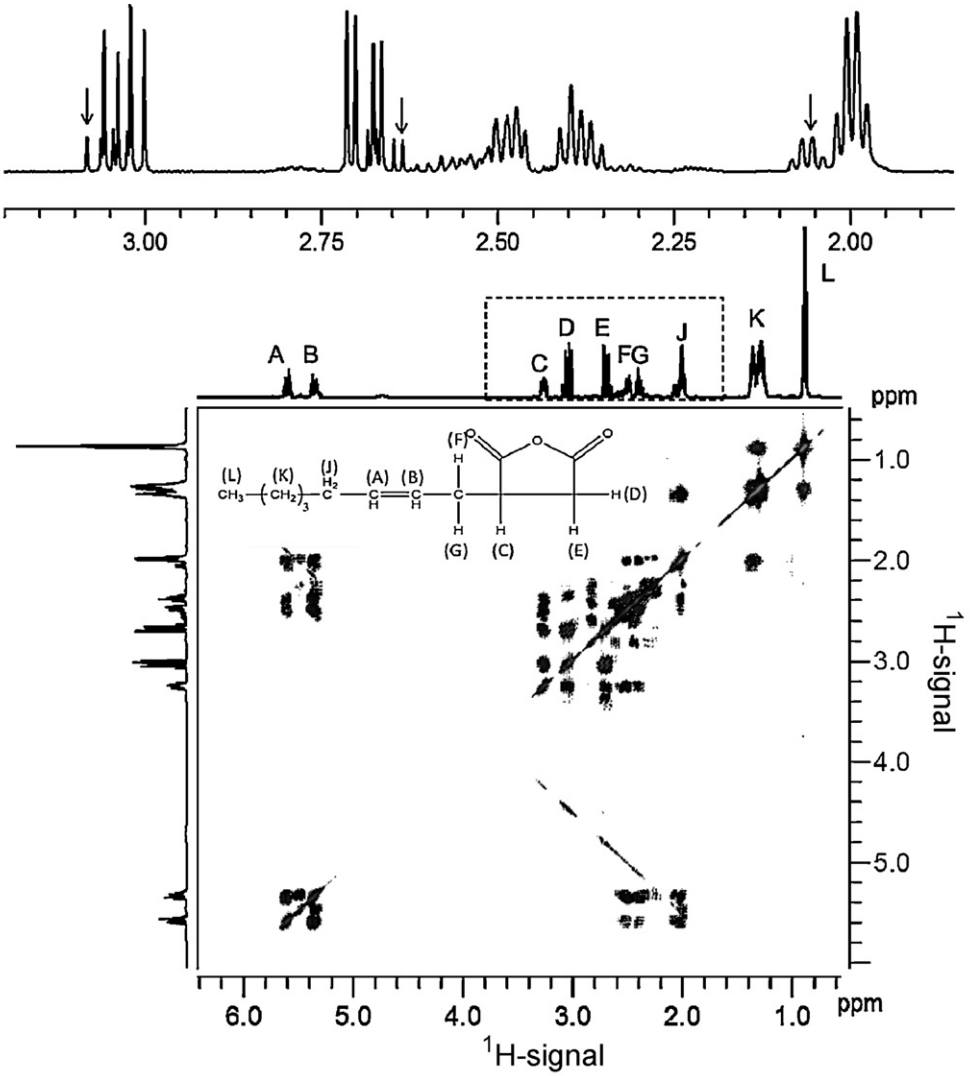
The modified starches were prepared for NMR experiments by following the method of Xu and Seib (1997) with modifications. OS waxy maize starch or native waxy maize starch (2–3 g) and α-amylase (Liquozyme SC DS) (10 μl) were mixed in 30 ml of water. In some cases, sodium acetate (0.3 g) was added. The slurry was heated in a water bath at 85 °C with shaking for 2 h to hydrolyze the starch, and then placed in a boiling water bath for 30 min to denature the enzyme. After cooling to room temperature, the hydrolyzed starch was freeze-dried. The freeze-dried hydrolyzed starch (0.2 g) was dissolved in 1 ml of D_2O and freeze-dried again, and the procedure was repeated once. The D_2O-exchanged starch (0.05 g) was dissolved in D_2O (0.50 ml) for NMR experiments. The OSA reagent was dissolved in CD_3OD (10%, v/v) for NMR analysis.

1D ^{1}H and broadband proton decoupled ^{13}C NMR spectra were recorded on a Varian 500 NMR System (Varian Inc., Palo-Alto, CA) at 25 °C. The NMR spectrometer was equipped with a 3-mm diameter, triple-resonance, inverse-detection, pulse-field-gradient probe operating at 499.85 MHz for ^{1}H and 125.70 MHz for ^{13}C. The ^{1}H spectra were collected in 128 individual scans with a sweep width of 16 ppm and a delay time of 1 s. The ^{13}C spectra of the native and OSA-modified starches were collected with a sufficient number of scans for good resolution, typically 16,000 scans and a delay time of 1 s. A delay time of 15 s was used to obtain the spectrum of the OSA reagent in methanol. 2D ^{1}H–^{1}H homonuclear correlation spectrum (COSY) was recorded with a single transient per t_1 increment with a sweep width of 3242.8 Hz in both dimensions. 2D ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) experiments also were performed using 512 transients and 16 scans per transient. The pulse sequence used was a part of the "Bio-pack" provided by Varian Inc. Sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (TSP) was used as a reference (0 ppm). Chemical shifts are reported in parts per million (ppm).

3. Results and discussion

3.1. Structure of OSA reagent

A 2D ^{1}H–^{1}H COSY spectrum of the OSA reagent in methanol is shown in Fig. 2. Resonance of the terminal methyl protons is close to 0.87 ppm, and the protons on the C–C double bond are in the region of 5.27 to 5.63 ppm (Guillén & Ruiz, 2001; Knothe & Kenar, 2004; Pavia, Lampman, Kriz, & Vyvyan, 2008). After those peaks were identified, the remaining peaks were assigned with the assistance of the COSY spectrum (Fig. 2) by tracing out the connectivity through cross-peaks, which arise from coupling between the protons (McIntyre & Vogel, 1990). Proton assignments are noted on each peak in Fig. 2. Our assignments generally agree with the spectrum of *trans*-(2-octenyl) succinic anhydride (CAS no. of 81949-84-0) in the Spectral Database for Organic Compound (SDBS, 2010), a Web site organized by the National Institute of



Assignment	D-trans	D-cis	E-trans	E-cis	F	G	J-cis	J-trans
δ (ppm)	3.02	3.05	2.71	2.66	2.47	2.39	2.05	2.00

Fig. 2. Two-dimensional (2D) ^{1}H–^{1}H COSY spectrum of OSA reagent in methanol. Structure of 3-(oct-2-enyl)dihydrofuran-2,5-dione is inserted and assignments for protons are listed on the top of the corresponding resonances. The region from 3.2 to 1.8 ppm is expanded and shown above the COSY spectrum. The arrows point to the small peaks at ≈2.06, 2.31, 2.58, 2.66 ppm and 3.44 ppm that have splitting patterns similar to those of their corresponding adjacent peaks, and are attributed to the *cis* isomer. A detailed assignment in this region is shown in the table below the COSY spectrum.

Advanced Industrial Science and Technology, Japan. For easy comparison in this paper, we labeled protons in the OSA the same as in the database. However, we noted extra side peaks in our ^{1}H NMR spectrum (Fig. 2). The small peaks at ≈2.06, 2.31, 2.58, 2.66, and 3.44 ppm have splitting patterns similar to those of their corresponding adjacent peaks. We attributed these minor peaks to the *cis* isomer. The ^{13}C NMR discussed below further confirmed these assignments.

To determine the configuration of the protons at the double bond (A, B in Fig. 2), we calculated their coupling constant. The coupling constant $^3J_{(H–H)}$ of the proton pair at the double bond was 15.21 Hz, indicating the *trans* configuration was the predominate form. The ^3J coupling constant for protons that are *cis* to each other would have a smaller value, close to 10 Hz (Pavia et al., 2008).

The 2D ^{1}H–^{1}H COSY spectrum (Fig. 2) was also helpful in determining the position of the double bond. The alkene proton B-*trans* (5.33 ppm) was coupled with methylene protons labeled F (2.47 ppm) and G (2.39 ppm), whereas proton A-*trans* (5.58 ppm) was coupled with the protons labeled J (2.00 and 2.05 ppm), suggesting the double bond was between the methylene groups (F, G and J) and the OSA reagent used in the experiment was 3-(E-oct-2-enyl)dihydrofuran-2,5-dione (Fig. 1 a and b), not 3-(E-oct-1-enyl)dihydrofuran-2,5-dione (Fig. 1c and d). Moreover, the ratio of the area of the resonances that arose from the methylene protons labeled K (1.39–1.22 ppm) to that of the methylene protons labeled J (2.00 and 2.05 ppm) was about 3:1, further confirming our assignment of the double bond position. The 2-ene position on the side chain of OSA agrees with the mechanism of formation of OSA by the Diels–Alder reaction of 1-octene with maleic anhydride (Royals, 1954).

With the assistance of the 2D ^{1}H–^{13}C HSQC spectrum (Fig. 3), which shows the correlations between a ^{13}C atom and its directly bonded protons (McIntyre & Vogel, 1990), the resonance of the ^{13}C NMR spectrum of the OSA in methanol were assigned (Table 1).

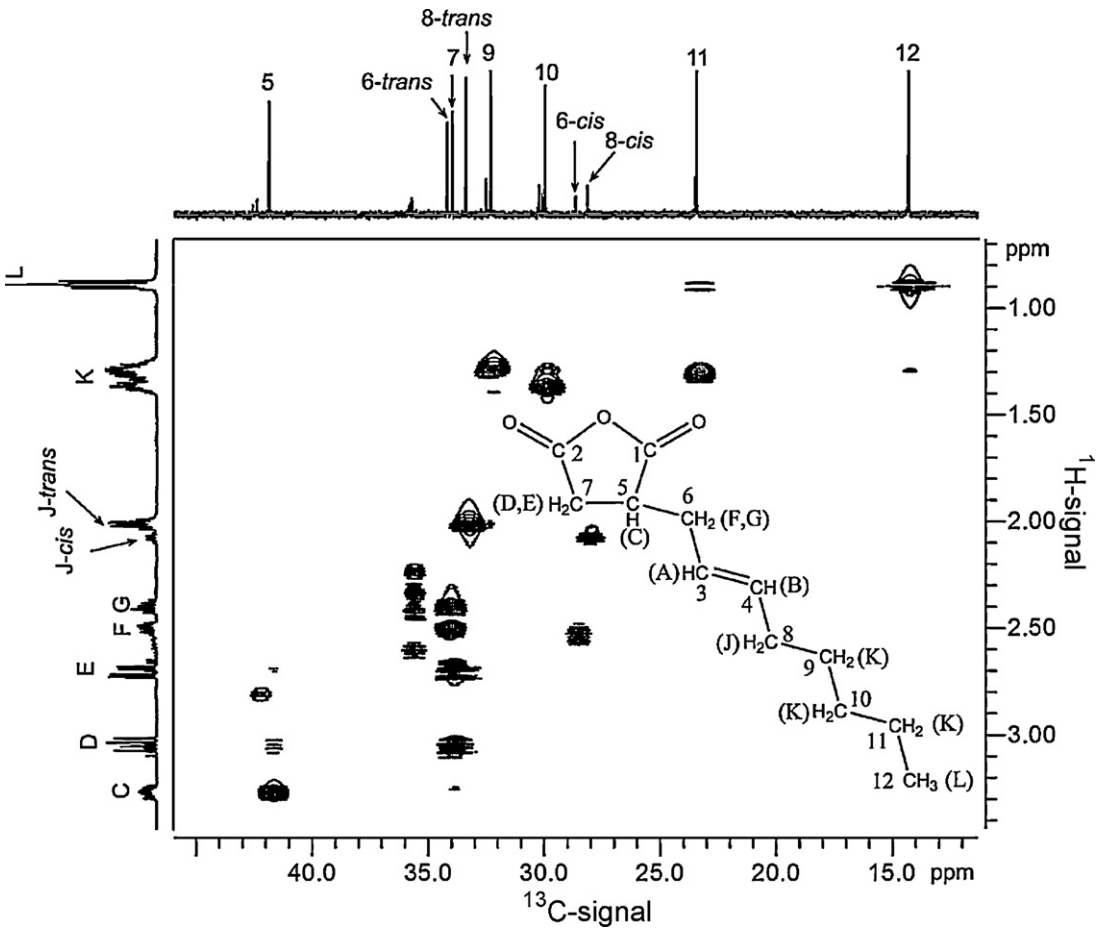


Fig. 3. Expanded region of two-dimensional (2D) ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) spectrum (45–12 ppm) of OSA reagent in methanol.

Identification of the *trans* and *cis* isomers of OSA was achieved on the basis of the extensive investigation of fats, oils, and unsaturated fatty acids by ^{13}C NMR (Gao et al., 2009) and the 2D ^{1}H–^{13}C HSQC spectrum (Fig. 3). Carbons of a *cis*-double bond occur upfield (Barton, Himmelsbach, & Burdick, 1975; Gao et al., 2009; Pfeffer, Luddy, Unruh, & Shoolery, 1977). Not only are the chemical shifts of the ene-carbons of a *cis* and *trans* double bond different, but also the chemical shifts of the allylic carbons adjacent to a *cis* or *trans* double bonds are different (Gao et al., 2009; Lie Ken Jie & Mustafa, 1997; Pfeffer et al., 1977). The allylic carbons adjacent to a *cis*-double bond resonate ~5.35 ppm to higher field than those adjacent to a *trans* double bond. In studying a mixture of methyl oleate (methyl *cis*-9-octadecenoate) and methyl elaidate (methyl *trans*-9-octadecenoate) in $CDCl_3$, Pfeffer et al. (1977) reported that allylic carbon resonances for the *cis* and *trans* isomers occur at 27.2, 27.3 ppm, and 32.5 and 32.6 ppm, respectively. On the basis of that information on fats and fatty acids, in this study, the resonances at 28.0 and 33.2 ppm were assigned to the allylic carbon (C-8) adjacent to the *cis* and *trans* double bonds, respectively. Once the allylic carbon (C-8) resonances were identified, we were able to determine their connected protons using the HSQC spectrum, and then to calculate the ratio of *cis* to *trans* isomers in the OSA reagent. According to the expanded HSQC spectrum (Fig. 3), the peak at 33.2 ppm of the *trans* form corresponded to the peak at 2.00 ppm (J-*trans*) in the proton spectrum, whereas the peak at 28.0 ppm of the *trans* form corresponded to the peak at 2.05 ppm (J-*cis*) in the proton spectrum. By calculating the ratio of the integrals of the *trans*-J proton to that of *cis*-J proton, which are shown to be clearly resolved signals in Fig. 2, we estimated that the ratio of the *trans* to *cis* isomers in the OSA reagent to be about 5:1.

Table 1
^{13}C NMR spectrum assignment of octenyl succinic anhydride (OSA).

Assignment	^{13}C chemical shift (ppm)
1	175.6
2	172.3
3-*trans*	136.5
3-*cis*	135.1
4-*trans*	125.3
4-*cis*	124.6
5	41.8
6-*trans*	34.1
7	33.9
8-*trans*	33.2
9	32.2
10	29.8
6-*cis*	28.6
8-*cis*	28.0
11	23.3
12	14.2

3.2. NMR spectroscopy of OSA-modified starch

^{1}H NMR spectra of α-amylase digests of native waxy maize starch and OSA-modified starches (DS = 0.019 and 0.056, respectively) are shown in Fig. 4. Peaks arising from the glucose in starch were assigned according to the literature (Gidley, 1985; McIntyre, Ho, & Vogel, 1990). Compared with the native starch, the OSA-modified starches showed several additional resonances due to OS substitution. The assignments are listed in Table 2. By comparing the intensities of the methyl protons on OS substituents to that of anomeric protons on glucosyl units, the DS of OSA-modified starch

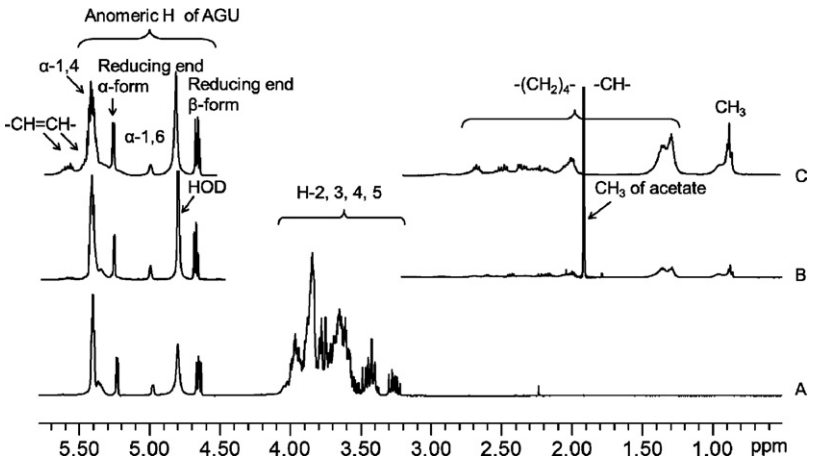


Fig. 4. ^{1}H NMR spectra of α-limit dextrins of waxy maize starch (A) and OSA-modified starches with DS of 0.018 (B) and 0.056 (C).

Table 2
^{1}H NMR spectrum assignment of octenyl succinic anhydride (OSA)-modified starch (DS = 0.056).

Functional groups		^{1}H chemical shift (ppm)
OSA[a]	Glucose unit	
A		5.54
B		5.43
	Internal H-1	5.38
	Reducing end α-form	5.22
	H-6 (α-1,6)	4.96
		4.77 (H–O–D)
	Reducing end β-form	4.64
	H-2,3,4,5	4.10–3.18
C, D, E, F, G, J		1.93–2.99
K		1.50–1.20
L		1.05–0.82

[a] The capital letters for protons in OSA group follow the notation in Fig. 3.

Table 3
^{13}C NMR spectrum assignment of octenyl succinic anhydride (OSA)-modified starch (DS = 0.056).

Functional groups		^{13}C chemical shift (ppm)
OSA[a]	Glucose unit	
2-carboxylate		186.8
2-carboxylate acid		185.0
1-ester		182.6
3		136.3
4		129.5
	C-1	102.6
	C-1 α-form	98.6
	C-1 β-form	94.6
	C-2, 3, 4, 5	81.7–70.5
	C-6	63.5
5		48.6
7		42.8
6		37.9
8-*trans*		34.6
9		33.6
10		31.3
8-*cis*		29.4
11		24.7
12		16.3

[a] The numbers for carbons in OSA group follow the notation in Fig. 3.

can be calculated (Bai & Shi, accepted for publication). ^{1}H NMR is also a useful technique for determining the DS of OSA-modified hyaluronic acid (Eenschooten, Guillaumie, Kontogeorgis, Stenby, & Schwatch-Abdellaouli, 2010).

Compared with the ^{1}H NMR spectrum of OSA (Fig. 2), the modified starches had a shoulder at ≈0.97 ppm adjacent to the sharp signals from the methyl protons at 0.90 ppm (Fig. 4). It is possible that terminal methyl protons in some substituted octenyl succinate groups, which are hydrophobic, might associate and aggregate in aqueous media (Eenschooten et al., 2010), and cause shifting of the methyl peak. Another possibility is that some methyl protons interact with starch molecules, and the interaction between the starch molecules and substituted OS could cause those methyl protons to shift downfield. Interestingly, the shoulder disappeared when the α-limit dextrin of the OSA-modified starch was examined in dimethyl sulfoxide-d_6 (data not shown), suggesting that the shoulder was caused by hydrophobic associate in aqueous

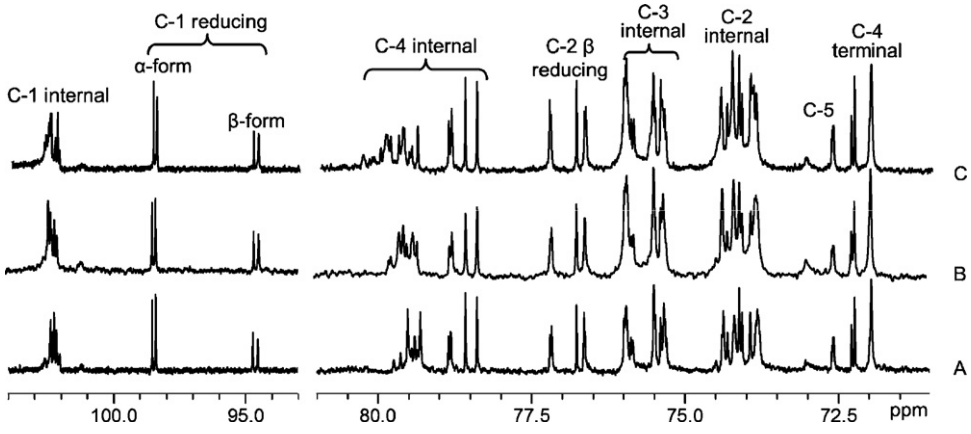


Fig. 5. ^{13}C NMR spectra of α-limit dextrins of native waxy maize starch (A) and OSA-modified starches with DS of 0.019 (B) and 0.056 (C).

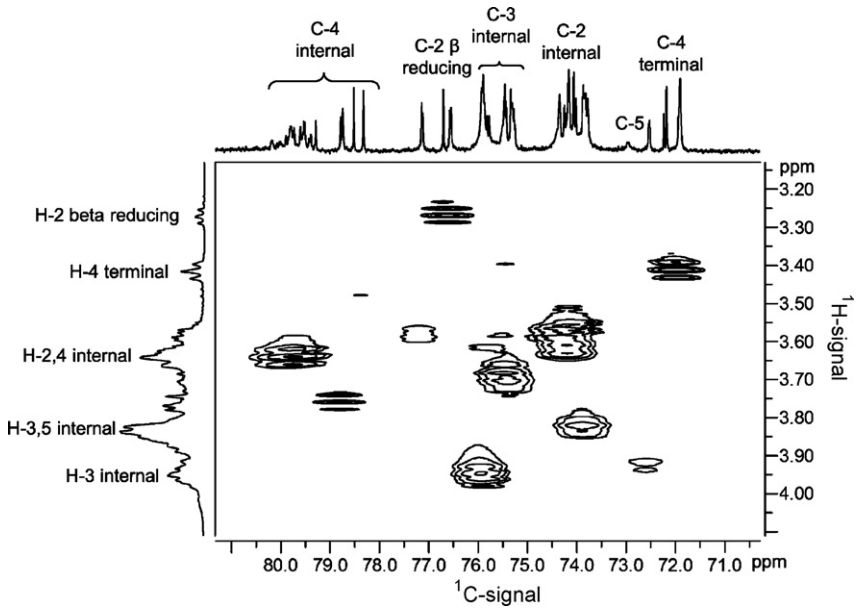


Fig. 6. Heteronuclear single quantum coherence (HSQC) ^{1}H–^{13}C spectrum of α-limit dextrin of 15% OSA-modified starch (DS = 0.056).

media. In addition, the resonance of the protons in the OS chain (0.54–3.21 ppm) became broader and less resolved (Fig. 4) compared to that of the same protons in the OSA reagent (Fig. 2). The peak broadening was likely a result of mixed molecules. Similar to the amylolytic enzyme action on phosphorylated starch (Kasemsuwan & Jane, 1996), the α-amylase probably does not hydrolyze glucosidic bonds near glucosyl units substituted with OS groups. As a result, the α-limit dextrins of OSA-modified starches contain molecules of various chain lengths. The protons on the OS chains are perturbed by differences in backbone structure of the maltodextrins and by the different positions of substitution on any one of those maltodextrins, so the ^{1}H-signals on the different OS substituents do not coincide. Peak broadening was also observed in ^{31}P signals of dextrins prepared from phosphorylated wheat starches (Sang, Seib, Herrera, Prakash, & Shi, 2010). It is also interesting to note that the peak (≈5.38 ppm) for the anomeric proton of internal α-1,4-linkages became broader as the level of OSA substitution increased (Fig. 4), presumably because of substitution of OS at the O-2 position.

^{13}C NMR spectra of the α-limit dextrins of native waxy maize starch and OS starches (DS = 0.019 and 0.056, respectively) had a large number of signals over a wide range of chemical shifts (Fig. 5), which provided spectral resolution that allowed us to determine the position of OS substitution. The assignments of the ^{13}C signals in the spectrum of digested OSA-modified starch (DS = 0.056) are shown in Table 3. The C-1 of glucose in the α-1,4-repeat units of starch appears at ≈100 ppm (Chi et al., 2008; Dais & Perlin, 1982; Peng & Perlin, 1987). The C-1 signals of the internal glucosyl units of OSA-modified waxy maize starches (DS = 0.019 and 0.056) became broader than those of the native waxy maize starch (Fig. 5), suggesting that the OS substitution occurred at O-2 and that the O-2 substituent affected the chemical shift of the neighboring C-1. Moreover, additional resonances were noted between 80.0 and 81.0 ppm, the resonance due to C-4 of internal glucosyl units in starch (Chi et al., 2008; Dais & Perlin, 1982; Peng & Perlin, 1987), suggesting that OS groups were substituted at the neighboring O-3 position in granular waxy maize starch.

To further determine the positions of substitution in starch modified by OSA, we closely examined the resonances of C-2, C-3, C-4, and C-5 (81–71 ppm) in modified starches (Fig. 5). With the assistance of the HSQC spectrum (Fig. 6) and based on peak assignments for the carbons of starch (Chi et al., 2008; Dais & Perlin, 1982; Peng & Perlin, 1987) and protons of maltodextrin (McIntyre et al., 1990), we assigned the multiplets at 78.2–80.2 ppm, 73.6–74.6 ppm, and 75.1–76.0 ppm, respectively to C-4, C-2 and C-3 of internal α-1, 4-linked glucosyl units. Line broadening at 73.9 and 76.0 ppm was observed, confirming the substitution at O-2 and O-3. Interestingly, no change was observed in the signal at 63.5 ppm, which arose from C-6. We concluded that OS substitution occurs at O-2 and O-3 and not at O-6. However, calculating the molar substitution at O-2 and O-3 is not possible because the signals due to OS modification were not well resolved.

4. Conclusions

Complete assignments of ^{1}H and ^{13}C NMR spectra of the OSA reagent were achieved by 1D and 2D NMR techniques. The OSA reagent used in this study was a 5:1 mixture of the *trans*:*cis* isomer of the 2-octenyl side chain. The systematic name of the *trans* isomer of the OSA reagent is 3-[(E)-oct-2-enyl]oxolane-2,5-dione. OS substitution occurred mainly at the O-2 and O-3 positions of the anhydroglucosyl units in the OSA-modified granular starch. Future work is needed to study the relationship between the structure and properties of OSA-modified starch.

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