Carbohydrate Polymers 83 (2011) 520–527

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Structure and preparation of octenyl succinic esters of granular starch, microporous starch and soluble maltodextrin

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ARTICLE INFO

Article history:
Received 4 May 2010
Received in revised form 3 August 2010
Accepted 9 August 2010
Available online 14 August 2010

Keywords:
Octenyl succinic anhydride
Waxy maize starch
Microporous starch
Maltodextrin

ABSTRACT

The reactions of octenyl succinic anhydride (OSA) with three physical forms of starch—insoluble granular waxy maize starch, granular microporous starch, and soluble maltodextrin—were investigated and compared. Effects of OSA level (3%, 9%, 15%, and 50% based on the weight of starch) on reaction efficiency (RE) also were examined. As OSA concentration increased, degree of substitution (DS) of all OSA-modified starches increased, but RE decreased. Octenyl succinic maltodextrin had a greater DS and RE than OS granular waxy maize starch prepared at the same conditions, indicating that maltodextrin had more reaction site available. DS was the highest (0.27) for OS maltodextrin but only 0.12 for granular waxy maize starch. Increasing the surface area of starch granules by creating pinholes increased RE at OSA concentrations of 9%, 15%, and 50%. Extensive ^{13}C and ^{1}H nuclear magnetic resonance analysis indicated that structures of OSA-modified granular starch and maltodextrin were different. In granular starch, most OS substitution occurred at the O-2 and O-3 positions; in the maltodextrin, O-2, O-3, and O-6 positions were substituted, even at the reducing end.

1. Introduction

Starch is modified to improve its functional properties (Wurzburg, 1986). By attaching hydrophobic groups to the repeat glucose units, the modified starch molecules become amphiphilic and they function as emulsion stabilizers (Trubiano, 1986). Octenyl succinic (OS) starch, prepared by reaction of starch with octenyl succinic anhydride (OSA), is one of the most important amphiphilic derivatives because it is approved for food use by legal regulations in the U.S. OS starch also has several uses in the pharmaceutical, personal care, and biodegradable plastics industries (Jane, Robert, Nidolov, & Roque, 1991; Trubiano, 1986).

Since Caldwell and Wurzburg (1953) first patented the reaction of starch with substituted cyclic dicarboxylic acid anhydrides, a number of researchers have investigated the OSA reaction of (i) starches of different botanical origin (Bao, Xing, Phillips, & Corke, 2003; Bhosale & Singhal, 2006, 2007; Billmers & Mackewicz, 1997; Han & BeMiller, 2007; He, Song, Ruan, & Chen, 2006; He, Liu, & Zhang, 2008; Jeon, Viswanathan, & Gross, 1999; Liu et al., 2008; Nilsson & Bergenstahl, 2007; Ruan, Chen, Fu, Xu, & He, 2009; Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2008; Shih & Daigle, 2003; Shogren, Viswanathan, Felker, & Gross, 2000; Song, He, Ruan, & Chen, 2006; Thirathumthavorn & Charoenrein, 2006), and of (ii) phytoglycogen (Scheffler, Wang, Huang, Gonzalez, & Yao, 2010) and hyaluronic acid (Eenschooten, Guillaumie, Kontogeorgis, Stenby, & Schwatch-Abdellaouli, 2010). Kim, Sandhu, Lee, Lim, and Lim (2010) prepared OS waxy rice amylodextrins by dry heating. Typically, OS starch is prepared by suspending starch granules in water and mixing them with OSA reagent under alkaline conditions (Trubiano, 1986). The reaction between starch granules and OSA in aqueous media is heterogeneous. Native starch granules are partially crystalline, and OS substitution is believed to occur in the amorphous regions of starch granules (Shogren et al., 2000; Song et al., 2006).

To be used in beverage applications, OSA-modified starch must be water soluble. One approach to solubilization is to react OSA with granular starch; after which the OSA-modified starch can be degraded and made water soluble (Wurzburg, 2006). Another approach could be to react OSA directly with converted soluble starch. Compared with the extensive research on the reaction of OSA with insoluble granular starch, very limited research has been reported on the reaction of OSA with soluble starch or degraded starch (Morehouse, 1994). We hypothesized that the reaction of OSA with soluble starch would have different patterns of substitution on starch molecules and a different reaction efficiency (RE), compared with OSA-modified granular starch. Thus the OS product obtained would function differently when used as an emulsion stabilizer.

Microporous starch with pinholes in starch granules can be generated by digesting granules with glucoamylase and α-amylase (Paramahans & Tharanathan, 1982; Takaya, Sugimoto, Wako, &

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doi:10.1016/j.carbpol.2010.08.012

Fuwa, 1979; Whistler, 1991). However, it is not known whether OSA reacts differently with microporous starch than with native starch. Would increasing the starch's surface area by creating pinholes allow more OSA to penetrate into the granules and result in a higher reaction efficiency? Objectives of this work were to investigate the reaction of OSA with starches having different physical forms, to determine whether there is any advantage to starting with microporous starch and soluble starch, and to study the structures of OSA-modified granular starch compared to that from soluble maltodextrin.

2. Materials and methods

2.1. Materials

OSA and waxy maize starch (Amoica TF) were obtained from National Starch Food Innovation (Bridgewater, NJ). Maltodextrin (MALTRIN 100) with $DP_n \sim 10$ was obtained from Grain Processing Corporation (Muscatine, IA). Glucoamylase (Spirizyme) and α-amylase (Liquozyme SC DS) were provided by Novozymes (Franklinton, NC). The enzyme activity of the glucoamylase was 750 amyloglucosidase units (AGU)/g as provided by Novozymes. One AGU is defined as the amount of enzyme that cleaves 1 μmol of maltose per minute under standard assay conditions (37.0 °C; 0.1 M, pH 4.3 acetate buffer; 23.2 mM maltose; incubation time of 5 min). The enzyme activity of the α-amylase was 240 Kilo Novo Units (KNU)-S/g. One KNU is defined as the amount of enzyme which, under standard conditions (i.e. at 37.0 °C; 0.0003 M $Ca.^{2+}$; and pH 5.6) dextrinizes 5.26 g of starch (Merck Amylum soluble) per hour. Other chemicals used in the study were analytical grade.

2.2. Preparation of microporous waxy maize starch

Waxy maize starch (35 g, dry weight) was suspended in 65 mL of acetate buffer (0.01 M) including $CaCl_2$ (0.0005 M) at pH 5.0. Glucoamylase (30 μL) and α-amylase (2.8 μL) were added to the slurry. The starch slurry was heated and held in a water bath at 45 °C for 2 h. The mixture was filtered, washed with water, and air-dried to obtain the microporous starch. The yield of the microporous starch was about 84%.

2.3. Preparation of OS starches

Waxy maize starch, microporous starch, or maltodextrin (100 g, dry weight) was placed in 150 mL of water and mixed to homogeneity by an overhead stirrer at 25 °C. The pH of the slurry was adjusted and controlled at 7.5%, 8.5%, or 9.5% with 3% (w/w) NaOH solution. OSA (3%, 9%, 15%, and 50% based on starch weight) was added at different rate with a burette. In the 15% and 50% OSA reaction, Na_2SO_4 (5% or 20%) was added to the waxy maize starch slurry. For microporous starch, 20% Na_2SO_4 was added when OSA concentration was 50%. During a reaction, the pH of the reaction was maintained with a pH controller (Model 501-3400, Barnant Co., IL). After addition of OSA, and after the pH was stable for 30 min the reaction was terminated by adding 1.0 M HCl to pH 6.0.

The insoluble granular starch was recovered by filtration, and then the starch cake was washed with 300 mL of water. The starch cake was dried in an oven at 35 °C for 48 h and ground with an analytical mill. In separate experiments designed to examine how the rate of OSA addition affected RE, OSA (3.00 g) as is or predissolved in ethanol (10 mL) was added to the granular starch slurry by adding all the reagent at once or by adding it dropwise over 4 h.

For soluble maltodextrin, the effect of maltodextrin level (30%, 40%, 50% initial concentration) on RE was examined, and each OS product was recovered by freeze-drying. The powdery freeze-dried OSA-modified maltodextrin (10 g) was washed with 80 mL of methanol to remove unreacted OSA in the sample. The washed sample was air-dried and saved for nuclear magnetic resonance (NMR) analysis.

2.4. Determination of bound and free OS content

Bound OS content of modified granular starches was determined by a titration method (Kweon, Choi, Kim, & Lim, 2001) with some modifications. Unless otherwise noted, OS starch (5.00 g dry weight) was washed with 20 mL of methanol twice to remove unreacted OSA. Methanol-washed OS starch was suspended in 20.0 mL of 0.1 M HCl and stirred for 30 min. The suspension was filtered, and the solids washed with water until no Cl^- was detected by 0.1 M $AgNO_3$ solution. Starch was resuspended in 300 mL of water containing 30 μL of α-amylase and heated in a boiling water bath for 20 min. The starch solution was titrated with 0.100 M NaOH solution using phenolphthalein as an indicator. Native waxy maize starch was titrated as a control. Bound OS (%), degree of substitution (DS), and RE were calculated by using following equations:

$$\%\,\mathrm{OS} = \frac{(V_1 - V_2)\ \times\ 0.100 \times 21}{W}$$

$$\mathrm{DS} = \frac{162 \times \%\,\mathrm{OS}}{210 - 209 \times \%\,\mathrm{OS}}$$

$$\mathrm{RE} = \frac{\%\,\mathrm{OS\ of\ OS\ starch}}{\%\,\mathrm{OSA\ added\ to\ the\ starch}} \times 100\%$$

where % OS is the weight percentage of OS in OS starches, V_1 is the titration volume of NaOH (mL) of OS starch, V_2 is the titration volume of NaOH (mL) of native starch, and W is the dry weight (g) of the OS starch.

Total OS content in granular starches was also obtained by titrating modified starches without methanol extraction. Unreacted (free) OS content was determined by subtracting bound OS content from total OS content.

OS content in modified soluble maltodextrin was determined by a NMR method described in the following section.

2.5. NMR spectroscopy

OSA-modified granular starches were converted to α-limit dextrins for NMR experiments by following the method of Xu and Seib (1997). OS waxy maize starch (2–3 g) was washed twice with methanol (10 mL) to extract free OS and the residue was air-dried prior to α-amylase hydrolysis. For comparison, OS starch (DS = 0.088) without methanol extraction was also analyzed. Samples of soluble maltodextrin were exchanged with D_2O twice, freeze-dried and dissolved in D_2O (10 wt.%) for analysis.

^1H and broadband proton decoupled ^{13}C NMR spectra were recorded on a Varian 500 NMR System (Palo Alto, CA, USA) 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 ^1H and 125.70 MHz for ^{13}C. The ^1H 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.

DS was calculated from the ^1H NMR spectra by using the following equation:

$$\mathrm{DS} = \frac{I_{0.85}/3}{I_{5.36} + I_{5.22} + I_{4.96} + I_{4.63} - I_{5.52}}$$

where $I_{0.85}$, $I_{5.52}$, $I_{5.36}$, $I_{5.22}$, $I_{4.96}$, and $I_{4.63}$ are the integrals of methyl protons, one of the alkene proton, internal H-1 of α-1,4-linked glu-

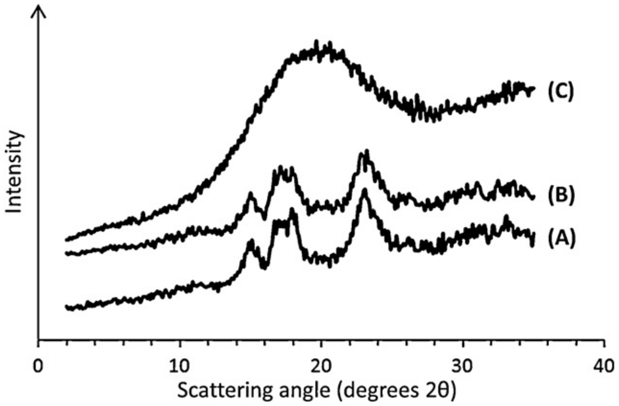

Fig. 1. X-ray diffraction pattern of native waxy maize starch (A), microporous starch (B) and maltodextrin (C).

cose units plus one of the alkene proton, H-1 of α-aromeric reducing end, H-1 of branching point, and reducing H-1 of β-aromeric reducing end, respectively.

2.6. Wide-angle X-ray diffraction

The starch samples were equilibrated to about 20% moisture at 25 °C in a glass-enclosure containing water. X-ray diffraction patterns of starches and maltodextrins were obtained with an X-ray diffractometer (APD 3520, Philips, Netherlands). The instrument was operated at 35 kV, 20 mA with Cu-Kα radiation, a theta-compensating slit, and a diffracted beam monochromator. Data were recorded between the diffraction angles (2θ) of 2° and 35°.

2.7. Scanning electron microscopy

The starch samples were coated with ≈18 nm Au/Pt and examined by a scanning electron microscope (LEO 1530VP, Zeiss, German) with the field emission gun operating at 3 kV.

2.8. Statistical analysis

Each experiment was performed in duplicate. Analysis of variance was performed with the SAS program (version 9.1.3, SAS Institute Inc., Cary, NC). Least significant differences for comparison of means were computed at $p < 0.05$.

3. Results and discussion

3.1. Morphology and X-ray diffraction patterns of starches and maltodextrin

Both native waxy maize starch and microporous starch had an A-type X-ray diffraction pattern (Fig. 1), giving strong reflection at 15°, 17°, and 23°. The % relative crystallinity of the microporous starch (38%) was about the same as the native waxy maize starch, reflecting localized hydrolysis of starch granules by the enzymes. Maltodextrin showed no crystalline pattern, reflecting its amorphous character.

The surface morphology of granular waxy maize starch, microporous starch, and maltodextrin before and after OSA modification, was examined by scanning electron microscopy. Microporous starch had numerous pinholes from surface to interior (Fig. 2). OSA modification did not change the surface morphology of granular starch. Both native waxy maize starch and microporous starch were birefringent when viewed under polarized light, whereas maltodextrin showed no birefringence.

Table 1
Degree of substitution (DS) and reaction efficiency (RE) of 3% octenyl succinic anhydride (OSA) modified waxy maize starch when OSA was added slowly and predissolved in ethanol (3/10, w/v)[a].

OSA addition method	DS	RE
Poured	0.0191a	80.5a
Slowly added	0.0194b	81.7b
Slowly added and predissolved in ethanol	0.0194b	81.9b

[a] Reactions were conducted at pH 7.5, 3% OSA concentration, and 40% starch solids. DS and RE were determined by the titration method. Each result is the average of values of samples from two reactions. Numbers in the same column followed by a letter in common are not significantly different at $p < 0.05$.

3.2. Optimum OSA reaction conditions

Before we compared the reaction of the granular starches and the maltodextrin with different levels of OSA, we examined the effects of the rate of OSA addition, pH, and solids content on the OSA reaction.

3.2.1. Effect of addition rate and predissolving OSA in ethanol

Slow addition of OSA and predissolving OSA in ethanol marginally increased the DS and RE of the 3% OSA modification reaction with native waxy maize starch (Table 1). We found that reaction was highly reproducible. We conducted 3 reactions using 3% OSA and waxy maize starch and found that the variation in bound OS was less than 6%. For modifications using 15% or higher OSA concentrations, the pH of the reaction slurry decreased quickly, and it was difficult to maintain pH if OSA was added too quickly into the slurry. Therefore, dropwise addition of OSA was preferred for OSA concentrations higher than 15%. Similar phenomena were observed for reactions of OSA with microporous starch and maltodextrin.

Several researchers (He et al., 2006; He et al., 2008; Ruan et al., 2009; Song et al., 2006) reported the use of predissolved OSA in ethanol, but in many cases, did not compare the results with those obtained by adding OSA directly. In our study, RE did not increase when OSA was predissolved in ethanol. The RE was high (about 80%) even without predissolving OSA in ethanol (Table 1).

3.2.2. Effect of reaction time

The rate of reaction varied when 3% OSA was reacted with different physical forms of starch (Table 2). At 30 min, the RE for waxy maize starch and microporous starch was ca. 58% and 53%, respectively, but was greater than 90% for the soluble maltodextrin, suggesting a much faster esterification rate for the dispersed starch molecules. Considering the possible side reaction of OSA with water, it was remarkable to find that RE was almost 100% for maltodextrin after 1.5 h. Reactions of both waxy maize starch and microporous starch were judged complete at about 1.5 h because

Table 2
Effect of reaction time on degree of substitution (DS) and reaction efficiency (RE) of 3% OSA modification[a].

Time (h)	Waxy maize starch		Microporous starch		Maltodextrin	
	DS	RE (%)	DS	RE (%)	DS	RE (%)
0.5	0.0137a	58.4a	0.0125a	53.4a	0.0232	92.7
1.0	0.0189b	79.5b	0.0176c	74.2c	0.0235	94.0
1.5	0.0191b	80.5b	0.0185d	78.3d	0.0241	99.3
2.0	0.0187b	78.9b	0.0166b	70.3b		

[a] Reactions were conducted at pH 7.5, 3% OSA concentration, and 40% starch solids. DS and RE were determined by titration for OS waxy maize and microporous starches. Results are the average of two measurements. DS and RE of OS maltodextrin were determined by the NMR method, and one measurement was performed. Numbers in the same column followed by a letter in common are not significantly different at $p < 0.05$.

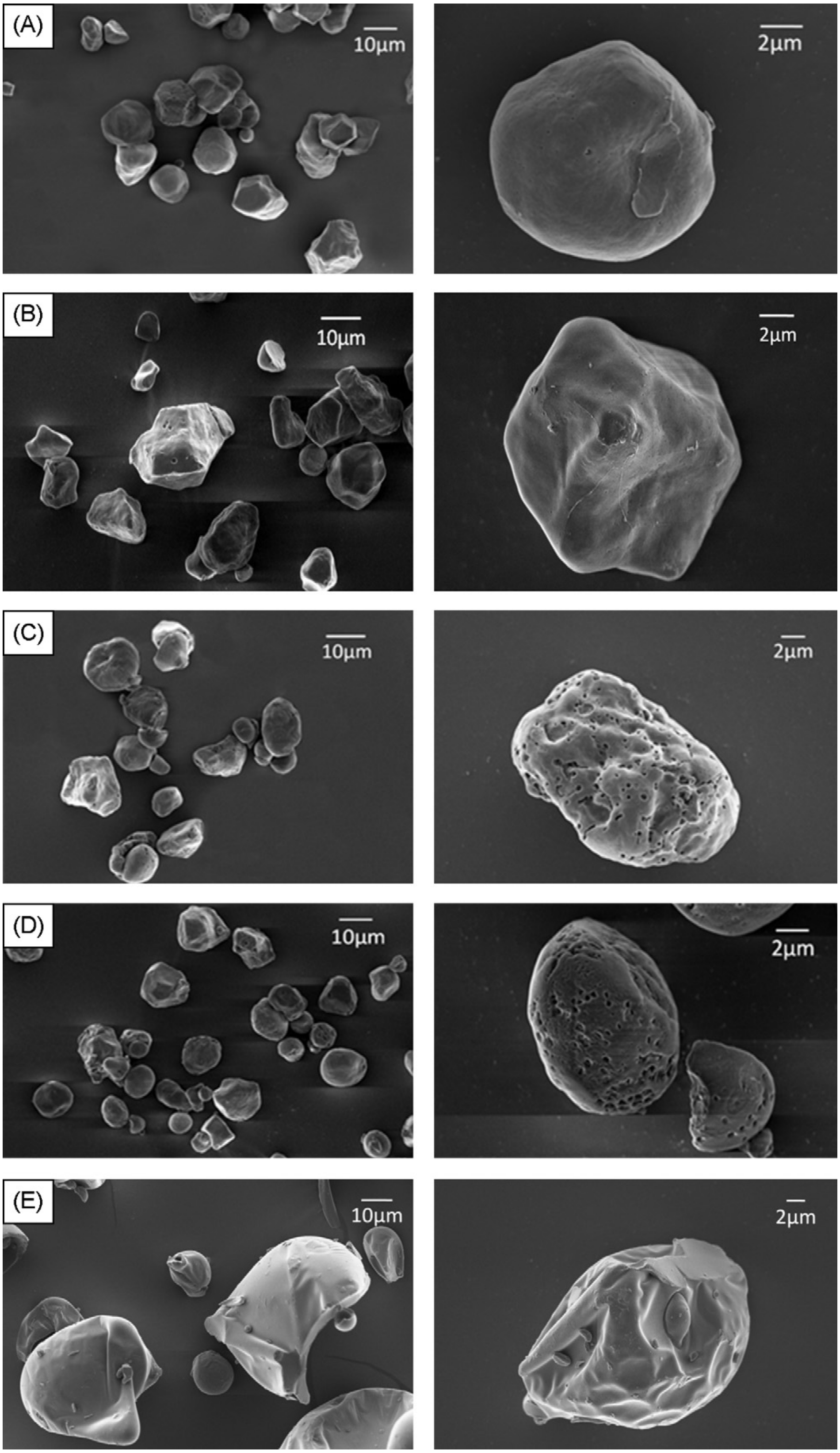

Fig. 2. Scanning electron microscopy (SEM) images of waxy maize starch (A), 15% OS waxy maize starch (B), microporous waxy maize starch (C), 15% OS microporous starch (D) and maltodextrin (E).

no NaOH was consumed after that time. Reactions required a longer time to complete when higher levels of OSA were used. When 9% OSA was used, it took about 6, 5, and 3 h for waxy maize starch, microporous starch, and maltodextrin, respectively, to complete the reaction. High levels of OSA caused starch granules to swell as discussed in Section 3.3 and Na_2SO_4 was added to prevent the starch granules from swelling. At 50% OSA, it took 4 h to complete the reaction with maltodextrin. In contrast, approximate 12 h

Table 3
Effect of pH on degree of substitution (DS) and reaction efficiency (RE) of 3% OSA modification of waxy maize starch and maltodextrin[a].

pH	Waxy maize starch		Maltodextrin	
	DS	RE (%)	DS	RE (%)
7.5	0.0191a	80.5a	0.0241a	99.3a
8.5	0.0195a	81.5a	0.0237a	97.1a
9.5	0.0173b	73.0b	0.0242a	101.2a
pH not controlled	0.0043c	18.6c	N/D	N/D

N/D, not determined.

[a] Reactions were conducted at 3% OSA and 40% starch solids. DS and RE of OS waxy maize starch were determined by the titration method. DS and RE of OS maltodextrin were determined by the NMR method. Each result is the average of values of samples from two reactions. Numbers in the same column followed by a letter in common are not significantly different at $p < 0.05$.

was needed to complete the reaction of OSA with the microporous starch.

3.2.3. *Effect of reaction pH*

Effects of reaction pH on 3% OSA modification of waxy maize starch and maltodextrin are shown in Table 3. When pH was controlled at 7.5, the RE for waxy maize starch was 80.5%. Increasing pH to 8.5 did not significantly affect RE or DS. However, increasing pH to 9.5 decreased RE to 73.0%. When pH was not controlled by incremental addition of NaOH, the reaction pH dropped to approximately 3, and RE was only 18.6%. Therefore, the preferable pH for preparing the starch ester with granular waxy maize starch was ca. 7.5–8.5.

At pH 7.5–9.5, the RE for maltodextrin was higher than that for waxy maize starch. RE for maltodextrin was 99.3% at pH 7.5. Increasing the pH to 8.5 and 9.5 did not significantly affect RE for maltodextrin.

Effects of pH on OSA reactions with granular starch have been reported previously (Bhosale & Singhal, 2006, 2007; He et al., 2006; Jeon et al., 1999; Liu et al., 2008; Ruan et al., 2009; Song et al., 2006). However, caution is needed when comparing results of different studies because reaction time and solids content, both of which affect reaction efficiency, were not the same in all studies. In some cases, it is not clear whether pH was kept constant during the reaction by incrementally adding NaOH. Nevertheless, the reported optimum pH is in the range of 7.5–9.0. As Jeon et al. (1999) explained, high pH (greater than 9) favors hydrolysis of OSA, whereas low pH (less than ~8) does not sufficiently ionize hydroxyl groups of starch for nucleophilic attack on the anhydride moieties. In the present study, we kept pH constant during the reaction and stopped the reaction 30 min after NaOH consumption ceased.

3.2.4. *Effect of slurry solids*

Maltodextrin solutions were prepared at 30%, 40%, and 50% solids. The solution of 60% solids was too viscous to stir and thus was not examined. As maltodextrin concentration increased from 30% to 50%, DS increased from 0.0236 to 0.0242 when starch was reacted with 3% OSA. RE was essentially 100% when the maltodextrin slurry solids' level was 40% or 50%. Because the starch slurry of waxy maize was difficult to stir at 50% solids, 40% solids content was used to compare the granular and dispersed reactions.

Effects of starch concentration on the reaction of OSA with granular starch have been extensively investigated (Bhosale & Singhal, 2006, 2007; He et al., 2006; Jeon et al., 1999; Liu et al., 2008; Ruan et al., 2009; Song et al., 2006). Increasing starch level generally gave a high RE. Thus, we used a high starch level (40%) for granular reactions in this study.

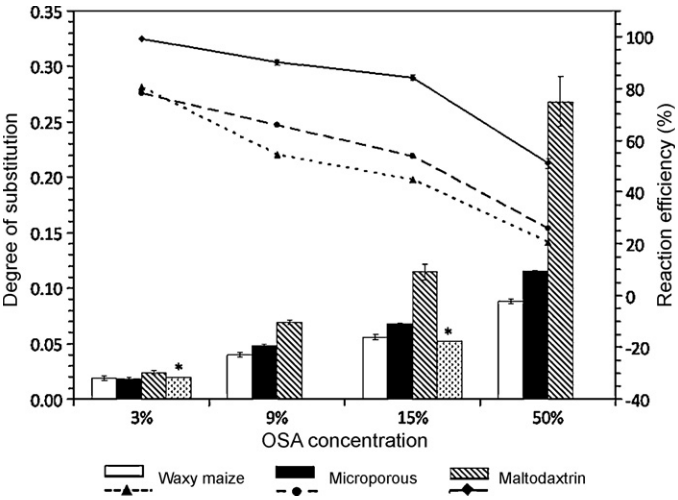

Fig. 3. Effect of OSA concentration on degree of substitution (bar graphs) and reaction efficiency (dotted lines). Reactions were conducted at pH 7.5 and 40% starch solids. DS and RE of modified waxy maize and microporous waxy maize starches were determined by the titration method. Bars with star notation were DS of modified waxy maize starches which was measured by the NMR method. DS and RE of modified maltodextrin were determined by the NMR method. Results were the average of two reactions.

3.3. *Comparison of OSA reaction with different physical forms of starches*

Granular waxy maize starch, microporous starch, and soluble maltodextrin were reacted with four levels of OSA (3%, 9%, 15% and 50%). As OSA concentration increased, DS increased but RE decreased for all three types of starches (Fig. 3). However, OS maltodextrins had a higher DS and RE than granular starches. When 3% OSA was used, RE was essentially 100% for maltodextrin, but as OSA concentration increased to 50%, RE decreased to 50%. The highest DS of OS maltodextrin obtained in this study was 0.27. For granular waxy maize starch, RE was 80.5% when 3% OSA was used, but as OSA concentration increased to 50%, RE decreased to 20.5%. The highest DS for granular waxy maize starch was 0.088, and the starch granules were partially swollen even in the presence of Na_2SO_4. For microporous starch, RE was only 78.3% when OSA concentration was 3%, and as OSA concentration increased to 50%, RE decreased to 26.0%. The highest DS for granular microporous starch was 0.12, and that modified starch did not swell in the presence of Na_2SO_4.

Our results suggest that only a limited substitution is attainable from OSA modification of granular starch via the aqueous slurry reaction. On the other hand, the maltodextrin used in this study was soluble in water, and all the hydroxyls along the oligomers were available for reaction, and they gave high DS and RE. In contrast, the reaction of OSA with insoluble granular starch is heterogeneous and occurs predominantly in the amorphous region (He et al., 2006; Shogren et al., 2000; Song et al., 2006).

High levels of OSA caused starch granules to swell, but the effects were different for granular waxy maize starch and microporous starch. At 9% OSA, the modified granules of waxy maize starch started swelling, and the reaction mixture became difficult to filter. The swollen starch granules lost their Maltese cross when viewed by the microscope (microphotographs not shown). Adding Na_2SO_4 to the starch slurry before the reaction prevented the modified starch granules from swelling. For 9% and 15% OSA modification of granular waxy maize starch, 5% Na_2SO_4 (based on the weight of starch) was enough to prevent starch from swelling and did not affect RE. However, when 50% OSA was used, starch swelling could not be prevented even when 20% Na_2SO_4 was added. In contrast, modified microporous waxy maize starch did not swell when reacted with 15% OSA, and at 50% OSA, starch swelling was avoided by using 20% Na_2SO_4 and the products were still birefringent when viewed under polarized light. These results suggest that granular waxy maize starch has a greater tendency to swell in the OSA reaction than microporous starch at high OSA concentrations. For future research, it would be interesting to examine how the levels of micropores (number and size of pinholes) affect swelling of starch granules and RE.

Comparing the RE of waxy maize with that of microporous starch at different levels of OSA modification revealed interesting results (Table 2 and Fig. 3). At 3% OSA, the RE for microporous starch was lower at an early reaction time but became similar to that of waxy maize starch after 1.5 h (Table 2). However, as OSA concentration increased, the reaction required a longer time to complete, and the DS and RE of the OS microporous starch were higher those that of the OS waxy maize starch (Fig. 3), indicating that creating pinholes and increasing the surface area of starch granules allowed more reagent to diffuse inside the granules. These results suggest that OSA distribution inside microporous starch granules may be different from that in waxy maize starch granules. Further study is needed to determine the locations, and to relate OSA distribution to functional properties such as emulsion stability.

Content of free OS in modified starches is shown in Table 4. As the OSA concentration in the reaction was increased, free OS content increased in the isolated modified granular starches and maltodextrin. However, the OS maltodextrins had a lower free OS content than granular starches because the RE of OSA modification of the maltodextrin was higher than that of the granular starches. Filtering and washing with water removed only part of the free OS. Previously, gas chromatography–mass spectrometry was used to quantify the content of total and unreacted OS in commercial OS starches (Park & Goins, 1995). In the present study, titration and NMR methods were used. Further research is needed to determine the effects of residual unreacted OSA on emulsification properties.

Table 4
Free OS content of OSA-modified waxy maize starch, microporous starch, and maltodextrin[a].

OSA concentration	Free OS content based on starch weight (%)		
	Waxy maize starch	Microporous starch	Maltodextrin
3%	0.29a	0.11a	0.00a
9%	1.87b	1.11b	0.40b
15%	1.91c	2.10c	1.11c
50%	4.80d	5.73d	4.80d

[a] Reactions were conducted at pH 7.5 and 40% starch solids. Results are the average of duplicates of reactions. Free OS content of OS waxy maize starches and microporous starches was determined by the titration method. Free OS content of OS maltodextrin was determined by the NMR method. Numbers in the same column followed by a letter in common are not significantly different at $p < 0.05$.

3.4. Structure of OS waxy maize starch and maltodextrin

In a previous study (Bai, Shi, & Wetzel, 2009), we confirmed formation of esters in the OSA-modified starches with Fourier transform infrared (FT-IR) microspectroscopy. In the present study, we examined the structure of OSA-modified starches with NMR. Detailed NMR assignments of the OSA reagent and modified starches are described in a separate study (Bai, Shi, Herrera, & Prakash, 2010).

3.4.1. Structure of OSA-modified granular starches

^1H NMR spectra of α-amylase digests of native waxy maize starch (S_0), and 50% OSA-modified starches with (S_2, DS = 0.088) and without methanol extraction (S_2') are shown in Fig. 4. Assignments are noted on each peak (Bai et al., 2010). Compared with the native starch (S_0), OSA-modified starches had several additional signals at 0.7–3.0 ppm, which are from the OS group. The broad peak at 0.8–1.0 ppm was from the methyl protons of the OS group. It is worth noting that the triplet at 0.85 ppm reduced significantly

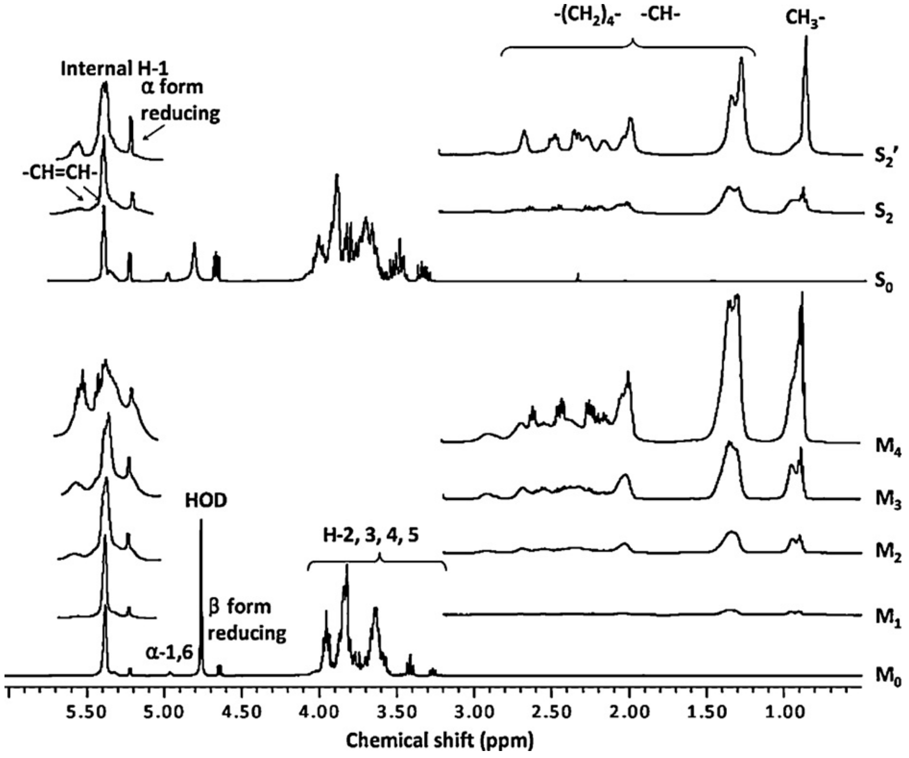

Fig. 4. ^1H NMR spectra of maltodextrin (M_0), OS maltodextrins with DS = 0.024 (M_1), DS = 0.069 (M_2), DS = 0.11 (M_3) and DS = 0.27 (M_4) and α-limit dextrins of the native waxy maize starch (S_0), 50% OSA-modified starch with DS = 0.088 (S_2) and 50% OSA-modified starch without methanol extraction (S'_2).

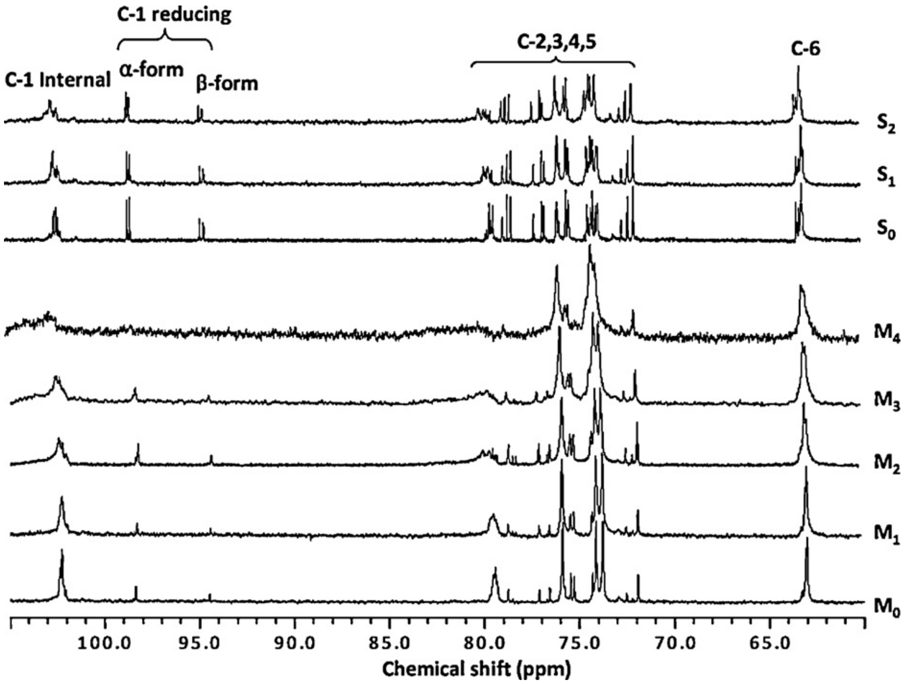

Fig. 5. ^{13}C NMR spectra of maltodextrin (M_0), OS maltodextrins DS = 0.024 (M_1), DS = 0.069 (M_2), DS = 0.11 (M_3) and DS = 0.27 (M_4) and α-limit dextrins of the native waxy maize starch (S_0), OSA-modified starch with DS = 0.040 (S_1) and DS = 0.088 (S_2).

but did not disappear after 50% OSA-modified granular waxy maize starch was thoroughly washed with methanol (Fig. 4). Therefore, the peak at 0.85 ppm was contributed by both free and bound OS. When the NMR method is used to calculate the DS of OS starch in D_2O, peaks at 0.94 and 0.85 ppm should be counted. In addition, a peak from one of the alkene protons was embedded under the peak of the anomeric proton on the internal repeat units of starch α-1,4 internal protons. To accurately calculate DS by using the NMR spectra, we subtracted the integral of the signal at 5.52 ppm from the other signal for the H-1 protons of repeat units, because the intensity of the double bond proton embedded with the H-1 glucose signal at δ 5.36 ppm is equal to the intensity of that at δ 5.52 ppm.

The DS of 3% OSA-modified starch was 0.0195 as obtained from the NMR method, comparing with 0.0191 of the titration method (Fig. 3). For the 15% OSA-modified starch, DS obtained from the NMR method and the titration method was 0.052 and 0.056, respectively (Fig. 3). Therefore, the DS calculated by using ^{1}H NMR was consistent with values obtained by using the titration method.

^{1}H and ^{13}C NMR spectra also were helpful in determining the position of OS substitution on starch. We (Bai et al., 2010) attributed peak broadening at 5.38 ppm (H-1 of α-1,4-linked repeated units) was due to substitution of OS at the O-2 position. The ^{13}C NMR spectra (Fig. 5) revealed that OS waxy maize starches (DS = 0.040 and 0.088) showed shoulder peaks at both 102.6 and 80.5 ppm, and the signals also became broader between 73 and 76 ppm, suggesting that the OS groups mostly were substituted at the O-2 and O-3 positions in granular waxy maize starch. As the DS increased to 0.088, there was no change for the peak at 63.5 ppm (arising from C-6), suggesting the hydroxyl group at C-6 was not substituted by the OS group.

3.4.2. Structure of OSA-modified maltodextrins

Maltodextrin and OSA-modified maltodextrin (DS = 0.024, 0.070, 0.11, and 0.27) were analyzed by ^{1}H and ^{13}C NMR (Figs. 4 and 5). When reacted with the soluble maltodextrin, OSA might have reacted at many different hydroxyl groups along the maltodextrin chain. At a low level of OSA modification (DS = 0.024, M_1 in Fig. 5), peak broadening was observed for resonances at 102.6 (C-1) and 79.5 ppm (C-4), indicating the reaction occurred at the O-2 and O-3 positions which slightly shifted the signals of C-1 and C-4. As OS modification level increased (DS = 0.070, 0.11, and 0.27; M_2, M_3 and M_4, respectively, in Fig. 5), resonances at 98.3 (C-1) and 63.5 ppm (C-6) became broad, too, indicating that OSA reacted on the reducing end and at the O-6 position. In addition, signals between 73.9 (C-2) and 76.0 ppm (C-3) became broader, further suggesting that hydroxyl groups at the O-2 and O-3 positions were substituted. However, quantifying the level of the OS groups at O-2, O-3, O-6, and the reducing end was not possible.

Our NMR results suggest the structure of OSA-modified maltodextrin was different from that of OSA-modified granular starch. When OSA reacts with a granular starch, the reaction is heterogeneous at the molecular and granular levels. Using FT-IR microspectroscopy, we found that OS concentration varied between waxy maize starch granules, suggesting the reaction was not uniform (Bai et al., 2009). Shogren et al. (2000) reported that more OSA reacted on the surface of starch granules. Because native granular starch is partially crystalline, only certain zones, most likely the glucose units in amorphous regions, can be reached by OSA. In contrast, the maltodextrin we chose to study is water soluble, and all glucose molecules are available for reaction. However, we do not suggest that the reaction is uniform, even on soluble maltodextrin. Certain positions, such as the glucose at the reducing end, may be more reactive than other glucose units.

4. Conclusions

Physical form of starch affected OSA modification. A water soluble maltodextrin has all of its glucose units available for reaction; its OS product gave a higher DS and RE. In contrast, the reaction of OSA with insoluble granular starch is heterogeneous and occurs predominantly in the amorphous region leading to a lower RE. For the 3% OSA treatment, RE was almost 100% for soluble maltodextrin, but about 80% for granular waxy maize starch. Creating pinholes and increasing the surface area of starch granules allowed more

OSA reagent to diffuse to reaction site in the granules, resulting in a higher DS and RE when OSA was above 9%. In the aqueous slurry reaction, the highest DS of OSA-modified waxy maize granular starch, microporous starch, and maltodextrin were 0.088, 0.12, and 0.27, respectively. OSA substitution occurred mostly at the O-2 and O-3 positions in granular starch, whereas, substitution in the maltodextrin occurred at O-2, O-3 and O-6 internal and the reducing end glucose units. Further research is needed to study the effects of OS distribution on starch function properties, such as emulsification.

Acknowledgments

We thank Dr. Paul Seib for helpful discussions and reviewing the manuscript, Dr. Yijun Sang for preparing the microporous starch, Dr. Om Prakash and Mr. Alvaro Herrera for obtaining some of the NMR spectra, and Dr. Leila Maurmann for training on the operation of the NMR spectrometer.

This is contribution 10-200-J from the Kansas Agricultural Experiment Station.

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