PREPARATION, STRUCTURE AND PROPERTIES OF OCTENYLSUCCINIC ANHYDRIDE MODIFIED STARCH

by

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B.S. China Agricultural University, 2006
M.S. Kansas State University, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

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Abstract

The reaction of starch and octenylsuccinic anhydride (OSA) produces lipophilic starch that has the ability to stabilize oil-in-water emulsions. The functional properties of octenylsuccinate (OS) starch depend on its molecular structure and distribution of OS groups. Structures of OSA and OS starches were investigated by NMR spectroscopy. In granular OS starches, OS groups were substituted at O-2, O-3 positions, but not the O-6 position. Distribution of OS groups was investigated by enzyme hydrolysis followed by chromatography analysis. OS substitution predominantly occurred at the amorphous region of the starch granules. OS starch of degree of substitution (DS) 0.018 had OS groups located close to the branching points, whereas the OS substitution in OS starch of DS 0.092 occurred near non-reducing ends as well as the branching points. OS starches with different substitution patterns were prepared from two approaches. OS starches from the first approach had OS substitution near the branching points or non-reducing ends, whereas OS starches from the second approach had OS groups distributed randomly throughout the starch chains. A method of preparing OS starch by dry heating a mixture of waxy maize starch and OSA was developed. The optimum reaction was investigated and found to be pH 8.5 by addition of 3% NH_4HCO_3, 180 °C and 2 h. Reaction efficiency of ca. 90% was obtained at OSA levels from 1 to 6%. The OS starch had a DS of 0.0202 with 98% solubility when reacted with 3% OSA. Transglucosidation occurred during the reaction. The OS starch had a degree of branching of 19.8 %. The highly debranched OS starch showed excellent emulsification property for vitamin E and vitamin A.

The structural changes of insoluble native waxy maize starch granules to cold water-soluble pyrodextrin during dextrinization under acidic conditions were investigated. We proposed that the starch was hydrolyzed by acid in the amorphous regions. Unwinding of the double helices also occurred, and crystallite size decreased. Starch molecules were hydrolyzed into small molecule fractions but remain in a radial arrangement. Glycosyl linkages including α-(1→2), α-(1→6), β-(1→2), and β-(1→6) linkages were formed and the majority starch chain terminals were 1,6-anhydro-β-D-glucopyranose. Transglucosidation occurred during dextrinization and the resulted pyrodextrin was highly branched.

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Table of Contents

List of Figures

List of Tables

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Dedication

To

My grandparents Xiuhua Li and Hongkui Wang

My parents Qin Wang and Fenglian Bai

For their love and support.

Chapter 1 - Introduction

Octenyl succinic anhydride (OSA) modified starch was developed by Cardwell and Wurzburg in 1953 (Caldwell & Wurzburg, 1953). Polysaccharides are hydrophilic in nature. After introducing hydrophobic groups from chemicals such as OSA, starch becomes amphiphilic and shows surface active properties. Preparation and properties of octenylsuccinate (OS) starch was reviewed by Sweedman et al. (2003). Unique from other starch derivatives, octenylsuccinate (OS) starch was approved for food use by US FDA in 1972. It is widely used in beverage emulsions, flavors, clouding agents, salad dressings, creams as well as other applications in pharmaceutical and industrial areas (Trubiano, 1986).

Preparation of OS starch

OS starch is prepared from esterification reaction (Figure 1.1). Polysaccharides include starch, gelatinized or ungelatinized were suggested to be reacted with OSA in an aqueous slurry system, dry state or organic suspension (Caldwell & Wurzburg, 1953). OS starch is prepared by a standard esterification reaction where OSA and the starch suspended in water and mixed under alkaline conditions (Trubiano, 1986). Reaction parameters including pH, reaction time, temperature, starch concentration and OSA concentration affect the degree of substitution (DS) and reaction efficiency (RE) of OS starch. For the 3% OSA modification, DS obtained from granular starch in aqueous slurry reaction under optimum condition was in the range of 0.017 to 0.020. Reaction efficiency was in the range of 72 to 82%.

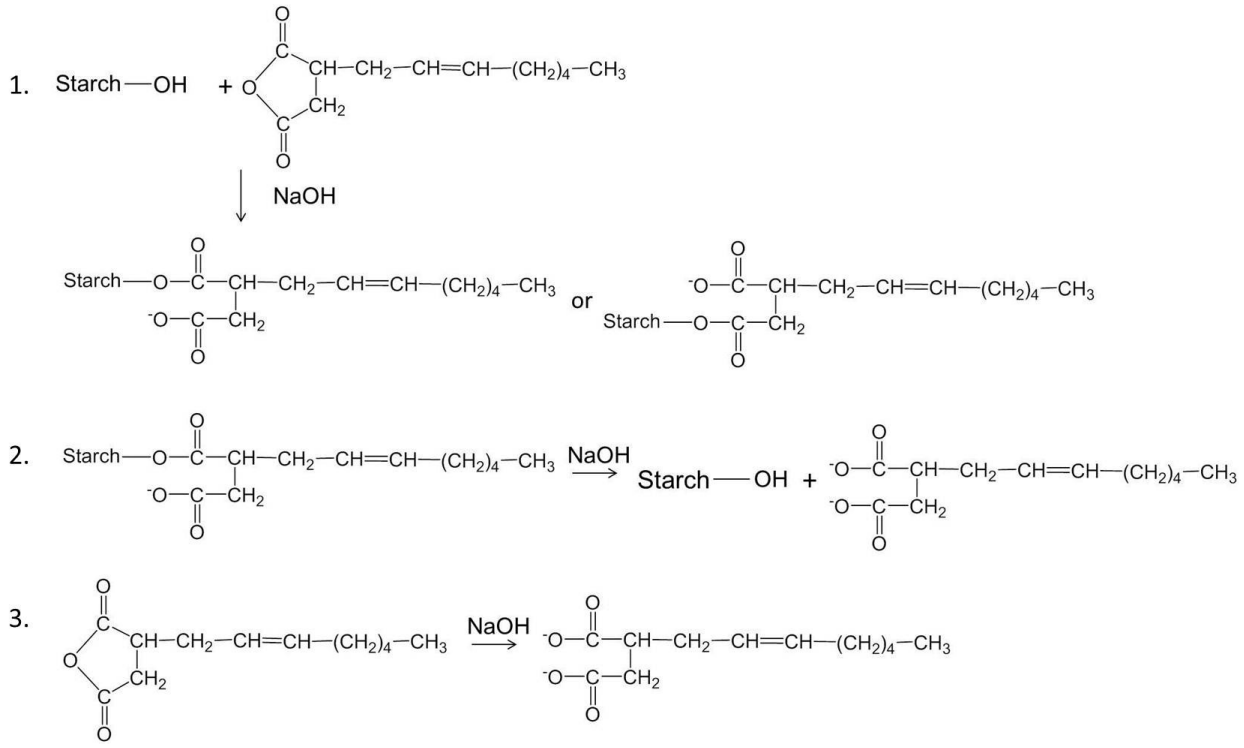
Reaction conditions

Reaction pH

OSA reaction was reported to be optimum at 8.0 ± 0.5 (Abdollahzadeh, Mehranian & Vahabzadeh, 2008; Bai & Shi, 2011; Bhosale & Singhal, 2006; Jeon, Lowell & Gross, 1999; Liu et al., 2008; Ruan, Chen, Fu, Xu & He, 2009; Song, He, Ruan & Chen, 2006; Zhu, Xie, Song & Ren, 2011). pH below 7.5 was suggested to be not efficient to activate the hydroxyl groups of starch for nucleophilic attack of the anhydride moieties (Jeon, Lowell & Gross, 1999). And pH above 9.5 was suggested to favor the side reactions as shown in Figure 1.1 of reaction 2 and 3 (Song, He, Ruan & Chen, 2006) and probably cause swelling of starch granules. Dilute NaOH

2-3% (wt%) was added to maintain pH during the reaction with sufficient stirring (Bai & Shi, 2011; Jeon, Lowell & Gross, 1999; Liu et al., 2008; Song, He, Ruan & Chen, 2006). High concentration of NaOH would cause locally high alkalinity and result in starch swelling. Maintaining the insolubility of the starch granule during chemical reaction and purification is very important. Therefore for aqueous process, low alkalinity and dilute NaOH to control pH were preferred (Tessler & Billmers, 1996). However concentration of NaOH lower than 2% (wt%) was not favorable because it would dilute the starch slurry and reduce the reaction efficiency.

Figure 1.1 Chemical reaction of OSA and starch.



Reaction time

Reaction time of OSA modification varied from 1.5 to 18.7 h (Abdollahzadeh, Mehranian & Vahabzadeh, 2008; Bai & Shi, 2011; Bhosale & Singhal, 2006; Jeon, Lowell & Gross, 1999; Liu et al., 2008; Ruan, Chen, Fu, Xu & He, 2009; Song, He, Ruan & Chen, 2006). Long reaction time was used in many studies as it was believed to favor the diffusion and adsorption of the reactants between the modifying agents and starch molecules (Khalil, Hashem & Hebeish, 1995). However, by comparing OS starches obtained from reaction of 1.5 to 18.7 h, DS was marginally better for the long reaction time. In addition, reaction time was closely related to the speed of

OSA addition. Slowly addition of OSA was used in many studies (Abdollahzadeh, Mehranian & Vahabzadeh, 2008; Bhosale & Singhal, 2006; Jeon, Lowell & Gross, 1999; Liu et al., 2008; Ruan, Chen, Fu, Xu & He, 2009; Song, He, Ruan & Chen, 2006). However, it has been observed that the addition speed of OSA did not significantly affect the reaction efficiency at low level of OSA modification (3% OSA). But at high OSA level (above 9%), dropwise addition of OSA was beneficial for high reaction efficiency (Bai & Shi, 2011).

Reaction temperature

Reaction temperature from 15 to 60 °C was studied and 30 to 35 °C was reported to be most desirable temperature for OSA modification (Bhosale & Singhal, 2006; Jeon, Lowell & Gross, 1999; Liu et al., 2008; Song, He, Ruan & Chen, 2006; Zhu, Xie, Song & Ren, 2011). High temperature was expected to enhance the solubility of OSA in the aqueous phase, OSA diffusion into starch granule as well as swelling of starch granule to improve reaction efficiency. However, reaction efficiency of modification above 35 °C either remained constant or decreased (Bhosale & Singhal, 2006; Jeon, Lowell & Gross, 1999; Ruan, Chen, Fu, Xu & He, 2009; Song, He, Ruan & Chen, 2006). It was suspected that an increase in reaction temperature would enhance hydrolysis of OSA thus reduced the reaction efficiency (Jeon, Lowell & Gross, 1999). It might also be possible that high temperature would accelerate hydrolysis of OS starch and remove the substituted OS group and decrease the reaction efficiency.

Starch concentration

Starch concentration of 30 to 45% was suggested to be the optimum for OSA reaction (Bai & Shi, 2011; Jeon, Lowell & Gross, 1999; Liu et al., 2008; Ruan, Chen, Fu, Xu & He, 2009; Song, He, Ruan & Chen, 2006; Zhu, Xie, Song & Ren, 2011). High starch concentration might increase the chance of contact between OSA and starch granules (Song, He, Ruan & Chen, 2006). In addition, it might increase the starch-reagent reaction relative to reagent hydrolysis, which is a competitive process (Jeon, Lowell & Gross, 1999). Starch slurry of concentration over 40% becomes too viscous to agitate therefore is not recommended.

OSA concentration

OSA modified starch for food application is restricted to be 3% or below by US FDA and OS starch of higher DS may have other applications. It has been observed that below 3% OSA

modification level, DS and RE increased as OSA level increased, which was interpreted due to the greater availability of the OSA molecules in the proximity of the starch molecule (Bhosale & Singhal, 2006). As OSA level increase above 3%, DS continued to increase but RE decreased (Bai & Shi, 2011; Ruan, Chen, Fu, Xu & He, 2009; Song, He, Ruan & Chen, 2006). Decreasing in reaction efficiency was suspected due to the dilution of starch slurry by using high amounts of anhydride and alkaline reagents (Song, He, Ruan & Chen, 2006). In addition, it has been noticed that as DS passed certain level, starch granules started to swell and lose their birefringence (Bai & Shi, 2011). The results reflected that only limited amount of OSA can be reacted with starch without disrupting their granular structure. Decreasing in reaction efficiency might also due to the restriction and barrier of the starch granular structure. The highest DS obtained for granular waxy maize starch was 0.088 (Bai & Shi, 2011). Swelling of the starch granules is undesirable and should be prevented during the production. Sodium nitrate of 5 to 20% (wt%) was suggested to prevent starch swelling up to OSA level of 15% (Bai & Shi, 2011).

Starch recovery and purification

OS starch is normally recovered by filtration and then washed by water and alcohol. Water is able to remove the salt residues created during the reaction. However, it is not efficient to remove the free OSA. The free OSA content was 0.29% of OS granular starch as purified by water (Bai & Shi, 2011; Qiu, Bai & Shi, 2012). Free OSA is probably detrimental to the stability of the emulsion prepared from OS starch. Alcohol would be an efficient solvent to remove the free OSA.

Botanical source of starch

The optimum reaction condition was observed to be slightly different for starch from different origins (Bhosale & Singhal, 2006). Potato starch was observed to have relatively low reaction efficiency compared with waxy maize, early indica rice and amaranth starches (Ruan, Chen, Fu, Xu & He, 2009). The difference might be due to the morphology and granular structure of the starches that are from different botanical sources. It has been observed that amylose has a positive impact on OSA modification (He, Song, Ruan & Chen, 2006). The result was proposed due to the model of the starch granule that amylose domains are in amorphous regions and better accessible than crystalline lamellae.

OSA reaction on other materials

OSA modification was investigated on materials other than native granular starch, including inulin, zein protein, microporous starch and soluble maltodextrin (Morros, Levecke & Infante, 2011; Biswas, Sessa, Lawton, Gordon & Willett, 2005; Bai & Shi, 2011; Huang, Fu, He, Luo, Yu & Li, 2010). Optimum reaction condition varied for different materials and the products exhibit different physical and chemical properties. OSA modification on inulin was investigated to prepare a natural polymer based surfactants in colloidal systems (Morros, Levecke & Infante, 2011). OSA was reacted with zein protein to improve the water resistance of the protein and enhance its applications in food coatings and biodegradable materials (Biswas, Sessa, Lawton, Gordon & Willett, 2005).

In our previous study, OSA reaction on native waxy maize, microporous starch and soluble maltodextrin was compared (Bai & Shi, 2011). Compare to native starch, DS was lower for microporous starches at OSA concentration of 3 to 9% (Bai & Shi, 2011). However, OSA was able to react with the starch to a higher DS of 0.12 without swelling of the starch granules (Bai & Shi, 2011). It has been suggested that OSA substitution occurred in the inner crystalline region of the enzyme treated starch (Huang, Fu, He, Luo, Yu & Li, 2010). Soluble maltodextrin is a starch derivative from α-amylase hydrolysis. OSA was suggested to react with maltodextrin with high reaction efficiency of 100% at 3% OSA level. The highest DS obtained from soluble maltodextrin is 0.27 (Bai & Shi, 2011). OSA modification on soluble polysaccharide was described in the original invention (Caldwell, Hills & Wurzburg, 1953). But very few researches were performed to investigate the preparation, structure and property of the starch.

Reaction types

Beside slurry reaction, dry-heating method of preparing OS starch was introduced by Kim et al. (2010). Granular starch was mixed with OSA that was hydrolyzed in water and pH was adjusted to 3 to 5. The dried starch was heated in the oven at 130 to 150 °C for 1 to 3 hours. The resulted products had DS in the range of 0.015 to 0.023 and the molecular weight decreased significantly from its native starch counterparts. It has been suggested that DS and dextrinization were affected mostly by pH as well as temperature and heating time (Kim, Sandhu, Lee, Lim & Lim, 2010). The modified starch showed different pasting profile from native starch and it

showed promising application as fat-replacing compound in diary cream and fat-reduced muffin (Chung, Lee, Han & Lim, 2010; Kim, Sandhu, Lee, Lim & Lim, 2010).

Analysis of OS starch

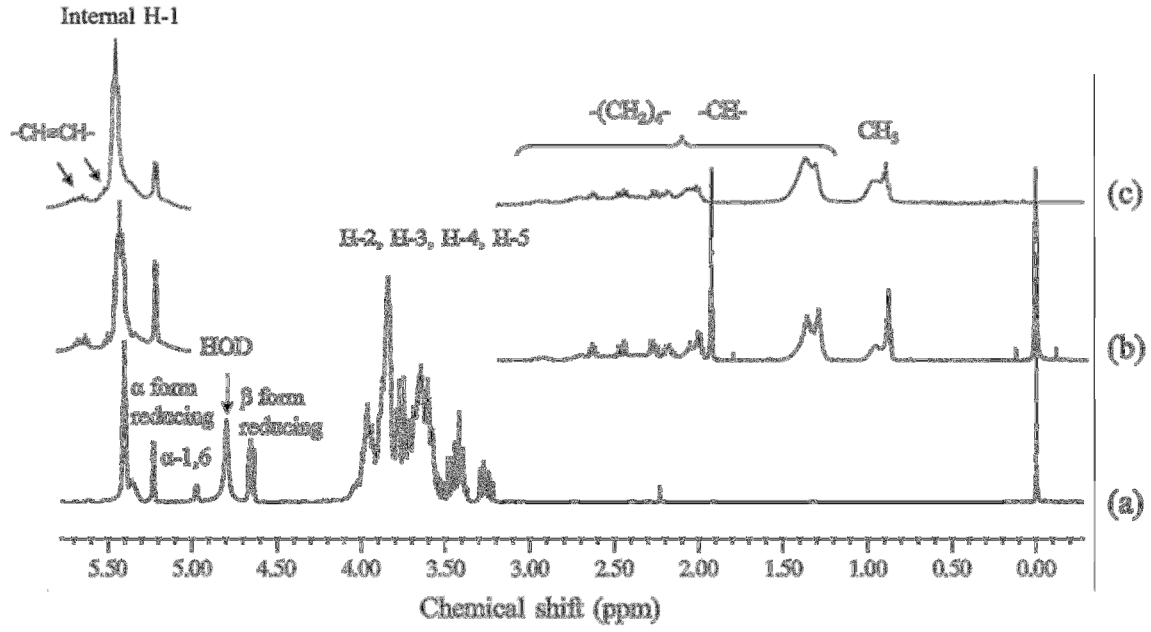
Bound and free OSA content are important characteristics for OS starches. Bound OSA content is used to calculate degree of substitution (DS). DS is a measure of the average number of hydroxyl groups on each D-glucopyranosyl unit (AGU) which are derivatized by substitution groups (Wurzburg, 1986). The maximum possible DS for starch is 3 since the majority of the AGUs have three hydroxyl groups available for substitution. Percentage OSA (%OSA) is expressed as the weight of OSA as a percentage of the total starch by substance. DS and % OSA can be determined by titration (Bai & Shi, 2011; Huang, Fu, He, Luo, Yu & Li, 2010; Song, He, Ruan & Chen, 2006; Kim, Sandhu, Lee, Lim & Lim, 2010), NMR spectroscopy and chromatography methods (Bai & Shi, 2011; Tizzotti, Sweedman, Tang, Schaefer & Gilbert, 2011; Nilsson & Bergensthl, 2007)

Traditionally, DS is determined by titration method. It is a fast and accurate method and has been used in many studies (Bai & Shi, 2011; Huang, Fu, He, Luo, Yu & Li, 2010; Song, He, Ruan & Chen, 2006). Titration method was also used to determine DS for partially soluble OS starch after modification (Kim, Sandhu, Lee, Lim & Lim, 2010).

^{1}H-NMR spectroscopy is another common method to determine DS. Typical ^{1}H-NMR spectra of α-limit dextrin of native waxy maize starch and OS starch with different DS are shown in Figure 1.2. A resonance at 0.87 ppm is assigned to methyl protons of the OS substituent. DS is calculated by the ratio of the integral from the methyl group to the sum of the starch H-1 peaks at 5.36, 5.22, 4.96 and 4.63 ppm (Bai & Shi, 2011). DS obtained from the NMR method is consistent with titration method with high accuracy and precision (Yanjie master thesis). In another method reported by Tizzotti et al (2011), starch without degradation was dissolved in DMSO-d_6. A very low amount of deuterated trifluoroacetic acid (d1-TFA) was added to the starch solution to shift the exchangeable protons of the starch hydroxyl groups to high frequency leading to a clear and well-defined ^{1}H NMR spectrum. The practice provides an improved way to determine the degrees of both branching and chemical substitution (Tizzotti, Sweedman, Tang, Schaefer & Gilbert, 2011).

Chromatography is used to quantify free and bound OSA in OS starches. Free and bound OS was determined by GC-MS and HPLC (Park & Goins, 1995; Qiu, Bai & Shi, 2012). Extraction of free and bound OSA is the key procedure to the two approaches. For the GC-MS approach, OS starch was dispersed in water for 3-4h at temperature below 80 °C then extracted by methanol to determine free OS. Total OSA was determined after alkaline hydrolysis of the ester bond of bound OSA and represented the sum of free and bound OSA (Park & Goins, 1995). Sample preparation of OS starch for HPLC was relatively easy compare to GC-MS. Free OSA was extracted by dispersing OS starch in methanol for 30 min. Total OSA was obtained by hydrolyzing OS starch with NaOH overnight. Adjust pH to acid is also critical for obtaining accurate results. In one study, HPLC identified six compounds of OSA and its hydrolyzed products which are 1-OS acid, *cis*-2-OS acid, *trans*-2-OS acid, 1-OSA, *cis*-2-OSA and *trans*-2-OSA (Qiu, Bai & Shi, 2012).

Figure 1.2 ^{1}H-NMR spectra of α-limit dextrin of native starch (a), 3% OS starch (b) and 15% OS starch (C)



Structure of OS starch

Location of OS substitution among and within starch granules and within the amylopectin and amylose molecules has been investigated. OS substitution was distributed throughout the starch granule at DS of 0.03 and 0.11 (Shogren, Viswanathan, Felker & Gross, 2000). A FT-IR

microspectroscopy study, which identified the OS substitution level on each single starch granule from random sampling, indicated that although most of the starch granules were accessible to OSA modification, the reaction was not uniform (Bai, Shi & Wetzel, 2009). The substitution location within starch granule was suggested to be heterogeneous. An X-ray photoelectron spectroscopy (XPS) study reported that the surface of starch granule is enriched with OSA group by a factor of 3-4 over that of the bulk granule (Shogren, Viswanathan, Felker & Gross, 2000). An interesting discussion was made in the publication about diffusion of OSA reagent into the starch granule through channels and cavity. A possible reaction mechanism was mentioned that OSA reagent was diffused into the starch granule followed by reaction with the starch (Shogren, Viswanathan, Felker & Gross, 2000). Another mechanism was also proposed. It was introduced that OSA in an aqueous slurry system may exist as a mixture of droplets and dissolved form as the low solubility of OSA in water (Shogren, Viswanathan, Felker & Gross, 2000). The droplets would react with the granule surface. OSA may travel into the starch granule and react when break into extremely fine particles (Shogren, Viswanathan, Felker & Gross, 2000). Details studies are required to investigate the mechanisms.

Granular starch is semi-crystalline, consisting of alternative lamellar of amorphous and crystalline regions. OS modification was suggested to be occurred in the amorphous regions of the starch granules since the wide-angle x-ray diffraction (WAXD) did not show any changes in the crystalline pattern of starch before and after modification (Bai & Shi, 2011; Bhosale & Singhal, 2007; Shogren, Viswanathan, Felker & Gross, 2000; Song, He, Ruan & Chen, 2006). However, the crystallinity of native starch was suggested to be slightly higher than their esters (He, Song, Ruan & Chen, 2006). After OSA modification, the gelatinization temperature and enthalpy decreased with increasing concentration of OSA for waxy maize and amaranth starch (Bhosale & Singhal, 2007). The results reflect that the granular structure of native starch was affected by OSA modification.

OSA substitution location on the starch anhydroglucose units (AGU) was investigated by NMR spectroscopy. Starch is a polysaccharide consists of glucose linked by α-1,4 and 1,6 linkages. It has been suggested that for granular reaction in an aqueous slurry system, OS substitution occurred primarily at O-2 and 3 positions but not at O-6. Substitution location on an AGU was different when reaction was carried out with soluble maltodextrin. OS substitution

occurred at O-2, 3 and 6 as well as the reducing end (Bai & Shi, 2011). Substitution location may affect the functional properties of OS starch.

Application of OSA modified starch

Emulsion stabilization mechanism of OS starch has been investigated in the past few years. Stabilization mechanism of OS starch was suggested to be steric hindrance, since little effect of pH, ionic strength and temperature on emulsion stability was observed (Charoen, Jangchud, Jangchud, Harnsilawat, Naivikul & McClements, 2011; Qian, Decker, Xiao & McClements, 2011). Gum arabic showed good stability to environment stress as well. However it produced larger droplet size and required higher hydrocolloid concentration comparing to OS starch (Qian, Decker, Xiao & McClements, 2011). OS starch was suggested to be adsorbed at the interface of oil/water emulsion (Nilsson & Bergenstahl, 2006). The adsorption is governed by the relationship between interfacial area and OS starch concentration. Very high surface load of OS starch was observed which was suggested to be caused by jamming at the interface due to lack for conformational changes and /or multilayer adsorption (Nilsson & Bergenstahl, 2006). Besides stabilizing emulsion by forming a film at the oil/water interface, OS starch showed to have capability to modify viscosity of continuous phase. Apparent viscosity and physically stability of the emulsion system increased with increase in OS starch concentration. (Dokic, Krstonosic & Nikolic, 2012). Substitution level of the OS starch, which was prepared in an aqueous slurry system, showed little effect on the emulsion stability (Viswanathan, 1999). During the homogenization process, molar mass and root mean square radius of OS starch were significantly decreased (Modig, Nilsson, Bergenstahl & Wahlund, 2006).

OSA modified starch was also used as wall material for encapsulation. Microcapsules can be prepared from spray granulation, spray drying and freeze drying (Anwar & Kunz, 2011). Oil load of the infeed emulsion markedly influenced the properties of the infeed liquid and the characteristic of the resulting powder. Retention of the active material was well correlated with the emulsion droplet diameter of the infeed liquid (Paramita, Furuta & Yoshii, 2012).

Objectives

OS starches have shown excellent emulsifying properties in many industrial applications. However, the relationship between the starch structure and functional properties has not been well understood.

The objectives of this dissertation were to:

1. Determine the detailed structure of the OSA reagent, and differentiate 1- and 2-octenylsuccinic anhydride as well as their *cis* and *trans* isomer.
2. Understand the OSA reaction with a mixture of granular starch and soluble maltodextrin.
3. Elucidate the substitution of OS groups in starch chains from the evaluation of the enzyme hydrolysates of OS starch.
4. Prepare and characterize α-amylase-degraded OS starches with different OS distributions.
5. Develop a method of prepare soluble OS dextrins that can be used in beverage applications. Investigate the optimum reaction condition and characterize the structure OS dextrins.
6. Investigate the structural changes during thermal decomposition based on small angle X-ray scattering, wide angle X-ray diffraction, microscopy, gel permeation chromatography, and differential scanning calorimetry.
7. Analyze the structure of pyrodextrin using NMR spectroscopy. It is the first detailed study being conducted to interpret 1D and 2D-NMR spectra of pyrodextrin.

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Chapter 2 - Study of octenylsuccinic anhydride modified waxy maize starch by nuclear magnetic resonance spectroscopy[1]

Abstract

Granular waxy maize starch was reacted with two levels (3 and 15%, based on the weight of starch) of octenylsuccinic 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-glucose 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 the O-6 position.

Keywords

Starch, octenyl succinic anhydride, nuclear magnetic resonance spectroscopy

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Introduction

Starch is the reserve carbohydrate of higher plants and occurs as granules throughout the plant kingdom. However, only a limited number of plants including maize, wheat, potato, cassava, rice, sorghum, sago, and arrowroot are grown for commercial starch production (Daniel et al., 2007). Native starches are used in food and industrial applications (Daniel et al., 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 and Solarek, 1984; Trubiano, 1986; Wurzburg, 2006). In addition, it has been used in biodegradable plastics (Jane et al., 1991) and emulsified foods as fat replacers (Cho et al., 1999; Kim et al., 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 et al. 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 et al., 2003, Bhosale & Singhal, 2006, He et al., 2006; Hui et al., 2009; Jeon et al., 1999; Liu et al., 2008; Shogren et al., 2000) and nuclear magnetic resonance (NMR) (Čížová et al., 2007; Choi et al., 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 (Figure 2.1 a 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) (Figure 2.1 c and d). Some researchers stated that they used 1-octenyl succinic anhydride (Scheffler et al., 2009) and 1-octenyl succinic 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 glucose 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 homonuclear correlation and 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 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.

Materials and methods

Materials

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.

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 addition of OSA, the pH was stable in 30 min and the reaction mixture was adjusted to pH 6.0 by 1.0 N 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.

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 to 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 ºC 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.

^{1}H and ^{13}C NMR 1D spectra were recorded on a 500 MHz Varian NMR spectrometer at 25ºC. 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 ^{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. ^{1}H-^{1}H 2D homonuclear correlation spectroscopy (COSY) was recorded with a single transient per t1 increment with a sweep width of 3242.8 Hz in both dimensions. Heteronuclear single quantum coherence (HSQC) ^{1}H-^{13}C 2D 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. Sodium 3-(trimethylsilyl) - propionate-2,2,3,3-d4 (TSP) was used as a reference (0 ppm). Chemical shifts are reported in parts per million (ppm).

Results and discussion

Structure of OSA reagent

A ^{1}H-^{1}H COSY spectrum of the OSA reagent in methanol is shown in Figure 2.2. Resonance of the terminal methyl protons is close to 0.87 ppm, and chemical shifts of the protons on the C-C double bond are in the region of 5.27 to 5.63 ppm (Guillen and Ruiz, 2001; Knothe and Kenar, 2004; Pavia et al., 2008). After those peaks were identified, the remaining peaks were assigned with the assistance of the COSY spectrum (Figure 2.2) by tracing out the connectivity through cross-peaks, which arise from coupling between the protons (McIntyre and Vogel, 1990). Proton assignments are noted on each peak in Figure 2.2. 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), a Web site organized by the National Institute of 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 (Figure 2.2). 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 Figure 2.2), we calculated their coupling constant. The coupling constant ^{3}J (H-H) of the proton pair at the double bond was 15.21 Hz, indicating the trans configuration was the predominate form. The ^{3}J coupling constant for protons that are cis to each other would have a smaller value, close to 10 Hz (Pavia et al., 2008).

The ^{1}H-^{1}H COSY spectrum (Figure 2.2) 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 (Figure 2.1 a and 1b), not 3-(E-oct-1-enyl)dihydrofuran-2,5-dione (Figure 2.1 c and d). Moreover, the ratio of the area of the resonances that arose from the methylene protons labeled K (1.39 to1.22 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 ^{1}H-^{13}C HSQC spectrum (Figure 2.3), which shows the correlations between a ^{13}C atom and its directly bonded protons (McIntyre and Vogel, 1990), the resonance of the ^{13}C NMR spectrum of the OSA in methanol were assigned (Table 2.1). 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 ^{1}H-^{13}C HSQC spectrum (Figure 2.3). Carbons of a *cis*-double bond occur upfield (Barton II et al., 1975; Gao et al., 2009; Pfeffer et al., 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 (C8) adjacent to the *cis* and *trans* double bonds, respectively. Once the allylic carbon (C8) resonances were identified, we were able to determine their connected protons by 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 (Figure 2.3), 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 Figure 2.2, we estimated that the ratio of the *trans* to *cis* isomers in the OSA reagent to be about 5 to 1.

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 Figure 2.4. Peaks arising from the

glucose in starch were assigned according to the literature (Gidley 1985, McIntyre et al., 1990). Compared with the native starch, the OSA-modified starches showed several additional resonances due to OS substitution. The assignments are listed in Table 2.2. By comparing the intensities of the methyl protons on OS substituents to that of anomeric protons on glucose units, the DS of OSA-modified starch can be calculated (Bai & Shi, 2010 accepted). ^{1}H NMR is also a useful technique for determining the DS of OSA-modified hyaluronic acid (Eenschooten et al., 2010).

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Compared with the ^{1}H NMR spectrum of OSA (Figure 2.2), the modified starches had a shoulder at ≈0.97 ppm adjacent to the sharp signals from the methyl protons at 0.90 ppm (Figure 2.4). 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 was 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 media. In addition, the resonance of the protons in the OS chain (0.54 to 3.21 ppm) became broader and less resolved (Figure 2.4) compared to that of the same protons in the OSA reagent (Figure 2.2). 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 glucose units substituted with OS groups. As a result, the α-limit dextrins of OSA-modified starches contain molecules of various chain

lengths, so the 1H-signals of the protons 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 et al., 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 (Figure 2.4), 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 (Figure 2.5), 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 2.3. 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 glucose units of OSA-modified waxy maize starches (DS = 0.019 and 0.056) became broader than those of the native waxy maize starch (Figure 2.5), 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 glucose 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 to 71 ppm) in modified starches (Figure 2.5). With the assistance of the HSQC spectrum (Figure 2.6) 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 to 80.2 ppm, 73.6 to 74.6 ppm, and 75.1 to 76.0 ppm, respectively to C-4, C-2 and C-3 of internal α-1, 4-linked glucose 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.

Conclusions

Complete assignments of ^{1}H and ^{13}C NMR spectra of the OSA reagent were achieved by ^{1}D and ^{2}D 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 anhydroglucose 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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SBDS, Spectral Database for Organic Compounds, http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng

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Tables and figures

Table 2.1 ^{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

Table 2.2 ^{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 to 3.18
C, D, E, F, G, J		1.93 to 2.99
K		1.50 to1.20
L		1.05 to 0.82

[a]The capital letters for protons in OSA group follow the notation in Figure 2.3.

Table 2.3 ^{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 to 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 Figure 2.3.

Figure 2.1 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).

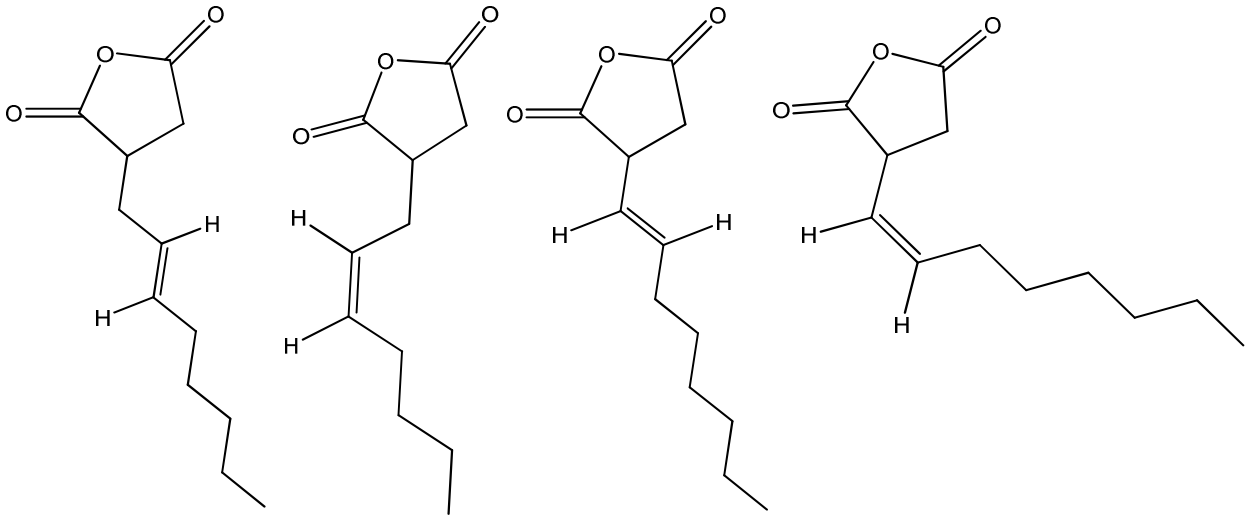
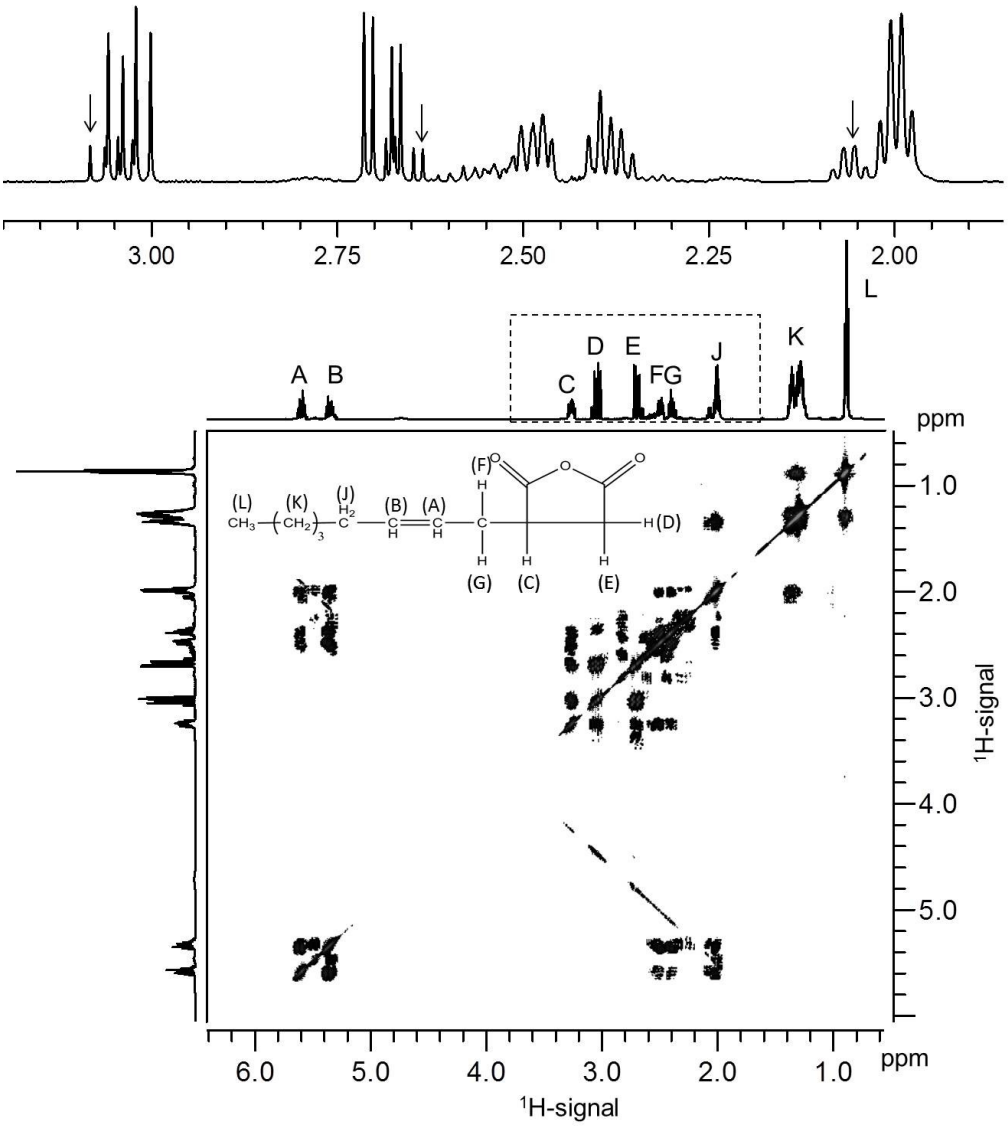


Figure 2.2 ^{1}H-^{1}H COSY spectrum of OSA reagent in methanol. Structure of 3-(oct-2-enyl) dihydrofuran-2,5-dione is inserted and assignments for protons are listed on the top of the corresponding resonances. The region from 3.2 to 1.8 ppm is expanded and shown above the COSY spectrum. The arrows point 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.



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

Figure 2.3 Expanded heteronuclear single quantum coherence (HSQC) spectrum (45 to 12 ppm) of OSA reagent in methanol.

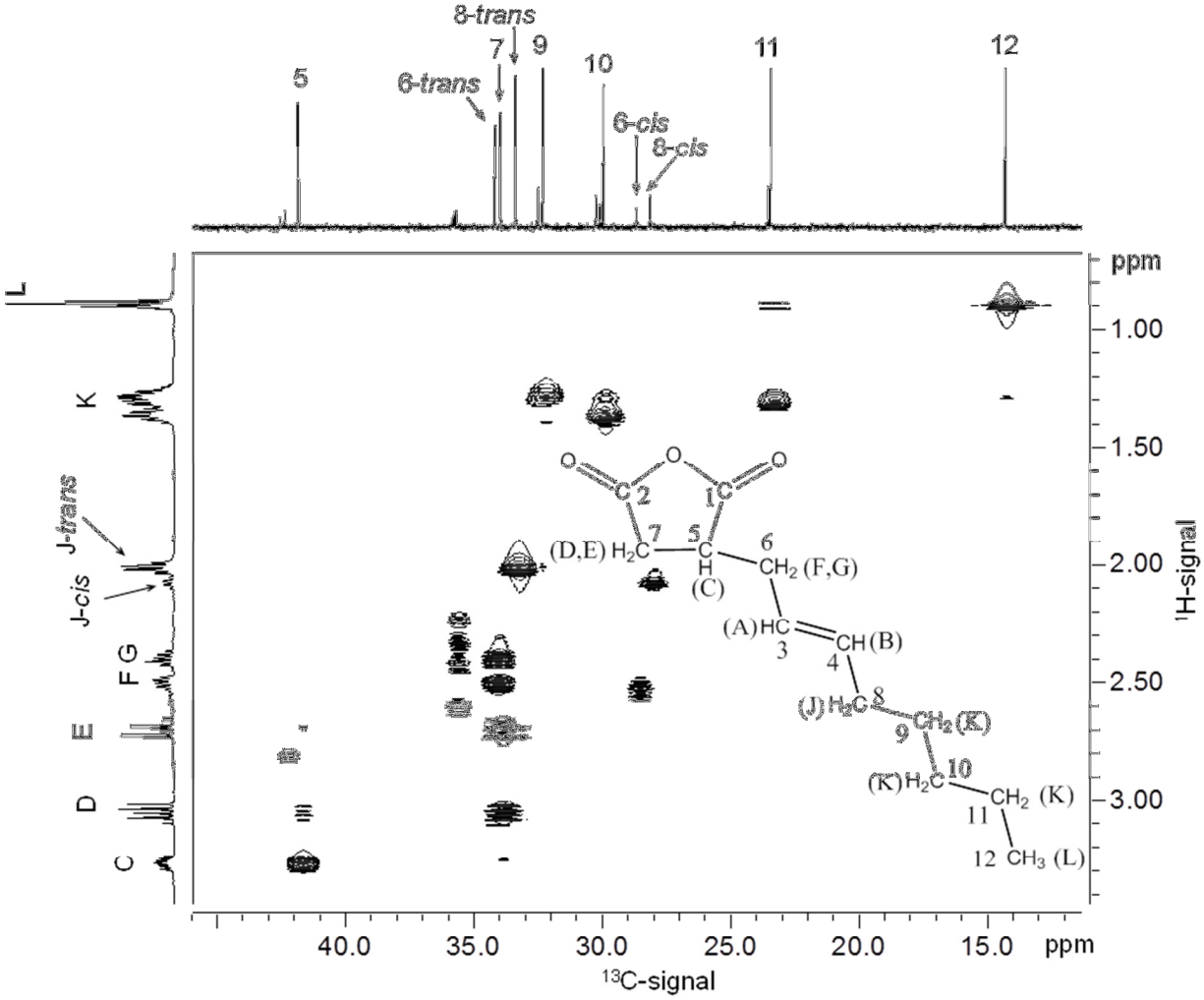


Figure 2.4 ^{1}H NMR spectra of α-limit dextrins of waxy maize starch (A) and OSA-modified starches with DS of 0.018 (B) and 0.056 (C).

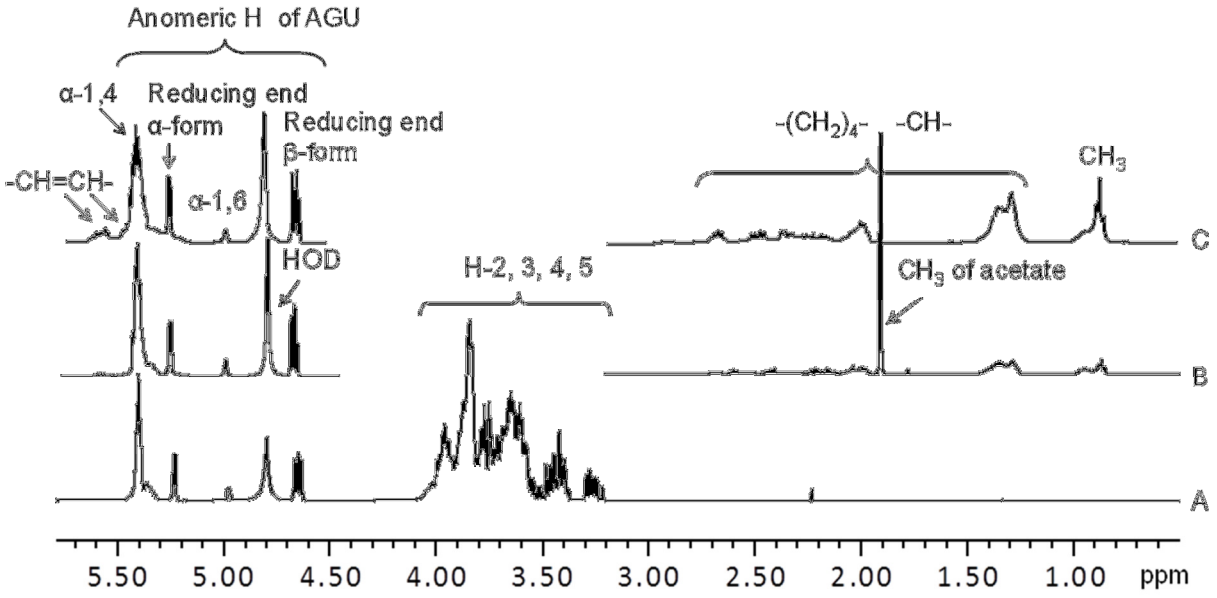


Figure 2.5 ^{13}C NMR spectra of α-limit dextrins of native waxy maize starch (A) and OSA-modified starches with DS of 0.019 (B) and 0.056 (C).

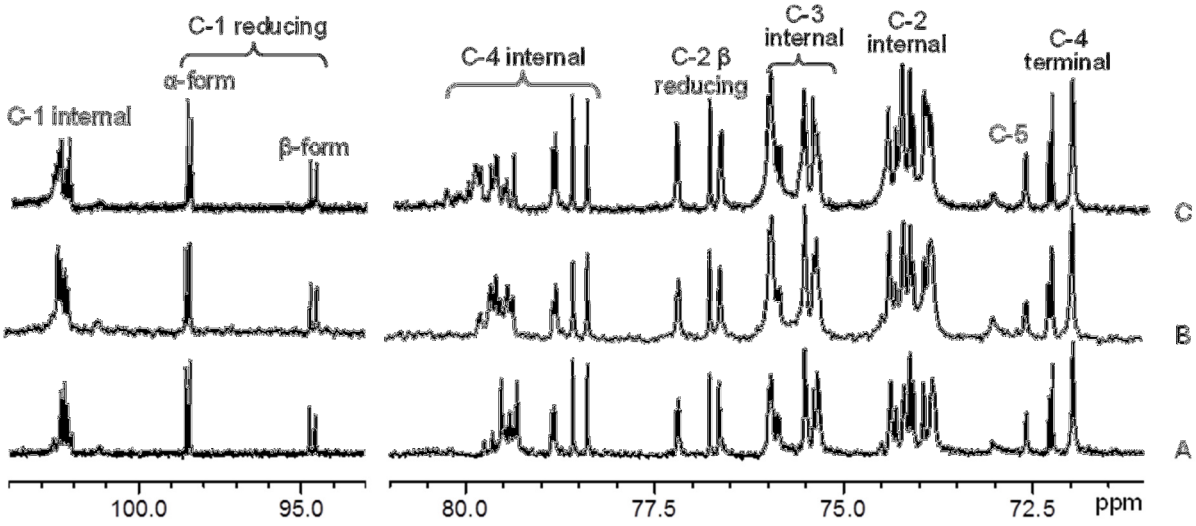
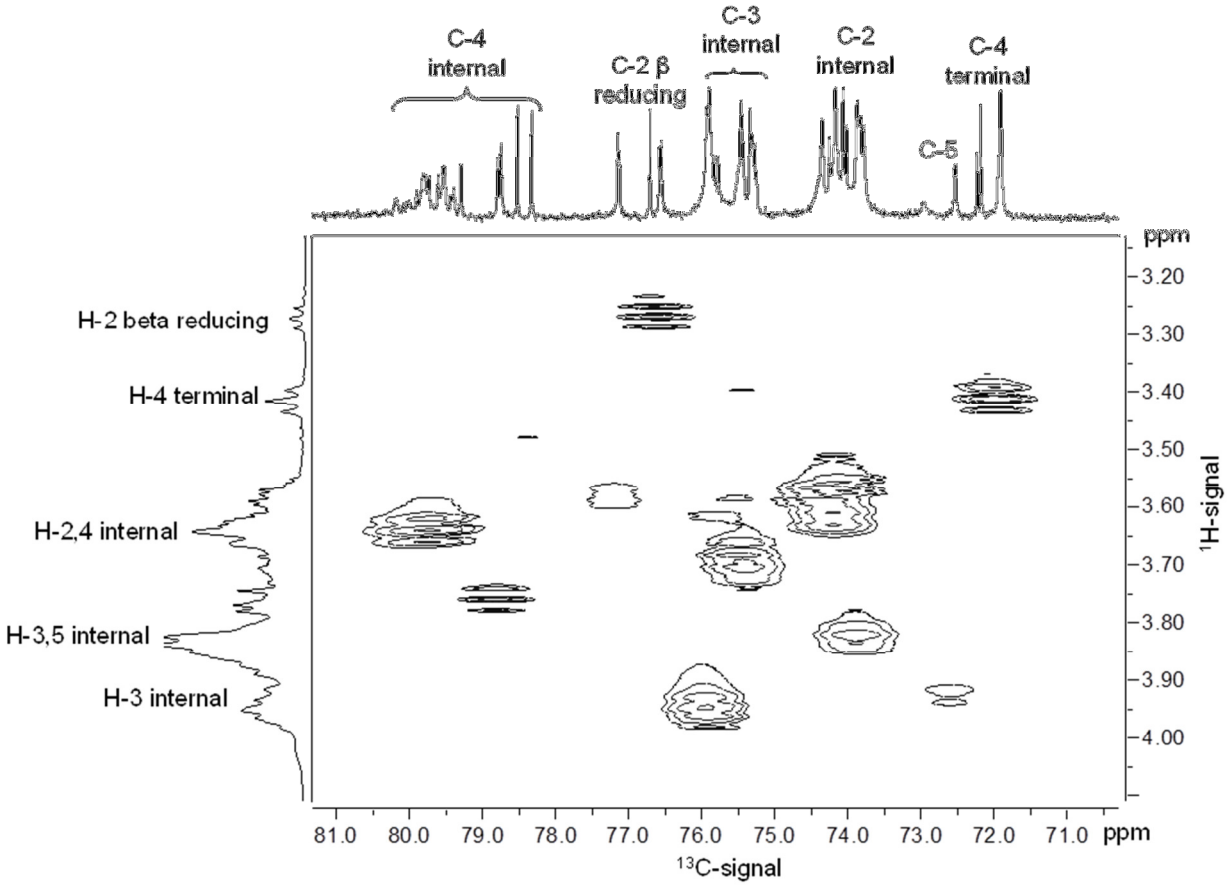


Figure 2.6 Heteronuclear single quantum coherence (HSQC) ^{1}H-^{13}C spectrum of α-limit dextrin of 15% OSA-modified starch (DS = 0.056)



Chapter 3 - Reaction of octenylsuccinic anhydride with a mixture of granular starch and soluble maltodextrin[1]

Abstract

The reaction of octenylsuccinic anhydride (OSA) with a mixture of granular waxy maize starch and soluble maltodextrin was investigated. OSA was reacted with a 1:1 (w/w) mixture of the granular starch and maltodextrin at OSA levels of 1.5, 3, 9, and 15% (wt% based on starch weight). After the first 0.5 h of the reaction, degree of substitution (DS) on maltodextrin reached 0.021, 0.033, 0.080, 0.10 for 1.5, 3, 9, and 15% OSA, respectively, whereas DS for granular starch was only 0.0020, 0.0087, 0.014, and 0.016. At 2 h of the reaction, the bound OS ratio of maltodextrin to granular starch was 10.8 when OSA concentration was 1.5% and decreased to ca. 5 at higher OSA concentrations. OSA preferred to react with maltodextrin than semi-crystalline granular starch when both existed in the system. OSA reacted with maltodextrin at a much faster rate and to a greater extent than with granular starch, but a significant amount of OSA reacted with granular starch at 3 to 15% OSA concentrations.

Keywords: Starch, maltodextrin, octenylsuccinic anhydride

[1] This chapter has been submitted to Carbohydrate Polymers for publication.

Introduction

Octenylsuccinic anhydride (OSA) reaction with starch has been known for decades (Caldwell & Wurzburg, 1953), but interest in it is increasing, as evidenced by the number of published papers in recent years (Sweedman, Tizzotti, Schäfer & Gilbert, 2013). The product of this reaction, octenylsuccinate (OS) starch, functions as a emulsion stabilizer and has wide food, pharmaceutical, and industrial applications (Trubiano, 1986). The reaction is an esterification reaction normally performed in aqueous solution under alkaline conditions (Trubiano, 1986). OSA reaction has been carried out with granular starch from different botanical sources, including waxy maize (Bai & Shi, 2011; Bhosale & Singhal, 2006; Shogren, Viswanathan, Felker & Gross, 2000; Zhu, Xie, Song & Ren, 2011), normal maize (Park, Chung & Yoo, 2004), potato (Ruan, Chen, Fu, Xu & He, 2009), rice (He, Song, Ruan & Chen, 2006; Song, He, Ruan & Chen, 2006), and amaranth (Bhosale & Singhal, 2006). In general, optimum reaction pH is 7.5 to 8.5 (Bai & Shi, 2011; Bhosale & Singhal, 2006; Song, He, Ruan & Chen, 2006; Zhu, Xie, Song & Ren, 2011) and optimum temperature is 30 to 35 ºC (Bhosale & Singhal, 2006; Song, He, Ruan & Chen, 2006; Zhu, Xie, Song & Ren, 2011). Degree of substitution (DS) increases with OSA treatment level (Bhosale & Singhal, 2006; Song, He, Ruan & Chen, 2006) and starch concentration (Bai & Shi, 2011; Song, He, Ruan & Chen, 2006; Zhu, Xie, Song & Ren, 2011); however, reaction time may vary from 2 to 24 h depending on the speed of OSA addition, other reaction parameters, and the botanical source of the starch (Bai & Shi, 2011; Bhosale & Singhal, 2006; Song, He, Ruan & Chen, 2006).

In our previous study, we examined OSA reaction with granular waxy maize starch and soluble maltodextrin and found higher reaction efficiency (RE) and a faster rate for soluble maltodextrin (Bai & Shi, 2011). In addition, the substitution pattern on hydroxyl groups appeared to differ between OS maltodextrin and granular OS starch. However, it is not clear how OSA would react in a mixture of soluble maltodextrin and granular starch. In this study, instead of examining the reaction of OSA with granular starch and soluble maltodextrin separately, we investigated the reaction of OSA reaction with a mixture of granular starch and soluble maltodextrin.

Materials and Methods

Materials

OSA and waxy maize starch (Amoica TF) were obtained from National Starch LLC (Bridgewater, NJ). Maltodextrin (MALTRIN 100) with degree of polymerization (DPn) ~10 was obtained from Grain Processing Corporation (Muscatine, IA). Other chemicals used in the study were analytical grade.

OSA reaction

A mixture of granular waxy maize starch (50 g dry weight) and soluble maltodextrin (50 g dry weight) was dispersed in 150 mL water and mixed with an overhead stirrer for 15 min. pH was adjusted to 7.5 by 3% (wt%) NaOH solution. OSA of 1.5, 3, 9, and 15% based on total weight of the mixture of the starch and maltodextrin was added to the slurry, and the pH was controlled at 7.5 during the reaction. Samples were taken during reaction at a time interval of 30 min. Each sample was vacuum-filtered through a filter paper. The maltodextrin fraction in the filtrate was recovered by freeze-drying. The starch cake was washed by distilled water (400 mL) three times and then by methanol (400 mL) three times to remove soluble maltodextrin residue and unreacted OSA. Starch was dried in an oven at 40 °C for 24 h.

In a set of separate experiments, OSA reaction was performed with granular starch alone at concentrations of 3, 9, 15, and 50% (based on starch weight). Sodium sulfate (5% based on starch weight) was added in the reactions of 15 and 50% (based on starch weight) OSA concentration.

Bound OS content determination

DS and bond OS were determined by NMR. Granular OS starch was hydrolyzed by α-amylase for NMR experiments as previously described (Bai, Shi, Herrera & Prakash, 2011). The freeze-dried maltodextrin (0.2 g) was washed by methanol (1mL) three times and vacuum-dried. OS starch and maltodextrin products were exchanged with D_2O once, dissolved in D_2O at 10% (wt%), and analyzed by NMR.

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 35kV,

20mA with Cu-Ka radiation, a theta-compensating slit, and a diffracted beam monochromator. Data were recorded between the diffraction angles (2θ) of 2 and 35°.

Statistical analysis

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

Results and discussion

OSA reacted significantly more with maltodextrin than granular starch when both existed in the aqueous system (Table 3.1). At the 1.5% OSA level, the amount of OSA that reacted on maltodextrin was 10.8 times greater than on the granular starch (Figure 3.1). As the OSA level increased to 9 and 15%, we still observed more substitution on the maltodextrin, but the ratio of the bound OS in the maltodextrin to that in the waxy maize starch dropped from 10.8 to 4.5 and 5.3, respectively (Figure 3.1). It seems that most OSA reacted with soluble maltodextrin initially, but the reaction rate decreased after OS groups were substituted on maltodextrin.

We used 3% OSA and reacted it with (i) waxy maize starch, (ii) soluble maltodextrin, and (iii) a mixture of the starch and maltodextrin. It is interesting to compare the reaction results (Table 3.1). The RE was almost 100% when 3% OSA was reacted with maltodextrin (Table 3.1) and DS was 0.023, whereas RE was ca. 80% for the granular starch with DS of 0.019. In comparison, when 3% OSA was reacted with a mixture of the granular starch and maltodextrin (1/1, w/w) for 2 h, the RE was 84.4% (Table 3.1). Among the total OSA reacted, 69.0% reacted on the maltodextrin, resulting in 4.1% OS on the maltodextrin, In contrast, only 15.4% reacted on the granular starch (Table 3.1). DS of maltodextrin fraction was 0.033, which was more than 4 times higher than the granular starch fraction.

The reaction rate of OSA modification on maltodextrin appeared different from granular starch. After the first 0.5 h of the reaction, DS on maltodextrin reached 0.021, 0.033, 0.080, and 0.10 for 1.5, 3, 9, and 15% (wt% based on starch weight) OSA, respectively, whereas DS for granular starch was only 0.0020, 0.0087, 0.014, and 0.016. These results indicate that OSA reacted much faster on maltodextrin than granular starch. At high OSA concentrations of 9 and 15% (wt% based on starch weight), interesting results were observed. At 9% OSA, the DS of the maltodextrin continued to increase from 0.5 h to 1.5 h, remained constant from 1.5 to 2.0 h, and

decreased from 2.0 h to 2.5 h. The reaction stopped after 1.5 h was probably because of the increase in hydrophobicity after OS substitution. In contrast, the DS of the granular starch increased from 0.5 h to 1.0 h, then remained constant up to 2.5 h (Figure 3.2). A similar trend was observed for 15% (wt% based on starch weight) OSA concentration (Figure 3.3). These results suggest that the OSA reaction rate was fast at the early stage of the reaction for both granular starch and soluble maltodextrin, but it slowed as the reaction progressed. Reaction rate on granular starch decreased much faster than maltodextrin. The fast decrease in reaction rate was probably due to the steric hindrance in granular starch for OSA to react as well as the increase in starch hydrophobicity after OS substitution. Granular starch appeared to have much less available space for OS substitution than maltodextrin.

OSA reaction was performed on granular starch alone, and the granular structure change was investigated by wide angle X-ray diffraction. Native waxy maize starch showed an A-type crystalline pattern. At DS of 0.019 and 0.039, crystalline patter of OS starch appeared to be the same as native starch (Figure 3.4). As DS reached 0.074, the crystalline pattern started to lose definition, and peak broadening was observed (Figure 3.4). These results suggest that at low level of OSA modification, OS substitutions occurred primarily at the amorphous region. For granular waxy maize starch without starch swelling, the maximum DS was about 0.088 (Bai & Shi, 2011), reflecting limited reaction space in the amorphous regions.

Therefore, the difference in OSA reaction on granular starch and maltodextrin was due to that the granular starch was partially crystalline, whereas maltodextrin was soluble in water. OSA reacted preferably with granular starch at the amorphous region and granular surface (Shogren, Viswanathan, Felker & Gross, 2000; Song, He, Ruan & Chen, 2006). The crystalline region of granular starch was tightly packed and was not readily accessible for starch modification (Richardson, Nilsson, Cohen, Momcilovic, Brinkmalm & Gorton, 2003). In contrast, maltodextrin, which was completely amorphous and soluble in water, had all the molecules available, or, in other words, had more sites for reaction; therefore, it appeared to have a faster reaction rate than granular starch.

When both maltodextrin and granular starch existed in one reaction system, OSA preferably reacted with maltodextrin and granular starch in its amorphous region, but the reaction was more profound in maltodextrin. As the reaction progressed, OSA reaction on maltodextrin and starch slowed; however, due to a much lower reaction rate on granular starch, OSA reacted

with maltodextrin, which had more sites available for reaction, until no available OSA in the system.

Conclusions

In an aqueous system mixed with the granular starch and maltodextrin, OSA preferably reacted with the soluble maltodextrin at OSA concentration of 1.5 to 15% (wt%, based on starch weight), but a significant amount of OSA reacted with granular starch at 3 to 15% OSA concentration. The initial reaction rate of maltodextrin with OSA was much faster than that of the granular starch. OSA preferably reacted with granular starch in its amorphous region and with soluble maltodextrin.

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Tables and figures

Table 3.1 Characterization of octenylsuccinate (OS) starch or maltodextrin when prepared in a reaction system of a mixture of waxy maize starch and maltodextrin (1/1, w/w) as well as starch only and maltodextrin only.

OSA level (%)	Reaction Time (h)	Starch fraction			Maltodextrin fraction		
		Degree of substitution	%OS	% reacted based on total OSA	Degree of substitution	%OS	% reacted based on total OSA
1.5	0.5	0.0020	0.26	8.7	0.021	2.7	89.3
	1.5	0.0018	0.24	8.0	0.021	2.6	86.3
3.0	0.5	0.0087^{c}	1.1^{b}	18.5^{c}	0.033^{a}	3.7^{a}	67.7^{a}
	1.0	0.0080^{b}	1.0^{b}	16.7^{b}	0.034^{a}	4.2^{a}	67.9^{a}
	2.0	0.0072^{a}	0.9^{a}	15.4^{a}	0.033^{a}	4.1^{a}	69.0^{a}
		Starch only *			**Maltodextrin only ***		
3.0	0.5	0.014^{a}	1.75^{a}	n/a	0.023	2.92	n/a
	1.0	0.019^{b}	2.39^{b}	n/a	0.023	2.95	n/a
	1.5	0.019^{b}	2.42^{b}	n/a	0.024	2.99	n/a
	2.0	0.019^{b}	2.37^{b}	n/a	n/a	n/a	n/a

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

* Data from Bai and Shi (2011) are incorporated here for comparison.

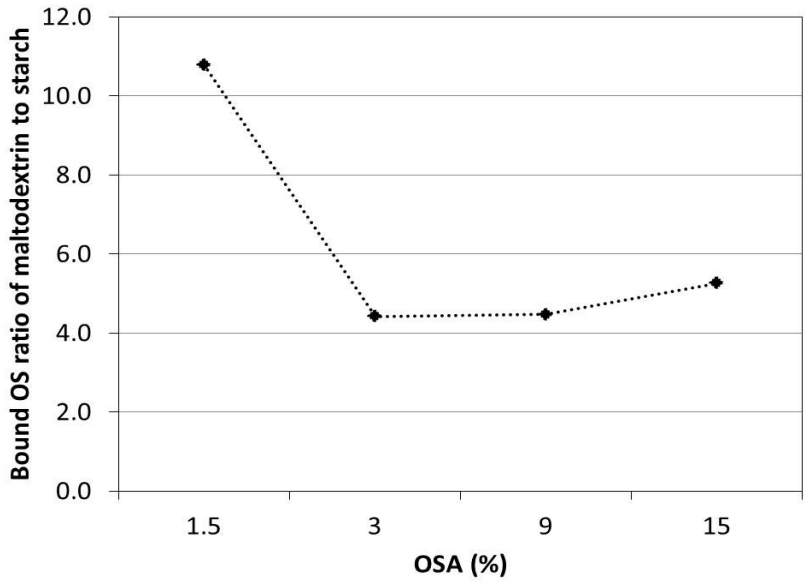

Figure 3.1 Ratio of octenylsuccinic anhydride (OSA) reacted on maltodextrin to waxy maize starch at different levels of OSA.

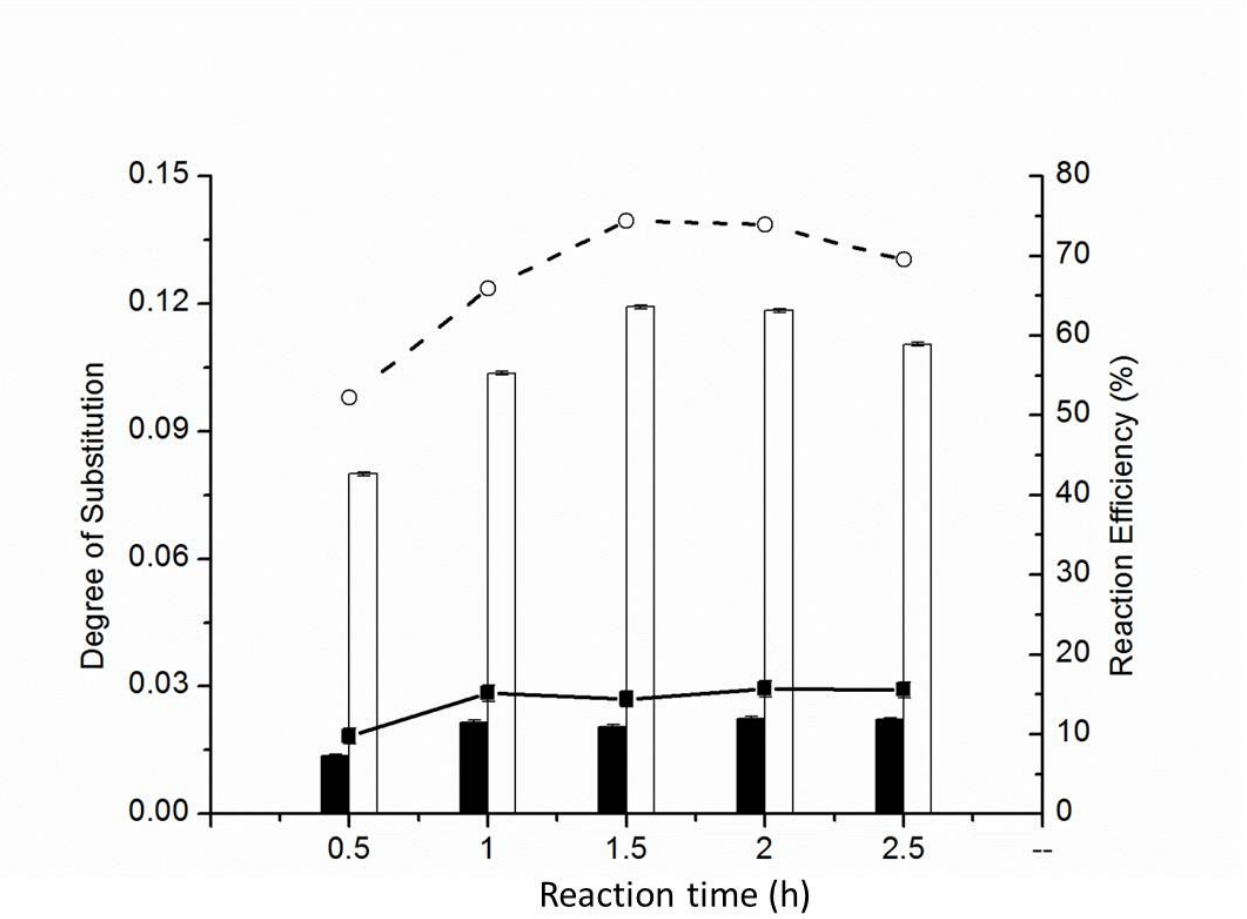

Figure 3.2 Degree of substitution (bar graph) and reaction efficiency (line graph) of 9% octenylsuccinic anhydride modification on the mixture of waxy maize starch (black bars and solid lines) and maltodextrin (white bars and dashed lines).

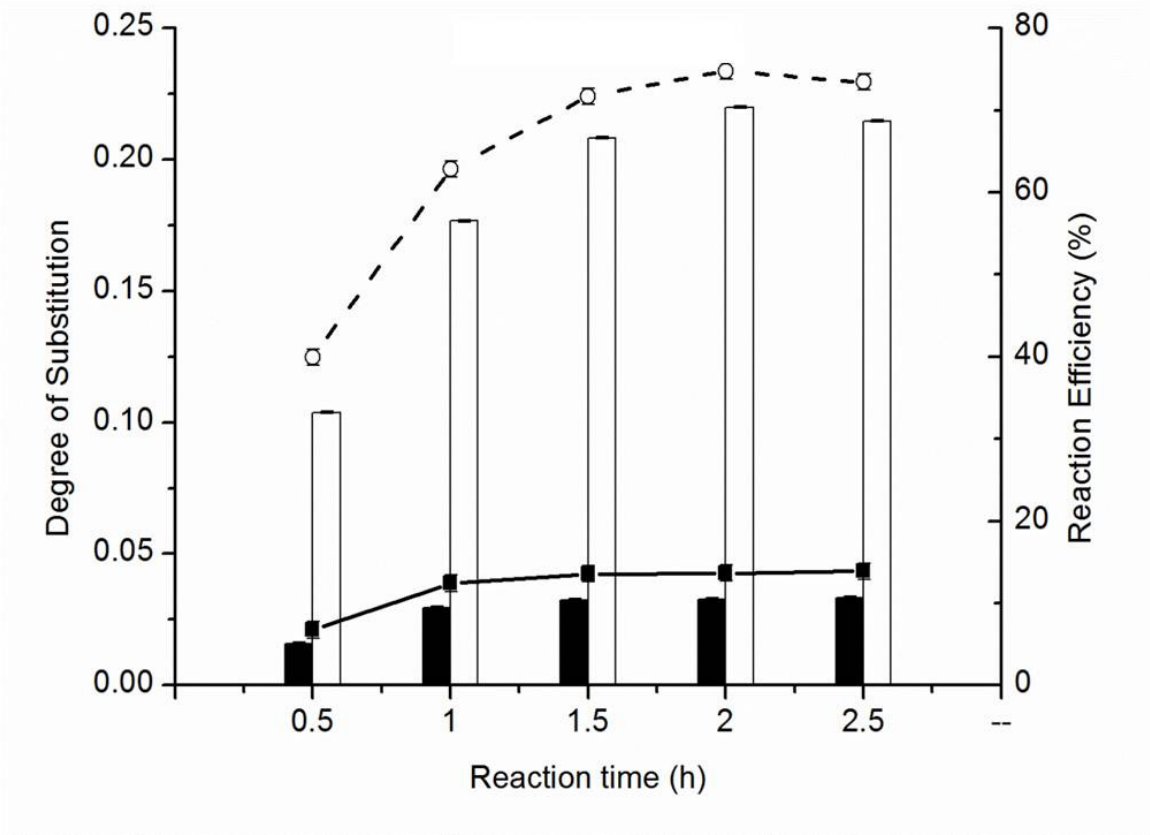

Figure 3.3 Degree of substitution (bar graph) and reaction efficiency (line graph) of 15% octenylsuccinic anhydride modification on the mixture of waxy maize starch (black bars and solid lines) and maltodextrin (white bars and dashed lines).

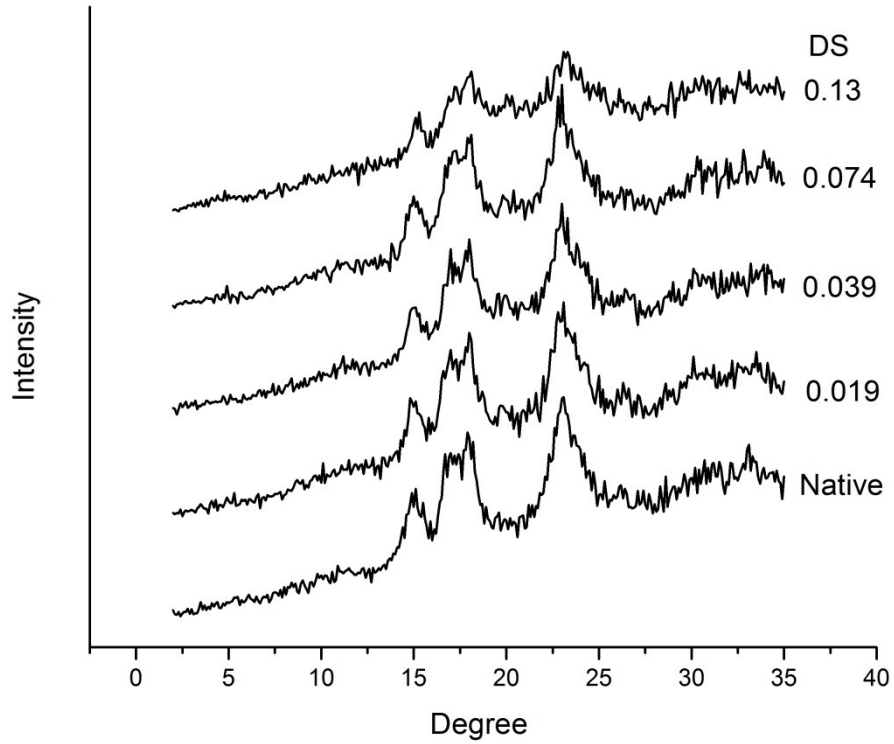

Figure 3.4 Wide-angle X-ray diffraction patterns of native waxy maize starch and octenylsuccinate starches of degree of substitution (DS) 0.019, 0.039, 0.074, and 0.13.

Chapter 4 - Position of Modifying Groups on Starch Chains of Octenylsuccinic Anhydride-Modified Waxy Maize Starch

Abstract

Octenylsuccinic anhydride (OSA)-modified starches with a low and high degree of substitution, DS=0.018 (OS-S-L) and 0.092 (OS-S-H), were prepared from granular native waxy maize starch in an aqueous slurry. The position of OS substituents along the 1, 4-linked chains was investigated by enzyme hydrolysis followed by chromatographic analysis. Amyloglucosidase, β-amylase, and isoamylase were used separately or in combination to hydrolyze the starches. High-performance anion-exchange chromatography, gel permeation chromatography, and size exclusion chromatography with a multi-angle light scattering detection were used to analyze the enzyme hydrolysates. Native starch, OS-S-L, and OS-S-H had β-limit values of 55.9, 52.8, and 34.4%, respectively. The weight-average molecular weight of the β-limit dextrin of OS-S-L was close to that of native starch, but for OS-S-H, it was approximately 7 times the β-limit dextrin of the native starch. Debranching of OS starches was incomplete compared with native starch; the β-amylolysis limits of debranched OS-S-L and OS-S-H were ca. 91 and 70%, respectively. Analytic results were consistent with the structure of OS-S-L having OS groups located on repeat units close to the branching points in amylopectins, whereas the OS substituents in OS-S-H occurred both near the branching points and the non-reducing ends.

Keyword: Substitution distribution, octenylsuccinic anhydride, modified starch, starch structure

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 characterized at three levels: universal, granular, and molecular (Huber & BeMiller, 2009). At the universal level, substituted starch is characterized by the degree of substitution (DS) and molar substitution (MS), which reflects the overall extent of modification. At the granular level, substituted starch is characterized 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 characterized 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 OSA treatment with up to 3 wt% reagent, and is commonly prepared by reacting granular starch with OSA in an aqueous system. OSA modification affects many physical and chemical properties of starch, including gelatinization behavior (Carlos-Amaya, Osorio-Diaz, Agama-Acevedo, Yee-Madeira, & Bello-Perez, 2011; Thirathumthavorn & Charoenrein, 2006), rheological properties (X. Y. Song, Zhu, Li, & Zhu, 2010; Thirathumthavorn & Charoenrein, 2006), and digestibility (Carlos-Amaya, Osorio-Diaz, Agama-Acevedo, Yee-Madeira, & Bello-Perez, 2011; Han & BeMiller, 2007; J. He, Liu, & Zhang, 2008). Knowledge of the structure of OS starch is helpful in understanding its physical behavior (Shogren, Viswanathan, Felker, & Gross, 2000).

The structure of OS starch has been investigated at all three levels. The overall extent of substitution, or DS, has been determined by titrimetric (X. Song, He, Ruan, & Chen, 2006) and nuclear magnetic resonance (NMR) methods (Bai & Shi, 2011; Bai, Shi, Herrera, & Prakash, 2011; Tizzotti, Sweedman, Tang, Schaefer, & Gilbert, 2011). 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). However, the distribution of the OS groups is not uniform among starch granules at DS 0.05, as determined by FT-IR microspectroscopy (Bai, Shi, & Wetzel, 2009). For individual starch granules, the surface concentration of OS was found to be approximately 3 to 4 times that of the bulk (Shogren, Viswanathan, Felker, & Gross, 2000). In addition, OS substitution occurred primarily in the amorphous region (Bai & Shi, 2011; G. Q. He, Song, Ruan, & Chen, 2006; Shogren, Viswanathan, Felker, & Gross, 2000; X. Song, He, Ruan, & Chen, 2006). 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, Shi, Herrera, & Prakash, 2011); whereas for the modified maltodextrin, OS groups were substituted at OH-2, OH-3, OH-6, and at reducing ends (Bai and Shi, 2011).

One question that is still unknown is how the OS substituents are distributed along a typical starch chain. Substitution distribution along the starch chain has been studied for various chemically modified starches and is often obtained by analyzing the limit dextrins of a modified starch after treatment by a single enzyme or a combination of enzymes. Limit dextrins are oligomeric or polymeric saccharides that remain after exhaustive treatment of starch with a hydrolytic enzyme. 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; Steeneken & Woortman, 1994). Therefore, the substitution distribution can be inferred by comparing the structures of enzyme hydrolysates of a native starch with that of its modified components. Enzymes including α-amylase, β-amylase, amyloglucosidase, isoamylase, and pullulanase are those most commonly used for starch structure characterization (Hizukuri, Abe, & Hanashiro, 2006; Richardson & Gorton, 2003). Enzymatic methods have been used successfully to characterize chain substitution patterns for methylated (Steeneken & Woortman, 1994; van der Burgt, Bergsma, Bleeker, Mijland, Kamerling, & Vliegenthart, 2000a; van der Burgt, Bergsma, Bleeker, Mijland, van der Kerk-van Hoof, Kamerling, et al., 1999, 2000a), oxidized (Zhu & Bertoft, 1997), cationic (Manelius, Buleon, Nurmi, & Bertoft, 2000; Manelius, Nurmi, & Bertoft, 2000; Richardson, Nilsson, Cohen, Momcilovic, Brinkmalm, & Gorton, 2003), acetylated (Chen, Huang, Suurs, Schols, & Voragen, 2005; Chen, Schols, & Voragen, 2004; J. Huang, Schols,

Klaver, Jin, & Voragen, 2007; J. R. Huang, Schols, Jin, Sulmann, & Voragen, 2007; Wang & Wang, 2002) and hydroxypropylated (Biliaderis, 1982; Hood & Mercier, 1978; Kavitha & BeMiller, 1998; Richardson, Nilsson, Bergquist, Gorton, & Mischnick, 2000) starches.

In this study, OS starch was prepared and hydrolyzed exhaustively by various enzymes including amyloglucosidase, isoamylase, and β-amylase followed by chromatographic analysis as shown in Figure 4.1. The distribution of OS groups along starch chains were deduced from analyses of the enzyme hydrolysates.

Materials and Methods

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 5,000 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 1320 ± 90 DP°/g as indicated by the product brochure. Isoamylase (EC 3.2.1.68) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The enzyme activity was 1.41 X 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.

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 stabilize 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).

Amyloglucosidase hydrolysis

Waxy maize starch or OS starches (0.1 g) was dispersed in 5 mL acetate buffer (0.05M, pH 4.5) and the slurry 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 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 incubated 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:

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

β-amylase hydrolysis

Waxy maize starch or OS starch (0.5 g) was dispersed in 20 mL acetate buffer (0.01M, pH 5.5) and the mixture 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 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 values were calculated as follows:

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

Isoamylase hydrolysis and successive β-amylolysis

Waxy maize starch or OS starch (0.5 g) was dispersed in 25 mL acetate buffer (0.05M, 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 GPC and for reducing sugar

(dextrose equivalents), and for 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 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.

Gel permeation chromatography (GPC)

GPC analysis was performed as previously described (Cai, Shi, Rong, & Hsiao, 2010). Starch hydrolysates (4 to 16 mg) were dissolved in DMSO (4 mL) by stirring at room temperature for 12 h, and solutions were injected after filtering through a 2 μm filter (Millex-AP, Millipore, Billerica, MA). GPC results were analyzed using Cirrus™ GPC Software Version 3.0 (Agilent Technologies, Santa Clara, CA). Molecular weight was relative to the dextran standards

Size exclusion chromatography with multi-angle light scattering (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-806M 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 normalized with bovine serum albumin (BSA). SEC-MALS experiments were performed at a column temperature of 55 °C with 0.1 M $NaNO_3$ as eluent and a flow rate of 0.5 mL/min. Starch solution (10 mg/mL) was filtered through a 1 μm filter before injecting. The injection volume was 100 μL. Data was analyzed using Astra 6 software.

High performance anion-exchange chromatography (HPAEC)

HPAEC was done as described by Cai and Shi (2010) 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 malti oligosaccharides of dp 3-6.

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:

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

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 $p < 0.05$.

Results and discussion

Amyloglucosidase hydrolysis

Native starch was 96.2% converted to glucose by amyloglucosidase (Table 4.1), which agreed with the results of other workers (Hood & Mercier, 1978; Richardson, Nilsson, Cohen, Momcilovic, Brinkmalm, & Gorton, 2003). In contrast, OS starch of DS 0.018 (OS-S-L) and 0.092 (OS-S-H) gave 84.7 and 58.0% hydrolysis, respectively (Table 4.1). 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 units, 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, Nilsson, Cohen, Momcilovic, Brinkmalm, & Gorton, 2003), then the glucose units released by amyloglucosidase would contain no OS substituents, whereas the residual dextrins would contain all the OS groups. Based on the amount of glucose released by glucoamylase

(Table 4.1), and invoking the cluster model of amylopectin, the OS-S-L product contained almost no OS groups near the non-reducing ends as opposed to the OS-S-H products which did.

β-amylase hydrolysis

Native starch, OS-S-L, and OS-S-H were hydrolyzed exhaustively by β-amylase, and the limiting β-amylolysis value was 55.9, 52.8 and 34.4%, respectively (Table 4.1). The β-limit value for the native starch is in agreement with that previously reported (Bertoft, 1989; Manners, 1989). For OS-S-L and OS-S-H, 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, Nilsson, Cohen, Momcilovic, Brinkmalm, & Gorton, 2003; Zhu & Bertoft, 1997). β-amylase is an exo-acting hydrolase that removes unsubstituted maltoglucosyl units from the non-reducing ends of amylopectin and leaving the inner part 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 (-3.2%) to that of the native starch, whereas that of OS-S-H product was considerably reduced (-22.2%), 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 x 10^6 (Table 4.1). The Mw of the β-limit dextrin from OS-S-L was 1.26 x 10^6, which was only slightly higher than that of the native starch (Table 4.1), indicating that OS substitutions were probably close to the outer branching points of the starch molecules in OS-S-L. In contrast, the β-limit dextrin of OS-S-H had an Mw of 7.04 x 10^6 which was much larger than that of native starch and OS-S-L (Table 4.1 and Figure 4.2). These results again indicate the OS groups in the OS-S-H product must be located near the non-reducing ends on the outer chains of its starch molecules.

Isoamylase hydrolysis and successive β-amylolysis

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 debranching of unmodified starch is a mixture of malto oligosaccharides varying in dp, mostly

below 80 anhydroglucose units (Robyt, 2009). The average DP of the debranched waxy maize starch, and the OS-S-L and OS-S-H products, were 22, 26, and 31, respectively (Table 4.1). The greater DP values of debranched OS-modified starches, as indicated by their low dextrose equivalent (DE) value, verifies 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 OS-S-L and 26.7% for OS-S-H (Table 4.1).

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 (Figure 4.3). Debranched waxy maize starch gave a bimodal distribution as observed by previous work (Bertoft, 2004; Biliaderis, 1982; Cai & Shi, 2010). Approximately 22 wt% 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 ~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 (Figure 4.3). Moreover, the average DP of Fraction 1 was much larger for debranched OS-S-H compared to 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 to 64 (Figure 4.4-A). The distribution pattern found for the waxy maize starch was similar to previous reports (Bertoft, 2004; Cai & Shi, 2010), 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. It has been suggested that the peak area from amperometric detection is not directly proportional to the molar concentrations of the maltooligosaccharides of different length (Ammeraal, Delgado, Tenbarge, & Friedman, 1991; Koizumi, Fukuda, & Hizukuri, 1991; Shi & Seib, 1992). However, when the total amount of injected dextrins is the same for each sample, as was the case, then a comparison can be made for each DP oligomer between samples. OS starches had reduced areas compare to the native starch over the entire unit-chain length profile (Figure 4.4-A). 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 less of the chain with that length was released. OS-S-H had more negative values in the range of DP 6 to 32 than OS-S-L (Figure 4.4-B). These results suggest that OS substitution occurred predominately on the A and B1 chains. The area differences for long chains (B2 and B3) was probably due to those long chains that carried A and B1 chains containing OS substituents.

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 4.1). HPAEC of the *Iβ* hydrolysate showed predominantly maltose with a low level of 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 4.1). These results further suggest that OS groups in OS-S-L 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 4.1), and that *Iβ* hydrolysate contained a large molecular fraction with an average DP of ca. 89, compared to 10 for OS-S-L (Figure 4.3). These results suggest the presence of OS groups near the non-reducing end of the starch chains in OS-S-H.

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, which is the location of the branching points of amylopectin (Steeneken & Smith, 1991; Steeneken & Woortman, 1994; van der Burgt, Bergsma, Bleeker, Mijland, Kamerling, & Vliegenthart, 2000a, 2000b; van der Burgt, Bergsma, Bleeker, Mijland, van der Kerk-van Hoof, Kamerling, et al., 1998; van der Burgt, et al., 1999; van der Burgt, Bergsma, Bleeker, Mijland, van der Kerk-van Hoof, Kamerling, et al., 2000b). Substitution has been reported to occur near the branching points as well as at the non-reducing ends for acetylated distarch phosphate made from smooth pea starch and for hydroxypropylated distarch phosphate made from waxy maize starch (Biliaderis, 1982). The non-reducing ends of amylopectin molecules may occur in tightly packed crystalline lamellae in granules, which limits their accessibility to chemical reagents. In agreement with the literature, 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 Figure 4.5). 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.

Conclusions

The distribution of OS groups along starch chains in OS starches was studied 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. As DS increased to 0.092, modifying groups were located near the branching points and on non-reducing ends.

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Tables and figures

Table 4.1 Structural characteristics of waxy maize starch and octenylsuccinated starches with degrees of substitution of 0.018 (OS-S-L) and 0.092 (OS-S-H).

Starch	*A*	*β*		*I*			*Iβ*
	% hydrolysis	β-limit value (%)	β-limit dextrin Mw ($x10^6$)	DE	DP	Resistant branches (%)	% hydrolysis
Native starch	96.2 ± 1.3c	55.9	1.08 ± 0.07a	4.5 ± 0.0c	22 ± 0a	--	100.0 ± 0.3c
OS-S-L	84.7 ± 2.3b	52.8	1.26 ± 0.02b	3.9 ± 0.1b	26 ± 1b	13.3 ± 0.3a	91.1 ± 0.1b
OS-S-H	58.0 ± 1.8a	34.4	7.04 ± 0.10c	3.3 ± 0.1a	31 ± 1c	26.7 ± 0.3b	70.6 ± 1.5a

A = amyloglucosidase hydrolysates

β = β-amylase hydrolysates

I = isoamylase hydrolysates

Iβ = isoamylase and successive β-amylase hydrolysates

DE = dextrose equivalent

DP = number average degree of polymerization

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

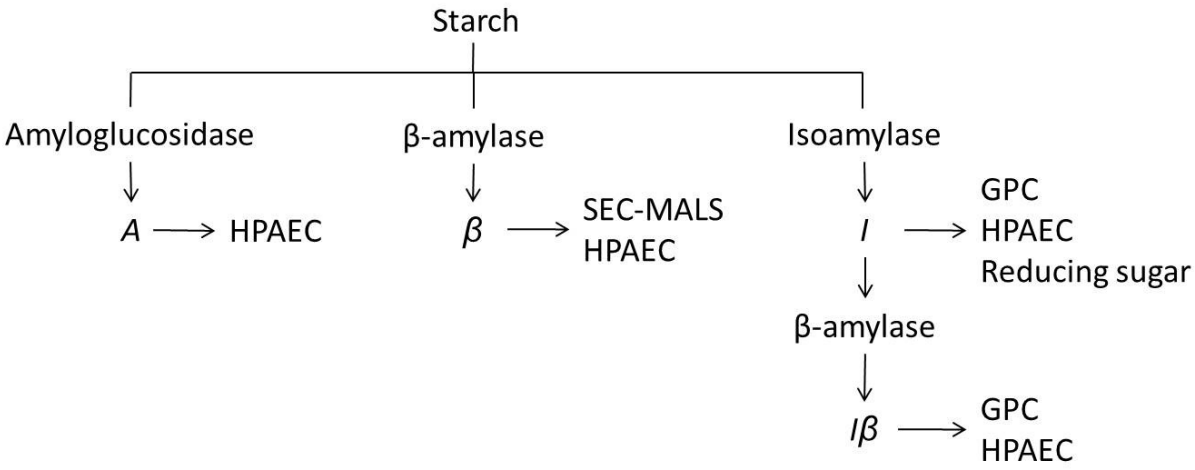


Figure 4.1 Enzymatic and analytical methods used to study the structure of octenylsuccinated starch.

A = amyloglucosidase hydrolysates

β = β-amylase hydrolysates

I = isoamylase hydrolysates

Iβ = isoamylase and successive β-amylolysis hydrolysates

HPAEC = high-performance anion-exchange chromatography

GPC = gel permeation chromatography

SEC-MALS = size exclusion chromatography with multi-angle light scattering

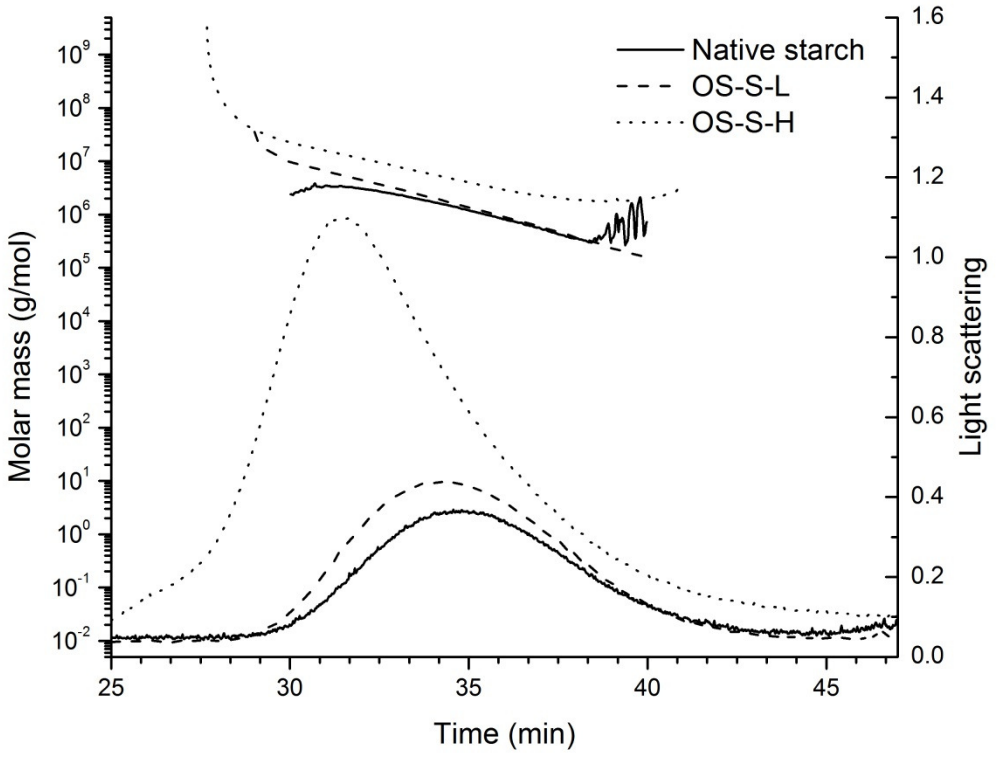
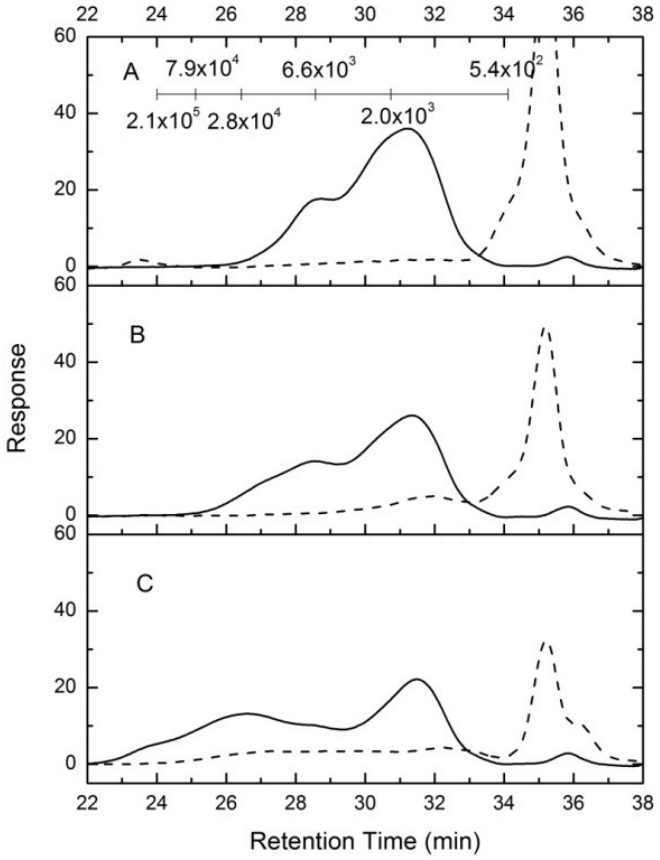


Figure 4.2 Molecular weight distributions (bottom) and molar masses (top) of β-limit dextrins of native waxy maize starch and octenylsuccinated starches with degrees of substitution of 0.018 (OS-S-L) and 0.092 (OS-S-H), all determined by SEC-MALS.



Treatment	*Sample*	Fraction 1		Fraction 2	
		DP	% Area	DP	% Area
I	Native	68	21.7	12	78.3
	OS-S-L	105	32.1	12	67.9
	OS-S-H	500	67.9	105	32.1
Iβ	Native	--	--	2	100.0
	OS-S-L	10	15.9	2	84.1
	OS-S-H	89	36.9	2	63.1

Figure 4.3 Molecular size distributions and degrees of polymerization (DP) of debranched starch before (*I*) (—) and after successive β-amylolysis (*Iβ*) (- - - - -) A = native waxy maize starch, B and C = octenylsuccinate starches with degrees of substitution, respectively, of 0.018 (OS-S-L) or 0.092 (OS-S-H) (C). DP = number average degree of polymerization.

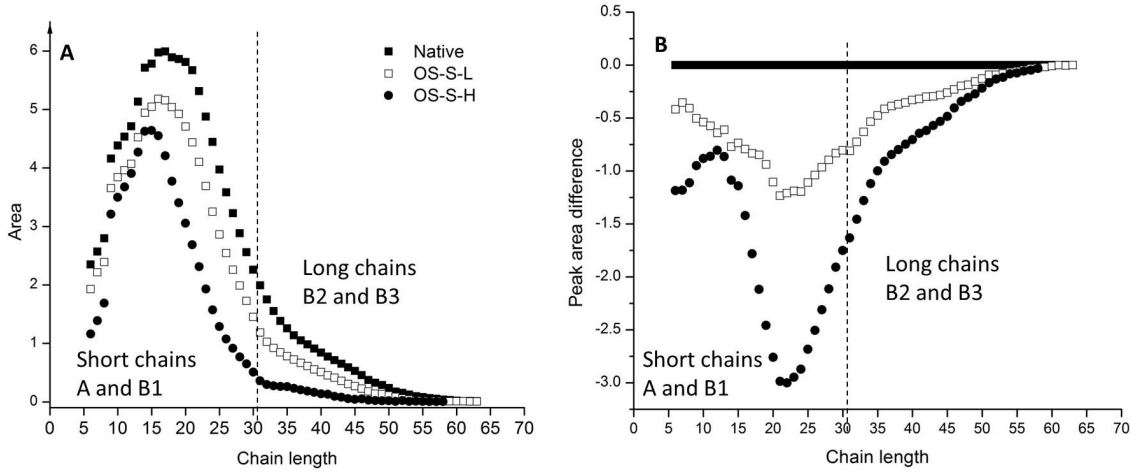


Figure 4.4 Chain length distributions (A) and peak area differences (B) of debranched waxy maize starch and octenylsuccinated starches with degrees of substitution (DS) of 0.018 (OS-S-L) and 0.092 (OS-S-H). Peak area differences were the area of each peak from an OS-substituted starch minus that of waxy maize starch.

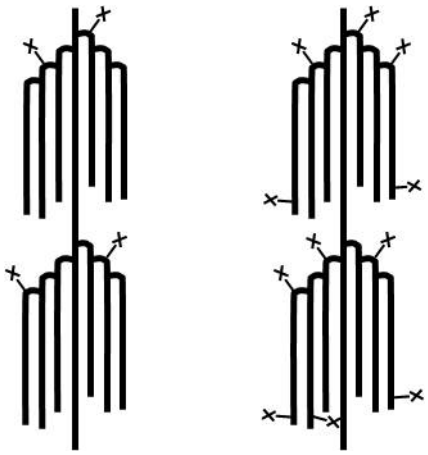


Figure 4.5 Proposed model structures for octenylsuccinated starches with degrees of substitution of 0.018 (left) and 0.092 (right).

Chapter 5 - Preparation and structure of α-amylase-degraded octenylsuccinate waxy maize starches with different substitution patterns

Abstract

The reaction of starch and octenylsuccinic anhydride (OSA) produces lipophilic starch that has the ability to stabilize oil-in-water emulsions. The functional properties of octenylsuccinate (OS) starch depend on its degree of substitution (DS), distribution of OS groups, and molecular structure. The objectives of this study were to prepare α-amylase-degraded OS starches with different OS distributions through two approaches and characterize the OS substitution distribution by enzyme hydrolysis followed by chromatography analysis. In the first approach, granular waxy maize starch was reacted with OSA and then cooked and hydrolyzed by α-amylase to produce maltodextrins (gOSMs) with ca. 7.5 dextrose equivalent (DE). In the second approach, granular starch was cooked and hydrolyzed by α-amylase to make a maltodextrin of DE 7.5 and then reacted with OSA for OS malrodextrins (sOSMs), which yielded OS maltodextrins with DS of ca. 0.02 and 0.09. Isoamylase action was significantly inhibited for gOSMs and sOSMs, indicating substitutions near the branching points of starch chains. Successive β-amylase conversion rates of gOSMs were significantly higher than sOSMs, suggesting that the OS substitution in sOSMs were more toward the non-reducing end than in gOSMs. Similar results were observed by amyloglucosidase hydrolysis. In addition, sOSMs were less converted by α-amylase than gOSMs. OS starches with different substitution distributions were prepared with two approaches. One product (gOSMs) had localized OS substitution near the branching points or non-reducing ends; the other product (sOSMs) had OS groups distributed randomly throughout the starch chains, and OS substitutions were found close to the branching points as well as the non-reducing ends.

Keywords: octenylsuccinic anhydride, substitution distribution, starch, maltodextrin

Introduction

Octenylsuccinic anhydride-modified starch (OS starch) is a chemically modified starch with broad applications in the food industry as an emulsion stabilizer (Trubiano, 1986). OS starches are traditionally obtained from granular starch in an aqueous slurry reaction system (Trubiano, 1986; Wurzburg, 2006). After modification, OS starch may be further cooked and hydrolyzed by enzymes for emulsification applications. In our previous study, we prepared OS esters from granular starch and soluble maltodextrin (Bai & Shi, 2011). The soluble OS maltodextrin had a different molecular structure from OS granular starch. OS starch prepared from maltodextrin exhibited substitution on O-2, O-3, and O-6 as well as reducing ends, whereas granular OS starch showed OS substitution on O-2 and O-3 positions for the granular OS starch. The substitution distribution for the two OS starches has not been investigated, although we believe it would be different. When OSA is reacted with granular starch, starch remains in its compact granular form and substitutions are localized in the amorphous region of the starch granule (Bai & Shi, 2011; He, Song, Ruan & Chen, 2006; Shogren, Viswanathan, Felker & Gross, 2000; Song, He, Ruan & Chen, 2006). However, in a dispersed system, all the starch molecules are available for reaction and result in OS starch with a different substitution distribution.

The objectives of this study were to prepare and characterize α-amylase-degraded OS starches with different OS distributions. Two approaches were designed. In the first approach, granular waxy maize starch was reacted with OSA, then cooked and hydrolyzed by α-amylase to produce maltodextrins (gOSMs) (Figure 5.1, approach 1). In the second approach, granular starch was cooked and hydrolyzed by α-amylase to make a maltodextrin, and the resulting soluble maltodextrin was reacted with OSA for OS maltodextrins (sOSMs) (Figure 5.1, approach 2). In another approach (Figure 5.1, approach 3), granular starch may be cooked and reacted with OSA followed by α-amylolysis. This technique was proposed to make cationic starch (Richardson, Cohen & Gorton, 2001); however, it requires very low solids for waxy maize starch due to high viscosity after cooking, so it was not used in this study. The substitution distribution of OS starches was investigated by enzyme hydrolysis of followed by chromatography analysis. Enzymes including α-amylase, β-amylase, isoamylase, and amyloglucosidase were used separately or combined to hydrolyze the OS starches. High-performance anion-exchange chromatography (HPAEC) and gel permeation chromatography (GPC) were used to analyze the

enzyme hydrolysates. Information on the substitution distribution of OS groups is needed to correlate the functional properties, such as emulsification performance, of the OS starches.

Materials and Methods

Materials

Waxy maize starch was obtained from National Starch LLC. (Bridgewater, NJ). *Bacillus* sp. α-amylase (A6380-100MG, type II-A), α-amylase from porcine pancreas (A3176-5MU, type VI-B), and β-amylase from barley (A-7130-10KU, type II-B) were purchased from Sigma-Aldrich (St. Louis, MO), and the enzyme activity was 839, 23, and 55.7 units/mg solid, respectively. The enzyme activity unit for α-amylase as suggested by Sigma-Aldrich is defined as one unit liberating 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. For β-amylase, enzyme activity is defined as one unit liberating 1.0 mg of maltose from starch in 3 min at pH 4.8 at 20 °C. α-amylase (Termamyl 120L) was obtained from Novozymes (Franklinton, NC); its enzyme activity was 120KNU-T/g. One KNU is defined as the amount of enzyme that dextrinizes 5.26g of starch (Merck Amylum soluble) per hour under standard conditions (37.0 °C, 0.0003MCa.$^{2+}$, and pH5.6). Glucose, maltose, and a series of oligosaccharides from maltotriose to maltoheptaose were purchased from Sigma–Aldrich (St. Louis, MO). Other chemicals were analytical grade.

Preparation of α-amylase degraded OS starch

Approach 1

Preparation of α-amylase-degraded OS starch from approach 1 is shown in Figure 5.1. Granular waxy maize starch was first reacted with OSA in an aqueous slurry system as previously described . Briefly, starch suspension (250 mL) of 40% solid content was adjusted to pH 7.5 by 3% (wt%) NaOH. OSA (3% or 15% based on the weight of starch) was added to the starch slurry while pH was maintained at 7.5 by 3% (wt%) NaOH during the reaction. After pH stabilized for 30 min, the reaction was terminated by adjusting pH to 6 with 1 N HCl. OS starch was recovered by filtration, washed by methanol (400 mL), and dried in an oven at 45 °C. Degree of substitution (DS) was determined by NMR spectroscopy.

The OS starches were converted to OS maltodextrins by α-amylase hydrolysis as described by Lumdubwong and Seib (2001) with some modifications. α-amylase (Termamyl 120L) (0.1% based on the weight of starch) was added to a starch slurry of 13% solids with 200 ppm Ca^{2+}. pH of the slurry was adjusted to 6.0–6.4 by 1 N NaOH. Starch hydrolysis was carried out at 94 °C with different reaction time depending on the DS of OS starches. α-amylolysis was stopped by adjusting pH to 3.0 by 1.0 N HCl. Starch slurry was held at 94 °C for another 10 min and cooled in an ice-water bath. After the temperature dropped below 60 °C, pH was adjusted to 6.0 by 1 N NaOH. The maltodextrin obtained was filtered and recovered by freeze-drying.

Approach 2

Granular waxy maize starch was first hydrolyzed by α-amylase as described in Approach 1. Different reaction time was used to achieve the same dextrose equivalent (DE) of the OS maltodextrins obtained from Approach 1. The freeze dried maltodextrin was then dispersed in water at 40% solid concentration and reacted with 1.89 or 12.20% OSA (wt% based on the weight of maltodextrin) to achieve the same DS of the OS maltodextrins obtained from Approach 1. The amount of OSA added in the reaction was calculated based on the reaction efficiency of OSA reaction as previously reported . The OSA modified maltodextrins were recovered by freeze drying, washed by methanol to remove the unreacted OSA and dried in a vacuum drier. DS was determined by NMR spectroscopy.

Characterization of OS starches

Determination of dextrose equivalent (DE)

DE of α-amylase hydrolyzed products was determined by the Nelson-Somogyi method (Somogyi, 1952).

NMR spectroscopy

OS starches were exchanged with D_2O twice, freeze-dried, and dissolved in D_2O (10% wt%) for analysis. NMR spectroscopy experiments were performed on a Varian (now Agilent; Santa Clara, CA) 500 MHz NMR System spectrometer. The NMR spectrometer is equipped with a cryogenic carbon enhanced 5 mm triple-resonance inverse detection pulse field gradient probe operating at 499.839 and 125.697 MHz for ^{1}H and ^{13}C, respectively. Temperature was set at 25 °C. The ^{1}H spectra were collected in 32 individual scans with a sweep width of 16 ppm and a

delay time of 1 s. The ^{13}C spectra maltodextrin and OS maltodextrin were collected in 2000 scans and a delay time of 1 s. The procedure for determining DS was performed as previously reported .

Gel Permeation Chromatography (GPC)

Maltodextrins (4 mg) were dissolved in DMSO (4 mL), stirred at room temperature for 12 h, and filtered through a 2 μm filter. GPC analysis was performed as previously described (Cai, Shi, Rong & Hsiao, 2010).

High performance anion-exchange chromatography (HPAEC)

HPAEC (Dionex ICS-3000, Dionex Corp., Sunnyvale, CA) was 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 as previously described (Cai & Shi, 2010). 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 as previously described (Cai & Shi, 2010). The column was qualitatively calibrated for linear dextrins with glucose, maltose, and a series of oligosaccharides from maltotriose to maltoheptaose.

Amyloglucosidase hydrolysis

Starch (0.1 g) was dissolved in 5 mL acetate buffer (0.05M, pH 4.5). Amyloglucosidase (1% based on the weight of starch) was added and incubated at 55 °C for 24 h. Another 1% amyloglucosidase was added to the starch solution and incubate for another 24 h. The solution was diluted and analyzed by HPAEC and GPC.

β-amylase hydrolysis

Maltodextrin or OS maltodextrin (0.1 g) was dissolved in 10 mL acetate buffer (0.05M, pH 4.8). β-amylase (1% wt% based on the weight of starch) was added. Starch solutions were incubated at 40 °C for 1.5 h followed by heating in a boiling water bath for 10 min to denature the enzymes. The hydrolysates were analyzed by HPAEC and GPC. β-limit values were

determined as the ratio of maltose generated in the β-amylase hydrolysis and the total content of maltose in the starch before hydrolysis.

Preparation of α-limit dextrin

α-limit dextrins of OS maltodextrin were prepared as described in Xu & Seib (1997) with a few modifications. OS maltodextrins (1.0 g), sodium acetate trihydrate (0.15 g), calcium chloride (5 mg), and Bacillus α-amylase (5 mg) were weighed into a 50 mL glass centrifuge tube. Water (15 mL) was added to the tube, and the mixture was shaken until the maltodextrin dissolved. The tubes were placed in a water bath at 37 ºC and shaken at 100 RPM. The temperature of the bath was raised to 80 ºC. After 2 h at 80 °C, the tubes were cooled to 45 ºC. Porcine pancreatic α-amylase (5 mg protein) was added, and the mixture was incubated at 45 ºC for 18 h. The enzyme was denatured by heating in a boiling water bath for 15 min. After cooling to room temperature, the aliquot was filtered through a filter paper (0.45 μm) and freeze-dried.

Debranching and successive β-amylolysis

Starch (0.5 g) was dispersed in 25 ml acetate buffer (0.05M, pH 3.5) and heated in a boiling water bath at 100 °C for 1 h. After the starch solution cooled to 50 °C, isoamylase (1% based on the weight of starch) was added, and the solution was incubated at 50 °C for 24 h. Samples of 10 mL were collected for β-amylase hydrolysis. The rest of the samples were diluted and analyzed by HPAEC.

Starch solution collected after debranching was cooled to 40 °C, and pH was brought up to 4.8 by 0.05 M sodium acetate. β-amylase (1% based on the weight of starch) was added, and the starch solution was incubated at 40 °C for 24 h. After complete β-amylase hydrolysis, the solution was freeze-dried and analyzed by HPAEC.

Results

Preparation of α-amylase-degraded OS waxy maize starches with different substitution patterns

The action of α-amylase on starch was altered after OS substitution. When α-amylolysis was performed under the same conditions (0.1% α-amylase, 1.5 h and 94 °C), the products from the native starch and OS starches with DS 0.018 and 0.092 had DE of 15.9, 10.7, and 5.0,

respectively (Table 5.1). To obtain maltodextrins with similar DE, α-amylolysis time was adjusted to 1.0, 1.25, and 3.0 h for native starch and OS starches of DS 0.018 and 0.092, respectively (Table 5.1). The resulted maltodextrin (M) and OS maltodextrins of DS 0.018 (gOSM-Low) and 0.092 (gOSM-High) had a DE of ca. 7.5 (Table 5.2) with the same molecular size range of 4.1E+02 to 7.2E+05 g/mol (Figure 5.2). In approach 2, OSA was reacted with the maltodextrin (DE 7.5) prepared from α-amylolysis of native waxy maize starch. OS maltodextrins of DS 0.018 (sOSM-Low) and 0.094 (sOSM-High) were obtained.

Structure of α-amylase degraded OS waxy maize starches with different substitution patterns

Substitution distribution on anhydroglucose units

^{13}C NMR spectrum of OS maltodextrin (DS 0.094) from approach 2 (sOSM-High) is shown in Figure 5.3. Peak broadening was observed for resonances at 102.6 (C-1), 98.3 (C-1 α-reducing), 79.5 (C-4), 76.0 (C-3), 73.9 (C-2), and 63.5 ppm (C-6), indicating that substitutions occurred at the O-2, O-3, and O-6 as well as the reducing ends as previously suggested . DE of sOSM-Low and sOSM-High were 7.1 and 6.5, respectively (Table 5.2). Because the molecular size of maltodextrin did not change after OSA modification as determined by GPC (data not shown), the decrease in DE reflected that OS substitution occurred at the reducing end. It was calculated that 7.7 and 12.2% of the reducing ends were substituted by OSA for sOSM-Low and sOSM-High, respectively (Table 5.1).

The OS substitution pattern on the anhydroglucose units (AGU) of OSA-modified maltodextrin prepared from normal maize starch was reported in our previous study . In this study, maltodextrin was prepared from α-amylolysis of waxy maize starch. The NMR results suggest that neither amylose nor the process of α-amylolysis affected the substitution location on AGU.

Amyloglucosidase hydrolysis

Maltodextrin was 99.4% converted to glucose by amyloglucosidase (Table 5.2), which was similar to conversions reported for native granular starch (Hood & Mercier, 1978; Richardson, Nilsson, Cohen, Momcilovic, Brinkmalm & Gorton, 2003). The conversion ratio for OS maltodextrin was significantly lower than that of maltodextrin (Table 5.2), indicating that

starch chains carried OS substitution groups and inhibited the action of amyloglucosidase. For OS maltodextrins prepared from approach 1, gOSM-Low and gOSM-High were 93.3 and 83.5% converted by amyloglucosidase, respectively. In comparison, the conversion ratio for sOSM-Low and sOSM-High was 90.8 and 77.2%, respectively (Table 5.2). Because amyloglucosidase is an exo-enzyme that hydrolyzes α-1,4 and α-1,6 linkages from the starch non-reducing ends, substitution groups in OS maltodextrins from approach 2 were closer to the starch non-reducing ends than the maltodextrins from approach 1.

α-amylase hydrolysis

GPC elution profiles of α-amylase hydrolysates of maltodextrin and OS-maltodextrins are shown in Figure 5.4. Maltodextrin after α-amylolysis had a peak at molecular weight of 4.30E+02 g/mol (Fraction 1) and comprised about 72% of the total starch molecules, primarily glucose, maltose, maltotriose, and maltotetraose. The rest of the molecules (Fraction 2) eluted from 6.63E+02 to 1.06E+04 g/mol were high molecular weight fractions with an average degree of polymerization (DP) of 12 (Fraction 1). The products from α-amylolysis were in agreement with those suggested in the literature . OS maltodextrins from approach 1 (gOSMs) had slightly different elution profiles from maltodextrin. The percentage of molecules eluted in Fraction 2 was 29.0 and 31.2%, which was slightly higher than that from maltodextrin, for gOSM-Low and gOSM-High, respectively. The increase was probably due to the starch molecules containing OS substitution groups that were resistant to α-amylase hydrolysis . Similar results were found for OS maltodextrins from Approach 2 (sOSMs); however, sOSMs had a more predominant peak at 4.09E+03 g/mol compared with gOSMs. The proportion of Fraction 2 increased to 32.9 and 40.6% for sOSM-Low and sOSM-High, respectively, and their corresponding DP was 16 and 18 (Figure 5.4). The results suggested that at the same DS, sOSM was more resistant to α-amylolysis than gOSM. It has been suggested that a minimum sequence length of two unsubstituted glucose residues is required for amylolysis to occur for methylated starch . Although the minimum sequence length required for OS starch might differ from the requirement for methylated starch, amylolysis clearly occurred at glucosidic bonds that were a few anhydroglucose units away from the substitution groups. Therefore, it is possible that the OS substitution groups in gOSMs were closer to each other than sOSMs, and the OS groups in sOSMs were distributed throughout the starch chains.

β-amylase hydrolysis

Maltodextrin and OS maltodextrin were hydrolyzed exhaustively by β-amylase; the β-limit values are listed in Table 5.2. Compared with maltodextrin, β-limit values for OS maltodextrins were significantly lower and decreased with the increase in DS. Results suggest that substitution groups in OS maltodextrin occurred at the outer starch chains and blocked the action of β-amylase. For OS maltodextrins prepared from approach 1, gOSM-Low and gOSM-High had β-limit values of 39.3 and 37.5%, respectively. In comparison, β-limit values for sOSM-Low and sOSM-High were 36.8 and 34.9, respectively. OS maltodextrins from approach 2 showed lower β-limit values than those from approach 1 at both low and high DS. β-amylase is an exo-enzyme that hydrolyzes α-1,4 linkages from the non-reducing end, and the action of β-amylase was blocked by starch branching points (Robyt, 2009) as well as chemical substitution groups (Hood & Mercier, 1978; Kavitha & BeMiller, 1998; Richardson, Nilsson, Cohen, Momcilovic, Brinkmalm, & Gorton, 2003; Zhu & Bertoft, 1997). Therefore, in agreement with the results from amyloglucosidase hydrolysis, OS maltodextrins from approach 2 had more substituents located closer to the non-reducing ends than the OS maltodextrins from approach 1.

Isoamylase debranching and successive β-amylolysis

The elution profiles of isoamylase debranched maltodextrin and OS maltodextrins as well as their β-limit dextrins from GPC are shown in Figure 5.6. Debranched maltodextrin had an elution profile from 28 to 36 min (Figure 5.5-A). It had a DE of 15.8, which was more than doubled from maltodextrin before debranching. The average DP of debranched maltodextrin was 6.3 (Table 5.2). Exhaustive β-amylolysis completely converted debranched maltodextrin to maltose (Table 5.2 and Figure 5.5-A), suggesting all linear-type molecules in the debranched maltodextrin.

For OS maltodextrins from approach 1 (gOSMs), gOSM-Low after debranching eluted from 27 to 35 min indicating that it contained larger molecular size than debranched maltodextrin. DE of debranched gOSM-Low was 14.2, which was 81.8% increase from 7.8 (DE before debranching) (Table 5.2). Compared with the 102.6% increase in maltodextrin (Table 5.2), much fewer starch chains were released by isoamylase from gOSM-Low. These results suggest that the OS substitution groups probably were close to the branching points of starch molecules that inhibited the action of isoamylase. Debranched gOSM-Low was further treated by β-amylase and was ca. 87% converted to maltose (Table 5.2). The elusion profile of β-limit

dextrin of debranched gOSM-Low had a peak at 32 min, which was absent in that of maltodextrin (Figure 5.5-B). In our previous study (Chapter 4), OS substitution occurred primarily near the branching points of the granular OS starch at DS 0.018. Therefore, the peak 32 min was most likely attributed to branching points containing OS substitution groups. Debranched gOSM-High eluted at 24 min (Figure 5.5-C), representing high molecular size fraction. DE of debranched gOSM-High was 7.6, which was 69.9% increase from DE before debranching (Table 5.2). Compared with the maltodextrin and gOSM-Low, fewer starch chains were released by isoamylase, suggesting that more OS substitution occurred near the branching points. β-amylase treated debranched gOSM-High eluted from 28 min, and only 71.4% of starch was converted to maltose. These results suggest that the β-limit dextrin of debranched gOSM-High contained starch molecules with high molecular size because some OS substitution probably occurred close to the branching points and some OS groups were close to the non-reducing ends. The results were consistent with our previous findings (Chapter 4).

For OS maltodextrins from approach 2 (sOSMs), GPC profiles of sOSM-Low and sOSM-High are shown in Figure 5.5. Compared with the debranched maltodextrin, a high molecular size fraction was observed for debranched sOSM-Low, suggesting that OS substitution was close to the branch points. DE of debranched sOSM-Low was 14.6. Compared with the DE before debranching, an increase of 103.4% was observed (Table 5.2). This value was similar to that of maltodextrin and higher than gOSM-Low, suggesting that fewer OS groups were located close to the branching points in sOSM-Low than in gOSM-Low. Debranched sOSM-Low was 88.4 % converted to maltose, which was slightly lower than gOSM-Low (Table 5.2). In addition, a high molecular size fraction at 28 min was observed for β-limit dextrin of debranched sOSM-Low. These results suggested that some OS groups in sOSM-Low were probably located close to the non-reducing ends of the starch chain. When DS in sOSM increased to 0.094, significant resistance to the debranching enzyme was observed. The elution profile of debranched sOSM-High showed that the sample contained a high molecular size fraction at 27 min (Figure 5.5-E). In addition, DE of debranched sOSM-High was 11.7, a 79% increase from before debranching. These results suggest that some OS substitution groups occurred close to the branching points of sOSM-High; however, compared with the OS maltodextrins from Approach 1, fewer OS groups were found close to the branching points. The β-limit value for debranched sOMS-High was 67.4, which was lower than that of gOSM-High

(Table 5.2) and suggests that more OS groups in sOSM-High occurred close to the non-reducing ends of the starch chains than in gOSM-High. Compared with the gOSM, OS substitution in sOSM appeared to be randomly distributed along the starch chains.

Discussion

Some have suggested that chemical substitution occurred preferentially in the amorphous region of starch granules where the branching points are located (Steeneken & Smith, 1991; Steeneken & Woortman, 1994; van der Burgt, Bergsma, Bleeker, Mijland, Kamerling, & Vliegenthart, 2000a, b; van der Burgt et al., 1998, 1999, 2000b). The outer chains of amylopectin were tightly packed into crystalline lamellae and were inaccessible to the chemical reagents. In our previous study, OS substitution was suggested to occur predominantly at the amorphous region of the starch granules. OS starch of DS 0.018 had OS groups located close to the branching points, whereas the OS substitution in OS starch of DS 0.092 occurred near non-reducing ends as well as the branching points. After the granular OS starches were converted by α-amylolysis, the location of the substitution groups should not change. The present study confirmed that OS maltodextrin from Approach 1 of DS 0.018 (gOSM-Low) had substitution groups located close to the branch points, whereas highly substituted OS maltodextrin of DS 0.092 (gOSM-High) had substitution groups close to the branching points as well as the non-reducing ends. In comparison, for OS maltodextrins from approach 2, OSA was reacted with maltodextrin, which is amorphous and completely soluble in water. All the starch molecules were available for reaction. As noted in the present study, OS substitution was not restricted in a certain part of the starch as in the granular OS starch. OS groups were randomly distributed along the starch chains. Even at low DS of 0.018, substituents were found close to the non-reducing ends of OS maltodextrin.

Conclusions

OS starches with different substitution distributions were prepared from two approaches. One product (gOSMs) had localized OS substitution near the branching points at low DS of 0.018 and near branching points as well as non-reducing ends at high DS of 0.092. The other product (sOSMs) had OS groups distributed randomly throughout the starch chains, and OS substitutions were found close to the branching points as well as the non-reducing ends at both low and high DS.

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Tables and figures

Table 5.1 Dextrose equivalent (DE) and conversion time for maltodextrin (M) and octenylsuccinate maltodextrins from approach 1 of DS 0.018 (gOSM-Low) and DS 0.092 (gOSM-High).

	DE after 1.5h α-amylolysis	*Conversion time (h) for DE 7.5*
M	15.9	1.00
gOSM-Low	10.7	1.25
gOSM-High	5.0	3.00

Table 5.2 Characterization of native starch converted maltodextrin (M) and octenylsuccinate maltodextrins from approach 1 of degree of substitution (DS) 0.018 (gOSM-Low) and 0.092 (gOSM-High) and approach 2 of DS of 0.018 (sOSM-Low) and 0.094 (sOSM-High).

Parameters	M	gOSM-Low	gOSM-High	sOSM-Low	sOSM-High
Degree of substitution	0	0.018	0.092	0.018	0.094
AMG hydrolysis (%)	99.4 ± 0.3	93.3 ± 0.0	83.5 ± 1.3	90.8 ± 0.3	77.2 ± 0.6
β-limit value (%)	41.7	39.3	37.5	36.8	34.9
Before debranching					
DE[a]	7.4 ± 0.3	7.7 ± 0.1	7.5 ± 0.1	7.1 ± 0.2	6.5 ± 0.2
DP_n[b]	12.8 ± 0.8	12.8 ± 0.2	13.0 ± 0.5	12.8 ± 0.8	12.8 ± 0.8
After debranching					
DE	15.8 ± 0.5	14.2 ± 1.7	13.1 ± 0.6	14.6 ± 0.2	11.7 ± 0.4
DP_n	6.3 ± 0.2	7.0 ± 0.8	7.6 ± 0.3	6.8 ± 0.1	8.5 ± 0.3
DE increased (%)[c]	102.6	81.8	69.9	103.4	79.3
Successive β-amylolysis	100.0 ± 0.0	86.9 ± 2.8	71.4 ± 0.2	88.4 ± 0.5	67.4 ± 2.0

[a] Dextrose equivalent.

[b] Degree of polymerization. The values of sOSM-Low and sOSM-High were adapted from the DP_n value of maltodextrin.

[c] Calculated by subtracting DE before debranching from DE after debranching and then divided by DE before debranching.

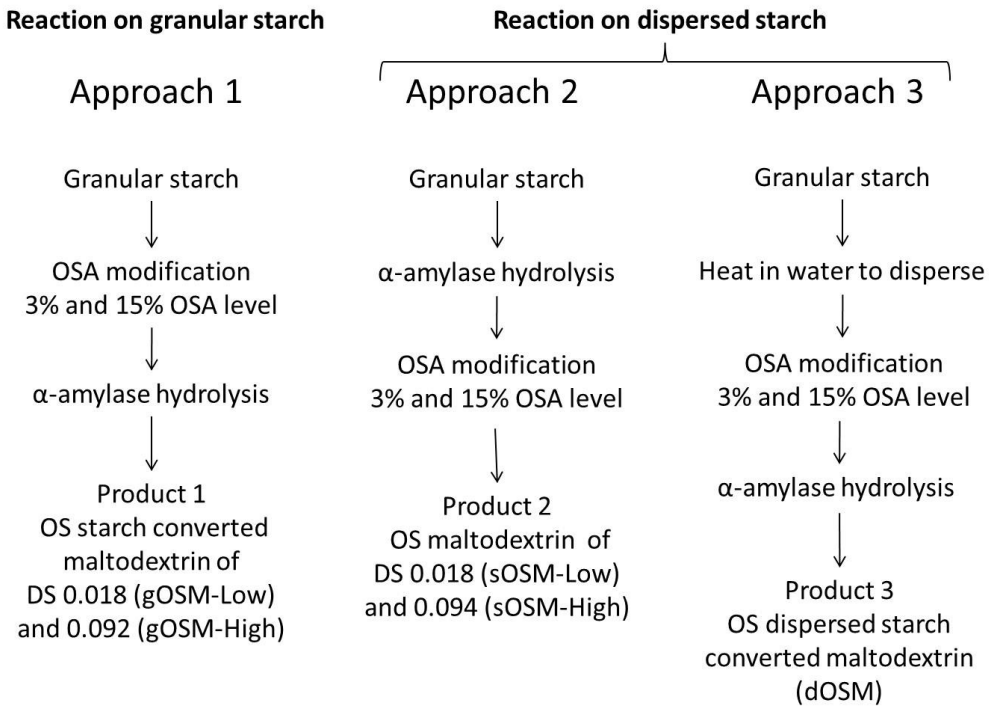


Figure 5.1 Three approaches to prepare α-amylase-degraded octenylsuccinic anhydride (OSA)-modified starch.

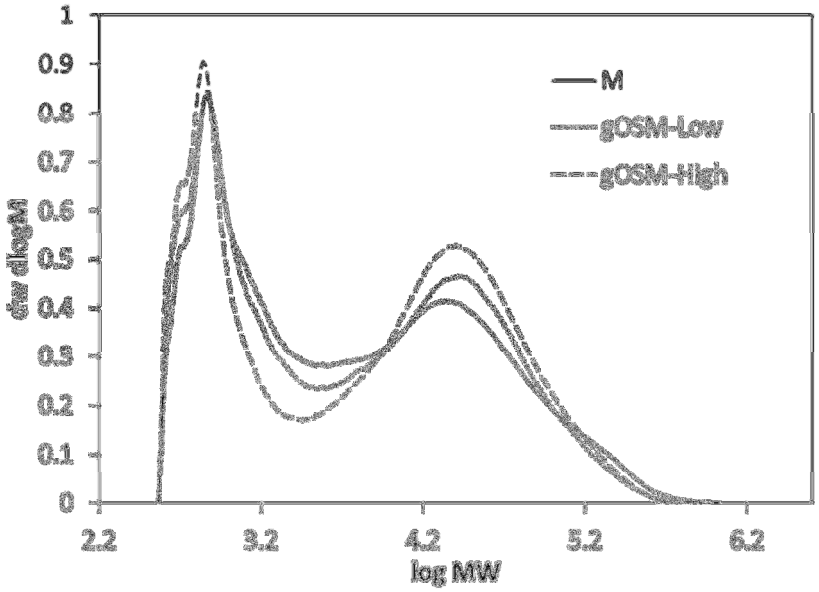


Figure 5.2 Molecular size distribution of maltodextrins with same dextrose equivalent (DE) from native starch (M) and granular octenylsuccinate starch with degree of substitution of 0.018 (gOSM-Low) and 0.092 (gOSM-High).

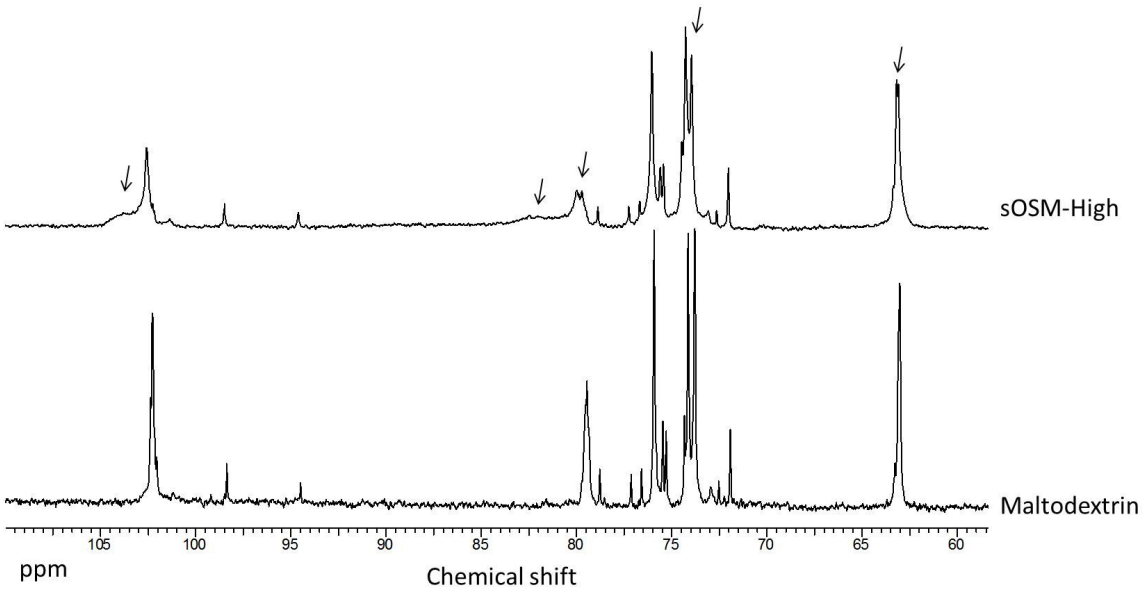
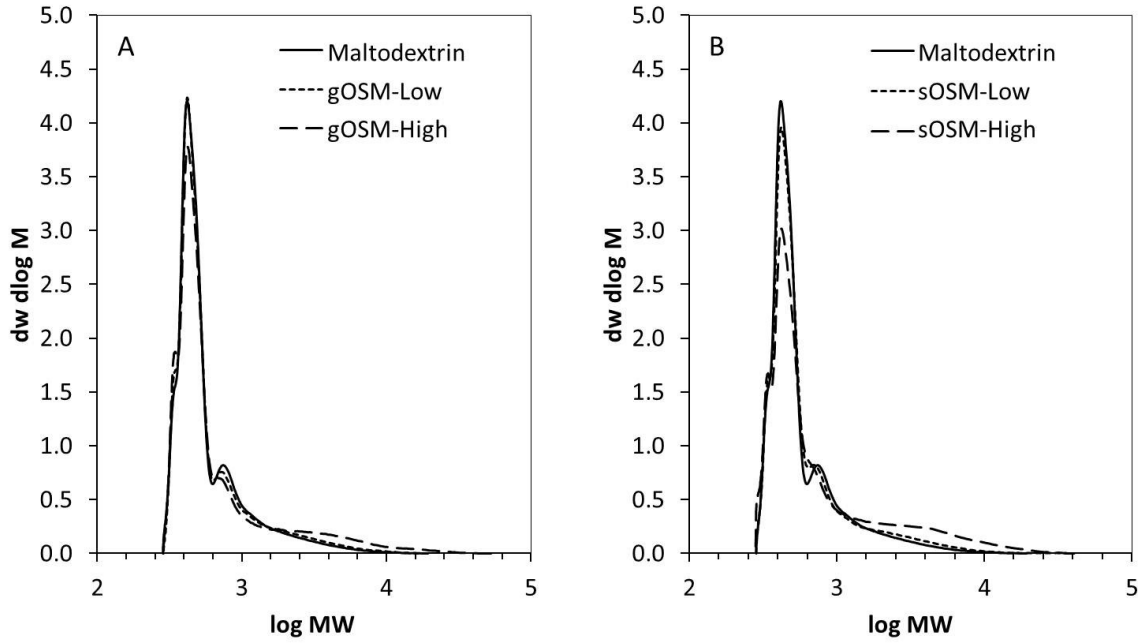


Figure 5.3 ^{13}C-NMR spectra of maltodextrin and octenylsuccinic (OS) maltodextrin from approach 2 with DS of 0.094 (sOSM-High).



Sample	α-amylase hydrolysis			
	Fraction 1		Fraction 2	
	DP	% area	DP	% area
M	2	72.0	12	28.0
gOSM-Low	2	71.0	13	29.0
gOSM-High	2	68.8	14	31.2
sOSM-Low	2	67.1	16	32.9
sOSM-High	2	59.4	18	40.6

Figure 5.4 Elution profiles and degree of polymerization (DP) of α-limit dextrins of maltodextrin (M) and octenylsuccinate maltodextrins obtained from Approach 1 (A) and Approach 2 (B).

gOSM-Low: OS maltodextrin from Approach 1 of degree of substitution (DS) 0.018.

gOSM-High: OS maltodextrin from Approach 1 of DS 0.092.

sOSM-Low: OS maltodextrin from Approach 2 of DS 0.018.

sOSM-High: OS maltodextrin from Approach 2 of DS 0.094.

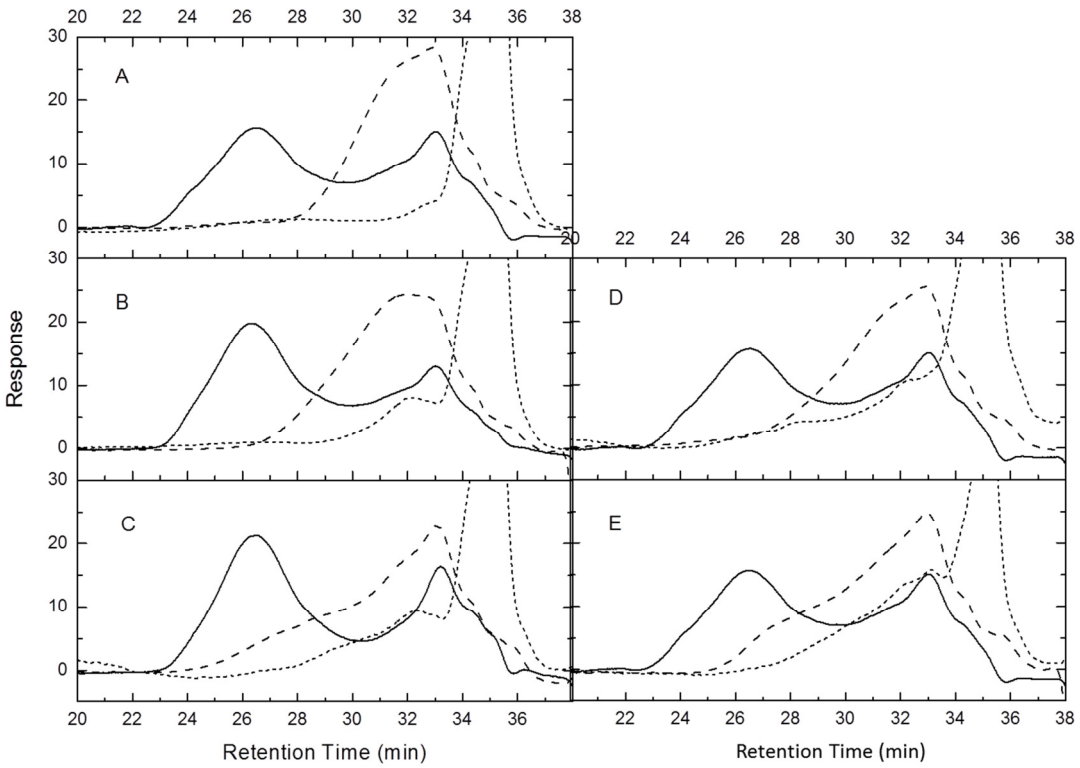


Figure 5.5 Elution profiles of starch before debranching (—), after debranching (- - - - -) and β-amylase hydrolysis after debranching (……) : maltodextrin (A), octenylsuccinate maltodextrins from approach 1 of degree of substitution (DS) of 0.018 (gOSM-Low) (B) and 0.092 (gOSM-High) (C) and approach 2 of DS of 0.018 (sOSM-Low) (D) and 0.094 (sOSM-High) (E).

Chapter 6 - Structural changes from native waxy maize starch granules to cold water-soluble pyrodextrin during thermal decomposition

Abstract

The structural changes occurring during the conversion of insoluble native waxy maize starch granules to cold water–soluble pyrodextrin under acidic conditions has been investigated. Starch granules were suspended in water and the pH of the slurry was adjusted to 2.5–3.0 by 0.5M HCl. The air-dried starch was thermally heated for different time intervals at 160 and 170 °C for 0.5 to 4 h. The pyrodextrins obtained had cold water solubility from 21% to 100%. Structural changes of starch granules during dextrinization were determined by multiple techniques, including synchrotron small-angle X-ray scattering (SAXS), wide-angle X-ray scattering (WAXS), differential scanning calorimetry (DSC) and gel permeation chromatography (GPC). In a mixture of water/glycerol (20/80, w/w), the SAXS characteristic peak at 0.6 nm^{-1} decreased in intensity as pyrodextrin solubility increased. The peak disappeared as pyrodextrin solubility reached 100%. In addition, starch crystal size as well as its melting enthalpy decreased as pyrodextrin solubility increased. The pyrodextrin molecular size decreased as solubility increased. Pyrodextrins had a granular shape identical to the native starch when observed in glycerol under a light microscope and showed strong birefringence under polarized light. It is proposed that the starch backbone is hydrolyzed by acid in the amorphous region. Unwinding of the double helices also occurs and crystallite size decreases. Starch molecules are hydrolyzed into small molecular fractions but remain in a radial arrangement.

Keywords: Pyrodextrin, dextrin, thermal decomposition, small-angle X-ray scattering, starch structure

Introduction

Thermal decomposition of dry starch usually causes depolymerization at temperatures below 300 °C.[1] A degraded starch product prepared in the dry state by heating, or through a combination of heat and acid, is commonly called a "pyrodextrin".[2] A closely related term "dextrin" broadly refers to all degraded starch products regardless of the method used in the process. Dextrins may be produced by hydrolysis of starch with enzymes or acids in water as well as by dextrinization or pyroconversion, or heating starch in its dry form.[2] Depending on the temperature, time, and level of acid used, pyrodextrins are generally classified into three categories: white dextrin, yellow or canary dextrin, and British gum;[2, 3] however, these broad classifications are arbitrary, and classifying a given dextrin product is not always easy. The properties of the products cover a wide range depending on pretreatment, level of acid used, and process conditions (e.g., temperature and time). [2] Yellow dextrin and more degraded British gum are soluble in cold water and become a viscous liquid with gummy and adhesive properties.[2] Traditionally, pyrodextrins have wide application in industry as adhesives, coatings, binders, and encapsulating agents.[1, 2, 4] The various applications and biological activities of pyrodextrins have been reviewed.[1] Pyrodextrin is suggested to be resistant to α-amylolysis and is considered as soluble dietary fiber, [4-10] which has many applications in the food industry. Preparation of indigestible dextrins by pyroconversion has been described.[11, 12]

Changes in molecular structure during the dextrinization process have been suggested to involve hydrolysis, transglycosidation, and repolymerization of the glucans.[2, 13] Starch molecules are significantly degraded by heat and acid, as reflected in a continual decrease in starch molecular weight, a progressive increase in solubility in water, decreased viscosity in water, and increased reducing value.[2, 4, 14] Transglycosidation results from the hydrolysis of the α-1,4 glucosidic linkages followed by a recombination of the fragments with nearby free hydroxyl groups to produce branched structures.[2] Transglycosidation and the branched structure of pyrodextrin were proposed from the evidence of methylation[15, 16] and reduced degree of β-amylolysis.[17] Repolymerization was proposed due to the slight increase in viscosity and the decrease in reducing sugar content.[2]

Compared with extensive studies in molecular structure, few attempts have been made to understand the precise long-range structural changes during dextrinization. Pyrodextrins look identical to native granular starch when viewed in glycerol under both normal and polarized light

microscopes,[2, 4, 18] and pyrodextrins have a wide-angle X-ray scattering (WAXS) pattern similar to native starch but with broader and weaker peaks.[5] However, the molecular arrangement in the pyrodextrin granules has not been thoroughly investigated; furthermore, it is not well understood why pyrodextrin is partially crystalline and retains its granular shape yet is readily soluble in water.

Small-angle X-ray scattering (SAXS), which probes larger length scales than WAXS, has been used to characterize the structure of native and modified starches.[19-29] Blazek and Gilbert (2011) [30] reviewed the application of SAXS to investigate starch structure. Native starch molecules are suggested to be arranged in a lamellar structure with a repeat spacing of ca. 9 nm, which corresponded to a SAXS peak at 0.6-0.7 nm^{-1}.[31] This SAXS peak disappears due to the loss of lamellar order in the case of starch gelatinization[22, 32] and acid hydrolysis [20, 27]. In this study, SAXS and WAXS were used to investigate the granular structure of pyrodextrin. We propose a model of starch structural changes during thermal decomposition based on scattering, microscopy, gel permeation chromatography (GPC), and differential scanning calorimetry (DSC). These findings enable a greater understanding of the granular structure of pyrodextrin and the implications to its functionality.

Materials and methods

Materials

Waxy maize (WM) starch (Amoica TF) was obtained from National Starch LLC (Bridgewater, NJ). Other chemicals used in the study were analytical grade.

Methods

Preparation of pyrodextrins

Waxy maize starch (100 g dry weight) was suspended in water (150 mL), and the pH of the slurry was adjusted to 3.0 by 0.5 M HCl and stirred for 30 min. Starch was filtered to a cake with moisture content at approximately 50%. The starch cake was broken and dried in an oven at 40 °C for 24 h to a moisture content of 10–15%. Starch pH was approximately 4.0 after air drying. The dried starch was ground and passed through a screen (100 mesh) and heated in an forced-air oven at 170 °C. Samples were collected at 0.5, 1, 2, 3, and 4 h during heating and were

kept at ambient temperature overnight. The resulting moisture content of the pyrodextrin samples was about 7%. In a separate experiment, the pH was adjusted to 2.5 and pyrodextrin was prepared as described above.

Light microscopy

Starch granules were observed in glycerol or a mixture of glycerol and water using an optical microscope with a digital camera (Model BX51, Olympus Co., Japan) under normal visible and polarized light.

Solubility

Solubility of OS starch was determined by a handheld refractometer (Fisher Scientific, Pittsburgh, PA). Starch (0.100 g) was dissolved in distilled water (0.9 mL) and centrifuged at 6708 x g for 3 min. Starch concentration in the supernatant was determined by the refractometer.

Differential scanning calorimetry (DSC)

Thermal properties of native starch and pyrodextrin products were measured using a Pyris-1 DSC (Perkin-Elmer, Norwalk, CT). Samples were mixed with solvents of distilled water or water/glycerol mixture (20/80, w/w) at ratio of 1:3 (starch : solvent, w/w) and were lightly stirred with a spatula. Pyrodextrin pastes were sealed in a centrifuge vial and allowed to stand for 12 h at 25 °C for hydration. Pyrodextrin pastes (40–55 mg) were accurately weighed with a microbalance and placed into large DSC stainless steel pans. The DSC pans were sealed and held at 10 °C for 1 min and heated to 160 °C at 10 °C/min. Onset (To), peak (Tp), and conclusion (Tc) temperatures as well as enthalpy (ΔH) were determined. An empty pan was used as a reference. Native waxy maize starch was included as a control. Samples were analyzed in duplicate.

SAXS and WAXS

Pyrodextrin paste in water, water/glycerol mixture of 20/80 and 40/60 (w/w), or glycerol at ratio of 1:1 (w/w) was prepared by manually mixing with a spatula followed by equilibrium at room temperature for 15 min. Pyrodextrin paste in water, water/glycerol mixture of 20/80 and 40/60 (w/w), or glycerol at ratio of 1:1 (w/w) was prepared by manually mixing with a spatula followed by equilibrating at room temperature for 15 min.

Native starch and pyrodextrin pastes were examined by SAXS and WAXS at the X27C beamline at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL). The details of the experimental setup at the X27C beamline have been reported elsewhere [33-36]. The wavelength of the X-ray was 1.371 Å. Two-dimensional WAXS and SAXS images were collected with an X-ray CCD detector (MarUSA) with resolution times of 30 and 60 s, respectively. The sample-to-detector distance was 155.6 mm for WAXS and 2018.5 mm for SAXS, respectively. The scattering angle in WAXS was calibrated using a polypropylene standard and an Al_2O_3 standard from the National Institute of Standards and Technology (Gaithersburg, MD). The scattering angle in SAXS was calibrated by an AgBe standard. Pyrodextrin samples were sealed in a sample holder and placed on the hot stage (Instec Inc., Boulder, CO). The WAXS and SAXS spectra were background-subtracted. The average d-spacing was calculated by:

$$d = 2\pi/q$$

where d (nm) is the lamellar repeat distance and q(1/nm) is the scattering vector, which is defined as:

$$q = (4\pi\sin\theta)/\lambda$$

where λ (nm) is the wavelength of the x-ray source and 2θ is the scattering angle.

Starch crystallinity was calculated as described elsewhere.[37] The approximate average size (D) in nm of crystallites in the samples was calculated from the Scherrer formula:

$$D = \frac{0.89\times\lambda}{\beta\times\cos\theta}$$

where β is the angular width in radians at the half maximum intensity corrected for instrumental line broadening and θ is half of angular position of the peak in radians. In the present case, such broadening has negligible influence compared to the width of the peaks observed.

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 $P<0.05$.

Results and discussion

Solubility and molecular weight distribution

The solubility and degree of degradation was affected by process parameters including pH, temperature, and heating time. At a pH of 2.5 and 170 ºC, starch became 100% soluble after heating for 0.5 h. At pH 3 and 170 ºC, pyrodextrins with solubility from 21 to 100% were produced by heating from 0.5 to 4 h (Table 6.1). Starch solubility increased rapidly during the first 2 h of the conversion. As the conversion continued, relatively little change occurred in the solubility. In contrast, at pH 3 and 160 ºC, starch was only 42.0% soluble after being heated for 4 h (Table 6.1). Molecular breakdown was indicated by a shift in molecular size distribution profile (Figure 6.1). The molecular weight of the waxy maize starch decreased rapidly at the beginning (0.5 to 1 h) of the conversion and slowed after 2 h. At the latter stage of dextrinization, starch molecular size remained in the range of 3.6×10^5 to 4.3×10^4 (Figure 6.1). These results suggest that starch molecules were hydrolyzed by acid and heat during dextrinization. Molecular scission was promoted by low pH, high temperature, and long reaction time, which is in agreement with previous studies [2, 5, 14]. To investigate the structural changes of starch from insoluble granules to water-soluble pyrodextrin, we selected the samples prepared by heating at pH 3 and 170 °C from 0.5 to 4 h, because their solubility ranged from 21 to 100%.

Birefringence of pyrodextrin

After dextrinization, starch granules remained and products had a characteristic yellow color. When examined under a microscope, pyrodextrins in glycerol appeared identical to native starch (Figure 6.2). Under polarized light, the pyrodextrins showed strong birefringence (Figure 6.2), indicating a radial orientation of molecules; however, when the pyrodextrin was suspended in water, the starch granules ruptured quickly. Starch granules disappeared as starch molecules dissolved. Similar results have been reported in other studies [2, 38]. It has been suggested that the crystallites of starch granules were oriented together in parallel fashion and underwent significant changes upon dextrinization.[17] To fully understand these changes in granular structure, we used SAXS and WAXS to characterize the crystalline and amorphous regions and lamellar structure of pyrodextrins.

Small-angle X-ray scattering

The SAXS lamellar peak of starch evolves only upon proper hydration,[28, 39] but the solubility of pyrodextrins in water increased after dextrinization (Table 6.1), and starch granules of pyrodextrins with high solubility disintegrated in water and lost their structural information. To effectively probe the structural changes of starch granules during dextrinization by SAXS and WAXS, we used a different ratio of glycerol to water as a plasticizer. Glycerol is a less effective plasticizer than water, and pyrodextrins retain their granular shape in glycerol (Figure 6.2). Using different ratios of glycerol to water, we were able to change the degree of plasticization and fully elucidate the structure of pyrodextrins with different water solubility.

Native waxy maize starch and pyrodextrins showed no SAXS characteristic peak in glycerol at room temperature (Figure 6.4); however, a well-defined SAXS peak at 0.62 nm^{-1} was observed for the native starch in a mixture of water and glycerol (20/80 or 40/60, w/w) (Figure 6.3 and Figure 6.4), which was attributed to the periodic lamellar arrangement of semi-crystalline starch with a repeat distance of ca. 9 nm.[31] Compared with the native starch, pyrodextrins showed significantly different SAXS patterns in water/glycerol (20/80, w/w) (Figure 6.3). A characteristic SAXS peak was observed for starch heated for 0.5 and 1 h, but the scattering intensity at ca. 0.6 nm^{-1} was both lower and less resolved than for native starch, suggesting a partial reduction in lamellar periodicity. There also appears to be a slight shift in peak position to higher q (from 0.62 to 0.65 nm^{-1}) indicating a possible decrease in the repeat distance of the alternating crystalline and amorphous lamellae. Such a decrease in lamellar spacing may result from the hydrolysis of amorphous lamellae and a partial disruption in the crystalline lamellae, resulting in a closer periodic arrangement. However, since the peak width is also broader, this re-arrangement is certainly not uniform. The 9 nm scattering peak was not observed after 2 h of dextrinization (Figure 6.3); this indicates that the lamellar structure of granular starch was significantly disrupted during the dextrinization process and there is a corresponding reduction in long-range order leading to a broad feature in the scattering after 2 h of dextrinization.

Increasing the ratios of water to glycerol from 20/80 (w/w) to 40/60 (w/w) reveals interesting results (Figure 6.4). For native starch, the size and position of the peak at ca. 0.62 nm^{-1} were little changed, but the peak for the 0.5-h pyrodextrin sample became more prominent. Yet, no peak was observed for the 4-h pyrodextrin sample, which indicates the loss of periodic lamellar structure.

Increasing the ratio of water to glycerol from 20/80 (w/w) to 40/60 (w/w) reveals interesting results (Figure 6.4). For native starch, the size and position of the peak at ca. 0.62 nm^{-1} were little changed, but the peak for the 0.5-h pyrodextrin sample became more prominent. Yet, no peak was observed for the 4-h pyrodextrin sample, which indicates the loss of periodic lamellar structure.

In addition to changing the ratio of water to glycerol (i.e., the degree of plasticization), we also heated the native starch and pyrodextrin in a mixture of water and glycerol (20/80, w/w) and examined their changes by SAXS (Figure 6.5). The peak ca. 0.62 nm^{-1} was little changed when the native waxy maize starch was heated from 25 to 100 °C. For the 0.5-h pyrodextrin sample, the intensity of the peak ca. 0.62 nm^{-1} became stronger from 25 to 60 °C but decreased after 60 °C, and the peak almost disappeared at 100 °C. These changes indicate that heating initially provides mobility and promotes the alignment of the periodic lamellar structure but weakens that structure at high temperatures.

Water plays dual roles when added to the pyrodextrins, functioning either as a plasticizer or solvent depending on the level of water used and the structure of the pyrodextrin. Glycerol alone is not able to solvate starch granules, and no SAXS peak is observed due to the lack of long-range order.[39, 40] When water is added, self-assembly transforms the disordered structure of dry starch into an ordered semctic-like lamellar structure as previously suggested[39] and observed in this study (Figure 6.3). Conversely, in the absence of water, the branches exhibit greater disorder and lamellar scattering is reduced. A water to glycerol ratio of 20/80 (w/w) is sufficient to allow the evolution of the 9 nm lamellar peak for starch granules at room temperature.[39] In this study, for the 0.5-h pyrodextrin, the SAXS peak ca. 0.62 nm^{-1} increased as the water ratio increased from 0/100 to 40/60 (w/w) (Figure 6.4); however, for the 4-h pyrodextrin, no SAXS peak was observed by increasing the level of water (Figure 6.4) or heating (Figure 6.5), indicating permanent loss of any significant long-range order of the alternating lamellar structure. The 4-h pyrodextrin retained its granular shape and was birefringent in glycerol (Figure 6.2), but in the mixture of water and glycerol (40/60, w/w), it lost both its birefringence and granular shape (data not shown), indicating that its granular structure was so weak that it was disrupted by the mixture of water and glycerol. Water, acting as a solvent, was able to completely dissolve the 4-h pyrodextrin sample (Table 6.1).

We believe that the loss of the periodic lamellar structure is due to the hydrolysis of the amorphous regions of starch and partial disruption of crystalline lamellae by heat. Acid primarily hydrolyzed the amorphous regions of the starch granule.[2] After a significant number of glycosidic bonds are cleaved and crystalline lamellae are disrupted, it is likely that it is no longer possible to form a periodic lamellar structure.

Comparing the SAXS results in this study (heating starch in dry form with acid) with the findings on the acid hydrolysis of starches in water is revealing.[20, 27] When hydrolyzed by acid in water, the lamellar peak intensity increased during the early stages of acid hydrolysis followed by a decrease in the latter stages of hydrolysis. In studying the acid hydrolysis of pea starch in water, Wang et al.[27] also reported that the position of the lamellar peak was essentially constant within the first 6–12 days of hydrolysis but shifted to higher q after 35 days of hydrolysis. Relative crystallinity increased after the amorphous regions were selectively hydrolyzed in water and removed during filtration. In contrast, hydrolyzed products remained inside starch granules in this study, and the crystallinity decreased during dextrinization (Figure 6.6) as discussed in the next section.

Using fractal analysis,[41] we also found changes in the starch during dextrinization and changes in fractal characteristics of the pyrodextrins when different ratios of glycerol to water were used. To establish the fractal relation, we re-plotted the SAXS data on a double logarithmic scale with intensity (I) as a function of the scattering vector (q) and calculated the slope or exponent (α) when there was a linear relationship between log I and log q (i.e. $I \propto q^{\alpha}$). A linear power-law behavior was observed over a range of q from 0.1 to 0.3 nm^{-1}, indicating that the native starch and pyrodextrins were fractal in nature.[41]

In glycerol, the native starch and pyrodextrin had similar α values of ca. -3.5 (Table 6.2). Such a value may be indicative of "surface fractal" behavior in which the surface of the starch and pyrodextrin is somewhat roughened in glycerol. As noted previously, both materials had an identical shape in glycerol when observed under a microscope (Figure 6.2). In a mixture of water and glycerol (20/80, w/w), the starch and 0.5-h pyrodextrin indicated rougher surface-fractal behaviour, but the slopes of the pyrodextrin decreased as dextrinization progressed from 1 to 4 h and exhibited slopes of between -1 and -3 (Table 6.2, Figure 6.3B). Those pyrodextrins became mass fractals that had a self-similar structure. These results indicate that starch granular structure was weakened after dextrinization and probably reflect increased water-solubility as reaction

time increased (Table 6.1). As the water level increased to 40%, all the starch and pyrodextrins appeared to be mass-fractal, because the slopes were in the range of -1.9 to -2.8 (Table 6.2).

Wide-angle X-ray diffraction

Native starch and pyrodextrins showed A-type crystalline WAXS patterns in the water/glycerol (20/80, w/w) mixture (Figure 6.6). Pyrodextrins showed broadened peaks with reduced intensity at 13, 16, and 21 degrees 2θ compared with the native starch. Similar results have been reported previously.[5] Crystallinity and crystal size were estimated[42] and are shown in Table 6.3. Crystallinity of the native waxy maize starch was approximately 43%, which is close to previous literature values.[42] Heating at 170 ºC for 0.5 and 4.0 h reduced crystallinity to 31.4 and 14.0%, respectively. In addition, crystal size was reduced from 9.6 nm of native starch to 7.8 and 7.0 nm of pyrodextrins. These results indicate a loss of structural organization in a direction lateral to the semi-crystalline lamellae due to the dextrinization process; however, for the 4-h pyrodextrin, WAXS still showed an A-type crystalline pattern in a mixture of water and glycerol (20/80, w/w), indicating the existence of crystalline structure. Therefore, the crystalline region of starch was not completely destroyed, but crystallites became smaller during the dextrinization process. Weak crystallites after dextrinization also were noted when the starch and pyrodextrin samples were heated in the mixture of water and glycerol (20/80, w/w) and changes by WAXS (Figure 6.7). Crystallinity of native waxy maize starch changed little when it was heated from 26 to 80 °C. In contrast, the crystallinity of the 0.5-h pyrodextrin was significantly reduced at 80 °C, and 4-h pyrodextrin became essentially amorphous at 80 °C.

Thermal properties

A reduction in the crystallinity of pyrodextrins from native starch is also reflected in the DSC data (Figure 6.8). Compared with native starch, 0.5-h pyrodextrins showed a lower onset temperature and smaller enthalpy of melting endotherm in water. Pyrodextrins with higher solubility did not have an endothermic peak, indicating the loss of starch crystallinity in water at room temperature. To further probe the structure and properties of pyrodextrin, native starch and pyrodextrins were analyzed in a mixture of water/glycerol (20/80 w/w) (Figure 6.8). The gelatinization endotherm for the native starch and 0.5-h pyrodextrin was shifted to higher temperatures relative to water. Similar results were reported by other studies [39, 43-46]; however, for the 0.5-h pyrodextrin, the gelatinization peak was much broader and the enthalpy was much

higher than that in water. Because glycerol was not suggested to affect the shape of the gelatinization endotherm,[39] the difference was probably due to changes in the crystalline region when hydrated in the two different solvents. Higher gelatinization enthalpy suggests higher double helical content.[47] Therefore, DSC results further suggest that part of the crystalline region was so weak that it dissolved in water at room temperature, resulting in a narrower gelatinization endotherm with a lower enthalpy value, but the weak crystalline region remained in the glycerol/water mixture. Glycerol has a large molecular weight and is not as effective as water as a plasticizer.[21] A broad melting peak also was observed for pyrodextrins of 2.0 and 4.0 h in the glycerol/water mixture (Figure 6.8), which supports this reasoning. The DSC results suggest that the crystalline region existed for all the pyrodextrin samples. Dextrinization affected the crystalline region and reduced the crystallinity and crystal size. The less perfect crystallites with short double helices were so weak that they disassociated in water at room temperature.

Native starch and pyrodextrins were also analyzed in 100% glycerol (Figure 6.9). The melting endotherm of each sample shifted to higher temperatures as expected. In addition, an exothermic peak was observed for all analyzed samples. The exothermic peak is suggested to result from a starch-glycerol "interaction," including the plasticization of amorphous lamellar regions that allow the crystalline lamellae to form a periodic lamellar structure and enhance crystallization.[39] Therefore, based on the model proposed by Perry and Donald [39], the DSC results suggest that the starch-glycerol interaction existed for pyrodextrin samples of different solubility. The process of rearranging crystalline lamellae to form a periodic lamellar structure upon plasticization, as well as crystallization, existed for all the pyrodextrins in glycerol during heating. It is also interesting to notice that when the native starch was heated by two heating-cooling cycles of 10-110-10-150 °C, the exothermic peak was observed in the first heating cycle but disappeared in the second heating cycle (Figure 6.9). In addition, little difference was observed for the endothermic peak between the two heating steps (Figure 6.9). The results suggest that the starch-glycerol interaction is irreversible and occurs prior to the melting of crystallites.

Based on WAXS and DSC results, we suggest that in addition to the effect of hydrolysis of the amorphous region, the changes in crystalline regions were another factor that affected the SAXS peak. For the 0.5-h pyrodextrin, although the crystalline region was affected by the dextrinization process, it still showed sufficient contrast with respect to the amorphous region to

exhibit a SAXS peak. However, in highly soluble pyrodextrins, the overall crystalline region was significantly altered by the process, and the contrast between crystalline and amorphous areas was significantly reduced. The radial arrangement of the starch molecules was not altered during the dextrinization process however.

Structural changes from native starch to pyrodextrin

Structural changes from native starch to pyrodextrin have been proposed in the literature[48] and are shown in Figure 6.10-A. In the traditional model, starch was converted by acid hydrolysis in combination with heat into smaller fragments. Starch molecules repolymerized into a branched structure; however, native starch granules are partially crystalline, which is not reflected in the model.

A new model describing long-range structural changes of starch during dextrinization is proposed based on the microscopic, NMR, GPC, SAXS, WAXS, and DSC results (Figure 6.10-B). As starch is heated at 170 °C for 0.5 h, the starch backbone is hydrolyzed by acid in the amorphous region. Unwinding of the double helices occurs as well, and the crystallite size decreases. Starch molecules are hydrolyzed into small molecule fractions but remain in a radial arrangement. The semi-crystalline lamellar structure still persists in the early stage of dextrinization. Upon proper plasticization, double helices tend to arrange in a periodic order; however, the periodicity is not as perfect as in native starch due to the hydrolyzed starch backbone and disrupted crystalline region.

As the dextrinization process continues, the starch backbone in the amorphous region is further hydrolyzed along with the crystalline region. Crystallinity decreases, crystallite size is reduced and starch molecules are hydrolyzed to a smaller molecular weight. While a crystalline arrangement may still have exist upon plasticization, due to the significant hydrolysis of the crystalline and amorphous region, a periodic structure of crystalline and amorphous lamellae could no longer be formed. Starch molecules still exist in a radial arrangement and result in birefringence.

Conclusions

In this chapter, the structural changes occurring during the conversion of insoluble native waxy maize starch granules to cold water–soluble pyrodextrin under acidic conditions has been investigated. It has been found out that during the dextrinization process, amorphous regions as

well as crystalline regions of starch granules are hydrolyzed by acid and heat. Starch molecules are hydrolyzed into small molecular fractions but they remain in a radial arrangement.

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Tables and figures

Table 6.1 Solubility of pyrodextrin in water prepared at different pH, heating temperature and heating time.

pH	Temperature (°C)	Time (h)	Solubility (%)
2.5	170	0.5	100.0 ± 0.9
3.0	160	4.0	42.0 ± 0.5
3.0	170	0.5	21.2 ± 0.6
		1.0	22.1 ± 1.2
		2.0	97.1 ± 1.8
		3.0	102.0 ± 1.0
		4.0	101.0 ± 1.2

Table 6.2 Slope (α) in SAXS of native waxy maize starch and pyrodextrins prepared at pH 3, 170 °C for 0.5 and 4 h in the double-log scale plot in solvents of water/glycerol at blending ratios of 40/60, 20/80, and 0/100.

Sample	Water/glycerol (w/w)		
	40/60	**20/80**	**0/100**
Native	-2.78	-3.31	-3.50
Dextrin 0.5 h	-2.40	-3.22	-3.49
Dextrin 4 h	-1.92	-2.16	-3.46

Table 6.3 Estimated crystallinity (%), crystal size (D), and full width at half maximum (FWHM) at 13.3 degree 2θ of samples in water/glycerol (20/80, w/w) mixture.

Sample	Time (h)	Crystallinity (%)	FWHM (Degree)	D (nm)
Native starch	n.a.	43.3	0.78	9.6
	0.5	31.4	0.96	7.8
	1.0	31.7	1.06	7.0
Dextrin	2.0	24.4	1.07	7.0
	3.0	20.3	1.08	7.0
	4.0	17.4	1.07	7.0

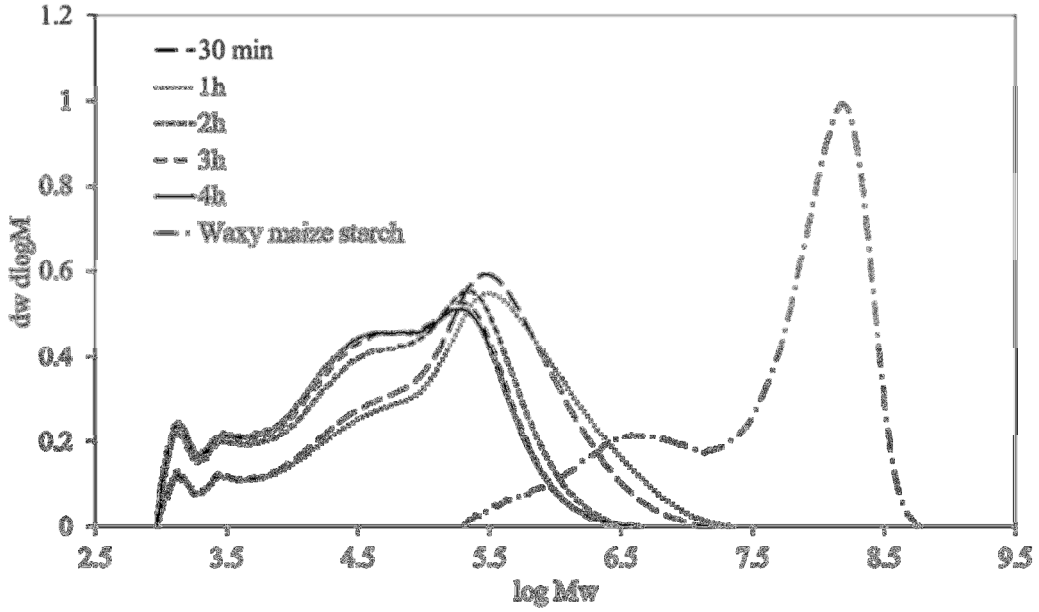


Figure 6.1 Molecular weight distribution of native waxy maize starch and pyrodextrins prepared from heating at pH 3 and 170 °C for 0.5 to 4 h.

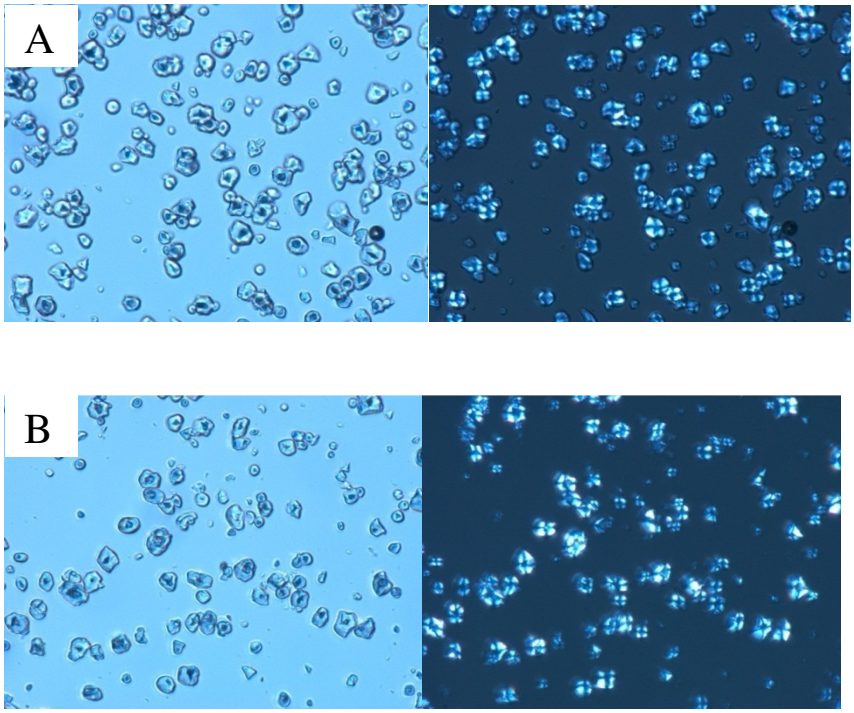


Figure 6.2 Microscopy images of (A) native waxy maize starch and (B) soluble pyrodextrin (100% solubility) in glycerol. Pyrodextrin was prepared at pH 3 and heated at 170 °C for 4 h. Scale bar in each graph represents 30 μm. Left images – unpolarized; right images – polarized.

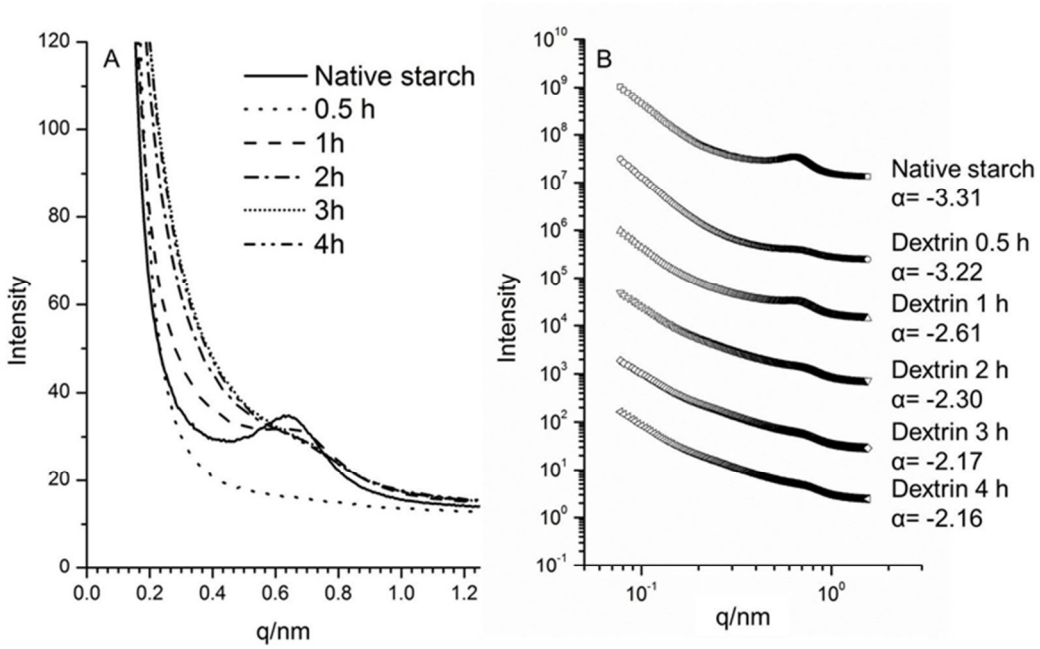


Figure 6.3 (A) Linear and (B) double-log scale SAXS plot of native starch and pyrodextrins in solvents of water/glycerol (20/80, w/w). Data shown in log-log plot have been shifted vertically for clarity.

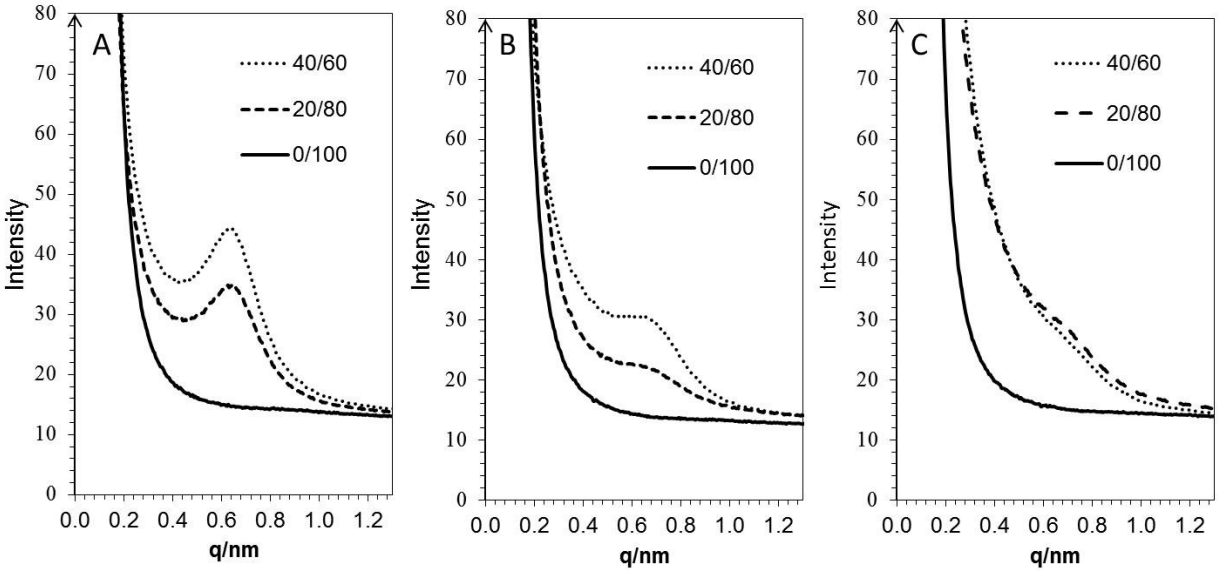


Figure 6.4 SAXS patterns of (A) native starch, (B) pyrodextrin after 0.5 h, and (C) pyrodextrin after 4 h in solvent of water/glycerol with ratios of 40/60, 20/80, and 0/100.

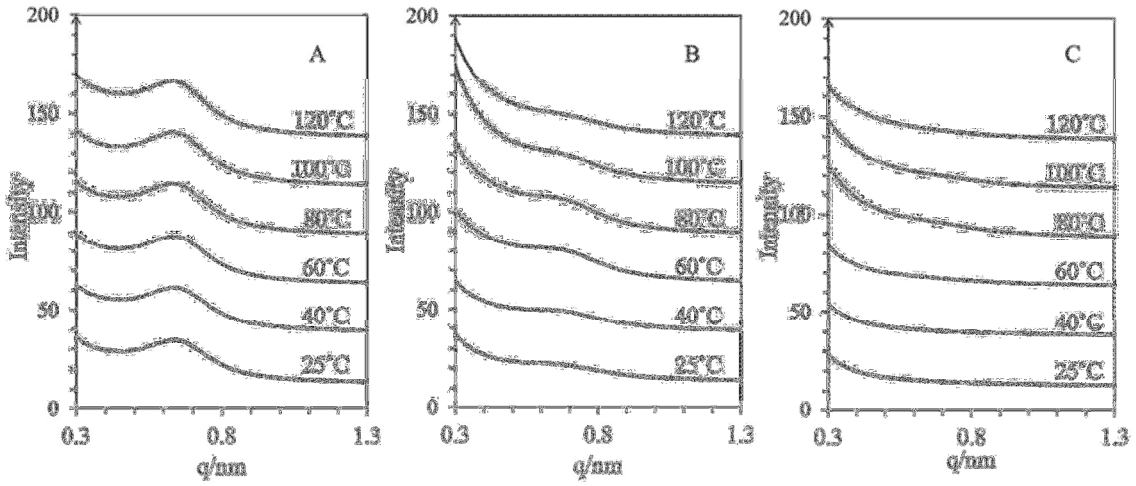


Figure 6.5 SAXS curves of native waxy maize starch (A), dextrin (pH 3, 170 °C, 0.5 h) (B), dextrin (pH 3, 170 °C, 4 h) (C) in a mixture of water/glycerol (20/80, w/w). Data have been vertically offset for clarity.

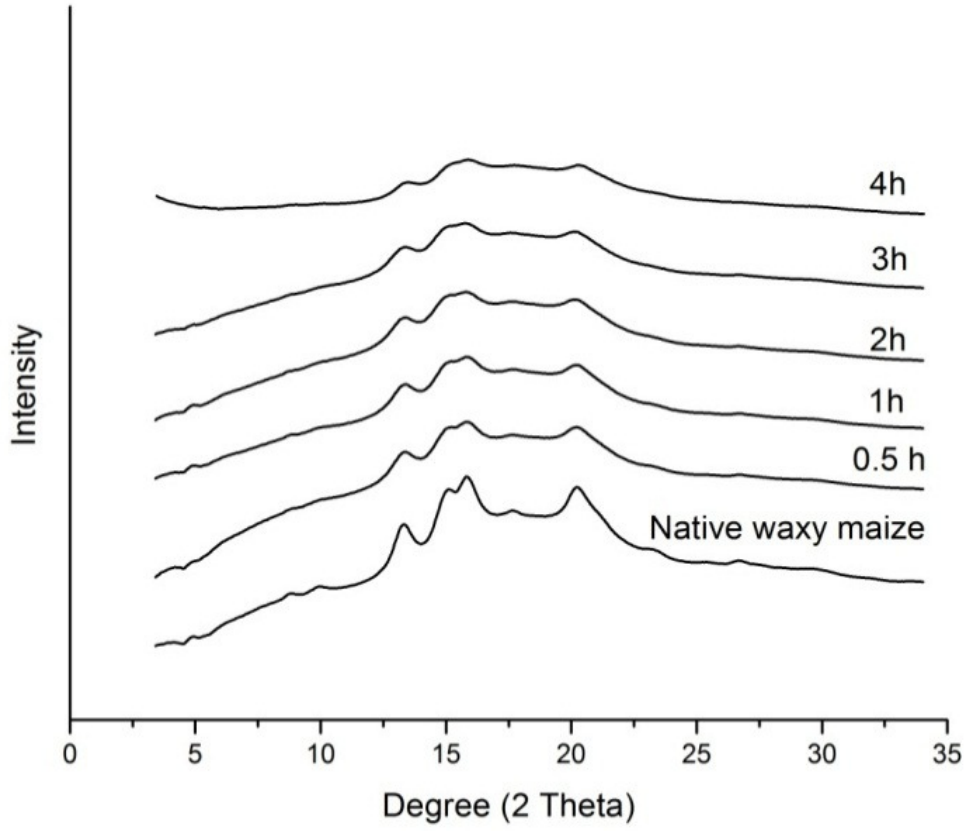


Figure 6.6 WAXS patterns of pyrodextrins (50%, w/w) in water/glycerol (20/80, w/w) mixture. Native waxy maize is shown as a reference. Pyrodextrins were prepared by heating waxy maize starch (pH 3.0) at 170 °C for 0.5, 1, 2, 3, and 4 h.

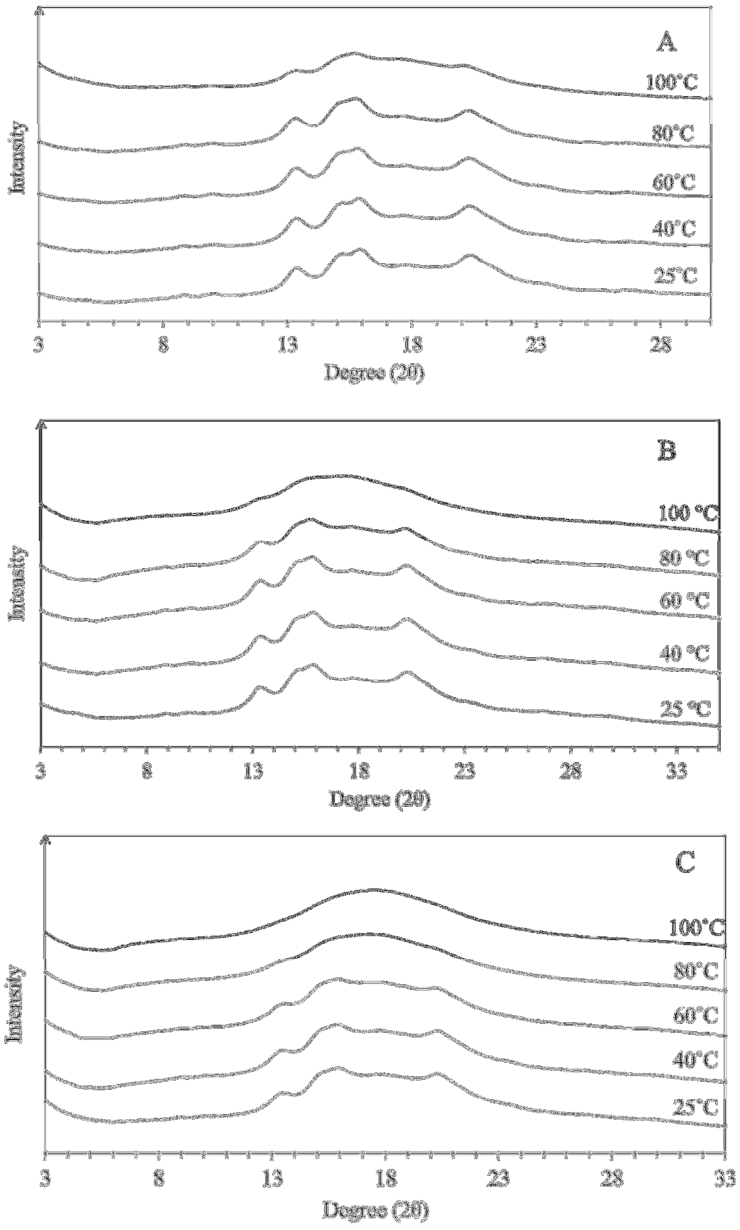
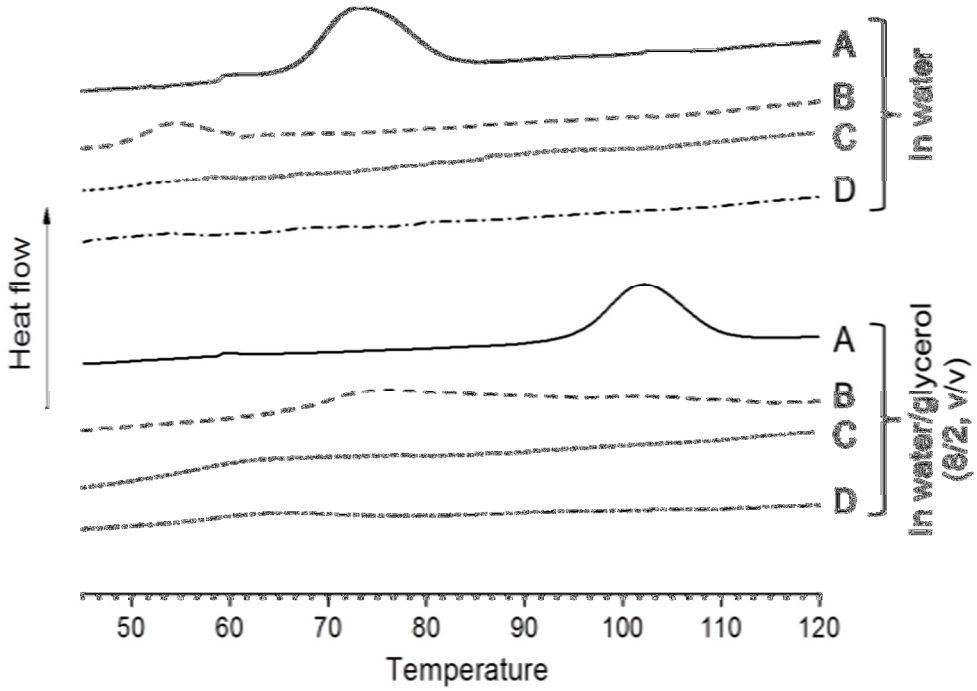
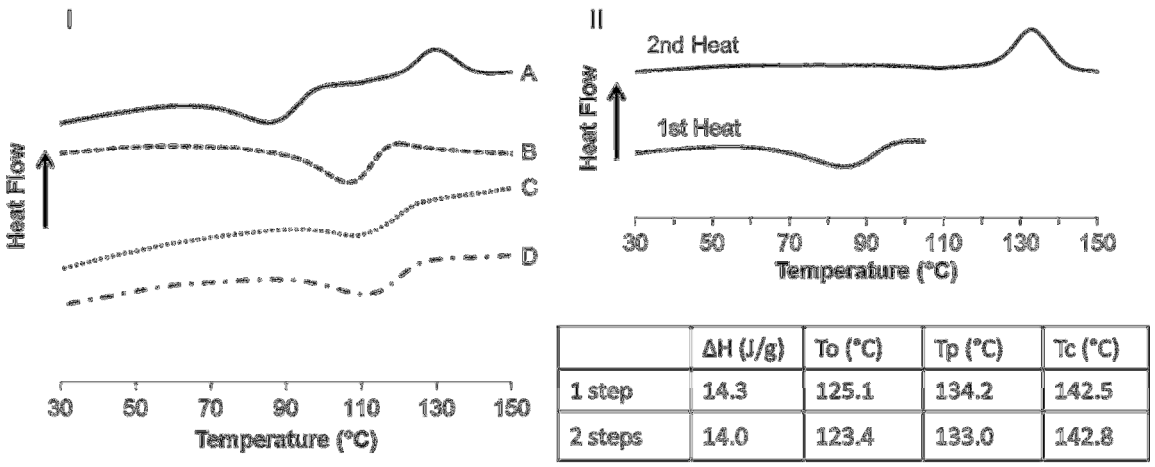


Figure 6.7 WAXS patterns of (A) waxy maize starch, (B) dextrin (solubility 21%) and (C) dextrin (solubility 100%) in a mixture of water/glycerol (20/80). Data have been offset vertically for clarity.



Solvent	Heating time (h)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Water	**Native**	65.2 ± 0.7	72.1 ± 0.1	81.4 ± 0.4	20.0 ± 1.6
	0.5	49.3 ± 2.2	53.2 ± 0.4	58.2 ± 2.7	6.9 ± 1.2
	2.0	No endothermic peak			
	4.0				
Water/glycerol mixture (20/80, w/w)	**Native**	93.7 ± 0.9	100.7 ± 0.8	109.0 ± 0.5	17.5 ± 0.2
	0.5	63.8 ± 0.3	74.2 ± 0.2	103.1 ± 1.1	14.7 ± 0.3
	1.0	63.1 ± 1.1	74.9 ± 1.1	98.7± 1.6	13.4 ± 2.0
	2.0	47.1 ± 2.2	61.9 ± 0.6	86.3 ± 1.1	10.1 ± 0.1
	3.0	52.4 ± 1.1	63.3 ± 1.2	79.7± 2.5	6.0 ± 0.0
	4.0	52.6 ± 0.3	63.2 ± 0.3	77.4 ± 2.8	4.6 ± 0.5

Figure 6.8 DSC profiles of (A) native starch and (B-D) pyrodextrins prepared at pH 3 and 170 °C for (B) 0.5 h, (C) 2 h and (D) 4 h.



	ΔH (J/g)	To (°C)	Tp (°C)	Tc (°C)
1 step	14.3	125.1	134.2	142.5
2 steps	14.0	123.4	133.0	142.8

Figure 6.9 DSC traces of (A) native starch and (B-D) pyrodextrins prepared at pH 3 and 170 °C for (B) 0.5 h, (C) 2 h and (D) 4 in glycerol in one heating cycle (I), and native starch in glycerol in two heating cycles (heating from 10 to 108 ºC, cooling to 10 ºC, and reheating to 150 ºC) (II).

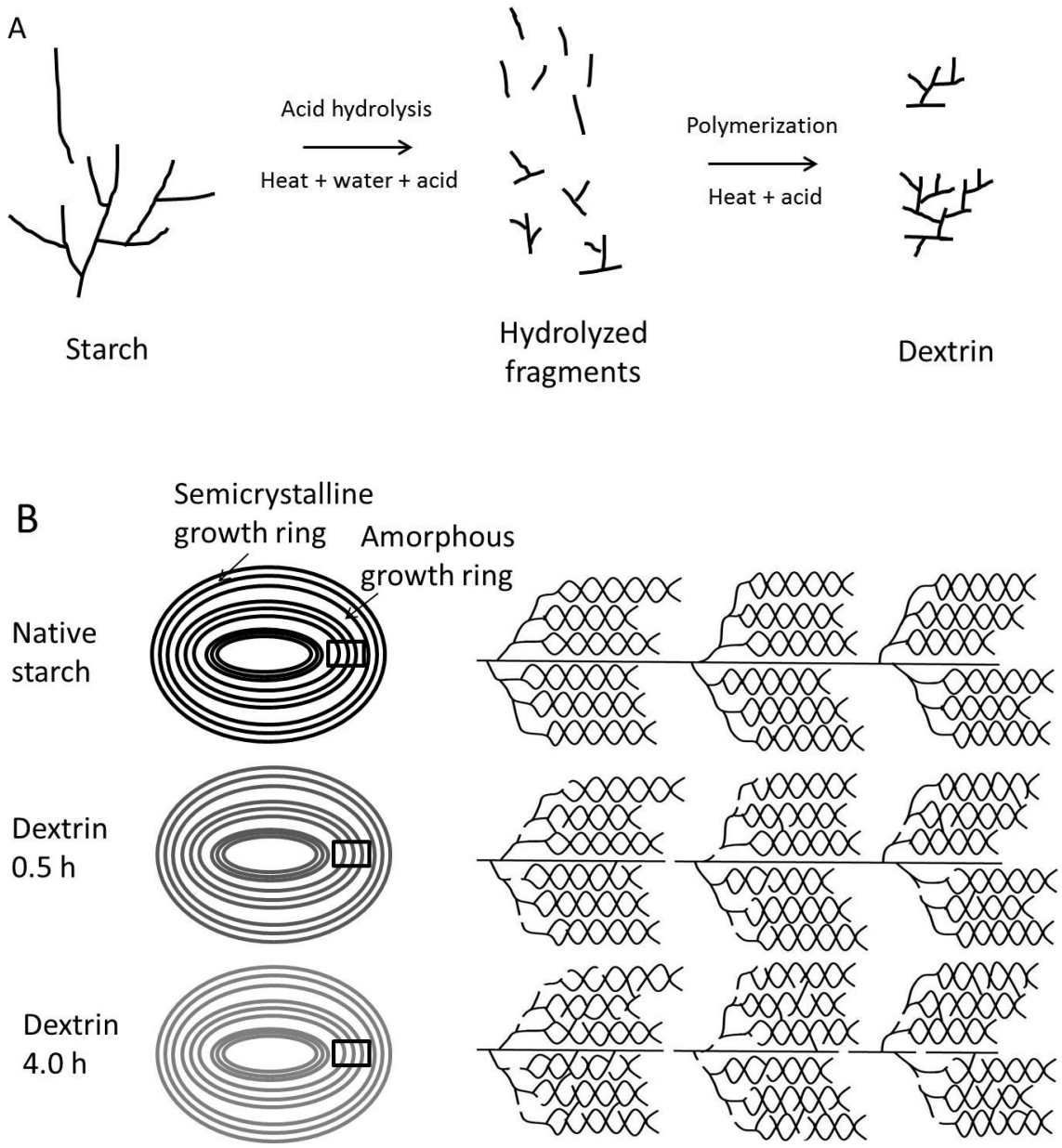


Figure 6.10 Structure changes from native starch to pyrodextrin. Part A is adapted from Rutenberg[48].

Chapter 7 - New insights into the structural changes of pyrodextrin during dextrinization by nuclear magnetic resonance spectroscopy

Abstract

The linkage type of pyrodextrin was characterized by NMR spectroscopy for the first time. Pyrodextrin was prepared by heating waxy maize starch at pH 3 and 180 °C for 4 h. ^{1}H and ^{13}C-NMR spectra of pyrodextrin were assigned with the assistance of 2D techniques including COSY, TOCSY, HSQC, and HMBC. During dextrinization, native waxy maize starch was hydrolyzed, and the resulted pyrodextrin became 100% soluble in water. There were 1.2% reducing ends (α-form) formed after starch hydrolysis, and 1,6-anhydro-β-D-glucopyranose was the major terminal group. Glycosyl linkages including α-(1→6), β-(1→6), α-(1→2), and β-(1→2) were formed. The degree of branching of pyrodextrin was 24.6%. Transglucosidation occurred during dextrinization, and the resulted pyrodextrin was highly branched.

Keywords

Pyrodextrin, dextrin, dextrinization, thermal decomposition, NMR

Introduction

Pyrodextrin is prepared by heat degradation of dry starch in the granular form, either with or without acid (Wurzburg, 2006). Pyrodextrins have been applied in the industry as binders, coatings, adhesives (Bhatt, Kumar & Soni, 2000) and dietary fiber (Lefranc-Millot, Wils, Roturier, Le Bihan & Saniez-Degrave, 2009; Ohkuma & Wakabayashi, 2001; Wurzburg, 2006). Depending on the preparation conditions, pyrodextrin is classified into three categories: white dextrin, yellow or canary dextrin and British gum (Tomasik, Wiejak & Palasinski, 1989; Wurzburg, 1986). The physical properties of pyrodextrin are often characterized by their color, solubility, alkali-lability, reducing sugar content, viscosity, β-amylolysis and etc. (Tomasik, Wiejak & Palasinski, 1989). The chemical reactions of dextrinization are complex and involve hydrolysis, transglucosidation and repolymerization. Glycosidic linkages of α-(1→4) and probably α-(1→6) are hydrolyzed during pre-drying and initial stages of dextrinization (Tomasik, Wiejak & Palasinski, 1989). The starch hydrolysis results in a decrease in the molar mass and viscosity, and an increase in reducing sugar and solubility. Transglucosidation means hydrolysis of the α-(1→4) glycosidic linkages followed by formation of new linkages with nearby free hydroxyl groups (Wurzburg, 1986). Considerable amount of transglucosidation was observed for British gum and pyrodextrins (Christensen & Smith, 1957; Geerdes, Lewis & Smith, 1957; Thompson & Wolfrom, 1958) . Formation of α-(1→6), β-(1→6), α-(1→2) and β-(1→2) linkages was reported for British Gum (Thompson & Wolfrom, 1958). Methylation studies suggested new bonds formation and branched structure of pyrodextrin (Brimhall, 1944; Christensen & Smith, 1957; Geerdes, Lewis & Smith, 1957). However, the details of the glycosidic bonds were not reported. The branched structure of pyrodextrin was suggested to be responsible for the increase in α-amylase resistance (Laurentin, Cardenas, Ruales, Perez & Tovar, 2003; Brimhall, 1944) . Repolymerization was also reported in pyrodextrin (Wurzburg, 1986). A slow increase in molecular weight during the latter stage of dextrinization was reported, which was suggested to be attributed to the formation of aggregate-like structure susceptible to repolymerization and transglycosylation (Terpstra, Woortman & Hopman, 2010). In addition, the formation of 1,6-anhydro-β-D-glucopyranose or levoglucosan type end groups was reported in British gum (Thompson & Wolfrom, 1958; Wolfrom, Thompson & Ward, 1959) and pyrodextrin (Katz, 1934; Kroh, Jalyschko & Haseler, 1996; Lowary & Richards, 1991). The anhydro end groups may act as an intermediate in reforming the polymer (Wolfrom, Thompson & Ward, 1959). However, the

formation of anhydro end group concept was opposed by Brimhall (1944), and a methylation study failed to support such a concept (Geerdes, Lewis & Smith, 1957).

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool to investigate the structure of carbohydrates and sugars. It has been successfully applied to characterize the glycosidic bonds of D-glucopyranose from analyzing ^{1}H-NMR and ^{13}C-NMR spectra (Roslund, Tähtinen, Niemitz & Sjöholm, 2008; Usui, Yamaoka, Matsuda, Tuzimura, Sugiyama & Seto, 1973).^{13}C-NMR spectrum of yellow potato dextrin was reported (McIntyre, Ho & Vogel, 1990) but was not fully characterized. No detail studies on the structure of pyrodextrin by NMR are reported. The aim of this study was to analyze the structure of pyrodextrin using NMR spectroscopy. It is the first detailed study being conducted to interpret 1D and 2D-NMR spectra of pyrodextrin. The study provides new insights into the structural changes of pyrodextrin during the dextrinization process.

Materials and methods

Materials

Waxy maize starch (Amoica TF) was obtained from National Starch LLC (Bridgewater, NJ). α-amylase (Termamyl ® 120L) was obtained from Novozymes (Franklinton, NC) and the enzyme activity was 120KNU-T/g. One KNU is defined as the amount of enzyme which, under standard conditions (37.0 °C; 0.0003MCa.$^{2+}$; and pH 5.6) dextrinizes 5.26 g of starch (Merck Amylum soluble) per hour. Other chemicals were analytical grade.

Preparation of pyrodextrin

Waxy maize starch (100 g dry weight) was suspended in water (150 mL) and the pH of the slurry was adjusted to 3.0 using 0.5 N HCl. Starch was filtered to a cake with approximately 50% moisture content. The starch cake was broken and dried in an oven at 40 °C for 24 h to a moisture content of 10 - 15%. The dried starch was ground, passed through a 100 mesh-screen, and heated in an oven at 180 °C. Samples were collected at 4 h and were kept at ambient temperature overnight. Moisture content of the pyrodextrins was about 7%.

Preparation of maltodextrin

Maltodextrin was prepared from native waxy maize starch as reported by Lumdubwong and Seib (2001) with some modifications. Briefly, α-amylase (1 mL) was diluted to 10 mL using 200 ppm Ca^{2+} solution. The enzyme solution (1.65 mL) was added to 400 mL $CaCl_2$ solution and pH was adjusted to 6.0 - 6.4 using 1 N NaOH. The solution was transferred to a three neck flask and heated in a water bath at 94 °C. Starch (150 g, dry weight) was suspended in 600 mL of 200 ppm Ca^{2+} solution. The pH of the solution was adjusted to 6.0 - 6.4 using 0.1 N NaOH. The starch solution was slowly added to the enzyme solution in 2 min with vigorous agitation. The hydrolysis lasted for 1 h and was stopped by adjusting pH to 3.0 using 1.0 N HCl and held at 94 °C for 10 min. The flask was then cooled in an ice-water bath. After the temperature dropped below 60 °C, the pH of the solution was adjusted to 6.0 using 1 N NaOH. The maltodextrin obtained was filtered through filter paper and freeze dried.

NMR spectroscopy

The pyrodextrin and maltodextrin samples (0.2 g) for NMR were prepared by exchanging with D_2O (1 mL) twice to reduce the effect of water peak. The D_2O-exchanged pyrodextrins were dissolved in D_2O at 10% (wt%) concentration and analyzed by NMR as previously described (Bai & Shi, 2011; Bai, Shi, Herrera & Prakash, 2011) .

The NMR spectra were recorded on a Varian 500 NMR System (Palo-Alto, CA) at 25 or 35 °C. The NMR spectrometer was equipped with a cryogenic carbon enhanced 5 mm triple resonance inverse detection pulse field gradient probe operating at 499.839 and 125.697 MHz for ^{1}H and ^{13}C, respectively. The ^{1}H spectra were collected in 32 individual scans with a sweep width of 16 ppm and a delay time of 1 s. The broadband proton decoupled ^{13}C spectrum was the accumulation of 500 scans. ^{1}H-^{1}H 2D homonuclear correlation spectroscopy (COSY) was conducted with 256 transients and 4 scans per transient. Total correlation spectroscopy (TOCSY) was performed with 256 transients and 16 scans per transient. Heteronuclear multiple bond correlation (HMBC) ^{1}H-^{13}C 2D experiment was conducted with 256 transients and 16 scans per transient. Heteronuclear single quantum coherance (HSQC) ^{1}H-^{13}C 2D experiment was conducted with 256 transients and 16 scans per transient in phase-cycling detection mode. The COSY, and HSQC pulse sequences used are part of “Bio-pack” provided by the Varian.

Tetramethylsilane (TMS) was used as an internal reference at 0 ppm. Chemical shifts are reported in parts per million (ppm).

Viscosity

Viscosity of pyrodextrin was determined by a Brookfield viscometer (RVDVII + Pro, Brookfield Engineering Laboratories, Inc., Middleboro, MA) with a CS4-18 spindle and a SC4-13 RPY chamber. Pyrodextrin solutions of 30% solid content were analyzed at spindle speed of 100 RPM at 25 °C.

Results and discussion

New bonds formation during dextrinization

The ^{1}H-NMR spectra of the maltodextrin, degraded from waxy maize starch by α-amylase, and the pyrodextrin, prepared by heating waxy maize starch at 180 °C for 4 h, are shown in Figure 7.1. Anomeric protons were well separated and resolved in the low-field region of the spectra between 4.4 and 5.5 ppm. All the other protons were overlapping in the crowded area between 3.5 and 4.0 ppm. By comparing the ^{1}H-NMR spectrum of the pyrodextrin to that of the maltodextrin, new peaks were observed at 5.44, 5.09, 4.75, 4.51-4.60, 4.4-4.5 and 4.1-4.2 ppm, indicating new bonds or linkages were formed during dextrinization. The peak at 4.75 ppm was overlapped with the water peak at 25 °C (Figure 7.1 B and C) but observed at 35 °C (Figure 7.1 D).

Formation of 1,6-anhydro-β-D-glucopyranose

The resonance at 5.44 ppm was correlated with a doublet at 4.01 ppm with a ^{3}J of 7.7 Hz in the TOCSY spectrum (Figure 7.2), indicating that they were in the same spin system. The proton at 5.44 ppm was correlated to the C1 at 103.72 ppm in the HSQC spectrum (Figure 7.3), while the proton at 4.10 ppm showed a correlation to the carbon at 67.77 ppm which was arisen from C6 (Gidley, 1985). The peak at 5.44 and 4.01 ppm were assigned to the H1 and H6 of the 1,6-anhydro-β-D-glucopyranose, respectively (Figure 7.5-A). Our assignment was consistent with the chemical shifts assigned to the 1,6-anhydro-β-D cellubiose (Koll, Borchers & Metzger, 1990).

The structural information was further investigated by analyzing HMBC spectrum of pyrodextrin, which detects the long range coupling (two-four bonds) relationships and rejects the one-bond relationship. The long range (<1 bond) heteronuclear couplings can be transmitted through oxygen and nitrogen as well as carbon. In the HMBC spectrum of pyrodextrin, no cross peak at 5.44 and 103.72 ppm was observed which was expected. The proton at 5.44 ppm showed three cross peaks at 67.77, 72.26 and 77.65 ppm (Figure 7.4). The strong correlation between H1 (5.44 ppm) and C6 (67.77 ppm) indicated the long range coupling and therefore confirmed the glycosyl bond formation between the hydroxyl groups on the primary carbon and anomeric carbon. Complete assignment of protons and carbons of 1,6-anhydro-β-D-glucopyranose was achieved by analyzing COSY, HSQC, TOCSY and HMBC spectra and is shown in Table 7.1.

Observing the low content of reducing ends in pyrodextrin was interesting because the pyrodextrin was 100% soluble in water (Table 7.2), which resulted from significant starch hydrolysis. The reducing ends in its α- and β-forms resonate at 5.20 and 4.63 ppm, respectively (Bai, Shi, Herrera & Prakash, 2011; McIntyre, Ho & Vogel, 1990) in the ^{1}H-NMR spectrum of maltodextrin (Figure 7.1). However, for pyrodextrin, the resonances at 5.20 and 4.63 ppm were observed only after the spectrum was significantly intensified (Figure 7.1-C). In the region of β-form reducing ends (4.63 ppm), multiple peaks were observed but not well resolved, which made the integration challenging. The content of α-reducing ends for the pyrodextrin was 1.2% compared to 4.2% for the maltodextrin.

The decrease in reducing sugar content was reported in the literature (Wurzburg, 1986). It has been proposed that 1,6-anhydro-β-D-glucopyranose/levoglucosan groups were formed as starch chain terminal (Thompson & Wolfrom, 1958). The structure of 1,6-anhydro-β-D-glucopyranose is shown in Figure 7.5-A. A glycosyl linkage was formed between the primary hydroxyl group on C6 and the anomeric carbon. The current study confirmed that very few reducing ends existed in pyrodextrin and 1,6-anhydro-β-D-glucopyranose was formed. The mechanisms of 1,6-anhydro-β-D-glucopyranose formation was proposed in the literature. One theory is that the primary hydroxyl group attacked the glycosyl linkage of the same D-glucose unit, resulting a rupture of the chain with formation of an anhydro end group (Thompson & Wolfrom, 1958). The other theory postulate that free radicals are formed after hydrolytic scission, the hydroxyl groups are attacked either by oxocarbenium ions or by free radicals (Tomasik, Wiejak & Palasinski, 1989).

Formation of α-(1→2) glycosyl linkage

A peak at 5.09 ppm with ^{3}J of 3.62 Hz was observed in the ^{1}H-NMR spectrum of pyrodextrin (Figure 7.1) and it showed a correlation to an anomeric carbon peak at 100.29 ppm in HSQC spectrum (Figure 7.3). The peak at 5.09 ppm in ^{1}H-NMR spectrum and 100.29 ppm in ^{13}C-NMR spectrum were assigned to anomeric proton (H1) and anomeric carbon (C1) of the α-(1→2) linkage, respectively (Figure 7.5-B). The assignment was made based on the chemical shift and coupling constant that were reported in the literature for anomeric proton and carbon of kojibiose (Roslund, Tähtinen, Niemitz & Sjöholm, 2008). Formation of α-1, 2 linkage was confirmed by HMBC spectrum (Figure 7.4). The peak at 5.09 ppm showed three correlation peaks at 73.44, 75.65 and 78.45 ppm in the HMBC spectrum (Figure 7.4). The peak at 73.44 and 75.65 ppm was assigned to C2 and C3/C5, respectively. In the HSQC spectrum (Figure 7.3), the peak at 78.45 ppm was correlated with a proton resonance at 3.68 ppm, which showed no correlation with the H1 at 5.09 ppm in TOCSY indicating that those two protons were not in the same coupling system. In addition, the peak at 3.68 ppm showed an one-bond coupling with the an anomeric proton at 5.36 ppm in the COSY spectrum (Figure 7.6). Therefore, the proton at 3.68 ppm was assigned to H2' of the α-(1→2) linkage and its connected carbon (C2') resonated at 78.45 ppm (Figure 7.3). Moreover, the long range coupling between H1 (5.09 ppm) and C2' (78.45 ppm) as suggested by the HMBC spectrum (Figure 7.2) was caused by the formation of α-(1→2) glycosyl linkage between two anhydroglucose units (AGUs). Peak assignments of the protons and carbons of α-(1→2) linkage are listed in Table 7.1. The assignments were achieved by analyzing COSY, TOCSY, HMBC and HSQC spectra.

Formation of α-(1→6) glycosyl linkage

The resonance at 4.93 ppm was assigned to the anomeric proton of α-(1→6) linkage as suggested in the previous work (Bai, Shi, Herrera & Prakash, 2011; Gidley, 1985; McIntyre, Ho & Vogel, 1990; Xu & Seib, 1997). However, significant peak broadening at 4.93 ppm was observed for pyrodextrin as compared to the ^{1}H-NMR spectrum of maltodextrin (Figure 7.1). The peak was found to be an overlap of two resonances as shown in TOCSY (Figure 7.2). The tail at 4.95 ppm was probably from the anomeric proton of the AGUs involving in two α-(1→6) linkages (Figure 7.7-B). It was possible that an anomeric carbon was originally involved in α-(1→4) linkage and the primary carbon in the same AGU was involved in α-(1→6) linkage. The

α-(1→4) linkage was hydrolyzed during dextrinization and the hydroxyl group on the anomeric carbon formed an α-(1→6) linkage with another AGU. Complete assignment of the protons and carbons of the AGUs that were involved in the α-1,6 glycosyl linkage was achieved from COSY, TOCSY, HSQC and HMBC, as shown in Table 7.1.

Formation of β-(1→6) and other glycosyl linkage

A new broad peak at 4.48 ppm was observed in the ^{1}H-NMR spectrum of pyrodextrin (Figure 7.1) and showed a strong correlation with a carbon at 71.01 ppm in HMBC spectrum (Figure 7.4). The carbon was attached directly to protons at 4.15 and 3.87 ppm as indicated in the HSQC spectrum (Figure 7.3), but those two protons were not in the same spin system with the proton at 4.48 ppm as suggested by TOCSY spectrum (Figure 7.2). The proton at 4.48 ppm was assigned to the anomeric protons that involved in β-(1→6) linkages and the primary carbon in the linkage (C6') resonated at 71.01 ppm. It has been reported that primary carbon in the β-(1→6) was downfield from that in the α-(1→6) linkage (Roslund, Tähtinen, Niemitz & Sjöholm, 2008). The assignment of the protons and carbons that were involved in β-(1→6) linkages are listed in Table 7.1.

A resonance at 4.75 ppm was observed when the HOD peak in the ^{1}H-NMR spectrum was shifted by increasing the temperature to 35 °C (Figure 7.1). Its directly attached carbon resonated at 77.68 ppm (Figure 7.3) and its long ranged coupled carbon resonated at 103.72 ppm (Figure 7.4) indicating that the proton was not anomeric. The proton was assigned to H-5 of D-glucuronic acid (Figure 7.5 E). Similar assignments were made in the literatures (Grasdalen, 1983; Grasdalen, Larsen, & Smidsrod, 1977, 1979, 1981).

In addition, multiple peaks were observed in the region from 3.74 to 4.66 ppm. The broad peaks in the region were the overlaps of multiple peaks and were not resolved (Figure 7.2). These peaks might be from the linkages of β-1,2, and β-1,4, because the anomeric proton of the β-linkages resonates in the region, as suggested in the literature (Roslund, Tähtinen, Niemitz & Sjöholm, 2008). Tentative assignment of the anomeric protons of the linkages are shown in Table 7.1 based on chemical shifts reported in the literature; however, complete peak assignment was challenging due to the low intensity and poor resolution.

A resonance at 4.75 ppm was observed when the HOD peak in the ^{1}H-NMR spectrum was shifted by increasing the temperature to 35 °C (Figure 7.1). In addition, multiple peaks were

observed in the region from 3.7 to 4.7 ppm. The broad peaks in the region were the overlaps of multiple peaks and were not resolved (Figure 7.2). These peaks might be from the linkages of β-1,2, β-1,3 and β-1,4 because the anomeric proton of the β-linkages resonates in the region as suggested in the literature (Roslund, Tähtinen, Niemitz & Sjöholm, 2008). Tentative assignment of the anomeric protons of the linkages were shown in Table 7.1 based on the literature reported chemical shift. However, complete peak assignment was challenging due to the low intensity and poor resolution.

Degree of branching

Degree of hydrolysis for pyrodextrin was reflected by its solubility and viscosity. Pyrodextrins that were prepared from heating for 4 h were 100% soluble in water and the viscosity was 63.5 cP (Table 7.2). The results suggest that native waxy maize starch was significantly hydrolyzed during dextrinization.

Degree of branching of pyrodextrin was calculated based on the assignment of the ^{1}H-NMR spectrum. However, due to the overlap of peaks that were assigned to different linkage types, quantification was made based on each resolved peak instead of the linkage type. Pyrodextrin prepared by heating at 180 °C for 4 h had 5.8% anomeric protons in the 1,6-anhydro-β-D-*glucopyranose.* Glycosyl linkages including α-(1→6), β-(1→6), α-(1→2), and β-(1→2) were formed. The total degree of branching (DB) was 24.6%. In comparison, the starch converted maltodextrin had DB of only 5.8% which was entirely α-D-(1→6) glycosyl linkage. The results indicated that transglucosidation occurred during the dextrinization and the resulted in a highly branched pyrodextrin.

Conclusions

^{1}H and ^{13}C-NMR spectra of pyrodextrin were assigned with the assistance of 2D-NMR techniques including COSY, TOCSY, HSQC and HMBC. During dextrinization, native waxy maize starch was hydrolyzed and the resulted pyrodextrin was 100% soluble in water. Only 1.2% reducing ends were formed after starch hydrolysis and 1,6-anhydro-β-D-glucopyranose was the major starch chain terminal. Transglucosidation occurred during dextrinization and the resulted pyrodextrin was highly branched. Glycosyl linkages including α-(1→6), β-(1→6), α-(1→2), and β-(1→2) were formed. The total DB was 24.6%. The highly branched structure of pyrodextrin

and transglucosidation would reduce the digestibility of starch which makes pyrodextrin a good source of soluble dietary fiber.

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Tables and figures

Table 7.1 Resonance assignments of 1H-NMR spectrum of pyrodextrin prepared at 180 °C and pH 3.0 for 4 h.

Proton	***H1***	***H2***	***H3***	***H4***	***H5***	***H6***	***H1'***	***H2'***	***H6'***
β-1,6 ahydro	5.44	3.53	3.68	3.81	3.74	4.10	na	na	na
α-1,2	5.09	3.55	4.03	3.75	3.64	3.88	5.36	3.68	na
α-1,6	4.93	3.54	3.71	3.98	3.69	3.81	5.36	nr	3.73
GA[a]	nr	nr	nr	nr	4.75	nr	na	na	na
β-1,2	4.61	3.72	nr	nr	nr	nr	na	na	na
β-1,4	4.58	3.72	nr	nr	nr	nr	na	na	na
β-1,6	4.48	3.31	3.38	3.75	3.45	3.61	5.36	na	4.15/ 3.87
Carbon	***C1***	***C2***	***C3***	***C4***	***C5***	***C6***	***C1'***	***C2'***	***C6'***
β-1,6 ahydro	103.72	72.26	77.65	79.31	77.65	67.77	na	na	na
α-1,2	100.29	73.44	75.65	79.31	75.65	63.04	102.24	78.45	67.77
α-1,6	101.24	72.96	75.96	75.96	75.96	63.04	102.24	na	67.77
GA[a]	103.72	73.41	nr	nr	77.68	nr	na	na	na
β-1,2	98.05	nr	nr	nr	nr	nr	na	na	na
β-1,4	nr	nr	nr	nr	nr	nr	na	na	na
β-1,6	105.15	75.65	75.32	78.64	nr	63.04	102.24	na	71.01

[a] D-glucuronic acid

Note: na: not assigned, nr: not resolved

Table 7.2 Chemical linkages, viscosity and solubility of maltodextrin and pyrodextrin prepared at 180 °C and pH 3.0 for 4 h.

	Chemical shifts (ppm)					*DB*[a]	*Viscosity (cP)*	*Solubility (%)*
	5.435	5.09	4.93	4.75	4.670-4.352			
	1,6-anhydro	α-1,2	α-1,6	β-1,3	β-1,2 β-1,6			
Maltodextrin	0	0	5.8	0	0	5.8	30	100
Pyrodextrin	5.8	3.2	8.5	5.1	12.9	24.6	63.5	104

[a] DB: degree of branching

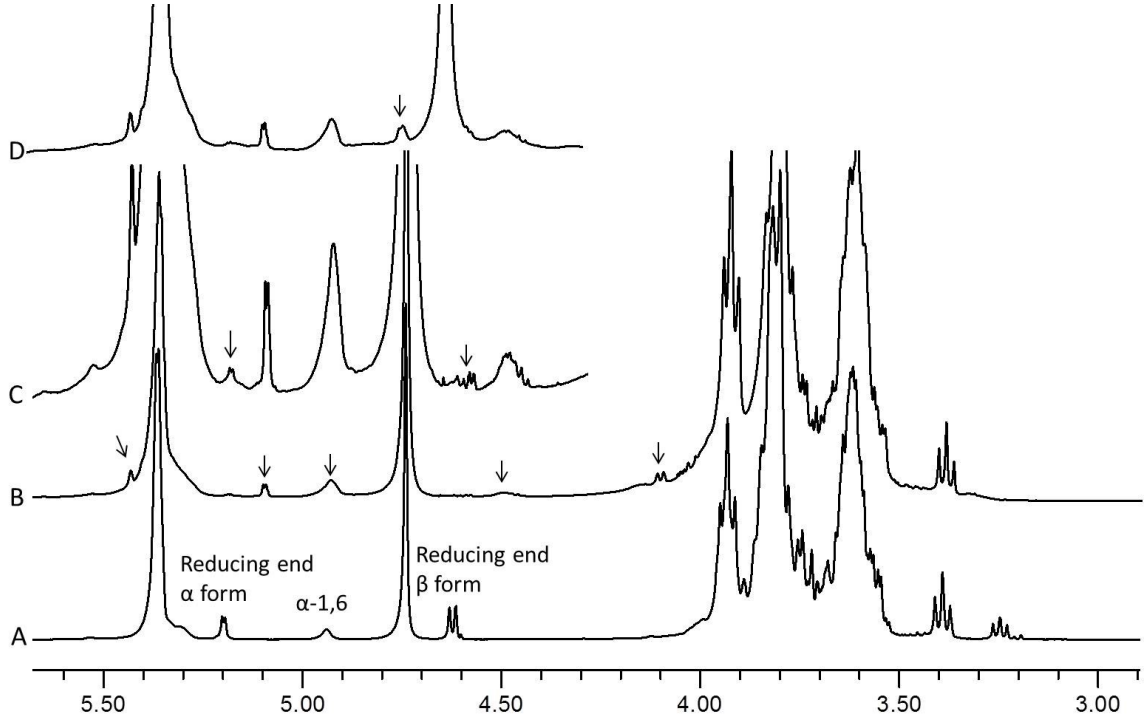

Figure 7.1 ^{1}H-NMR spectrum of maltodextrin (A), pyrodextrin (B) and its expanded region (C) and pyrodextrin recorded at 35 °C (D). ↓ indicates new peaks formed in the pyrodextrin.

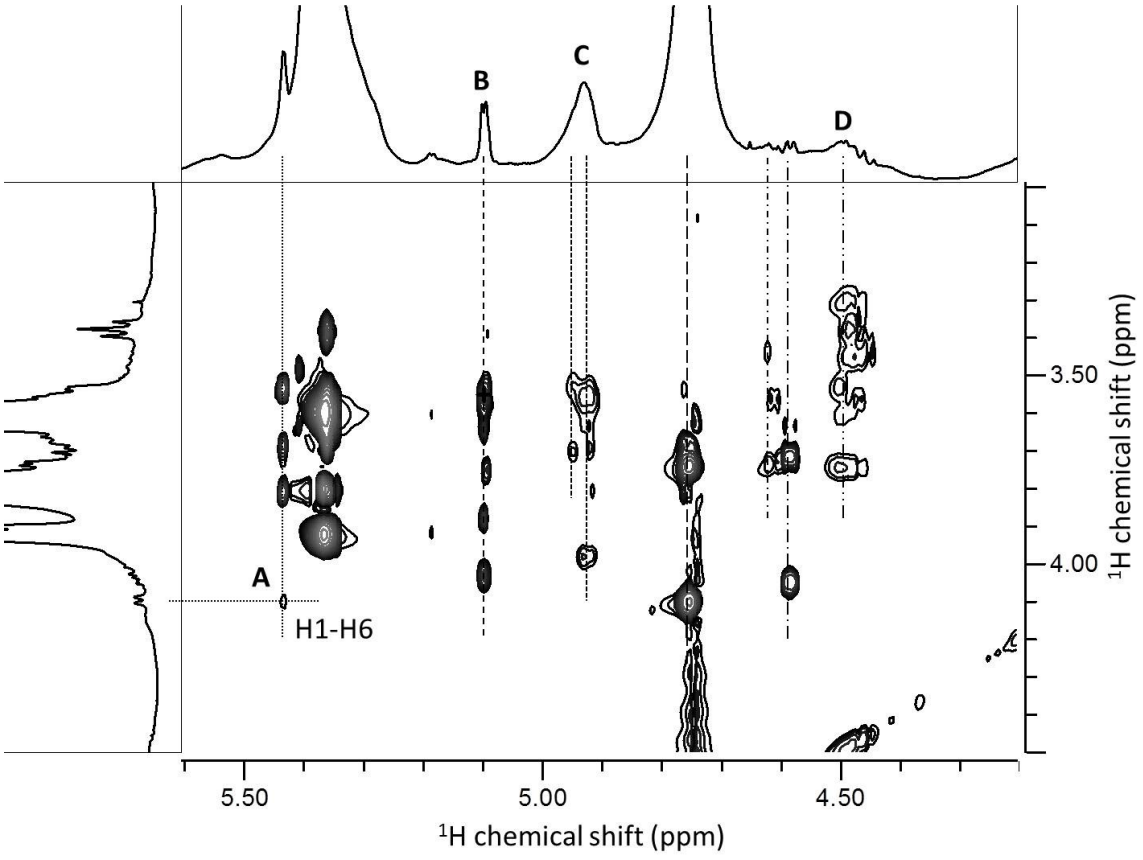

Figure 7.2 Total correlation spectroscopy (TOCSY) spectrum of pyrodextrin prepared at 180 °C and pH 3.0 for 4 h.

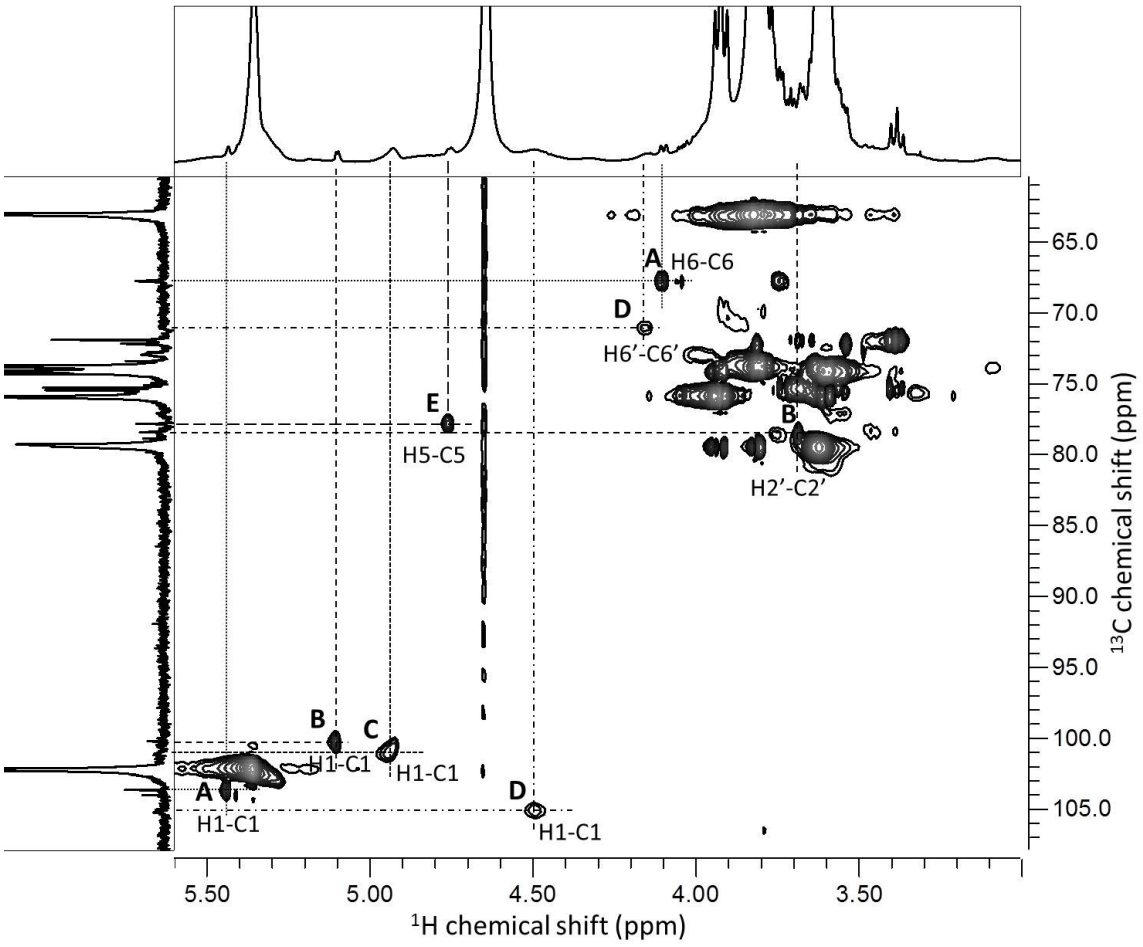

Figure 7.3 Heteronuclear single quantum coherence (HSQC) spectrum of pyrodextrin prepared at 180 °C and pH 3.0 for 4 h.

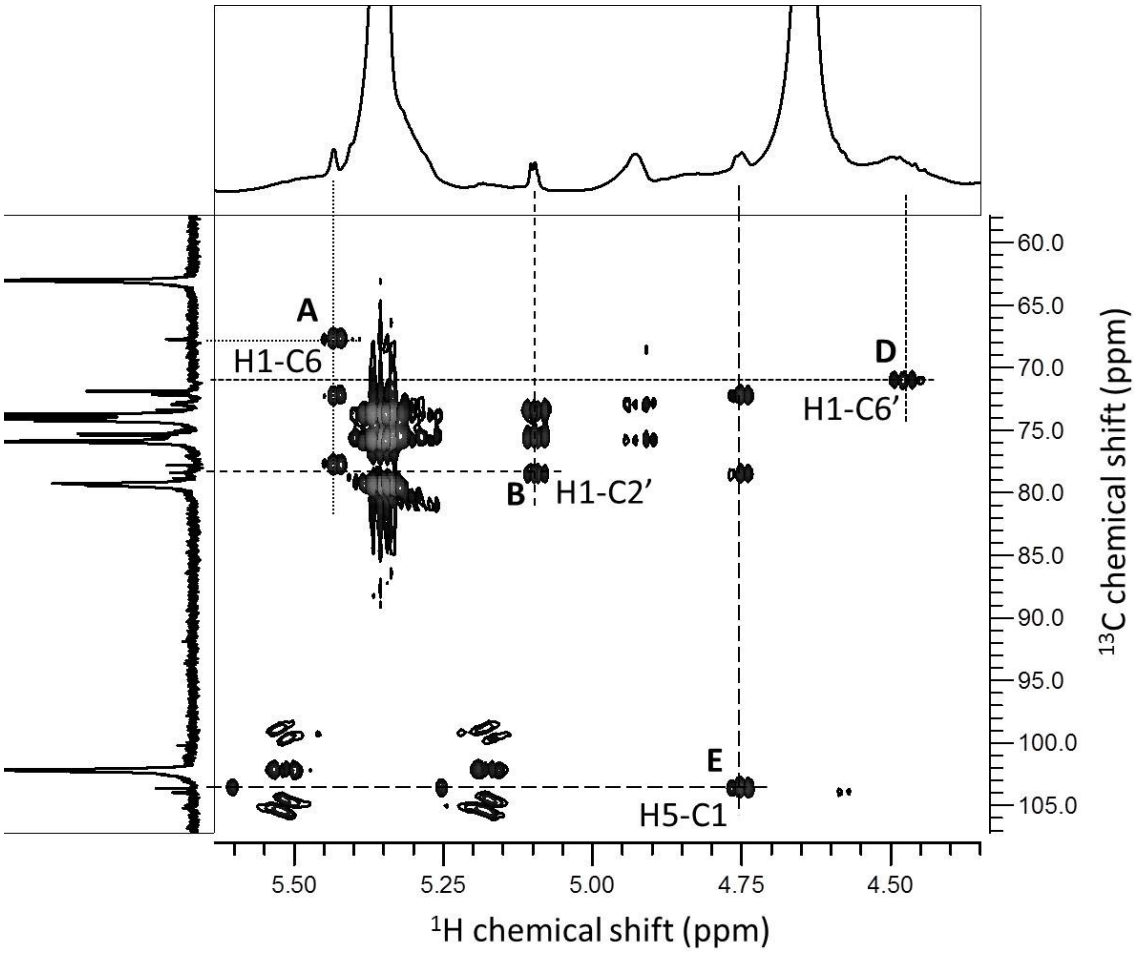

Figure 7.4 Heteronuclear multiple bond correlation (HMBC) spectrum of pyrodextrin prepared at 180 °C and pH 3.0 for 4 h.

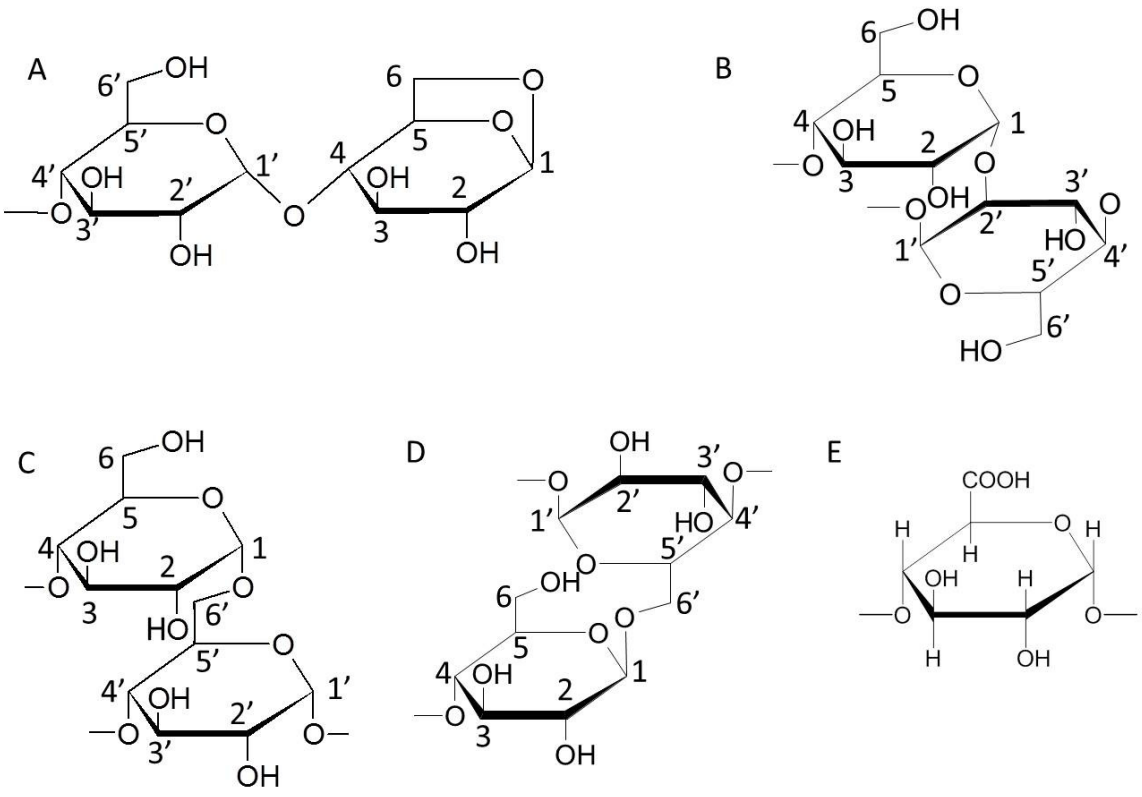

Figure 7.5 New chemical structures formed in the pyrodextrin prepared at 180 °C and pH 3.0 for 4 h.

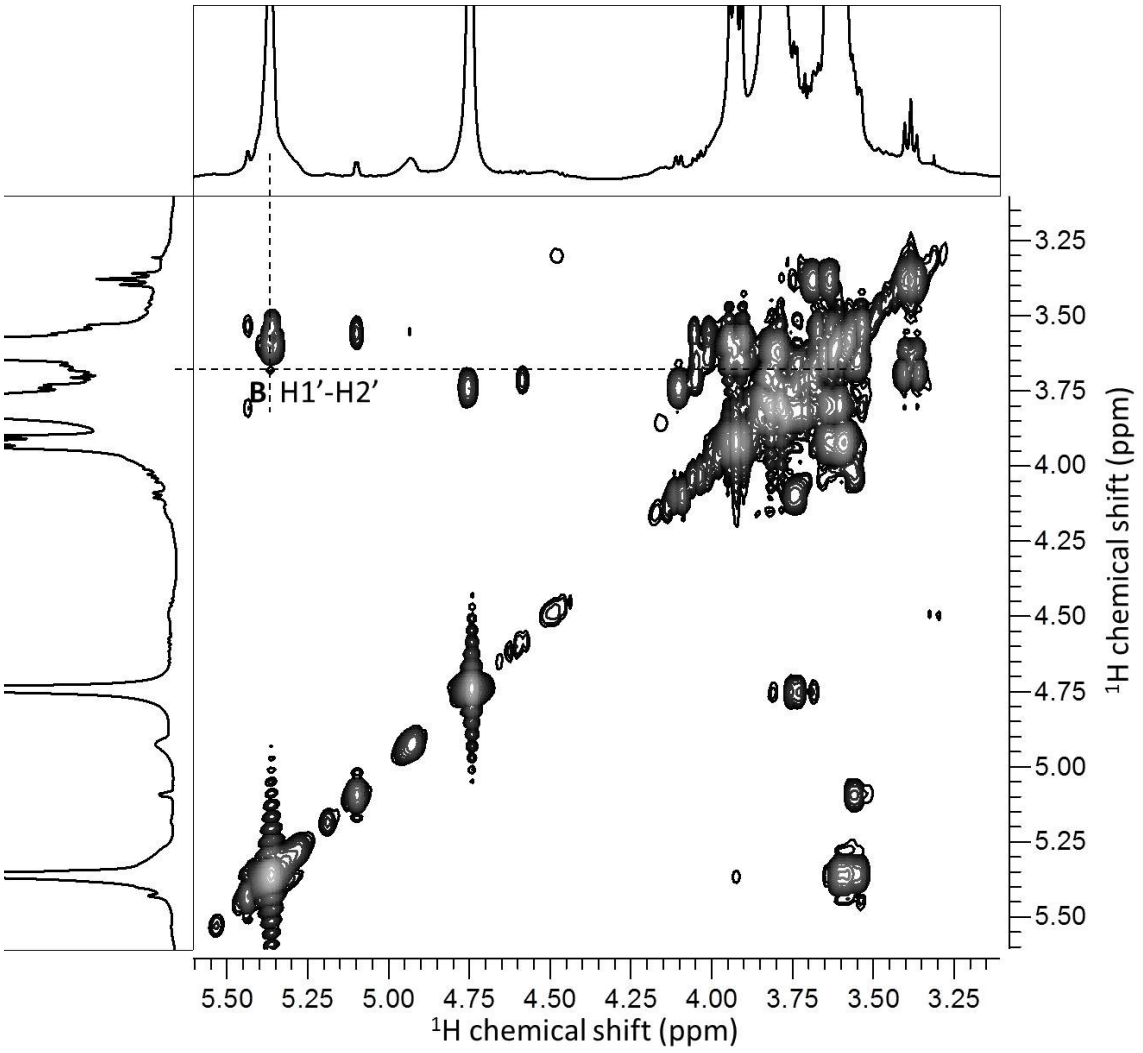

Figure 7.6 Correlation spectroscopy (COSY) spectrum of pyrodextrin prepared at 180 °C and pH 3.0 for 4 h.

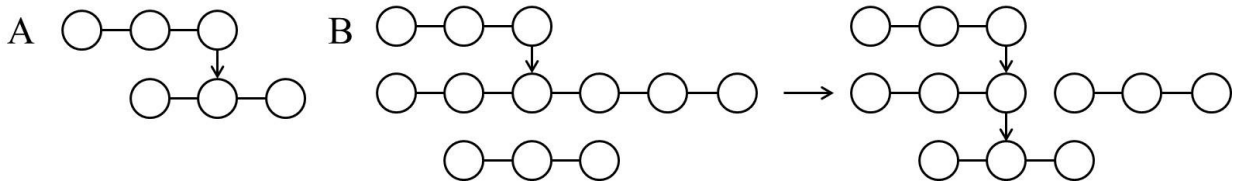

Figure 7.7 Schemes of α-(1→6) glycosyl linkages, ○ = anhydroglucose unit; – = α-1,4 linkage; ↓ = α-1,6 linkage.

Chapter 8 - Preparation and characterization of octenylsuccinate starch obtained from waxy maize starch by dry heating

Abstract

A method of preparing soluble octenylsuccinate (OS) starch with a significantly simplified production process, low cost, high yield, and excellent product emulsification properties was developed in this study. OS starch was prepared by dry heating a mixture of waxy maize starch and octenylsuccinic anhydride (OSA). Reaction conditions including pH, temperature, and time were studied to prepare OS starch with a high degree of substitution (DS), high reaction efficiency (RE), high solubility, and a light color. The optimum reaction conditions were found to be pH 8.5 attained by the addition of 3% NH_4HCO_3 and heating at 180 °C for 2 h. RE of ca. 90% was obtained at OSA levels from 1 to 6%. The OSA reaction did not change the granular appearance of the starch; however, the molecular weight of starch was significantly reduced after reaction. Heat and OSA reaction resulted in significant starch hydrolysis in the amorphous and crystalline regions of starch granules. OS substitutions probably occurred at the amorphous region of starch granules. Transglucosidation occurred during the reaction. Glycosyl linkages including α-(1→2), α-(1→6), β-(1→2), and β-(1→6) linkages were formed, and 1,6-anhydro-β-D-glucopyranose was formed at the starch chain terminals. OS starch had a degree of branching of 19.8%. The highly branched OS starch showed excellent emulsification property for vitamin E and vitamin A.

Keywords

Pyrodextrin, dextrin, octenylsuccinic anhydride

Introduction

Preparation of octenylsuccinate (OS) starch was first disclosed by Cardwell and Wurzburg in 1953. Traditionally, the reaction was carried out in an aqueous slurry system under alkaline conditions (Caldwell & Wurzburg, 1953; Sweedman, Tizzotti, Schäfer & Gilbert, 2013; Trubiano, 1986). Cardwell and Wurzburg (1953) mentioned dry reaction and organic dispersion methods in their patent. In the dry reaction examples, OSA was thinned with toluene, mixed with starch, and agitated for 3 days at 90–100 °F. Degree of substitution (DS) was not reported. Starch was not expected to be soluble in water or to be significantly degraded.

Kim et al. (2010) reported a method of preparing OS waxy rice amylodextrin by dry heating. OSA was hydrolyzed to free acid form in water and mixed with starch. The mixture was adjusted to pH 3.0–5.0, dried, and heated at 130–150 °C for 1–3 h. Molecular weight of starch was reduced, but the product still showed a high peak viscosity and significant breakdown in its pasting curve, indicating the swelling of starch granules during gelatinization. The OS dextrin produced from this approach was suggested as an effective substitute for the fat in dairy cream and muffins because it provided good foaming ability, storage stability, and soft texture (Chung, Lee, Han & Lim, 2010; Kim, Sandhu, Lee, Lim & Lim, 2010).

For OS starch to be used as an emulsifier in beverages, it must undergo enzyme or acid conversion to be completely soluble in water (Wurzburg, 1986). In 2008, we filed a provisional patent application describing an approach to prepare OS starch by dry heating the mixture of OSA and granular starch. Our goal was to prepare soluble OS dextrins that can be used in beverage applications. The method of preparing OS starch by dry heating is described in this study. Optimum reaction conditions were investigated to prepare OS starch with a high degree of substitution (DS), high reaction efficiency (RE), high solubility, and a light color. The structure and properties of the OS dextrins were evaluated. The method significantly simplifies the OS starch production process and has great potential for low-cost, high-yield industrial production of OS starches with excellent emulsification properties.

Materials and methods

Materials

Octenylsuccinic acid anhydride (OSA) was obtained from Gulf Bayport Chemicals L.P. (Pasadena, TX). Waxy maize starch (Amoica) and commercial OS starch were provided by

National Starch LLC (Bridgewater, NJ). α-amylase (BAN 480) was obtained from Novozyme (Bagsvaerd, Denmark) with an activity of 480 KNU-B/g. One KNU is defined as the amount of enzyme that dextrinizes 5.26g of starch (Merck Amylum soluble) per hour under standard conditions (37.0 °C, 0.0003MCa^{2+}, and pH5.6). All other chemicals were analytical grade. Vitamin E acetate (purity >98%) and vitamin A were obtained from Zhejiang NVB Co. Ltd (Xin Chang, Zhejiang, China)

Preparation of OS starch

The process of preparing OS starch is shown in Figure 8.1. Waxy maize starch (100 g) was suspended in distilled water (150 g) with agitation. The pH of the starch slurry was adjusted by adding NH_4HCO_3. The starch suspension was filtered through filter paper (P8, Fisher Scientific, Pittsburgh, PA), and the starch cake was mixed with 1–6% OSA (wt% based on the dry weight of starch) by a mixer (Model K45SSWH, KitchenAid, St. Joseph, MI) at 2nd speed for 15 min. The mixture was dried in an air-forced oven at 40 °C overnight. Starch mixture was ground by an analytical mill (A-10, Tekmar, Staufen, Germany) followed by sieving through a 110-mesh sifter. The powdered starch was thinly spread over an oven pan and heated at 120–190°C for 0.5–4 h. Degree of substitution (DS) was measured by titration, nuclear magnetic resonance spectroscopy (NMR), or high-performance liquid chromatography (HPLC).

Titration

DS of OS starches was determined by a titration method as previously described with some modifications (Bai & Shi, 2011). Starch (5.00 g dry weight) was suspended in methanol (20 mL) to remove the unreacted OSA. After filtration, the starch cake was re-suspended in a mixture of 0.100 M HCl and methanol (1:9, w/w) (20 mL) and stirred for 30 min. The starch was filtered and washed with a mixture of water and methanol (1:9, w/w) (40mL) and dispersed in water (300 mL). For the completely soluble starch, the starch solution was titrated by 0.100 M NaOH directly. For partially soluble starches, the suspensions were cooked in a boiling water bath for 20 min, cooled to room temperature, and titrated by 0.100 M NaOH. Phenolphthalein was used as an indicator. DS, %OS, and reaction efficiency (RE) were calculated as below:

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

Where % OSA is the weight percentage of OSA in OSA modified starch, V_1 is the titration volume of NaOH (mL) for OS starch, V_2 is the titration volume of NaOH (mL) for native starch, W is the dry weight (g) of the OS starch.

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

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

NMR spectroscopy

Starch samples (0.2 g) were exchanged with D_2O (1 mL) twice to reduce the effect of water peak. The D_2O-exchanged starch was dissolved in D_2O at 10% (wt%) concentration. The NMR spectra were recorded on a Varian 500 NMR System (Palo Alto, CA) at 25 °C. The NMR spectrometer is equipped with a cryogenic carbon enhanced 5-mm triple resonance inverse detection pulse field gradient probe operating at 499.839 and 125.697 MHz for ^{1}H and ^{13}C, respectively. The ^{1}H spectra were collected in 32 individual scans with a sweep width of 16 ppm and a delay time of 1 s. The broadband proton-decoupled ^{13}C spectra were the accumulation of 500 scans.

High-performance liquid chromatography (HPLC)

Determination of DS by HPLC was reported elsewhere (Qiu, Bai & Shi, 2012). Vitamin E was measured by an HPLC system (1100 Series, Agilent, Waldbronn, Germany) equipped with a quaternary pump, an automatic injector with a 100μl loop, and a diode array detector (DAD) and a Phenomenex Kinetex C18 column (2.6 μm, 100×4.6 mm; Torrance, CA) at 25 °C. Methanol was used as mobile phase with flow rate of 1.0 mL/min, and the detection wavelength was set at 285 nm. Concentration of substance in HPLC was determined from the peak area.

Solubility

Solubility of OS starch was determined by a handheld refractometer (Fisher Scientific, Pittsburgh, PA). Starch (0.100 g) was dissolved in distilled water (0.9 mL) and centrifuged at 6708 x g for 3 min. Starch concentration in the supernatant was determined by the refractometer.

Small-angle x-ray scattering (SAXS) and wide-angle x-ray diffraction (WAXD)

Starch in a mixture of water/glycerol (20/80, w/w) at a ratio of 1:1 (w/w) was prepared by mixing manually with a spatula followed by equilibrium at room temperature for 15 min. X-ray crystallographs were recorded at 25–75 °C. Native and OS starch were examined by SAXS and WAXD at the X27C beamline at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL). The details of the experimental setup at the X27C beamline have been reported elsewhere (Cai, Shi, Rong & Hsiao, 2010; Chen et al., 2006; Chen et al., 2007; Chu & Hsiao, 2001).

Color measurement

Starch color was measured by Chromameter (CR-210, Konica Minolta Sensing Americas, Inc., Ramsey, NJ). Color was reported in the CIE L*a*b color system. L is the lightness factor. The maximum L value is 100, representing a perfect reflecting diffuser, and the minimum L value is 0, representing black. a and b are the chromaticity coordinates; positive a is red, negative a is green, positive b is yellow, and negative b is blue.

Gel permeation chromatography (GPC)

GPC analysis was performed as previously described (Cai, Shi, Rong & Hsiao, 2010). OS starches (4 mg) were dissolved in DMSO (4 mL) and stirred at room temperature for 12 h. Samples were injected after filtering through a 2-μm filter (Millex-AP, Millipore, Billerica, MA). GPC results were analyzed using Cirrus^{TM} GPC Software Version 3.0 (Agilent Technologies, Santa Clara, CA). Molecular size was relative to the dextran standards.

Scanning electron microscope (SEM)

The starch samples were coated with ≈18nmAu/Pt and examined by a scanning electron microscope (LEO1530VP, Zeiss, German) with the field emission gun operating at 3kV.

Preparation of vitamin E emulsion

OS starch solution in water at 8% solid content was heated in a water bath at 60 °C for 2 h. Sodium benzoate of 0.04 g was added to the starch solution as a preservative. Vitamin E acetate of 8% (based on the weight of aqueous phase) was added while mixing with a portable homogenizer (Bamix, Mettlen, Switzerland) for 3 min. The starch solution was pre-homogenized

by a bench-top homogenizer (PRO 350, PRO Scientific Inc., Oxford, CT) at 6000 RPM for 2 min. The pre-emulsion was homogenized by a microfluidizer (M-110P, Microfluidics, Newton, MA) for 6 passes at 20,000 PSI. Particle size was measured 1 h after preparation of the emulsion by a laser diffraction particle size analyzer (LA-910, HORIBA, Ltd., Tokyo, Japan). Volume-surface mean diameter (d32) and volume-weighted mean diameter (d43) were calculated as suggested in the literature (Charoen et al., 2011; Jafari, He & Bhandari, 2007). Emulsions were stored in the dark at room temperature.

Preparation of vitamin A emulsion

Starch (30 g) was added to water (60 g) and heated in a 60 °C water bath for 30 min, then pH was adjusted to 6.0 by 1% (wt%) NaOH. Vitamin E (0.04 g) and sodium benzoate (0.04 g) were added to the starch solution as preservatives. The starch solution was pre-homogenized by a bench-top homogenizer (PRO 350, PRO Scientific Inc., Oxford, CT) at 8000 RPM for 5 min. Vitamin A (10 g) was added gradually (over a period of 3-5 min) to the starch solution while homogenizing at 8000 RPM. Then the speed was increased to 10,000 RPM for another 5 min. The pre-emulsion was homogenized by a microfluidizer for 7 passes at 20,000 PSI. The emulsion was analyzed by laser diffraction particle size analyzer (LA-910, HORIBA, Ltd., Tokyo, Japan) immediately after preparation. Emulsions were stored in the dark at room temperature.

Determination of vitamin E concentration of the emulsion

An internal emulsion sample (200 μL) was collected immediately or 7 days after preparation of the emulsion. The emulsion was mixed with 0.8 mL sodium acetate buffer (0.1 M, pH 5.2) and hydrolyzed by α-amylase (4μL) at 60 °C for 15 min, then diluted to 10 mL by ethanol. After centrifugation at 6708 x g for 3 min, the supernatant (0.1 mL) was diluted to 10 mL with methanol and analyzed by HPLC. The concentration of vitamin E in the emulsion (C1) was calculated from a vitamin E standard curve. Oil load (%) was calculated from the equation below:

$$\text{Oil load } (\%) = \frac{C1}{C2} \times 100\%$$

Where C2 is the concentration of vitamin E added to the emulsion

Statistical analysis

All tests were performed in triplicate except the color analysis. Color was measured once, and the average instrument error was reported. Means were compared with Student's t test. Least significant differences for comparison of means were computed at $p < 0.05$.

Results and discussion

Preparation of OS starch from dry heating

Effects of reaction pH

Reaction pH changed in three steps during the process (Figure 8.1, Table 8.1). First, pH of the starch slurry was adjusted to 8.0, 8.5, 8.5, and 8.8 (pH I) when NH_4HCO_3 was added at concentrations of 0.3, 3.0, 4.0, and 6.3% (wt% based on the dry weight of starch), respectively. Second, the starch pH decreased to ca.5 (pH II) after pre-drying, which was attributed to hydrolysis of OSA from anhydride form to acid form. Third, after heating at 180 °C, starch pH further decreased to 3 (pH III), which resulted from the decomposition of NH_4HCO_3. OSA reaction and dextrinization took place at pH III. It was observed that a different concentration of NH_4HCO_3 resulted in little difference in pH III, which was probably attributed to weak alkalibility of NH_4HCO_3 as well as thermal decompositon of NH_4HCO_3 at high temperatures.

DS and RE of OS starch were significantly affected by the concentration of NH_4HCO_3, but solubility and color were not (Table 8.1). As the concentration of NH_4HCO_3 increased from 0.3 to 3%, RE increased from 85.93 to 92.00%. Further increasing the concentration of NH_4HCO_3 resulted in decreased DS and RE. OSA appeared to require hydrolyzation to its acid form before the reaction to achieve high DS and RE (Kim et al., 2010). In addition, hydroxyl groups of starch needed to be activated for nucleophilic attack on OSA (Jeon, Lowell & Gross, 1999). NH_4HCO_3 probably played an important role in ionizing starch as well as hydrolyzing OSA, because DS and RE were very low without NH_4HCO_3 (Table 8.1). It was suspected that adding a small amount of NH_4HCO_3 limited starch ionization and OSA hydrolysis, but at a high concentration of NH_4HCO_3, reduced DS and RE was probably due to the reaction between OSA and NH_4HCO_3 during pre-drying. The reaction was optimum when 3% NH_4HCO_3 was added to adjust pH I to 8.5.

NH_4HCO_3 instead of NaOH and Na_2CO_3 was used in this study because it decomposed at high temperature. This property of NH_4HCO_3 has two advantages. On one hand, a lower pH III was achieved with NH_4HCO_3 than NaOH and Na_2CO_3, which was not only favorable for achieving high DS (Kim, Sandhu, Lee, Lim & Lim, 2010) but also for promoting starch conversion (Wurzburg, 1986). On the other hand, little residue was in the OS starch (<1ppm nitrogen content), which was desirable for food applications.

Effects of reaction temperature

The effects of temperature on DS, RE, solubility, and color of OS starch are shown in Table 8.2. DS increased as temperature increased from 120 to 180 °C and decreased as temperature increased to 190 °C. The solubility of OS starch was 0% when OSA and the starch mixture were heated at 120 and 140 °C for 2 h and increased to ca. 92% when heated at 170 °C. The highest solubility was obtained when the reaction was carried out at 180 °C. A further increase in temperature to 190 °C caused a slight decrease in solubility. The color of OS starch became yellowish as temperature reached 170 °C. High temperature resulted in products with higher b value and lower L value, reflecting a yellowish color, and the starch darkened. The yellow color was attributed to dextrinization of starch as well as the yellowish color generated by heating OSA.

Reaction temperature had a significant effect on OSA modification as well as starch dextrinization. Reaction efficiency was promoted at high temperature. However, low RE at 190 °C might be due to the evaporation of OSA, which has a boiling temperature in the range of 179-183 °C. Starch conversion was also promoted at high temperature. In the approached described by Kim et al. (2010), the reaction was carried out at 130–150 °C. The OS starch was not expected to be soluble in water, which was also observed in the current study. The optimum reaction temperature was suggested to be 180 °C.

Effects of reaction time

The effects of reaction time on DS, RE, solubility, and OS starch color are shown in Table 8.3. At 0.5 h reaction time, DS was 0.0185 and RE was ca. 78%. After reacting for 1 h, DS and RE reached 0.02 and 84.1%, respectively. Further increase in reaction time did not increase DS and RE. Solubility of OS starch was 0% at 0.5 h reaction time and increased to 98.5% after 2 h. Molecular size distribution of OS starch prepared at 1, 2, and 4 h is shown in Figure 8.2.

Starch molecules were significantly hydrolyzed during the reaction, and starch hydrolysis increased with reaction time. Even though OS starch prepared from 2-h and 4-h reactions showed no significant difference in solubility, the molecular size of the 4-h OS starch was smaller. Overall, results indicated that the OS substitution occurred in early stages of the reaction (0.5 to 1 h), whereas dextrinization and starch conversion occurred at later stages (1 to 4 h). Because longer reaction time resulted in starch with a darker color, the optimum reaction time was ca. 2 h.

OSA concentration

Properties of OS starch at different substitution levels are shown in Table 8.4. DS of OS starch increased with the OSA concentration. RE remained ca. 90% for all reactions. Solubility of the OS starch at the 1 and 2% OS modification level was 95%. Solubility increased to 97% when OSA concentration increased to 3% but dropped to 85% as OSA concentration increased to 6%. The increase in solubility was probably attributed to the low pH from the OS acid. It is interesting to note that after OSA concentration increased further to 6%, starch solubility decreased significantly. It was suspected that starch molecules were cross-linked at high OS concentration. Color of the OS starch was significantly darker than the other samples.

It is interesting to observe that RE of the OSA reaction by dry heating was significantly higher than the reaction between OSA and granular starch in an aqueous slurry system (Bai & Shi, 2011). In addition, RE remained at 90% when OSA concentration was 6% compared with only 80% for the slurry reaction (Bai & Shi, 2011). The results indicated that OSA reaction by dry heating was more effective than the slurry reaction when starch was in granular form.

Structure characterization of OS starches

OS starch prepared at 3% OSA concentration with pH 8.5 (adjusted by 3% NH_4HCO_3) and heated at 180 °C for 2 h was characterized by SEM, WAXS, DSC, SAXS, and NMR.

OS starch appeared to have the same granular shape and surface morphology as native starch (Figure 8.3), indicating that OSA modification did not change the appearance of starch granules.

OS starch showed an A-type crystalline pattern, but peaks were significantly broadened and peak intensities were reduced (Figure 8.4). As suggested in the previous study, native waxy maize starch had a crystallinity of 43.3% and the crystal size was 9.6 nm. After OSA reaction,

crystallinity and crystal size decreased to 22.1% and 7.1 nm, respectively. OS starch at elevated temperature showed a decreasing trend for peak broadening and peak intensity (Figure 8.4). OS starch became completely amorphous at 75 °C. The results indicated that the OSA reaction significantly disrupted the crystalline region of native waxy maize starch granules. Although starch crystals still existed, crystal size decreased and starch crystals were very weak and started melting at a relatively low temperature. Compared with OSA reaction in an aqueous slurry system, which did not affect the crystalline region of starch granules (Bai & Shi, 2011; Bhosale & Singhal, 2007; Shogren, Viswanathan, Felker & Gross, 2000; Song, He, Ruan & Chen, 2006), the OSA reaction in this study significantly hydrolyzed the crystalline regions of starch granules. The process affected the starch granules in a manner similar to dextrinization.

Reduced crystallinity of OS starch was also reflected by the DSC results (Table 8.5). Compared with the native starch, OS starch had a lower onset temperature and lower enthalpy value. As the substitution level increased, the melting peak range became narrower and enthalpy decreased, suggesting reduced crystallinity. Compared with the yellow dextrin that was prepared without OSA modification, the melting peak was narrower and the enthalpy was significantly lower. The results indicated that the crystalline regions of starch granules were affected more when OSA reaction and thermal degradation occurred simultaneously.

OS starch showed no SAXS lamellar peak when analyzed in glycerol/water (8/2, w/w) at room temperature and elevated temperatures. In contrast, native waxy maize starch showed a well-defined SAXS peak at 0.62 nm^{-1} (Figure 8.5). The SAXS characteristic peak is attributed to the periodic lamellar arrangement of semicrystalline starch granules with a repeat distance of ca. 9 nm. Starch dextrinization with a combination of heat and acid was studied by SAXS. Disappearance of the SAXS peak indicated that the lamellar structure of granular starch was completely destroyed during the process. Therefore, similar to dextrinization of starch, the lamellar structure of starch was destroyed by the OSA reaction at high temperature.

Glycosyl linkage type of OS starch

^{1}H-NMR spectrum of OS starch is shown in Figure 8.6. Resonances were observed in the region of 1.3 to 3.0 ppm, which were attributed to OS substituted groups as previously assigned (Bai, Shi, Herrera & Prakash, 2011). The starch peaks were in the region of 3.3 to 5.3 ppm. The resonances at 5.45, 5.11, 4.94, and 4.51 ppm were assigned to the anomeric protons of 1,6-

anhydro-β-D-glucopyranose, α-(1→2), α-(1→6), and β-(1→6) linkages, respectively, as suggested in the previous work for yellow dextrin. The content of α-(1→2), α-(1→6), and β-(1→6) were 3.35, 5.65, and 7.3%, respectively. ^{13}C-NMR spectrum of OS starch shows the OS peaks in the region of 15 to 40 ppm (Figure 8.7). The resonances at 101.85, 98.40, 99.32, and 103.44 ppm were assigned to the anomeric carbons of 1,6-anhydro-β-D-glucopyranose, α-(1→2), α-(1→6), and β-(1→6) linkages, respectively. These results indicate that during the dry reaction, starch hydrolysis and transglucosidation occurred. OS starch had a highly branched structure with a degree of branching [non-α-(1→4) bonds] of 19.8%.

Application in vitamin E and vitamin A emulsions

Vitamin E emulsion stability for lab-made OS starch and commercial starch is shown in Table 8.6. Fresh emulsion from lab-made OS starch has an average particle size (d32) of 1.0 μm. Commercial starch has a slightly higher average particle of 1.3 μm. Oil load for lab-made OS starch was 93.2%, which is almost 10% higher than that for commercial starch. After storage at room temperature for 7 days, average particle size and oil load decreased for both lab-made OS starch and commercial starch. However, lab-made OS starch showed superior stability. OS starch prepared by dry reaction has great potential for commercial application due to its excellent emulsifying properties.

Particle size distribution of the vitamin A emulsion is shown in Figure 8.8. Particle size of vitamin A emulsion prepared from lab-made OS starch was significantly smaller than that from commercial OS starch. A homogeneous emulsion system was obtained from lab-made OS starch, whereas the oil phase separated quickly when commercial OS starch was used (Figure 8.9). The results indicate that the OS starch had superior emulsification properties for vitamin A.

Conclusions

OS starch with high DS and solubility was prepared from dry heating granular starch with OSA. The best product with high solubility and DS and light color was obtained when 3% (wt% by starch weight) NH_4HCO_3 was added and the starch was heated at 180 °C for 2 h. The OS starch had a DS of 0.022, and the reaction efficiency was 90%. The OS starch was 100% soluble in water and had a light yellow color.

The structure of OS starch was investigated by SEM, WAXD, DSC, SAXS, and NMR. OSA modification by dry heating did not change the appearance of the granular starch, but the

molecular weight of starch was significantly reduced after the reaction. Heat and OSA reaction, which reduced the pH of starch, resulted in significant starch hydrolysis in the amorphous and crystalline regions of starch granules. The crystalline region was affected by OSA modification. The process of dry reaction was similar to dextrinization. A significant number of α-(1→2), α-(1→6), and β-(1→6) linkages were formed, and β-anhydro-D-glucopyranose was formed as starch chain terminals. OS starch obtained from dry reaction had a highly branched structure with degree of branching [non-α-(1→4) bonds] of 19.8%. OS starch prepared from a dry reaction showed excellent emulsification properties for vitamin E and vitamin A emulsions.

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Tables and figures

Table 8.1 Characteristics of octenylsuccinate (OS) starch prepared at different reaction pH. Reactions were carried out at 180 °C; heating time was 2 h.

NH_4HCO_3 (%)	pH[a]			DS[b]	RE[c] (%)	Solubility (%)	Color		
	I	II	III				L	a	b
0	4.7	3.9	2.5	0.0022	9.3	22.0	ND[d]	ND	ND
0.3	8.0	3.5	2.9	0.0202 ± 0.0004 b	85.93 ± 0.09 b	98.9 ± 1.7 a	90.04	-0.93	+12.76
3.0	8.5	5.7	3.0	0.0220 ± 0.0007 c	92.00 ± 2.36 d	98.3 ± 1.9 a	92.50	-1.37	+11.11
4.0	8.5	5.8	2.8	0.0214 ± 0.0001 c	88.60 ± 2.51 c	98.5 ± 3.8 a	91.19	-1.21	+11.41
6.3	8.8	5.1	3.0	0.0190 ± 0.0007 a	79.40 ± 2.51 a	95.0 ± 1.3 a	90.76	-1.24	+11.83

[a] pH I, II, III are described in Figure 8.1.
[b] DS: degree of substitution
[c] RE: reaction efficiency
[d] ND: not determined

Means with the same letter are not significantly different from each other ($P<0.05$)
Average error of color analysis was 0.01, 0.01, and 0.00 for L, a, and b values respectively.

Table 8.2 Characteristics of octenylsuccinate (OS) starch prepared at different reaction temperatures. Reactions were carried out at pH 8.5 (adjusted by 3% NH4HCO3); heating time was 2 h.

Temp (°C)	*DS*[a]	*RE*[b] *(%)*	*Solubility (%)*	*Color*		
				L	a	b
120	0.0156 ± 0.0010 a	66.10 ± 4.10 a	0.0 ± 0.0 a	94.63	-1.49	+2.45
140	0.0168 ± 0.0011 b	71.10 ± 4.71 b	0.0 ± 0.0 a	95.29	-1.43	+3.33
170	0.0220 ± 0.0001 c	88.35 ± 0.58 c	92.2 ± 1.9 b	93.48	-1.50	+7.46
180	0.0220 ± 0.0007 c	92.00 ± 2.36 d	98.2 ± 1.9 c	92.50	-1.37	+11.11
190	0.0206 ± 0.0010 c	86.90 ± 4.10 c	97.5 ± 2.0 c	88.43	-0.14	+16.89

[a] DS: degree of substitution
[b] RE: reaction efficiency
Means with the same letter are not significantly different from each other ($P<0.05$)
Average error of color analysis was 0.01, 0.01, and 0.00 for L, a, and b values respectively.

Table 8.3 Characteristics of octenylsuccinate (OS) starch prepared at different reaction time. Reactions were carried out at pH 8.5 (adjusted by 4 % NH4HCO3) and 180 °C with heating time of 2 h.

Time (h)	*DS*[a]	*RE*[b] *(%)*	*Solubility (%)*	*Color*		
				L	a	b
0.5	0.0185 ± 0.0010 a	78.03 ± 4.10 a	0.0 ± 0.0 a	92.64	-1.17	+7.71
1.0	0.0200 ± 0.0011 ab	84.07 ± 4.71 ab	22.2 ± 2.2 b	92.66	-1.31	+8.68
2.0	0.0214 ± 0.0001 b	88.60 ± 2.51 b	98.5 ± 3.8 c	91.19	-1.21	+11.41
4.0	0.0213 ± 0.0008 b	90.55 ± 2.26 b	101.1 ± 1.3 c	89.22	-0.61	+13.84

[a] DS: degree of substitution

[b] RE: reaction efficiency

Means with the same letter are not significantly different from each other ($P<0.05$)
Average error of color analysis was 0.01, 0.01, and 0.00 for L, a, and b values respectively.

Table 8.4 Characteristics of octenylsuccinate (OS) starch prepared with different levels of octenylsuccinic anhydride (OSA). Reactions were carried out at pH 8.5 (adjusted by 3% NH4HCO3) at 180 °C and a reaction time of 2 h.

OSA (%)	*DS*[a]	*RE*[b] *(%)*	*Solubility (%)*	*Color*		
				L	a	b
1	0.0067 ± 0.0006 a	89.40 ± 7.20 a	95.0 ± 1.7 a	91.92	-1.41	+9.83
2	0.0141 ± 0.0001 b	89.70 ± 6.30 a	94.7 ± 0.8 a	ND[c]	ND	ND
3	0.0220 ± 0.0007 c	92.00 ± 2.36 a	98.2 ± 1.9 b	92.50	-1.37	+11.11
6	0.0386 ± 0.0007 d	93.43 ± 2.36 a	85.0 ± 2.7 c	89.27	-0.72	+14.12

[a] DS: degree of substitution
[b] RE: reaction efficiency
[c] ND: not determined
Means with the same letter are not significantly different from each other (P<0.05)
Average error of color analysis was 0.01, 0.01, and 0.00 for L, a, and b values respectively.

Table 8.5 Differential scanning calorimetry (DSC) of native waxy maize starch, octenyl succinate (OS) starches, and pyrodextrin.

	OSA (%)	*To (°C)*	*Tp (°C)*	*Tc (°C)*	*ΔH (J/g)*
Native starch	0	93.7±0.9	100.7±0.8	109.0±0.5	17.5±0.2
Yellow dextrin	0	47.1 ± 2.2	61.9 ± 0.6	86.3 ± 1.1	10.1 ± 0.1
OS starches	1	55.0 ± 0.2	67.5 ± 1.2	91.6 ± 1.3	3.4 ± 0.2
	2	53.0 ± 1.1	66.1 ± 0.1	89.9 ± 0.9	3.5 ± 0.2
	3	54.1 ± 0.9	65.6 ± 0.2	84.7 ± 0.7	2.3 ± 0.1

Table 8.6 Emulsion characteristics of octenylsuccinate (OS) starch and commercial starch.

Storage time	*Starch*	d_{32}	d_{43}	*Oil load (%)*
Fresh	OS starch	1.0	1.28	96.1 ± 0.0
	Commercial	1.3	1.67	87.5 ± 0.7
7 days	OS starch	0.43	0.45	84.5 ± 0.9
	Commercial	0.47	0.53	71.7 ± 0.6

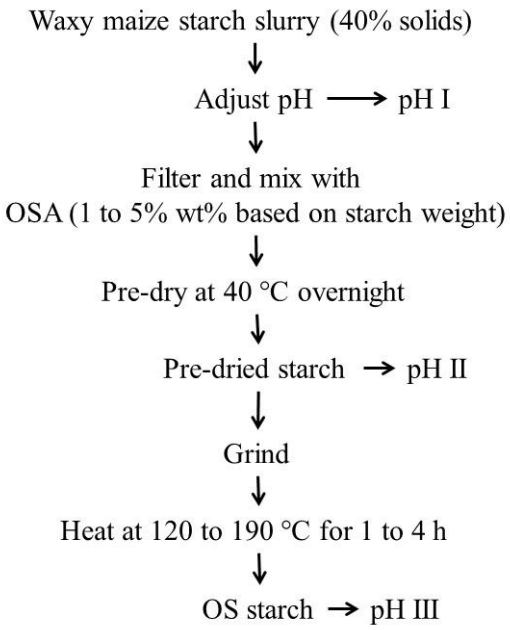


Figure 8.1 Process of preparing octenylsuccinate (OS) starch from dry heating

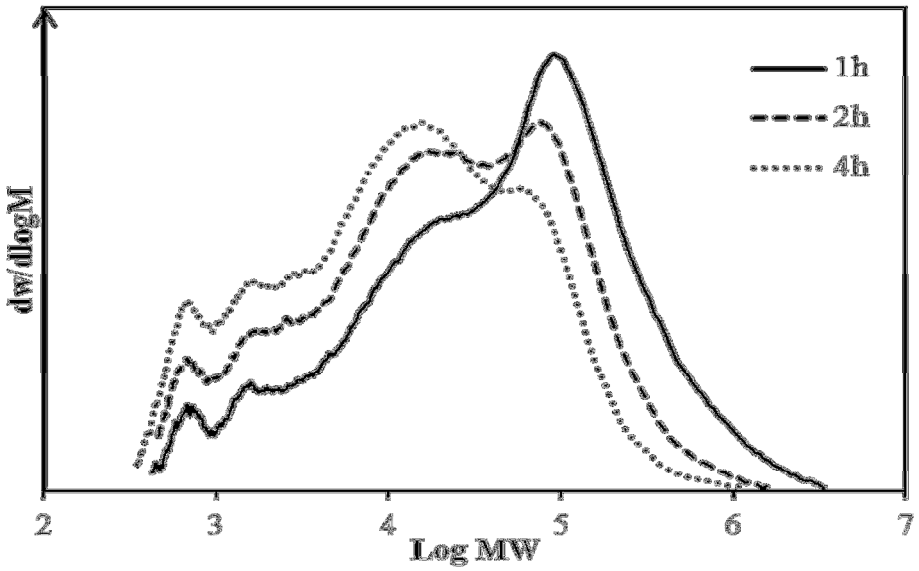


Figure 8.2 Molecular size distribution of octenylsuccinate (OS) starch prepared by heating at 180 °C for 1 to 4 h.

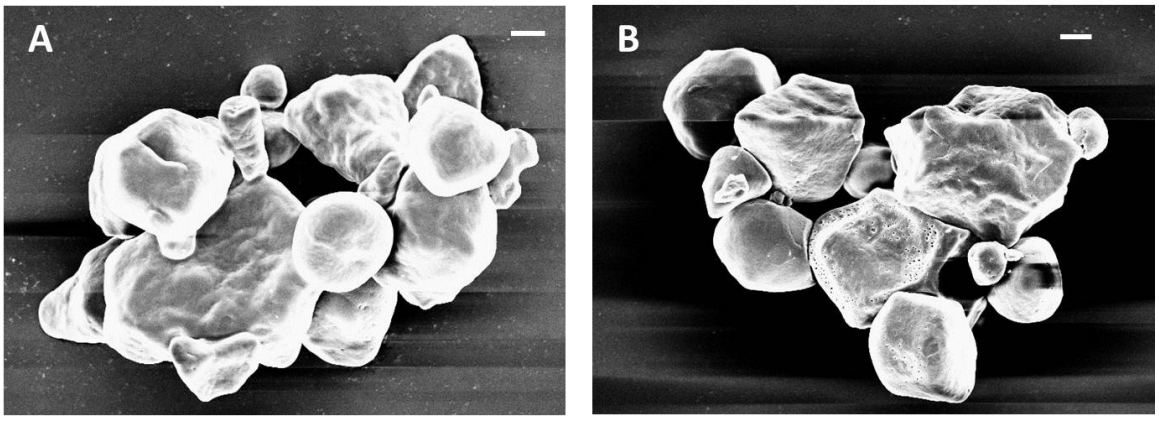
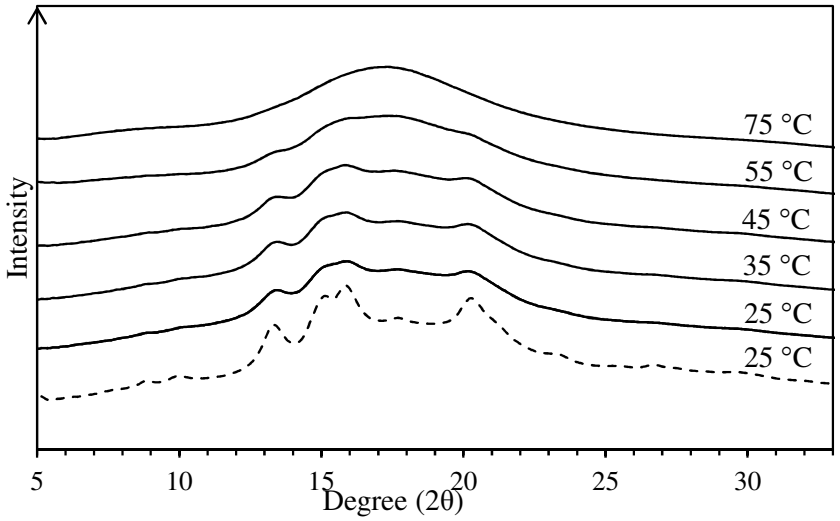


Figure 8.3 Scanning electron microscope (SEM) of native waxy maize starch (A) and octenylsuccinate (OS) starch prepared at 3% OSA, pH 8.5 (adjusted by 3% NH_4HCO_3), 180 °C and reaction time of 2 h. (B). The scale bar in each picture is 3 μm.



Sample	Crystallinity (%)	FWHM	D (nm)
OS starch (25°C)	22.1	0.98	7.1

Figure 8.4 Wide-angle X-ray diffraction patterns of native waxy maize starch (dotted line) and octenylsuccinate starch (solid line) prepared at 3% OSA, pH 8.5 (adjusted by 3% NH_4HCO_3), 180 °C and reaction time of 2 h and native waxy maize starch in glycerol/water (8/2, w/w).

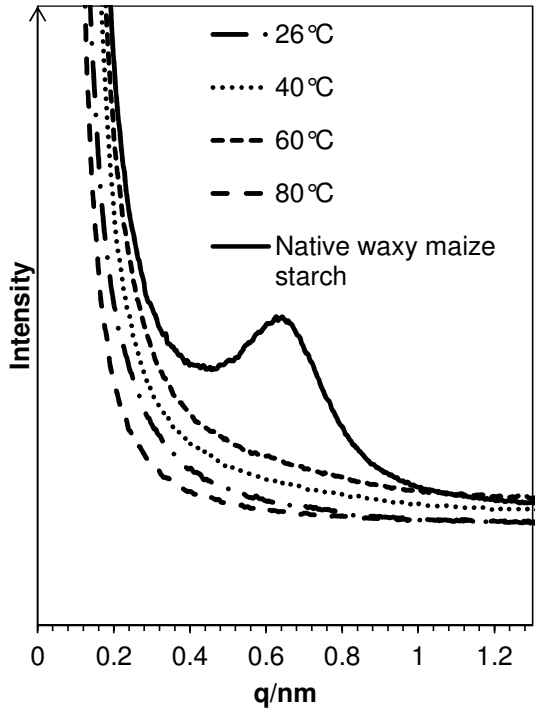


Figure 8.5 Small-angle X-ray scattering (SAXS) patterns of octenylsuccinate starch (dotted line) prepared at 3% OSA, pH 8.5 (adjusted by 3% NH_4HCO_3), 180 °C and reaction time of 2 h and native waxy maize starch (solid line) in glycerol/water (8/2, w/w).

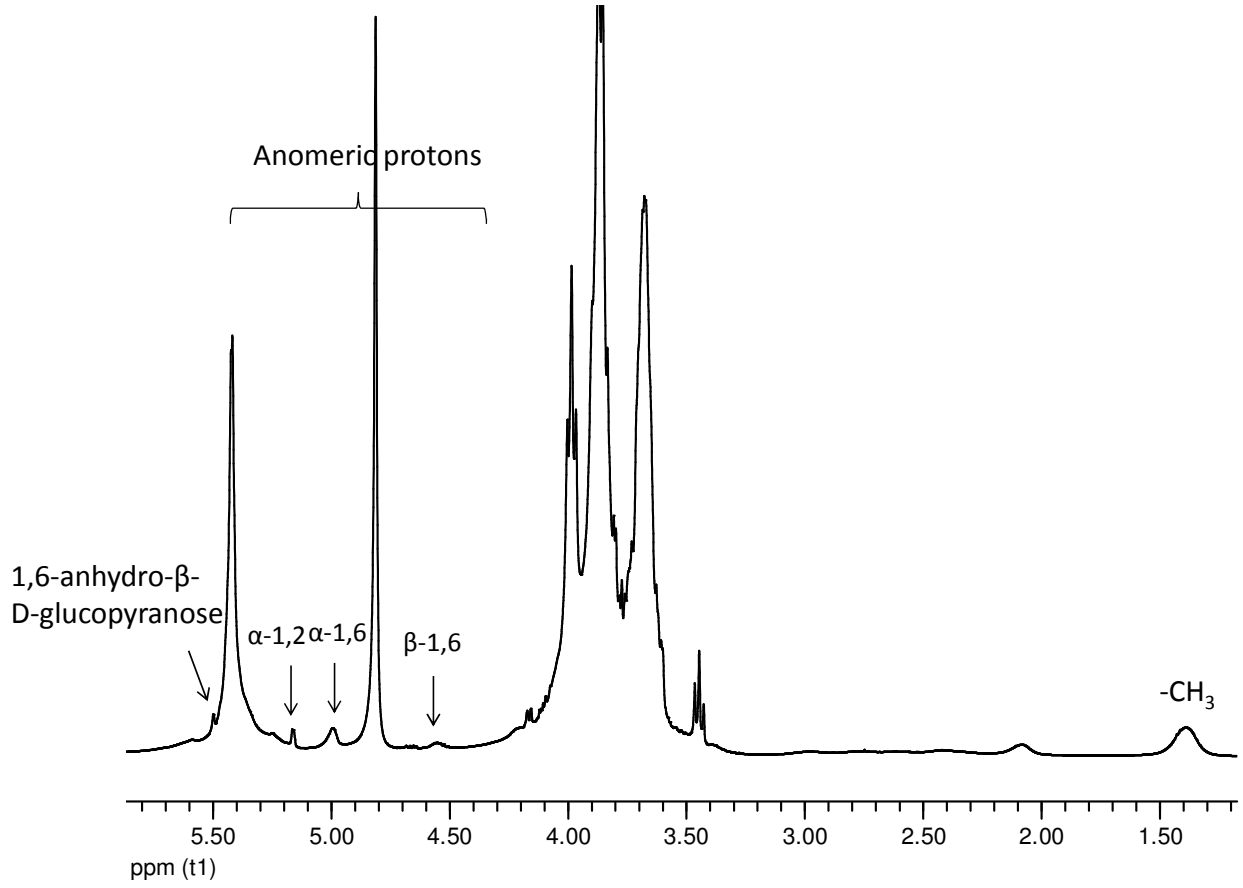


Figure 8.6 ^{1}H-NMR spectrum of octenylsuccinate (OS) starch prepared at 3% OSA, pH 8.5 (adjusted by 3% NH_4HCO_3), 180 °C and reaction time of 2 h

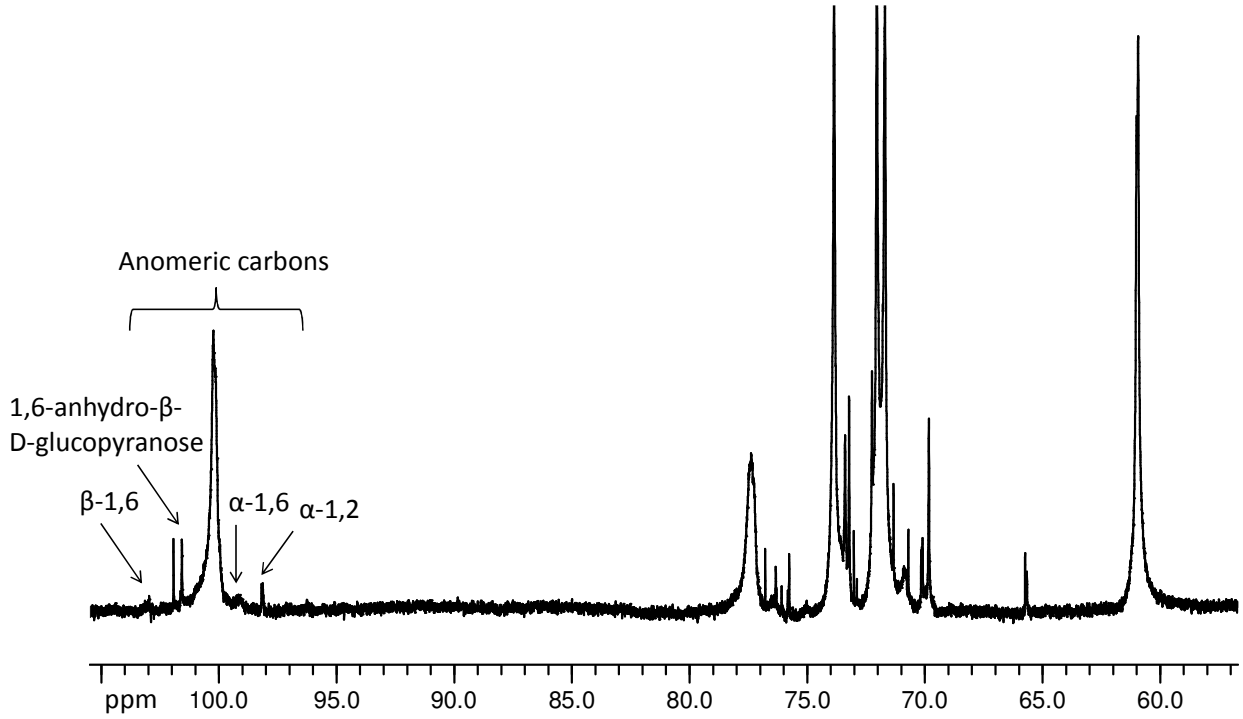


Figure 8.7 ^{13}C-NMR spectrum of octenylsuccinate (OS) starch prepared at 3% OSA, pH 8.5 (adjusted by 3% NH_4HCO_3), 180 °C and reaction time of 2 h.

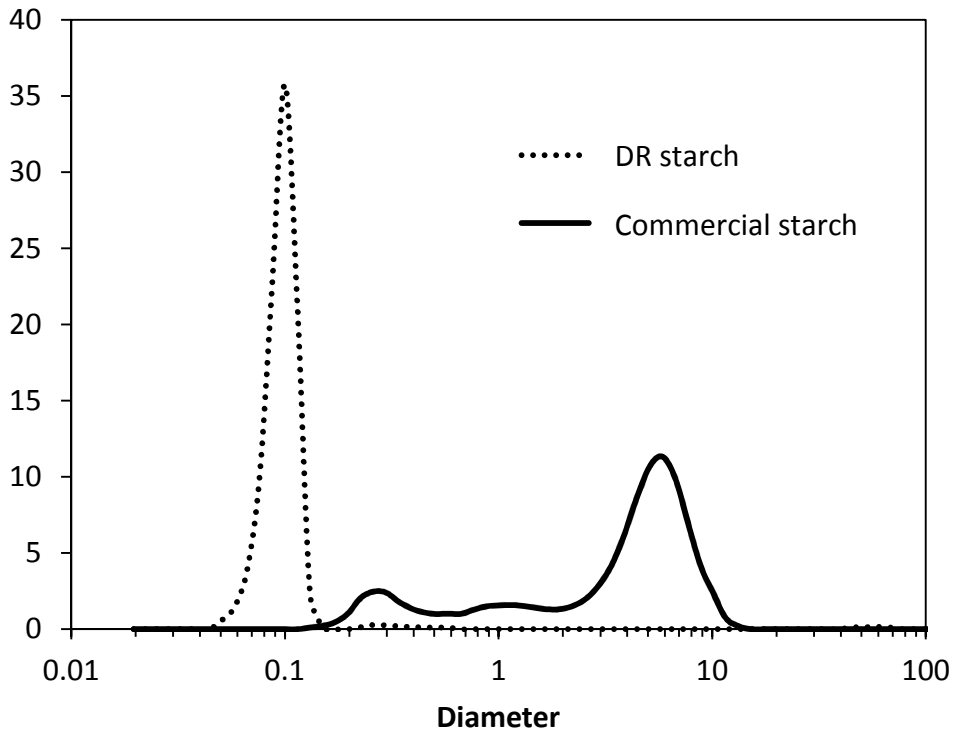


Figure 8.8 Particle size distribution of emulsion prepared by DR starch and commercial starch immediately after preparation.

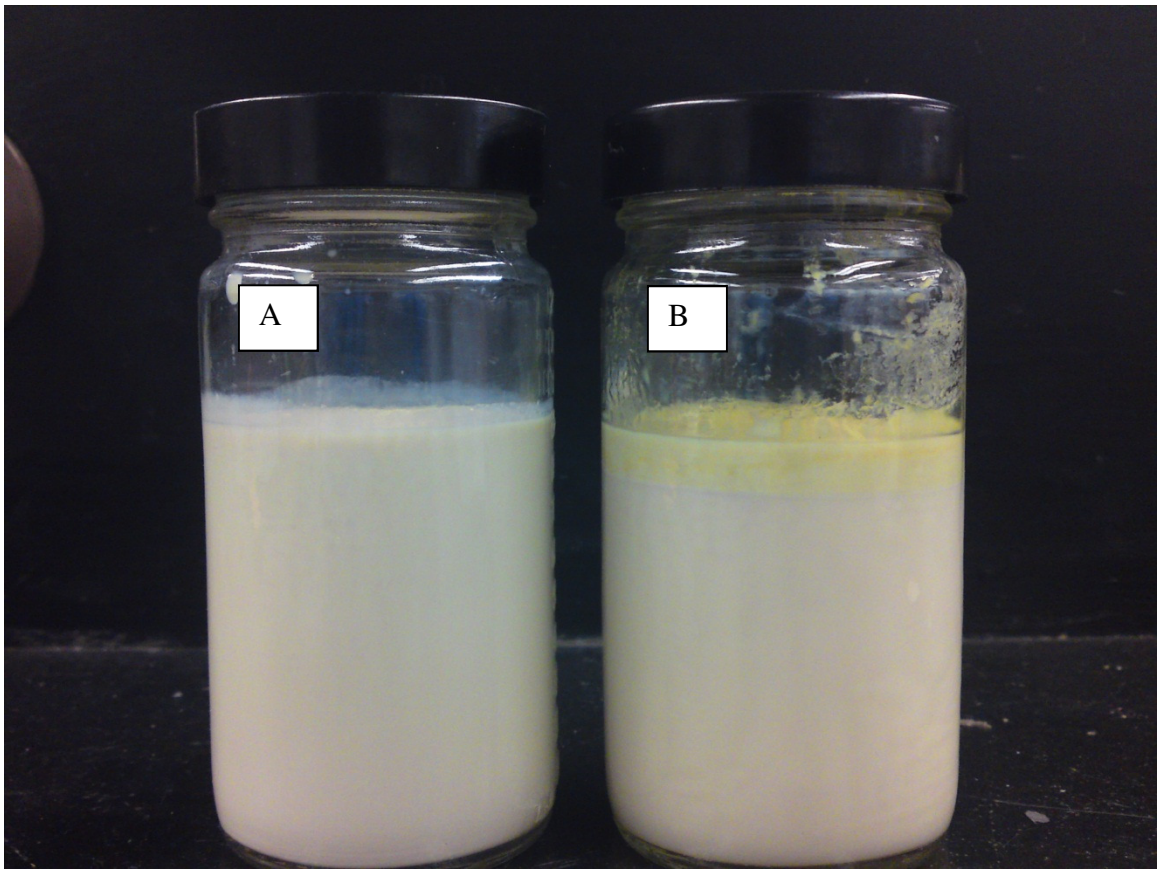


Figure 8.9 Vitamin A emulsion prepared by lab made OS starch prepared at 3% OSA, pH 8.5, 180 °C, and heating time of 3 h (A) and commercial starch (B).

Chapter 9 - Conclusions and future work

Conclusions

This dissertation reported the methods of preparing octenylsuccinate (OS) starch of different structures. Conformation of OSA, OS substitution location on anhydroglucose units, OS substitution distribution along starch chains, and emulsification property of OS starch were described. In a separate topic, a model of structural changes from native waxy maize starch to cold water soluble pyrodextrin was proposed and formation of new linkages was reported.

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. By applying the 1D and 2D NMR techniques, complete assignments of ^{1}H and ^{13}C NMR spectra of the OSA reagent were achieved. 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 anhydroglucose units in the OSA-modified granular starch.

The substitution distribution of OS groups was investigated by enzyme hydrolysis followed by chromatography analysis. When OS starch was prepared in an aqueous slurry system from granular waxy maize starch, OS substitution predominantly occurred at the amorphous region of the starch granules. OS starch with low substitution level (DS 0.018) had OS groups located close to the branching points, whereas the OS substitution in highly substitution OS starch (DS 0.092) occurred near non-reducing ends as well as the branching points. When OS starch was prepared in a starch solution with soluble maltodextrin, OS substitutions were found close to the branching points as well as the non-reducing ends. In comparison to OS maltodextrin obtained from granular reaction, OS substitution was more uniformly distributed along the starch chain, and more OS substitutions were found close to the non-reducing ends.

The reaction of octenylsuccinic anhydride (OSA) with a mixture of granular waxy maize starch and maltodextrin was investigated. OSA was reacted with a 1:1 (w/w) mixture of the granular starch and maltodextrin at OSA levels of 1.5, 3, 9, and 15% (wt% based on starch weight). OSA preferred to react with maltodextrin than semi-crystalline granular starch when

both existed in the system. OSA reacted with maltodextrin at a much faster rate and to a greater extent than with granular starch.

Structural changes of starch granules during dextrinization were investigated by multiple techniques including synchrotron small-angle X-ray scattering (SAXS), wide-angle X-ray diffraction (WAXD), differential scanning calorimetry (DSC) and gel permeation chromatography (GPC). The starch backbone was hydrolyzed by acid at the amorphous region. Unwinding of the double helices occurred as well and the crystallite size was decreased. Starch molecules were hydrolyzed into small molecule fractions but still remained in a radial arrangement.

Structure of pyrodextrin was characterized by NMR spectroscopy for the first time. Pyrodextrin was prepared by heating waxy maize starch at pH 3 and 180 °C for 4 h. ^{1}H and ^{13}C-NMR spectra of pyrodextrin were assigned with the assistance of 2D techniques including COSY, TOCSY, HSQC and HMBC. During dextrinization, native waxy maize starch was hydrolyzed and the resulted pyrodextrin became 100% soluble in water. There were 1.2% reducing ends formed after starch hydrolysis and 1,6-anhydro-β-D-glucopyranose was the major terminal group. Glycosyl linkages including α-(1→6), β-(1→6), α-(1→2), and β-(1→2) were formed. The degree of branching of pyrodextrin was 29.7%. Transglucosidation occurred during dextrinization and the resulted pyrodextrin was highly branched.

OS starch with high DS and solubility was prepared from dry heating granular starch with OSA. pH was preferably adjusted by NH_4HCO_3 than NaOH and Na_2CO_3. Best product with high solubility, DS and light color was obtained when 3% (wt% by starch weight) NH_4HCO_3 was added and heated at 180 °C for 2 h. The OS starch had a DS of 0.022 and the reaction efficiency was 90%. The OS starch was 100% soluble in water and had a light yellow color. Starch was significantly hydrolyzed in the amorphous and crystalline regions of starch granules during heating in the presence of OSA. Significant amount of α-(1→2), α-(1→6), β-(1→6) and β-(1→2) linkages were formed and 1,6-anhydro-β-D-glucopyranose was formed as starch chain terminals. OS starch obtained from dry reaction had a highly branched structure and showed excellent emulsification property for vitamin E and vitamin A.

Future works

Based on the results and conclusions from this dissertation, future research on the following subjects should be considered:

1. To investigate the emulsification properties of OS starches of different structures. Effect of substitution distribution along the starch chain, the molecular size of OS starch, the degree of branching, and DS of OS starch on the emulsion stability has not been investigated. It would be interesting to compare the emulsification properties of OS starches obtained from the two approaches as well as the dry heating method.
2. To explore the functionality of OS starch in emulsion and encapsulation for applications in food and drug delivery.
3. To further understand the structure and properties of OS starch prepared from dry heating method.
4. The effect of dextrinization conditions (ie. temperature, time and pH) on the formation of different linkage types.

Appendix A - Starch esters and method of preparation

Background of the invention

Field of the Invention

The present invention is generally directed towards starch that has been modified with an organic acid anhydride reagent and methods of preparing the same. In certain embodiments, starch produced according to the present invention exhibits good water solubility and emulsifying characteristics when compared with conventional starches. Further, methods of preparing modified starches according to the present invention do not require further treatment with acids, other than the organic acid anhydride reagent, or enzymes to make the starch water soluble.

Description of the Prior Art

Native starch is partially crystalline and not soluble in water at room temperature. Also, native starch molecules are hydrophilic and do not possess emulsifying properties. A number of references such as U.S. Patent No. 2,661,349 and U.S. Patent No. 6,037,466 disclose introducing hydrophobic groups to the starch by reaction with cyclic dicarboxylic acid anhydrides so that the starch can be used as an emulsion stabilizer.

Octenyl succinc acid anhydride (OSA) treated starch, prepared by adding up to 3% OSA, has been approved by the FDA for food use and can be used in food and beverage applications. Starch may also be reacted with greater than 3% OSA for non-food applications, such as in oil- and petroleum-based cosmetics, or pharmaceutical pastes, alcohol-based lotions, body deodorant sprays, and encapsulation of flavors, fragrances, vitamins, clouds, and oils. Conventionally, OSA-modified starch must undergo acid hydrolysis or enzymatic conversion in order to be rendered water soluble at room temperature. Modified starch that undergoes this further processing may contain acid or enzyme residues.

Summary of the invention

In accordance with one embodiment of the present invention, there is provided a method of preparing a lipophilic starch. First, a starch mixture having a pH of between about 7 to about 11 is formed. The starch mixture is then processed to obtain a starch cake. An organic acid anhydride reagent is added to the cake thereby forming a reaction mixture. The reaction mixture is dried to a moisture content of between about 0 to about 15% by weight. The dried reaction mixture is heated at a temperature of at least about 100°C for between about 1 minute to about 6 hours.

Detailed description of the preferred embodiment

The present invention provides a method for introducing a hydrophobic group onto starch molecules, degrading the starch, and making the starch water soluble. The starch to be modified according to the present invention may be native, converted, or derivatized. Exemplary starches include those derived from corn, potato, wheat, rice, tapioca, sago, sorghum, waxy maize, waxy wheat, waxy potato, or high amylase corn.

In certain embodiments, the process begins by preparing a starch mixture that has a neutral to basic pH. In one embodiment, the starch mixture may be in the form of a starch slurry prepared by mixing the starch with water, an alcohol, or other organic solvent, such as toluene. In one particular embodiment, the slurry is prepared with water and/or alcohol, wherein the alcohol is selected from the group consisting of methanol, ethanol, isopropyl alcohol, and mixtures thereof. The pH of the mixture or slurry is adjusted to between about 7 to about 11 by the addition of a base. In certain embodiments, the base is selected from the group consisting of metal and non-metal hydroxides, oxides, carbonates, and mixtures thereof. In further embodiments, the base is selected from the group consisting of sodium hydroxide, ammonium hydroxide, ammonium carbonate, ammonium carbonate, and mixtures thereof. In still other embodiments, the pH of the mixture or slurry is adjusted to between about 8 to about 11, or even to between about 8.5 to about 10.

Next, the starch mixture is processed to obtain a starch cake. In certain embodiments, this processing step involves removing liquid from the slurry, such as by filtration. To the starch cake, an organic acid anhydride reagent is added thereby forming a reaction mixture. In certain embodiments, the organic acid anhydride reagent has the general formula

wherein R is a dimethylene or trimethylene group and R' is a linear, branched or cyclic alkyl, alkenyl, aralkyl or aralkenyl group having 2 to 20 carbon atoms. In further embodiments, the organic acid anhydride reagent is octenyl succininc acid anhydride (OSA). The organic acid anhydride reagent is generally added to the cake at a level of between about 0.5% to about 100% by weight, based upon the dry weight of the starch. In still other embodiments, the organic acid anhydride reagent is generally added to the case at a level of between about 1% to about 25% by weight, or even between about 2% to about 9% by weight, based upon the dry weight of the starch. The organic acid anhydride may be added to the starch cake by any means known to those of skill in the art, such as, for example, by spraying.

After permitting the reaction between the starch and organic acid anhydride reagent to proceed for a predetermined period of time, the reaction mixture is dried to a moisture content of between about 0 to about 15% by weight. In other embodiments, the reaction mixture is dried to a moisture content of between about 0% to about 10%, or even between about 0% to about 6% by weight. In certain embodiments, the drying step comprises heating the reaction mixture to a temperature of less than about 140°C, or between about 25°C to about 90°C, or between about 30°C to about 60°C.

Once the desired moisture content is reached, the dried reaction mixture is heated at a temperature of at least about 100°C, or between about 100°C to about 200°C, or between about 140°C to about 180°C. This heating step may be carried out for between about 1 minute to about 6 hours, or between about 30 minutes to about 4 hours, or between about 1 to 3 hours. Both the drying and heating steps may be carried out with any suitable apparatus known to those of skill in the art, including forced air ovens, dextrinizers, and fluidized bed dryers.

In certain embodiments according to the present invention, the starch modified with the organic acid anhydride reagent does not undergo a subsequent acid hydrolysis step. Thus, the need to hydrolyze the modified starch with a mineral acid such as HCl or H_2SO_4 is eliminated. Likewise, the present invention eliminates the need for enzymatic conversion of the modified starch, such as with ?-amylase or any enzyme within the amylase family, in order to render it water soluble. Therefore, it is an advantage of certain embodiments of the present invention that the starch produced contains essentially no residues of such mineral acids or enzymes.

The modified starch prepared in accordance with the present invention may be water insoluble, partially water soluble, or completely water soluble. Water solubility of the modified

starch is measured by a refractometer. A 10% solids dispersion is prepared, centrifuged, and the supernatant is analyzed by the refractometer. In certain embodiments, the modified starch has a solubility in water at 25°C of greater than about 90%, or greater than about 95%, or even greater than about 98%.

As noted above, it is possible to prepare modified starch according to the present invention wherein the starch is degraded, so as to improve the water solubility thereof, without the addition of mineral acids or starch-degrading enzymes (e.g., amylase enzymes). Therefore, in certain embodiments, the modified starch will have a water solubility as described herein and contain less than about 0.5%, or less than about 0.1%, or even less than 0.01% by weight of mineral acid and/or starch-degrading enzyme residues. As used herein, the term "mineral acid residues" can refer to either the acid or a salt thereof, and the term "starch-degrading enzyme residues" can refer to the enzymes themselves or to denatured forms of the enzymes. In alternate embodiments, the water-soluble, modified starches are substantially free of mineral acid and/or starch-degrading enzyme residues.

Examples

The following examples set forth the effects of pH, temperature, and exposure times on degree of substitution, reaction efficiency, and solubility of the modified starch. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

Materials and Methods

Octenyl succinic acid anhydride ("OSA") was obtained from Gulf Bayport Chemicals L.P. (Pasadena, TX). Waxy maize starch (Amoica TF) was provided by National Starch and Chemical (Bridgewater, NJ). All other chemicals used in the following examples were analytical grade.

Titration Method 1

In the following examples, degree of substitution ("D.S.") was measured by titration. For the insoluble modified starches reacted with 3% OSA, 5.00 g dry weight of the starch was suspended in 20.0 mL of 0.100 M HCl, and stirred for 30 minutes. The suspension was filtered through a piece of No.2 filter paper (Whatman Internal Ltd.), and the residue was washed with

water until no Cl^- could be detected by 0.1 M $AgNO_3$ solution. The starch was then re-suspended in 300 mL water and heated in a boiling water bath for 20 minutes. After cooling down, the starch solution was titrated with 0.100 M NaOH solution, using phenolphthalein as an indicator. The control used is described in each example. The % bound octenyl succinate (OS), D.S., and reaction efficiency ("R.E.") were calculated using the following equations:

$$\% \text{ OS} = \frac{(V_1 - V_2) \times 0.1 \times 21}{W}$$

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

$$\text{D.S.} = \frac{162 \times \% \text{ OS}}{210 - 209 \times \% \text{ OS}}$$

$$\text{R.E.} = \frac{\%\text{OS of OS starch}}{\%\text{OSA added to the starch}} \times 100\%$$

Titration Method 2

The bound OS content for modified starches reacted with greater than 3% OSA, partially and completely soluble OS starches was determined, by first suspending 5.00g dry weight of the starch in 20.0 mL of methanol and filtered. The cake was re-suspended in a 20 mL mixture of 0.100 M HCl and methanol (1:9, w/w) and stirred for 30 minutes. The starch was filtered and washed with a 40mL mixture of water and methanol (1:9, w/w), and then dispersed in 300 mL water. For the completely soluble samples, the solutions were titrated by 0.100 M NaOH directly. For partially soluble starches, the suspensions were cooked in a boiling water bath for 20 minutes and the solutions were titrated after cooling down. Phenolphthalein was used as an indicator. The D.S., %OS and R.E. were calculated by the same equations above in method 1.

Solubility of OS starch

A potable refractometer was used to check the solubility of the OS starch. A 10% solids suspension was prepared, centrifuged, and the supernatant was analyzed by the refractometer.

Example 1

In this example, NaOH was used to adjust the initial pH of the starch slurry and the resulting effects on the degree of substitution ("D.S.") and reaction efficiency ("R.E.") of the starch were measured.

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. The pH of the starch slurry was adjusted to 7.5 and 9.5 with 3% (w/w) NaOH. The suspension was filtered and the starch cake was mixed with 3% OSA (based on the dry weight of starch) using a mixer (Model K45SSWH, KitchenAid, St. Joseph, MI) at 2nd speed for 15 minutes. The mixture was dried in a forced-air oven at 35°C overnight until the moisture content was below 12%. The starch mixture was spread over an oven pan (38cm ×26 cm) and heated at 160°C for 1 hour, 2 hours, or 4 hours. Native waxy maize starch was used as a control. Degree of substitution and reaction efficiency were measured by titration method 1. pH after treatment was measured by suspending a portion of the treated starch in water (10% solids by weight).

As shown in Table A.1, D.S. and R.E. were greater at a the higher pH and longer heat treatment times.

Table A.1 Sample adjusted to pH 7.5 and 9.5 by NaOH and heat treated at 160°C

No.	Adjusted	Temperature (°C)	Reaction	%OSA	D.S.	R.E.%	pH after
1-A	7.5	160	0h	N/A	N/A	N/A	2.68
			1h	0.61	0.0047	20.35	2.92
			2h	0.79	0.0062	26.43	2.74
			4h	0.93	0.0073	31.13	2.98
1-B	9.5	160	0h	N/A	N/A	N/A	3.45
			1h	1.17	0.0091	39.01	3.56
			2h	1.42	0.0111	47.44	3.91
			4h	1.67	0.0131	55.67	4.07

Example 2

In this example, Na_2CO_3 was used to adjust the initial pH of the starch slurry and the resulting effects on Degree of Substitution ("D.S."), reaction efficiency ("R.E."), and starch solubility were measured.

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. A weighed amount of Na_2CO_3 was added to the starch slurry. The experiments were carried out as described in Example 1, except that sample 2-A was heated at 160 °C for 4 hours, then was reheated at 190°C for 2 hours. In contrast, sample 2-B was heated at 190°C for 2 hours. D.S. and R.E. of the samples heat treated at 190°C were calculated using titration method 2. D.S. and R.E of other samples were determined by titration method 1.

As shown in Table A.2, the more basic Na_2CO_3-treated starch slurries generally produced higher D.S. and R.E. values. The highest D.S. was achieved at pH 10.3. In addition, solubility of the starch was found to be higher when the starch slurry combined with Na_2CO_3 was exposed to higher temperatures, such as in Samples 2-A and 2-B.

Table A.2 Samples adjusted to different pH values by Na2CO3 and heat treated at 160°C

No.	Adjusted pH	% Na_2CO_3 (w/w)	Reaction time	%OSA	D.S.	R.E.%	pH	%Solubility
2-A	4.71	0	0 h	N/A	N/A	N/A	3.89	N/A
			1 h	0.18	0.0014	5.94	2.73	N/A
			2 h	0.34	0.0027	11.46	2.84	7.5
			4 h	0.28	0.0022	9.34	2.47	22.0
			160°C 4 h +	1.23	0.0096	40.89	2.85	95.0
2-B	9.45	0.56	Before air dry	N/A	N/A	N/A	4.14	N/A
			0 h	N/A	N/A	N/A	3.17	N/A
			190°C 2 h*	1.86	0.0146	62.10	3.80	100.0
2-C	9.72	1	Before air dry	N/A	N/A	N/A	5.20	N/A
			1 h	1.39	0.0109	46.37	5.12	N/A
			2 h	1.58	0.0124	52.55	5.39	N/A
			4 h	1.62	0.0127	53.91	5.64	0.0
2-D	10.3	1.5	0 h	N/A	N/A	N/A	4.99	N/A
			1 h	1.76	0.0138	58.54	5.27	N/A
			2 h	1.90	0.0133	56.60	3.10	1.0
			4 h	1.88	0.0147	62.51	3.03	1.0
2-E	10.32	2.58	0 h	N/A	N/A	N/A	N/A	N/A
			1 h	1.40	0.011	46.68	9.34	N/A
			2 h	1.82	0.0143	60.65	8.09	5.0
			4 h	1.54	0.0121	51.30	6.67	5.0
2-F	10.51	4.57	Before air dry	N/A	N/A	N/A	9.52	N/A
			0 h	N/A	N/A	N/A	9.97	N/A
			1 h	1.23	0.0096	41.13	10.73	N/A
			4 h	1.74	0.0137	58.13	8.84	10.0

Example 3

In this example, NH_4HCO_3 was used to adjust the initial pH and the resulting effects on Degree of Substitution ("D.S."), reaction efficiency ("R.E."), and starch solubility were measured.

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. A weighed amount of NH_4HCO_3 was added to the suspension. The experiment was carried out as described in Example 1. Sample 3-A was heated at 160°C for 4 hours was reheated at 190°C for 2 hours. D.S. and R.E. were measured by titration method 2.

As shown in Table A.3, the use of NH_4HCO_3 was effective in achieving relatively high D.S. and R.E. values especially when the starch was heated for longer periods of time and/or at higher temperatures. In addition, it was shown that use of NH_4HCO_3 in conjunction with a higher heat treatment temperature (190°C) greatly increased the solubility of the sample.

Table A.3 Samples adjusted to pH 8.57 by NH4HCO3, and heat treated at 160 °C

No.	Adjusted pH	%	Reaction	%OSA	D.S.	R.E.%	pH	%Solubility
3-A	8.57	3.02	0 h	N/A	N/A	N/A	2.60	N/A
			1 h	1.38	0.0108	45.90	2.34	N/A
			2 h	1.56	0.0122	51.86	2.34	5.0
			4 h	1.27	0.0100	42.47	2.83	25.0
			+190°C 2 h	2.00	0.0158	66.75	2.57	92.0

Example 4-6

In these examples the weight of NH_4HCO_3 added to the starch slurry was varied and the effects on degree of substitution ("D.S."), reaction efficiency ("R.E."), and starch solubility were measured.

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. The pH of the starch slurry was adjusted to between 8.0 – 8.8 by the addition of varying amounts of NH_4HCO_3. The experiments were carried out as described in Example 1; however, the heating temperature was adjusted to 170°C, 180°C, or 190°C, and heating time was adjusted to ? hour, 1 hour, 2 hours, or 4 hours. A starch sample without adding OSA was prepared as a control. D.S. and R.E. were measured by titration method 2.

As shown in Tables A.4-6, increased amounts of NH_4HCO_3 and longer exposure to higher temperatures led to an increase in D.S., R.E., and solubility of the starch.

Table A.4 Samples adjusted to pH 8.0 by NH_4HCO_3 (0.27% by starch dry weight) and heat treated at different temperatures.

No.	Temp	Time	%OSA	D.S.	R.E.%	pH	Solubility
4-A	170°C	cake	N/A	N/A	N/A	4.51	N/A
		0h	0.71	0.0055	23.71	2.81	0.0
		1/2h	1.18	0.0092	39.48	2.58	0.0
		1h	1.50	0.0117	49.91	2.49	6.0
		2h	2.22	0.0175	73.90	2.72	72.0
		4h	2.20	0.0174	73.36	2.97	95.0
4-B	180°C	cake	N/A	N/A	N/A	5.54	N/A
		0h	N/A	N/A	N/A	3.42	N/A
		1/2h	0.66	0.0051	21.99	3.40	0.0
		1h	1.61	0.0126	53.60	3.09	2.0
		2h	2.50	0.0198	83.22	2.89	20.0
		4h	2.80	0.0222	93.45	2.94	92.5
4-C	190°C	1/2h	1.73	0.0136	57.70	2.77	5.0
		1h	2.59	0.0205	86.26	2.77	30.0
		2h	2.68	0.0212	89.36	2.82	100.0
		4h	2.70	0.0214	89.98	2.93	98.0

Table A.5 Samples adjusted to pH 8.5 by NH_4HCO_3 (3.85% by starch dry weight) and heat treated at different temperatures

No.	Temp	Time	%OSA	D.S.	R.E.%	pH	Solubility
5-A	170°C	cake	N/A	N/A	N/A	6.48	N/A
		0h	N/A	N/A	N/A	5.19	5.0
		1/2h	2.36	0.0186	78.56	2.82	5.0
		1h	2.29	0.0181	76.30	2.79	5.0
		2h	2.72	0.0216	90.61	2.81	85.0
		4h	2.61	0.0207	87.14	2.83	100.0
5-B	180°C	1/2h	2.33	0.0184	77.82	2.80	5.0
		1h	2.68	0.0212	89.36	2.76	80.0
		2h	2.57	0.0204	85.71	2.81	102.5
		4h	2.58	0.0204	86.04	2.85	85.0
5-C	190°C	1/2h	2.10	0.0165	69.96	3.24	6.0
		1h	2.72	0.0216	90.82	2.87	86.0
		2h	2.61	0.0206	86.90	2.91	100.0
		4h	2.38	0.0188	79.41	2.91	96.0

Table A.6 Samples adjusted to pH 8.75 by NH_4HCO_3 (6.29% by starch dry weight) and heat treated at different temperatures.

No.	Temp	Time	%OSA	D.S.	R.E.%	pH	Solubility
6-A	170°C	cake	N/A	N/A	N/A	N/A	N/A
		0h	0.61	0.0047	20.18	4.30	N/A
		1/2h	1.74	0.0137	57.99	3.61	2.5
		1h	2.10	0.0165	69.94	3.09	5.0
		2h	2.12	0.0167	70.70	3.00	7.5
		4h	2.79	0.0221	92.87	3.02	81.0
6-B	180°C	1/2h	2.22	0.0176	74.18	2.86	5.0
		1h	N/A	N/A	N/A	2.98	90.0
		2h	N/A	N/A	N/A	3.02	95.0
		4h	N/A	N/A	N/A	3.03	97.5
6-C	190°C	1/2h	N/A	N/A	N/A	2.92	0.00
		1h	N/A	N/A	N/A	2.80	95.0
		2h	N/A	N/A	N/A	2.81	101.0
		4h	N/A	N/A	N/A	2.78	100.0

Example 7

In this example, the viscosities exhibited by different starch samples from Examples 4, 5, and 6 were measured.

Viscosity of the starch samples was determined by a Brookfield viscometer (RVDVII + Pro, Brookfield Engineering Laboratories, Inc., Middleboro, MA) with a CS4-18 spindle and a SC4-13 RPY chamber at 25 °C. Starch solutions of 50% solids of lab made starches and a commercial starch were prepared and added to the chamber. The commercial starch is a converted (degraded), OSA-modified starch obtained from National Starch LLC, Bridgewater, NJ. The spindle speed (RPM) was selected. The reading of shear stress (SS), shear rate (SR), viscosity (cP) and % (torque) are shown in Table A.7.

Viscosity of a starch solution reflects the molecular weight of a starch sample. Compared with a commercial sample, the lab made starch sample showed higher viscosity indicating that the molecular size of the lab made sample was higher than that of the commercial sample.

Table A.7 Viscosity of OS starch and a commercial starch solution

Sample	Starch	SS	SR	CP	%	RPM
4-A, 4h	50	262.3	9.30	2825	56.5	10
5-A, 2h	50	146.9	2.33	6340	31.8	5
6-A, 4h	50	124.2	9.30	1340	26.7	10
Commercial	50	135.3	18.60	727.5	29.1	20
Commercial	30	43.2	93.0	46.5	9.3	100
5-B, 2h	30	96.7	93.0	104.0	20.8	100

Example 8

In this example, the effect of grinding the starch prior to heat treatment on solubility was measured.

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. The pH of the starch slurry was adjusted to 8.45 by different weights of NH_4HCO_3 (Table 8). The experiment was carried out as in Example 1. However, the heating temperature was adjusted to 170°C or 180°C, and heating time was 2 hours. For sample 7-D, the OSA-modified starch was sieved by a 200-mesh sifter after heating. For sample 7-E, the starch mixture before heating was ground by an analytical mill (A-10, Tekmar) and sieved through a 200-mesh sifter. The starch was thinly spread over an oven pan (38cm ×26 cm) and heated at 180°C for 2 hours. A starch sample without adding OSA was prepared as a control. D.S. and R.E. were measured by titration method 2.

Solubility of the OS starch dispersed in an aqueous medium (10%, w/w) was analyzed by a potable refractometer before centrifugation ("SBC"). The starch solution was centrifuged at 3500 rpm for 5 minutes and the supernatant was analyzed by the refractometer as well ("SAC").

As shown in Table A.8, grinding of the starch prior to heat treatment appeared to have a slight positive effect on the solubility of the starch product when compared to samples that had not undergone grinding.

Table A.8 Samples adjusted to pH 8.45 by NH_4HCO_3 (6.29% by starch dry weight) and heat treated at different temperatures.

No.	Wt of NH_4HCO_3 (g)	#200 mesh sieve	Temp (°C)	pH after heating	SBC (%)	SAC (%)	%OSA	D.S.	R.E.(%)
7-A	3.02	No	170	3.23	100	84.2?3.8	2.80	0.0222	93.19
7-B	4.00	No	170	3.19	90	N/A	N/A	N/A	N/A
7-C	3.85	No	170	3.25	72	N/A	N/A	N/A	N/A
7-D	3.85	Yes	180	2.81	102.5	94	2.57	0.0204	85.71
7-E	3.02*	Yes	180	3.01	N/A	99	2.68	0.0213	89.47

*Cake moisture: 50.20%

Example 9

This Example describes an embodiment of the present application directed to a non-food application using a 5% OSA treatment.

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. The pH of the starch slurry was adjusted to 8.45 with 3.02 g NH_4HCO_3. The suspension was filtered and the starch cake was mixed with 5% OSA (based on the weight of starch) by a mixer (Model K45SSWH, KitchenAid, St. Joseph, MI) at 2nd speed for 15 minutes. The mixture was dried in an air oven at 35°C overnight until the moisture content was below 12%. The mixture was then spread over an oven pan (38cm ×26 cm) and heated at 180°C for 2 hours. Afterwards, the starch mixture was ground by an analytical mill (A-10, Tekmar) and sieved through a 200-mesh sifter. A starch sample without adding OSA was prepared as a control. D.S. and R.E. were measured by titration method 1.

The bound OS content was 4.77%. The D.S. and R.E. of the OS starch were 0.0386 and 93.43%, respectively. Solubility of the OS starch was analyzed by a potable refractometer. The starch solution (10%, w/w) was centrifuged at 3500 rpm for 5 minutes. The solubility of the material was found to be 86%.

Example 10

In this example, wheat starch (100 g) was suspended in distilled water (150 mL) with agitation. The pH of the starch slurry was adjusted to 8.45 by adding 3.02 g NH_4HCO_3. The experiment was carried out as described in Example 9 except that 3% OSA was added. The bound OS content was 2.59%. The D.S. of the OS starch was 0.0205, and the R.E. was 94.33%. The solubility of the OS starch was 75%.

Example 11

In this example an emulsion of sample 7-E was prepared. OS starch (8.0g, dry basis) was mixed with sodium benzoate (0.1g), citric acid (0.2g) and water (50.40 mL) in a Waring blender (Model 31BL92, Dynamics Corporation of America). The mixture was blended at low speed (powerstat at 25-30) for 2 minutes. Orange oil (8g) was slowly added to the mixture over 30 seconds and the mixture was blended for an additional 30 seconds. The jar was then covered and blended at high speed (powerstat at 100) for 2 minutes. The emulsion solution was permitted to rest in the blender for 30 minutes and transferred to a tall glass jar (10 oz.). The jar was capped and heated at 45°C in an air oven for 24 hours. The emulsion was very stable after heating at 45°C for 24 hours. Microscope photographs of the emulsion were taken and are shown in Figures A.1 and depict a small oil droplet size.

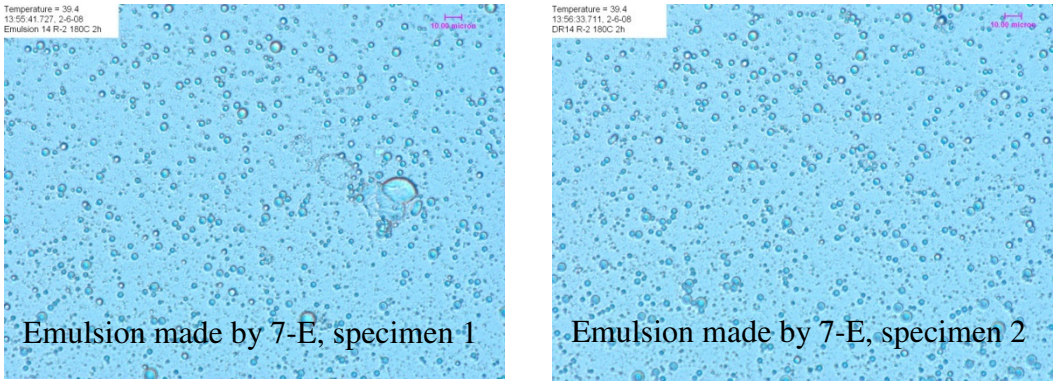


Figure A.1 Microscope pictures of emulsion prepared from sample 7-E after heat at 45ºC for 24h

Example 12

In this example, Samples 7-E and 5-B (both heat treated for 4 hours) were suspended in glycerol and viewed with a microscope under normal and polarized light. Figure A.2 depict

Sample 7-E and 5-B under normal and polarized light. Figure A.2 shows that starch granules of both samples had Maltese cross-like crystalline structures when viewed under polarized light. The results suggest that the molecular order of starch granules remained after OSA modification.

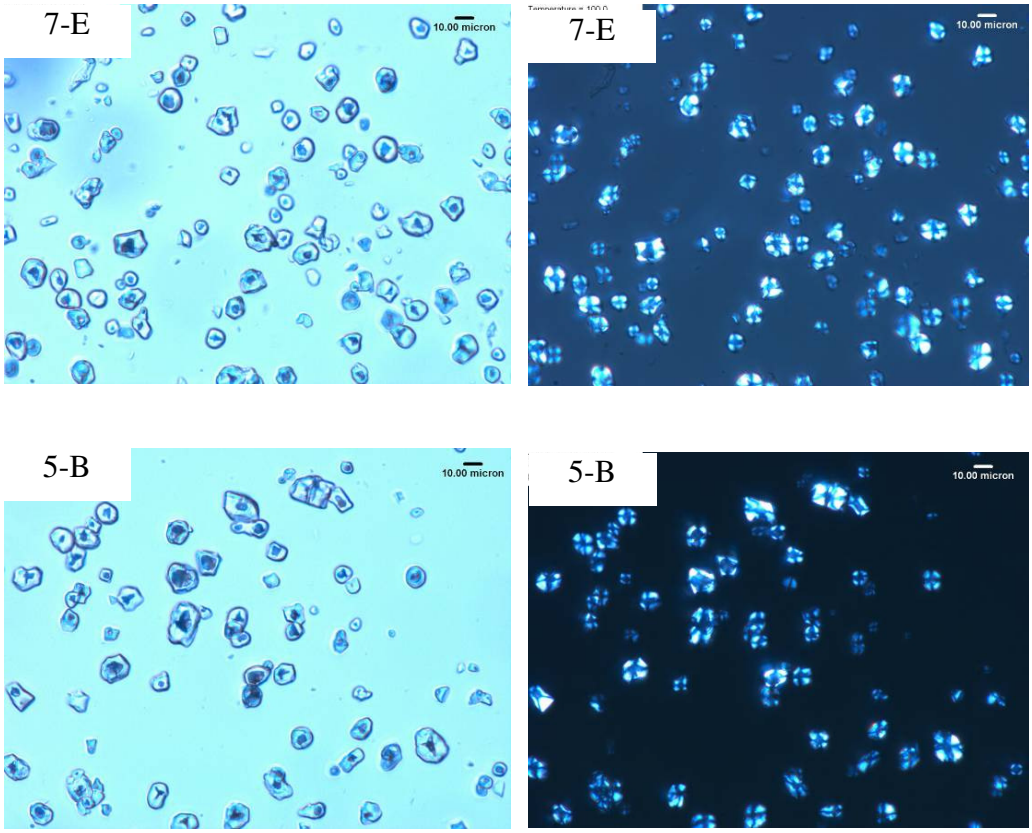


Figure A.2 Microscope pictures of sample 7-E and 5-B (4h)

Example 13

In this example, Sample 5-B (heat treated for 1 hour, 2 hours, and 4 hours) and a commercial sample (same as used in Example 7 above) were analyzed by gel permission chromatography (GPC). The starch (0.1% by weight) was dispersed in DMSO and heated in a boiling water bath for 1 hour. Then the solution was analyzed by the GPC. The results are shown in Figure A.3. The lab made OS samples exhibited larger molecular weights than the commercial sample.

Example 14

Corn starch (100 g) was suspended in distilled water (150 g) with agitation. The pH of the starch slurry was adjusted to 8.47, 8.51 and 8.70 by addition of NH_4HCO_3. The method is

described in Example 8. Heating temperature was adjusted to 170°C, 180°C or 190°C and heating time was 1 h, 2 h, 3h, 4 h and 5h. Solubility (Figure A.4) and pH (Table A.9) of the final product was measured. The method of measuring solubility is the same as example 8. DS and RE of two samples (Table A.10) were measured by the titration method 2. A starch sample without adding OSA was prepared as a control.

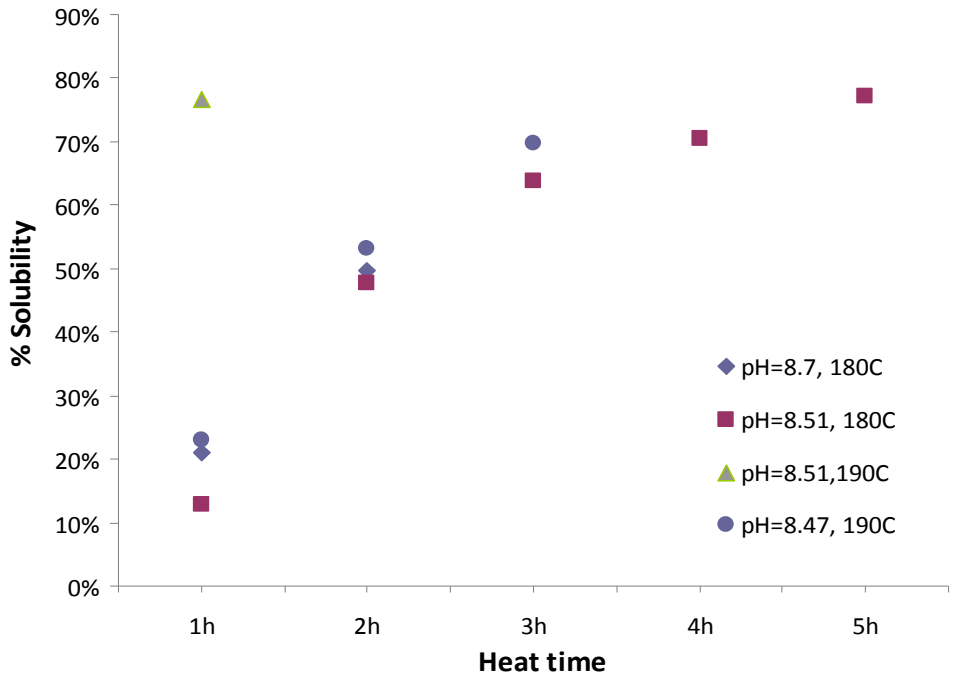


Figure A.3 Solubility of OS corn starch prepared by dry reaction.

Table A.9 Samples adjusted to pH 8.47, 8.51 and 8.70 by NH_4HCO_3 and heat treated at different temperatures.

Adjusted pH	Amount of NH_4HCO_3 added (g)	Cake moisture (%)	Temp. (°C)	pH of starch before heat	pH of heated starch
8.70	3.02	46.22	180	5.10	3.02
8.51	1.02	46.03	180	3.67	3.31
8.47	0.51	47.60	190	3.54	3.02

Table A.10 DS and RE of two samples of OS corn starch prepared by dry reaction

Heat time	Adjusted pH	pH of starch before heat	Heat temp. (°C)	Solubility (%)	DS	RE	%OSA
2h30min	8.70	5.10	180	50	0.0199	84.0	2.52
5h	8.51	3.67	180	77	0.0177	74.6	2.24

Example 15

Tapioca starch (100 g) was suspended in distilled water (150 g) with agitation. The pH of the starch slurry was adjusted to 8.62 and 8.72 by addition of NH_4HCO_3. The experiment is carried out as described in Example 8. Heating temperature was adjusted to 180°C or 190°C and heating time was 1 h or 2 h. Solubility of the final product was measured by using the same method in example 8. DS of the products is measured by the NMR method and is shown in Table A.11.

Table A.11 DS and RE of two samples of OS tapioca starch prepared by dry reaction

Adjusted pH	Amount of NH_4HCO_3 added (g)	Temp. (°C)	Time (h)	Cake moisture (%)	pH of starch before heat	pH of heated starch	Solubility (%)	DS
8.62	3.02	180	2	45.87	N/A	N/A	N/A	0.01792
8.72	1.33	190	2	46.73	3.90	2.88	99.57	0.01811

Example 16

Potato starch (100 g) was suspended in distilled water (150 g) with agitation. The pH of the starch slurry was adjusted to 8.59 by addition of NH_4HCO_3 (3.02 g). The experiment is carried out as described in Example 8. Heating temperature was adjusted to 180°C and heating time was 2 h. Solubility of the final product was 85.7% as measured by using the same method as in example 8. The value is 85.66%. DS of the product is measured by the NMR method and the value is 0.01956. the RE is 82.43%.

Example 17

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. The pH of the starch slurry was adjusted to 8.45 by 3.02 g NH_4HCO_3. The suspension was filtered

and the starch cake was mixed with 3% OSA (by the weight of starch) by a mixer (Model K45SSWH, KitchenAid, St. Joseph, MI) at 2nd speed for 15min. The mixture was dried in an air oven at 35°C overnight until the moisture content was below 12%. Starch mixture (31.12 g) was mixed with sodium aluminum phosphate (SAP) (3.09 g). pH of the starch sample was 4.90 with SAP. The starch was heated in the air-forced oven at 180 °C for 2 h. DS of the product is measured by the NMR method and the value is 0.01821. The RE is 76.86%.

Example 18

OSA (3.02 g) was stirred in 20 mL NaOH (3% wt %) for 2h. Hydrolyzed OSA was added to starch (100 g, dry weight) in ethanol (180 proof, 100mL) solution. pH of the solution was adjusted to 3.0 by 1N HCl. The starch slurry was dried in an air-forced oven at 35 °C until the moisture content was below 12%. Starch was ground by a coffee grinder and heated in oven at 180 °C for 0.5 h and then 165 °C for 2 h. pH of the final product was 3.3. DS was measured by the titration method and the value is 0.1152. RE was 49.03%.

Example 19

Waxy maize starch (100 g) was suspended in distilled water (150 mL) with agitation. The pH of the starch slurry was adjusted to 8.45 by 3.02 g NH_4HCO_3. The suspension was filtered and the starch cake was mixed with 3% OSA (by the weight of starch) and 100 mL ethanol. The mixture was dried in an air oven at 35°C overnight until the moisture content was below 12%. The starch was heated in the air-forced oven at 180 °C for 2 h. DS of the product is measured by the NMR method; the value is 0.01242.The RE is 52.84%.

Claims

1. A method of preparing a lipophilic starch comprising: forming a starch mixture having a pH of between about 7 to about 11; processing said starch mixture to obtain a starch cake; adding an organic acid anhydride reagent to said cake thereby forming a reaction mixture, said organic acid anhydride reagent having the formula wherein R is a dimethylene or trimethylene group and R' is a linear, branched or cyclic alkyl, alkenyl, aralkyl or aralkenyl group having 2 to 20 carbon atoms; drying said reaction mixture to a moisture content of between about 0 to about 15% by weight; and heating said dried reaction mixture at a temperature of at least about 100°C for between about 1 minute to about 6 hours.

2. The method according to claim 1, wherein said starch mixture is prepared by forming a starch slurry.

3. The method according to claim 2, wherein said starch slurry is prepared by dispersing a quantity of starch in a liquid medium, said liquid medium being selected from the group consisting of water, alcohol, toluene, or combinations thereof.

4. The method according to claim 2, wherein the pH of said starch slurry is adjusted by adding a base thereto, said base being selected from the group consisting of metal and non-metal hydroxides, oxides, carbonates, and mixtures thereof.

5. The method according to claim 4, wherein said base being selected from the group consisting of ammonium hydroxide, ammonium carbonate, and ammonium bicarbonate, and mixtures thereof.

6. The method according to claim 2, wherein said step of processing said starch mixture to obtain a starch cake comprises filtering said starch slurry.

7. The method according to claims 1 or 2, wherein said organic acid anhydride reagent comprises octenyl succinic anhydride.

8. The method according to claims 1 or 2, wherein said organic acid anhydride reagent is added to said cake at a level of between about 0% to 100% by weight, based on the dry weight of the starch.

9. The method according to claims 1 or 2, wherein said step of drying said reaction mixture comprises heating said reaction mixture to a temperature of less than 120°C until said moisture content of between about 0% to about 15% by weight is achieved.

10. The method according to claim 1 or 2, wherein said step of heating said dried reaction mixture comprises heating said dried reaction mixture at a temperature of between about 100°C to about 200°C.

11. The method according to claim 10, wherein said step of heating said dried reaction mixture comprises heating said dried reaction mixture at a temperature of between about 140°C to about 180°C for between about 30 minutes to about 3.5 hours.

12. A water-soluble, lipophilic starch comprising starch that has been modified with an organic acid anhydride reagent and which contains less than about 0.5% by weight of mineral

acid and/or starch-degrading enzyme residues, said lipophilic starch having a solubility in water at 25°C of greater than about 90%.

13. The water-soluble, lipophilic starch according to claim 12, wherein said organic acid anhydride reagent has the formula and wherein R is a dimethylene or trimethylene group and R' is a linear, branched or cyclic alkyl, alkenyl, aralkyl or aralkenyl group having 2 to 20 carbon atoms.

14. The water-soluble, lipophilic starch according to claim 13, wherein said organic acid anhydride reagent comprises octenyl succinic anhydride.

15. The water-soluble, lipophilic starch according to claim 12 or 13, wherein said lipophilic starch is substantially free of mineral acid and/or starch-degrading enzyme residues.

Abstract

A lipophilic starch is provided along with methods of making the same. The starch is prepared by modifying the starch with an organic acid anhydride reagent, such as octenyl succinic anhydride, drying the modified starch to a moisture content of less than 15% by weight, and then heat treating the dried starch at a temperature of at least 100°C for at least one minute.

Appendix B - Dry reaction of OSA reaction at low temperature

Method

Waxy maize starch (100 g) was suspended in distilled water (150 g) with agitation. The pH of the starch slurry was adjusted by adding NH_4HCO_3 of 3.02g. The suspension was filtered and the starch cake (50% moisture content) was mixed with 3% OSA (by the dry weight of starch) by a mixer (Model K45SSWH, KitchenAid, St. Joseph, MI) at 2nd speed for 15min. The mixture was dried in an air-forced oven at 35°C overnight. The starch mixture was spread over an oven pan (38cm ×26 cm) and heated at 120 °C or 140 °C for 2 h, 4 h, or 6 h. Native waxy maize starch was used as a control.

Solubility of the product is 0%. Titration results are shown in Table B.1.

Results

Table B.1 DS and RE of OS waxy maize starch prepared at low temperature

Temperature	*Time*	*%OSA*	*D.S.*	*R.E.%*
120 °C	2h	1.982	0.01560	66.07
	4h	1.863	0.01465	62.11
	6h	1.829	0.01437	60.98
140 °C	2h	2.132	0.01680	71.07
	4h	2.528	0.02000	84.26
	6h	2.547	0.02016	84.90

Appendix C - Hydrolysis of NH_4HCO_3 and spraying on the starch

Method

In previous experiments, starch was dispersed in water and NH_4HCO_3 was added to the starch slurry. After filtration the starch cake has a moisture content of ca. 50%. If 3% (wt% based on starch weigh) of NH_4HCO_3 was added then approximately 1.5% NH_4HCO_3 was left in the starch cake. In this experiment, NH_4HCO_3 solution in water (10%, wt%) was sprayed on dry starch to have NH_4HCO_3 to starch ratio of 1.5/100 (w/w) . The mixture was manually blended by hand. Then OSA dissolved in ethanol (37.5%, wt%) was sprayed on starch to have OSA to starch ratio of 3/100 (w/w). Starch and OSA was mixed by hand. The mixture was heated in an air forced oven at 40°C overnight then heated at 180ºC for 2h. Degree of substitution of the product obtained was measured by titration.

In another approach, OSA was mixed with NH_4HCO_3 solution (10%, wt%) for 10min and 1h, respectively. OSA was not completely soluble in NH_4HCO_3 solution after mixing for 10 mins. Whereas, after 1h mixing, OSA in NH_4HCO_3 solution became clear. Then the each mixture was sprayed on dry starch. Starch mixture was manually mixed by hand. The mixture was heated in an air forced oven at 40°C overnight then heated at 180ºC for 2h. Degree of substitution of the product obtained was measured by titration.

Solubility

Starch solution (1%, w/v) was heated at 85 ºC for 30 min then immediately cooled with an ice water bath. The solution was centrifuged at 5000X g for 10 min. The supernatant was decanted, heated at 60 ºC overnight. The residue was dried at 130 ºC for 1h. The solubility was calculated as: (dry matter in the supernatant/total dry weight of starch)*100%.

Results

Table C.1 Characteristics of OS starches prepared from OSA of differerent pre-treatments.

Sample name	*%OSA*	*D.S.*	*R.E.%*	*OSA Conc.*	*Solubility (%)*
OSA in NH_4HCO_3 for 10 min	1.666	0.01307	66.63	2.50	88.77
OSA in NH_4HCO_3 for 1h	1.976	0.01555	77.48	2.55	86.03*
OSA dissolved in ethanol 1	1.781	0.01399	55.66	3.20	88.37
OSA dissolved in ethanol 2	1.784	0.01401	53.57	3.33	88.01*

* average value

Appendix D - Wide angle X-ray diffraction and small angle X-ray scattering of pyrodextrins from waxy wheat and waxy potato starches

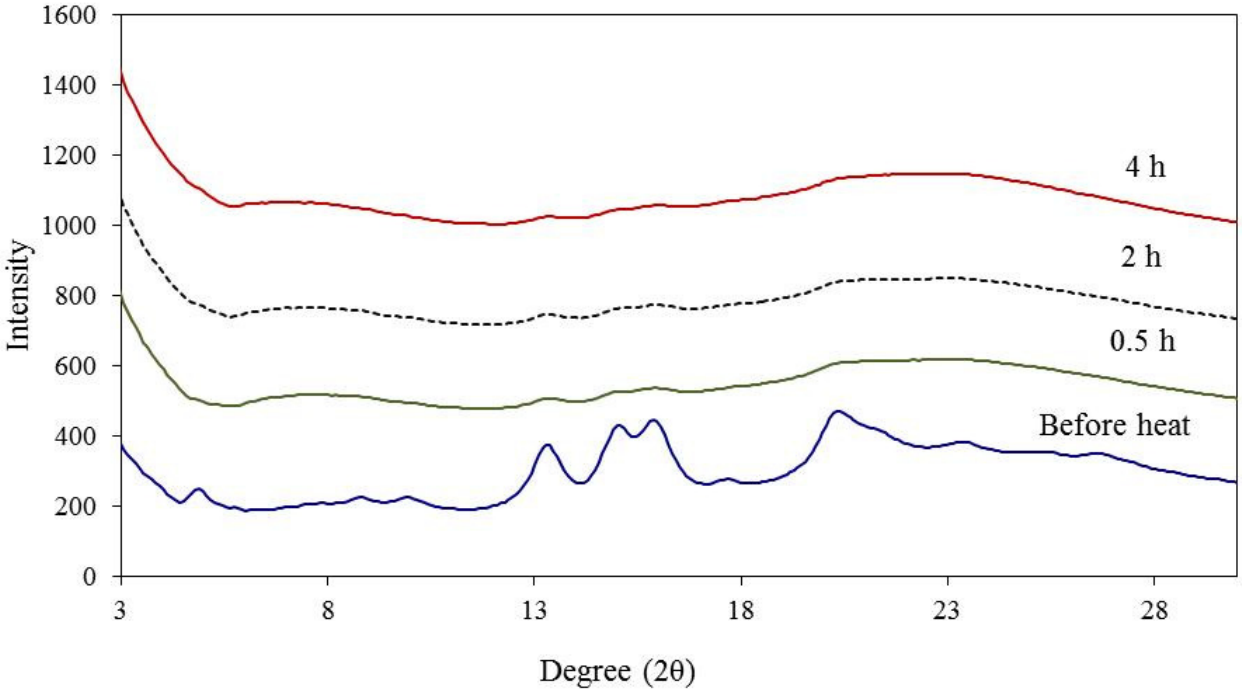


Figure D.1 Waxy wheat dextrins in H_2O

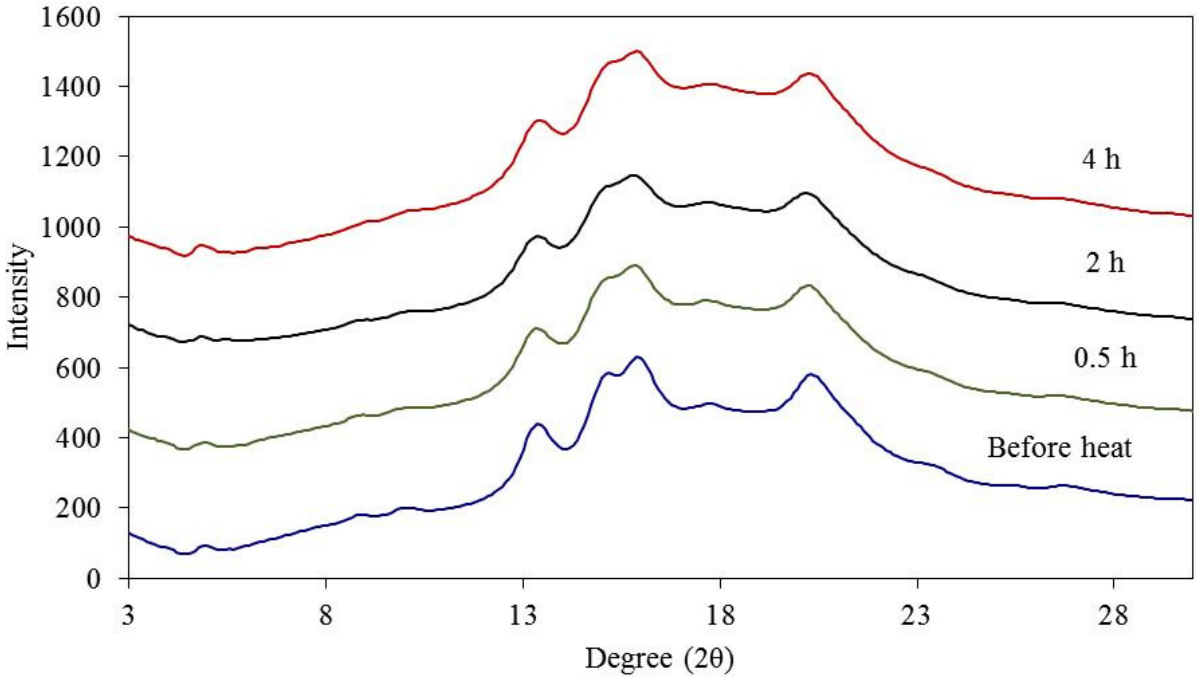


Figure D.2 Waxy wheat dextrins in glycerol/water (8/2)

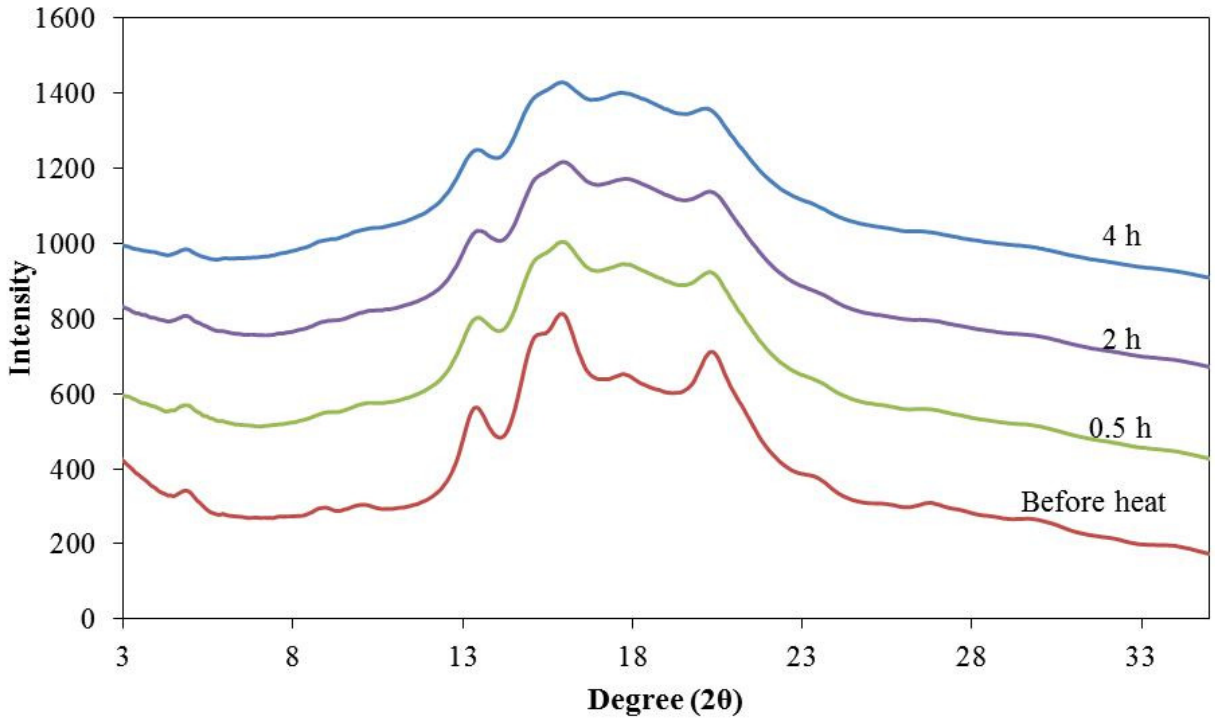


Figure D.3 Waxy wheat dextrins in glycerol/water (9/1)

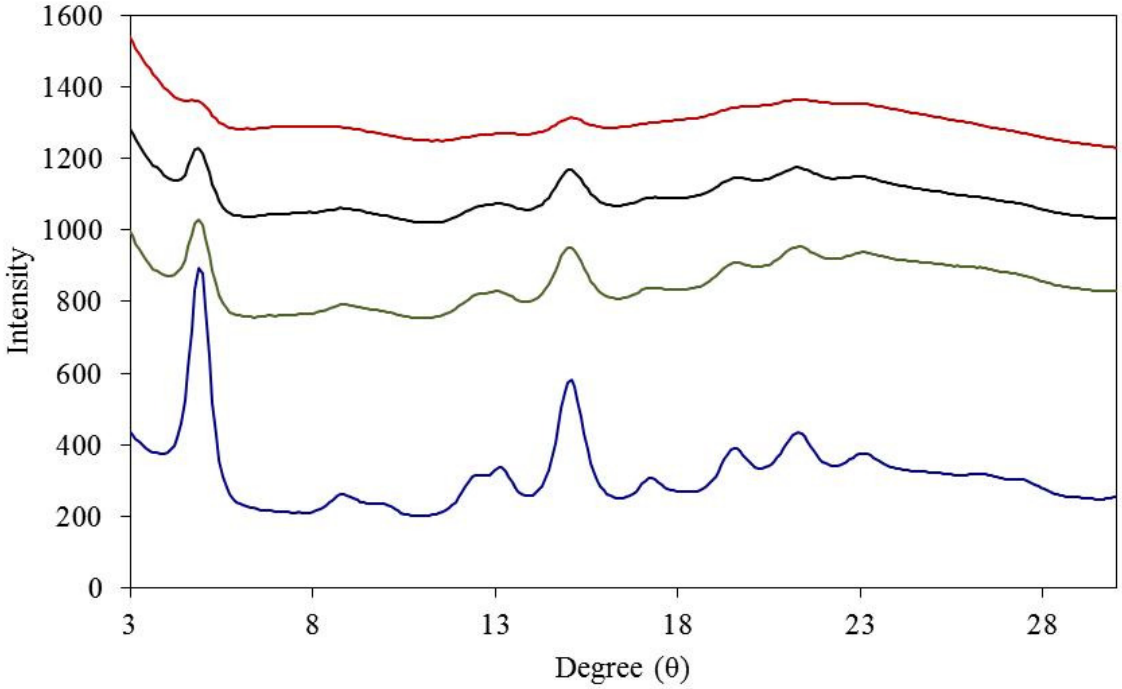


Figure D.4 Waxy potato dextrins in H_2O

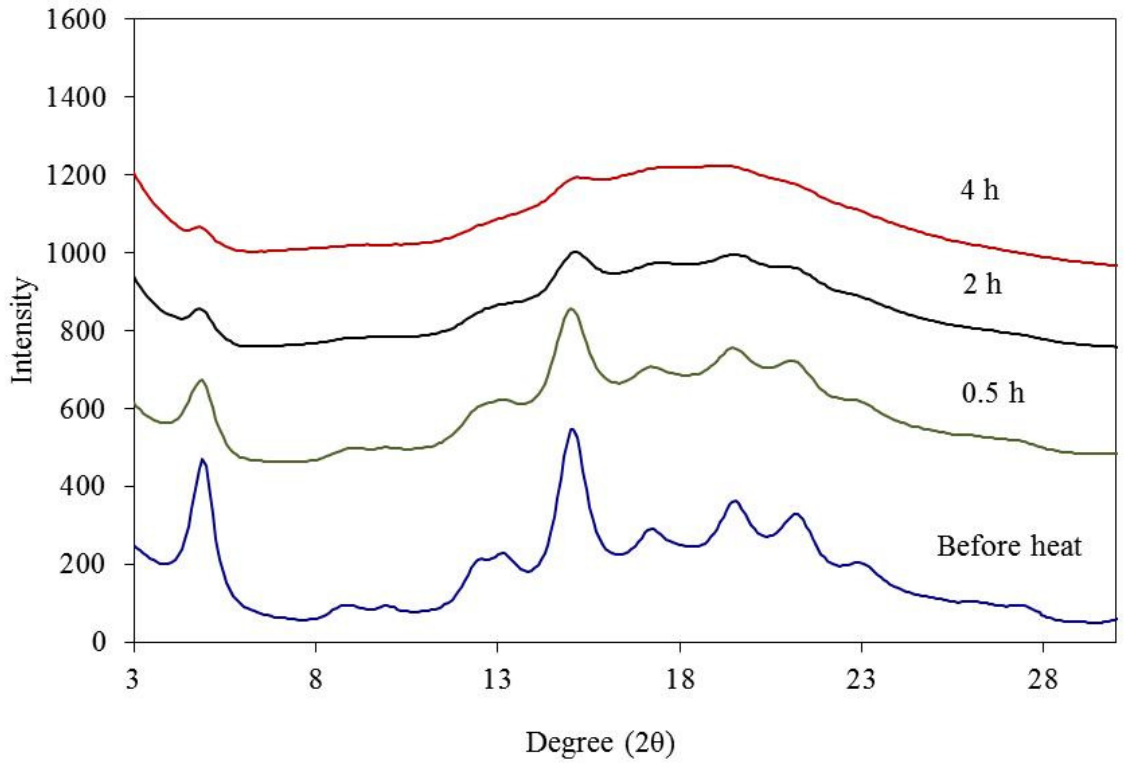


Figure D.5 Waxy potato dextrins in glycerol/water (6/4)

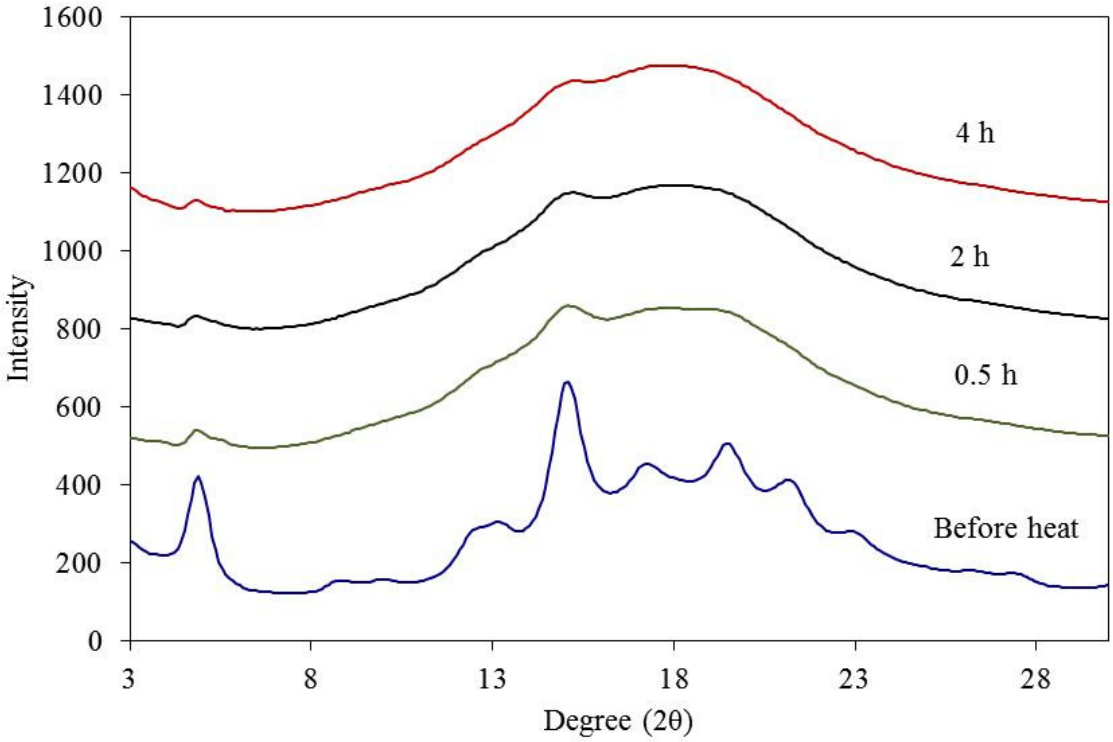


Figure D.6 Waxy potato dextrins in glycerol/water (8/2)

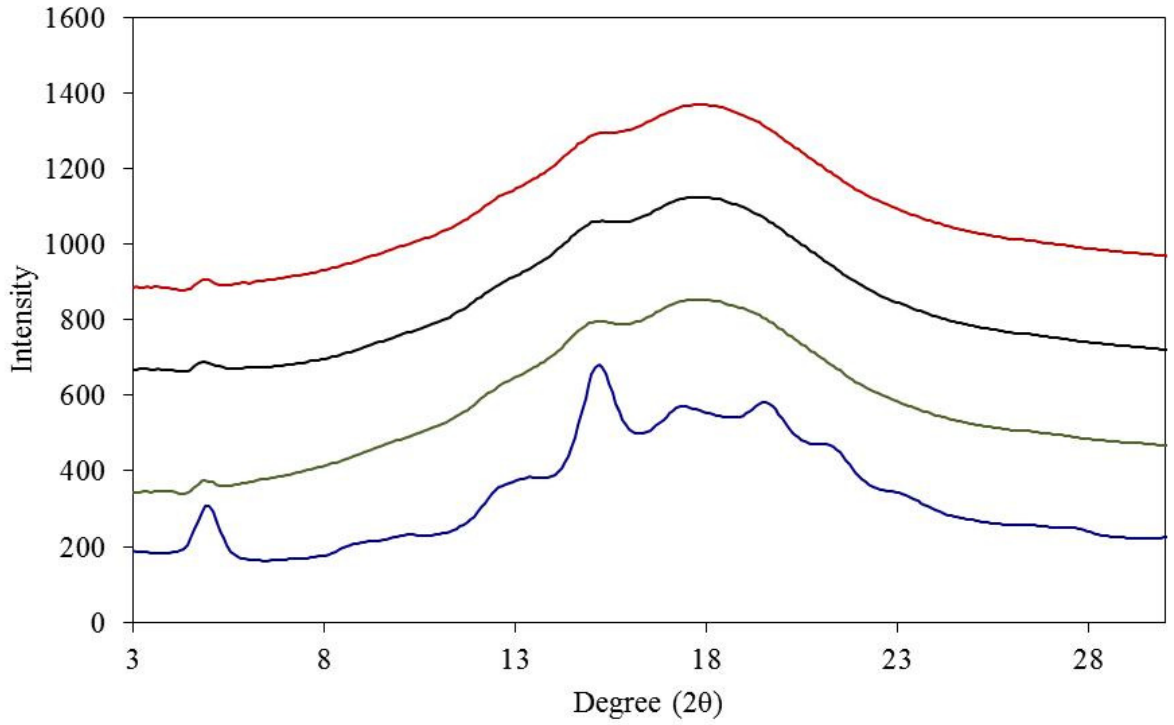


Figure D.7 Waxy potato dextrins in glycerol/water (9/1)

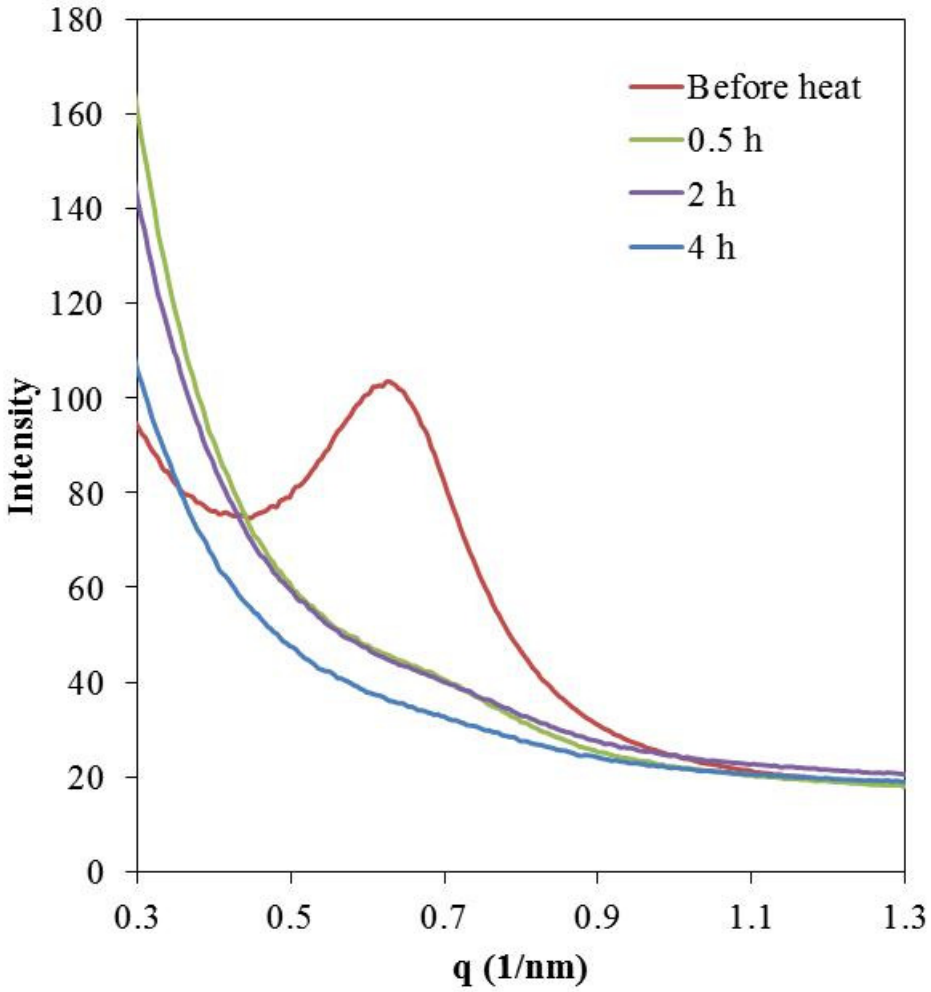


Figure D.8 Waxy wheat dextrins in H_2O

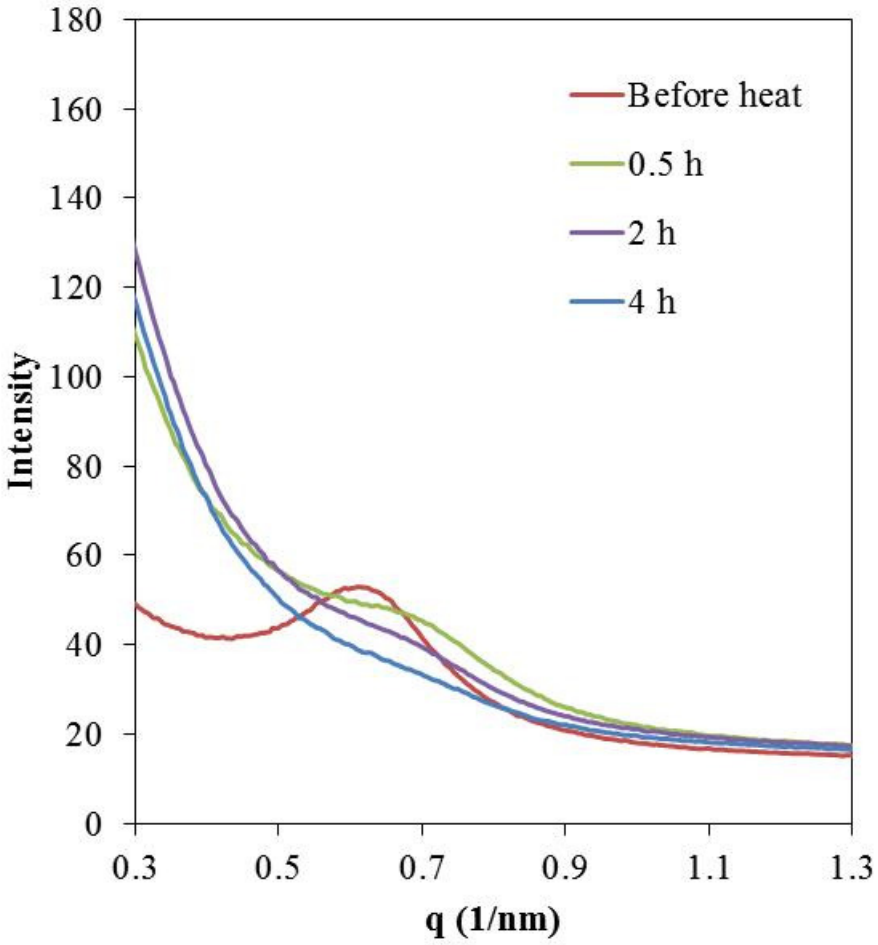


Figure D.9 Waxy wheat dextrins in glycerol/water (6/4)

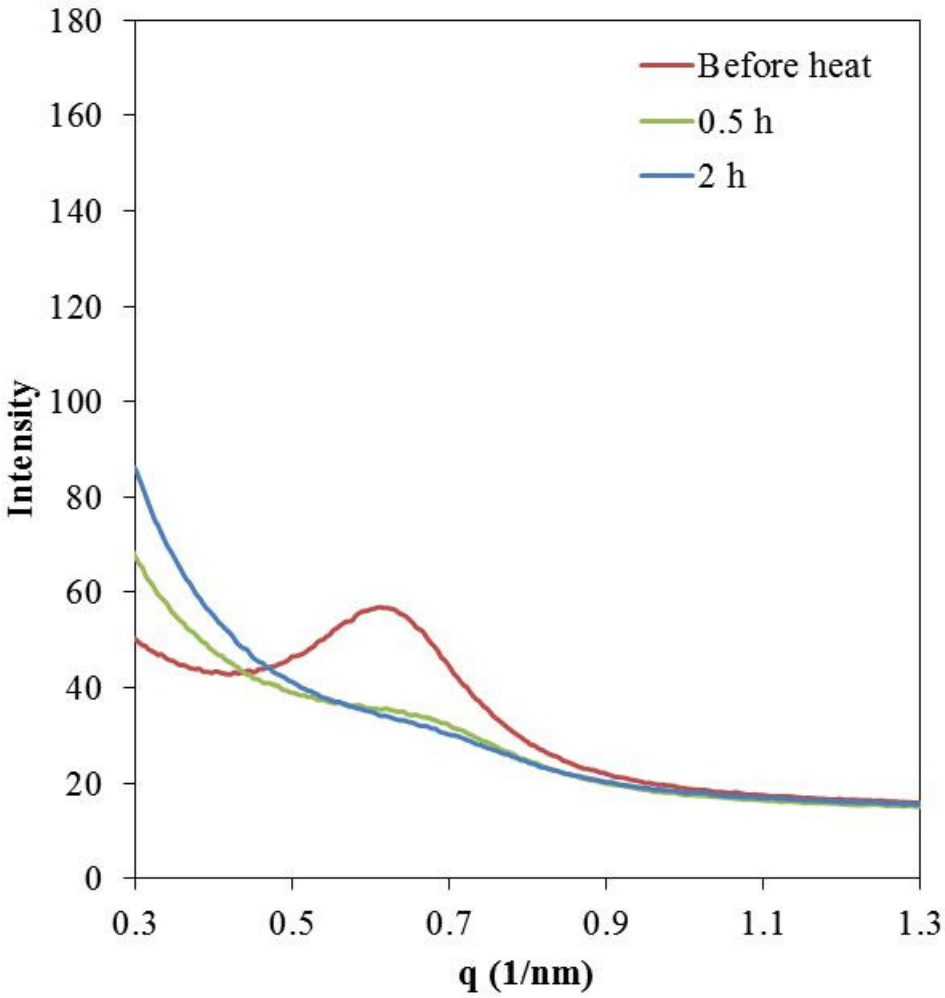


Figure D.10 Waxy wheat dextrins in glycerol/water (8/2)

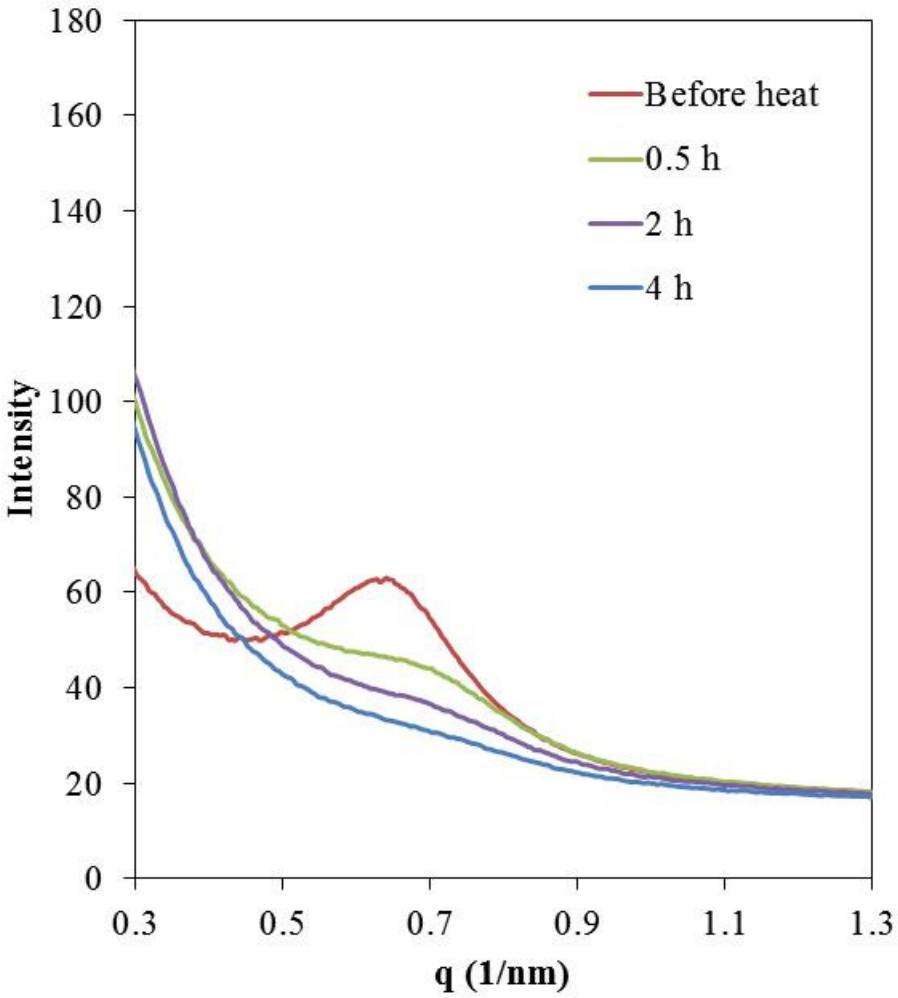


Figure D.11 Waxy wheat dextrins in glycerol/water (9/1)

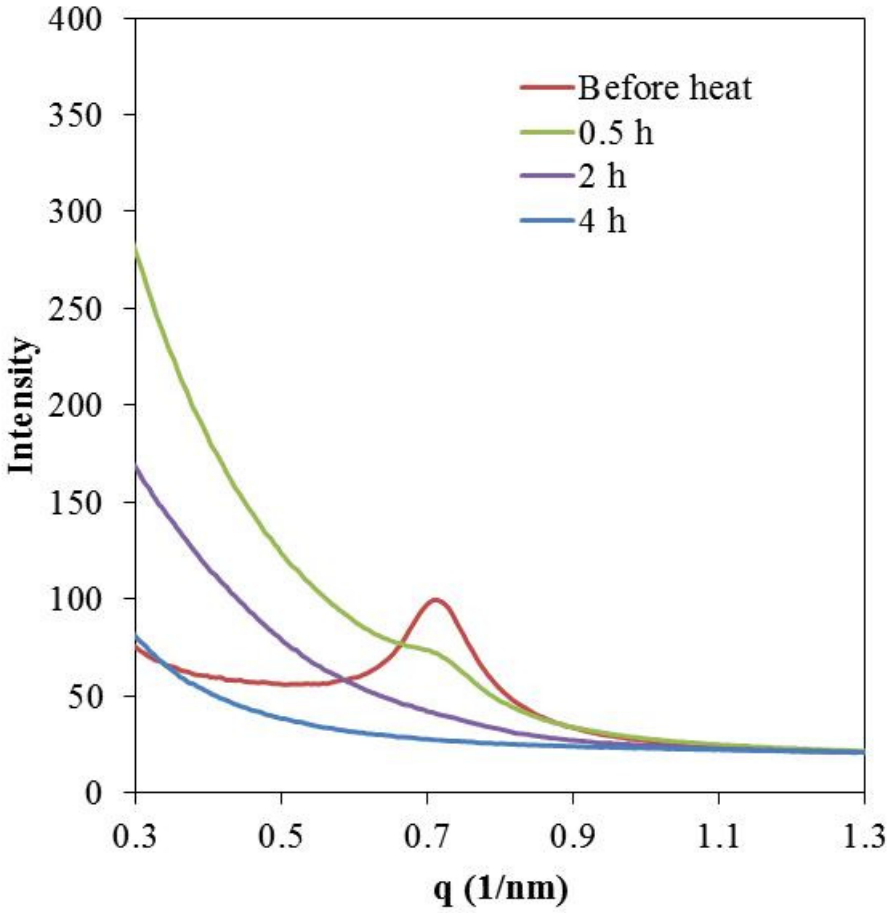


Figure D.12 Waxy potato dextrins in H_2O

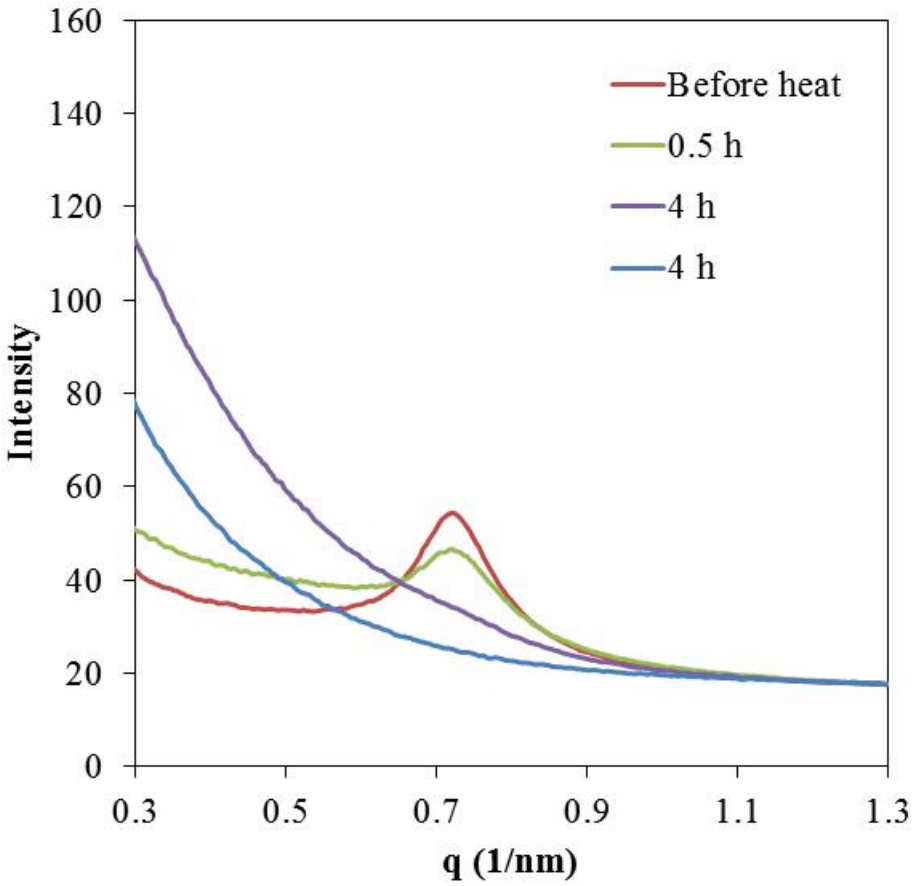


Figure D.13 Waxy potato dextrins in glycerol/water (6/4)

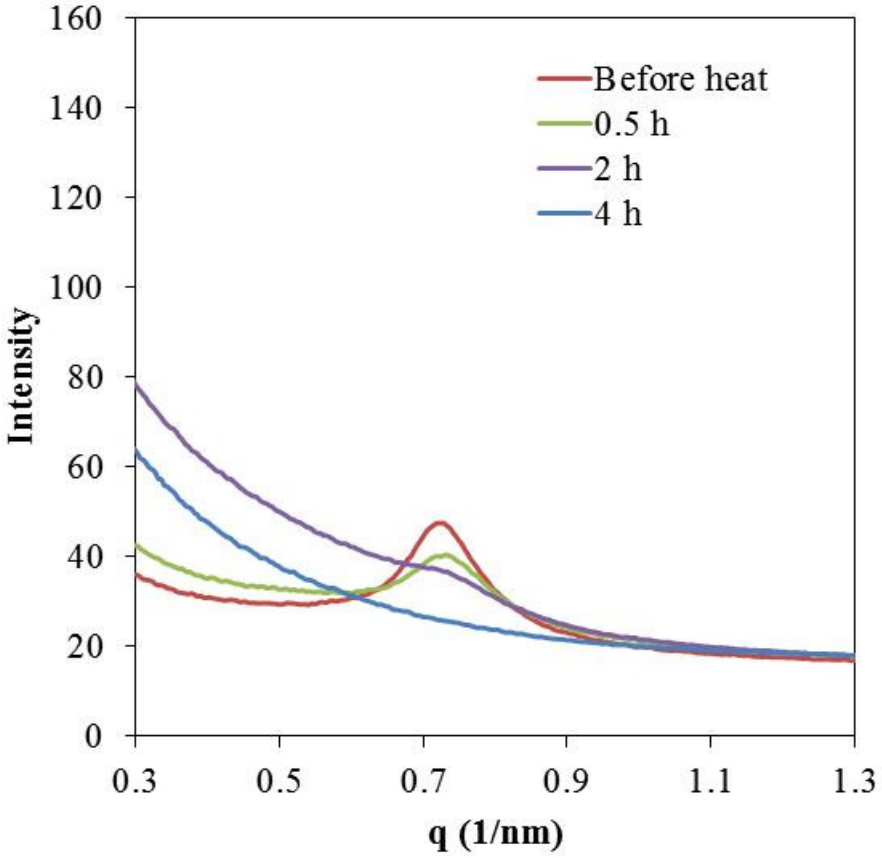


Figure D.14 Waxy potato dextrins in glycerol/water (8/2)

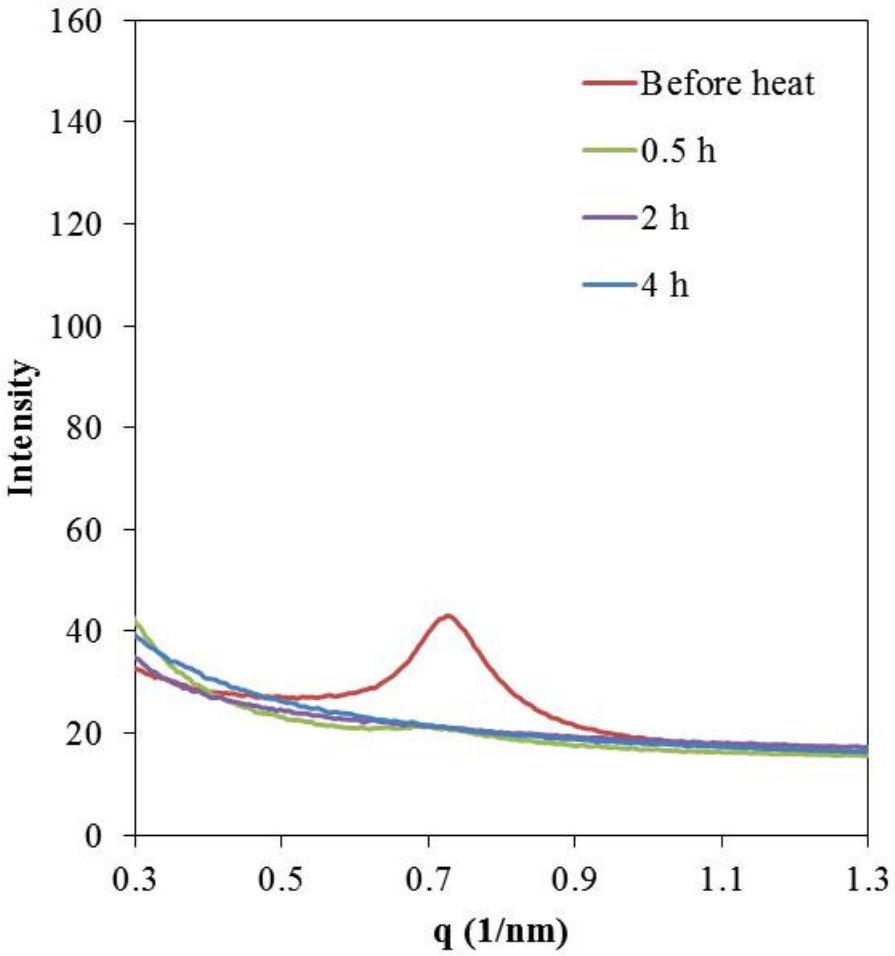


Figure D.15 Waxy potato dextrins in glycerol/water (9/1)