PREPARATION AND STRUCTURE OF OCTENYL SUCCINIC ANHYDRIDE MODIFIED WAXY MAIZE STARCH, MICROPOROUS STARCH AND MALTODEXTRIN

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Abstract

Octenyl succinic anhydride (OSA) modified starch is widely used in emulsion and encapsulation applications. The functionality of OS starch depends on its molecular structure. A systematic study was performed to investigate the reaction of OSA with granular waxy maize (WM) starch, microporous WM starch and soluble maltodextrin. OS starches were prepared in an aqueous slurry system, and the degree of substitution (DS) of OS starches was determined by titration and ^{1}H-NMR spectroscopy. For both 3% and 50% OSA treatment, OS maltodextrin had higher DS and reaction efficiency (RE) than OSA modified WM starch and microporous WM starch. The maximum DS of OSA modified granular WM starch was 0.14 and the highest DS of OS maltodextrin was 0.27. For the 3% OSA treatment, the RE for WM starch and maltodextrin was ~ 80% and ~100%, respectively. The structure of OSA modified WM starch and the locations of OS groups on anhydroglucose units (AGUs) were studied by ^{1}H-NMR and ^{13}C-NMR. As increasing OS substitution, ^{13}C - signal at C-1 shifted to upper field. In addition, the ^{13}C - signal at C-6 shifted to downfield when DS reached 0.073. The results suggested that OS groups were predominantly substituted at the O-2 position and started being substituted at O-6 position when DS was 0.073. FT-IR microspectroscopy was used to detect the heterogeneity OS starch products. Native WM starch, OSA modified WM starches (DS=0.019 and 0.073) and a starch blend with native starch to OSA modified WM starch (DS=0.073) ratio of 7:3 were examined. More than one hundred starch granules of each sample were analyzed one by one by FT-IR microspectroscopy. For the OS starch (DS=0.019), 7% starch granules showed carbonyl absorption. For the OS starch (DS=0.073), 99% starch granules showed carbonyl absorption but the intensity varied, indicating that OSA reacted with most starch granules when DS was 0.073. However, the OS contents of individual granules varied. For the starch blend, only 30% starch granules had carbonyl absorption. FT-IR microspectroscopy is a useful tool to detect heterogeneity of OS starch blends containing native starch.

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Dedication

This dissertation is dedicated to my family.

CHAPTER 1 - Introduction

Starch is the major food reserve material of plants and the main nutritional source for human beings. In the modern food industry, starch is isolated to be functional ingredients for altering and improving food processing purposes (Moore et al 1984). Starch is cheap and ubiquitous. It can be isolated from different plants, such as cereal grains (corn, wheat, and rice), tubers and roots (potato, tapioca, and arrowroot), fruits (green banana), stem-pitch (sago palm) and other seeds (beans, peas, lentils). Starch has very unique properties including pasting, gelatinization, retrogradation, glass transition and interaction with other components. Because of these properties, starch products are highly desired throughout industry. About 84% of starch is used to produce refinery products, including ethanol, glucose syrups, high-fructose syrups and maltodextrins. The rest of native starch is produced into starch products for applications in paper, food and textile industry. Food industries use about 20% of the starch products and starch acts as important food ingredient to provide bulk and body as well as improve texture and mouth feel (BeMiller 2007).

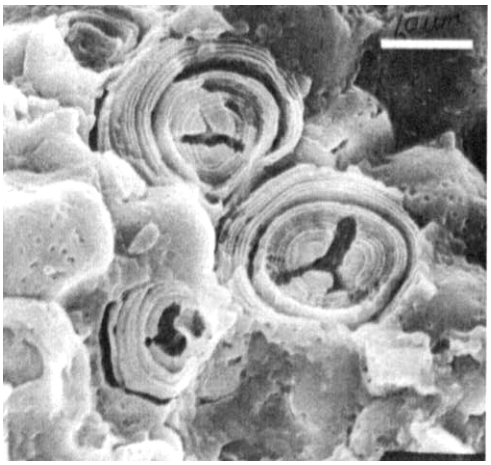
In the modern food industry, starch is required to tolerant wide range of processing techniques, various distribution, storage and final preparation conditions. However, after cooking in water, native starch becomes weak bodied, cohesive, rubbery pastes or gels (BeMiller 2007). As a result, native starch needs to be modified in order to improve its function properties and meet consumers' various demands. After modification, starch shows increased ability to resist heat, shear stress, and acids.

Starch is a macromolecular complex of two polymeric components, amylose and amylopectin. Amylose molecule is essentially a linear polymer with primary α-1, 4 linkages. Amylopectin is a highly branched molecule contains 4-5% α-1, 6 linked branch points. Hizukuri (1996) proposed the clustered model of amylopectin. The cluster model can explain many features of amylopectin such as why waxy starches are crystalline, why the viscosity of amylopectin is higher than glycogen.

Amylose and amylopectin molecules are packed in a starch granule. The size and shape of starch granules vary when starches are isolated from different botanical sources. The amorphous region is easier to be digested by α-amylase compared to the crystalline region

(Hoseney 1994). The ring patterns in Figure 1.1 indicate the alternative of amorphous and crystalline region. Low level chemical modification does not change the crystalline form of starch granules, therefore chemical modification only affects the amorphous region (Shogren 2000; Wang, et al., 2002; He et al., 2006).

Figure 1.1 Cross Section of a Sorghum Kernel Treated with α-amylase.



Note the concentric rings in the broken starch granules. Bar=10µm

(Reprinted from Hoseney 1994)

In a starch molecule, the end with an aldehyde group is called the reducing end, and the other end is called non-reducing end. The hydrophilic hydroxyl groups make starch soluble in water, and they are interactive with other chemicals. There are three hydroxyl groups available for reaction in an anhydroglucose unit (AGU) locating at C-2, C-3 and C-6 position.

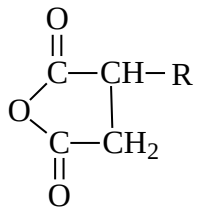
Four types of modifications can be used to modify starch. They are chemical reactions, enzymatic modifications, physical modifications and combinations of the above methods. The modified starch derivatives are the products of either glucosidic bond cleavage or forming new functional groups, or substitution of free available hydroxyl groups, or bridging of molecular chains by cross-linking reagent (Tharanathan 2005; Eliasson 2004). Chemical modifications include crosslinking, substitution or conversion. In most cases, starch is reacted with a small amount of chemical reagents. The degree of substitution (DS), the location of OS group in a starch granule and a starch molecule can affect the properties of modified starches. Physical modifications apply heat, shear stress and moisture to change properties of native starch. There are two main approaches of physical modification: pregelatinization and heat-treatment. Starch modification can be carried out at either dry solid state or in water slurry. The chemical and

physical modifications make starches better tolerate heat, acid and shearing of the severe industry processing.

Alkenylsuccination is one of the substitution reactions. The method of preparing polysaccharide derivatives of substituted cyclic dicarboxylic acids were patented by Caldwell and Wurzburg in 1953 (Caldwell & Wurzburg 1953). They invented a simple and economical method to prepare polysaccharides with amphiphilic property. The modified polysaccharides were able to form stable and permanent emulsion.

The structural formula of the alkenyl succinates is shown in Figure 1.2. The R group may be alkyl, alkenyl, aralkyl or aralkenyl, and can contain 5-18 carbon lipophilic chain. The polysaccharides for modification can be starch, gelatinized or ungelatinized, from sources including corn, tapioca, potato, wheat, sago, rice and waxy maize; as well as modified or thin-boiling starches and starch derivatives; and also cellulose and hemi-cellulose (Caldwell & Wurzburg 1953). The anhydride can form esters with hydroxyl groups in starch polymers in alkaline condition, and the esterification may take place in the aqueous slurry, dry state, or organic suspension or dispersion. The modified product was in the form of acid esters, however due to the alkaline conditions prevailing during the reaction, the final product is ordinarily produced in the form of the sodium or other salt of the acid ester (Caldwell & Wurzburg 1953).

Figure 1.2 Structural Formula of a Cyclic Dicarboxylic Anhydride



The alkenyl succination changes properties of native starch, such as pasting properties (Bhosale & Singhal, 2007; Park, Chung, & Yoo, 2004; Shih & Daigle, 2003), emulsifying properties (Nilsson 2007) and digestibility (Han & BeMiller, 2007). Alkenyl succinic starches are widely used emulsifiers in a variety of oil-in-water emulsions in food, pharmaceutical and industrial areas (Trubiano1986). The only approved alkenyl succiniate for food use was octenyl succinic anhydride with a side chain of eight carbons. OSA modified starch has been approved for food use in the US since 1972. The US Food and Drug Administration (FDA) restricts that the maximum level of OSA treatment is 3% and the amount of free OSA should be below 0.3%.

For more than 50 years, OSA modification has be studied extensively in the following areas: (i) materials and reaction parameters; (ii) distribution of OSA groups; (iii) physical and chemical properties and (iv) industrial applications.

Materials and Reaction Parameters

OS starch is produced from waxy corn, amaranth, rice, wheat, potato and barley starch (Bhosale & Singhal, 2006; Song, et al 2006; Bao et al 2003; Nilsson & Bergenstahl, 2007). Waxy maize starch is favored for many users because it imparts less cereal-type flavor to foods than common corn starch (Moore et al., 1984). Some cultivars, such as rice and amaranth which are widely grown in certain areas, lack the versatility for modern food industry. Researches were carried out for value-added and properties improvement purposes. On the other hand, the physical properties such as pasting of modified starches partially depend on the biological sources of its parent starch (Thirathumthanorn & Charoenrein, 2006; Bhosale & Singhal 2007).

OS starch is produced in an aqueous slurry system and the main reaction is shown in Figure 1.3. Besides esterification, there are two competitive side reactions (Figure 1.4) affecting the reaction efficiency (RE). The optimal reaction parameters for the main reaction and the side reactions are different. Therefore, RE can be improved by altering the reaction parameters.

Figure 1.3 OSA Reaction in an Aqueous Solution

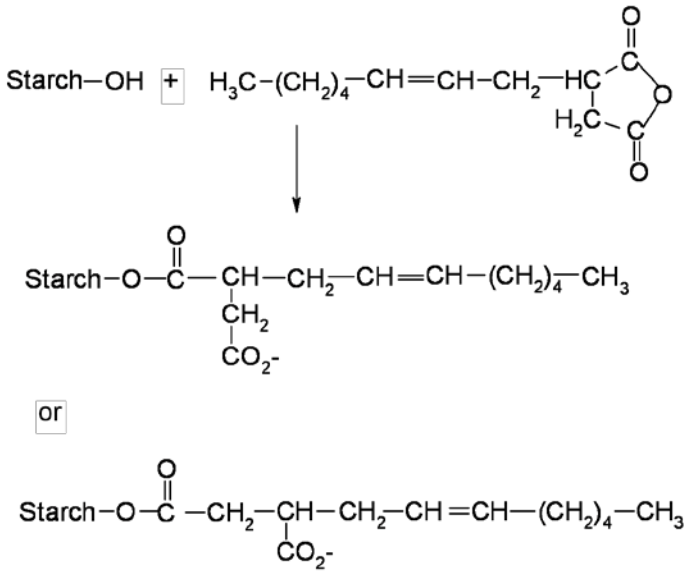
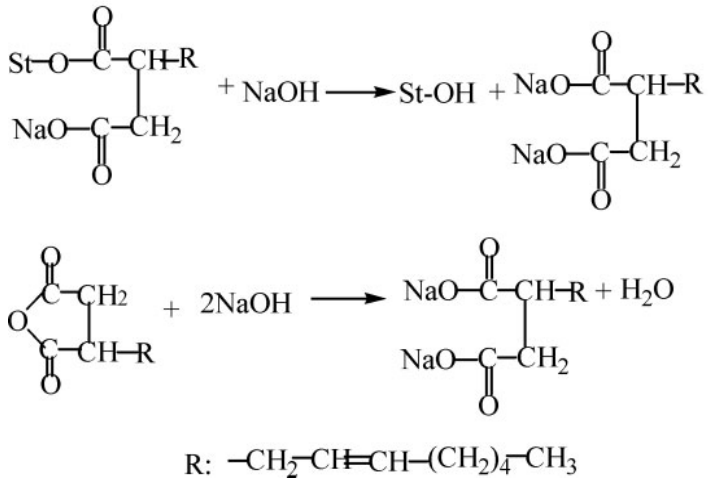


Figure 1.4 Side Reactions of OSA Modification



(Reprint from Song et al., 2006)

Reaction parameters such as pH, temperature, starch concentration, weight percentage of OSA and reaction time were analyzed to determine the optimum condition for producing OS starch with high DS and RE (Bhosale & Singhal, 2006; Song et al., 2006; Bao et al 2003; Nilsson & Bergenstahl, 2007). OS amaranth starch had the highest DS when it was prepared in following conditions: pH at 8.0, reaction temperature of 30°C, starch concentration of 25%, 3% OSA based on starch weight and 6 h reaction time (Bhosale & Singhal 2006). All parameters were the same for waxy corn starch, except for the reaction time of 24h. The RE increased as weight percentage of OSA (<3%) increased. The maximum DS for both samples was 0.02 (Bhosale & Singhal 2006). Similar research was done on early indica rice starch (Song, et al., 2006). It has been suggested that the optimum reaction condition was pH at 8.5, reaction temperature at 35°C, starch concentration of 35%, 3% OSA (based on the weight of starch) and 4 h reaction time. Under this condition, DS and RE was 0.018 and 78%, respectively. The amylose content of early indica rice positively affected the RE. As amylose content increased from 0 to 39.6%, DS increased from 0.024 to 0.030, and RE also increased from 62.8% to 77.5%. In addition to investigating OS starches for food use with DS below 0.02, OS starches with high DS of 0.047 (Bao et al., 2003) and 0.11 were also prepared (Shogren, 2000). However, the RE decreased to less than 40% when 15% OSA (based on the weight of starch) was used.

Recently, a method of preparing acid-treated starch was published (Thirathumthavorn & Charoenrein 2006). Rice and tapioca starch was dissolved in 1M HCl to give a starch concentration of 40%. The slurry was heated in a water bath at 35ºC with continuous stirring for

1 to 4 h. The acid hydrolysis reaction was stopped by adding diluted NaOH to pH 6.5. The acid hydrolyzed starch was reacted with OSA in an aqueous solution (Thirathumthavorn & Charoenrein 2006). DS of the OSA modified tapioca and rice starch was 0.0137 and 0.0128 respectively. After acid hydrolysis, degree of polymerization (DP) of the starch decreased significantly.

Similar reagents such as dodecenyl succinic anhydride (DDSA) were reacted with WM or normal corn starch (Jeon et al., 1999; Chi et al., 2007). Jeon et al (1999) suggested that the best reaction condition of DDSA modification with WM starch was pH between 8.5 and 9.0, temperature at 23ºC, 5% anhydride concentration and 35% starch slurry solids. Increased anhydride concentration from 5 to 10% decreased the RE. The chain length of alkenyl succinic increased from 8 to 12 had negative effect on RE. It has also been found that reaction condition of 30% starch slurry solids with 10% DDSA (based on starch weight) at pH 8.5-9.0 and at temperature of 40°C was the most effective one for modification of normal corn starch. DS of the product was 0.0256 and RE was 42.7% (Chi et al., 2007).

The researches (Bhosale & Singhal 2006; Song, et al., 2006; Jeon, Viswanathan, Gross, 1999; Chi et al., 2007) on reaction conditions agreed with the patent of Caldwell and Wurzburg (1953), which suggested that the pH of suspension should be between 7 and 10 and reaction temperature was preferably around room temperature. Tessler and Billmers (1996) suggested that in preparation of starch esters it is essential to maintain the insolubility of the starch granules during chemical modification and purification in the industry. The typical restrictions of an aqueous process are a low substitution level (DS<0.2), low alkalinity, dilute NaOH to control pH and low reaction temperature.

In order to remove the undesired components of OS products, filtration or centrifugation was used for starch purification (Song et al., 2006; Bao et al., 2003; Chi et al., 2007). It has been reported that the starch-OSA derivative was washed with water and centrifuged, and then repeated for three times (Bhosale and Singhal 2006). Instead of centrifugation, repeatedly filtration was also used (Park, Chung, & Yoo, 2004). The solvents used for starch purification include water, 95% ethanol, acetone and 70% aqueous alcohol. However, no research has compared the efficiency of purification methods.

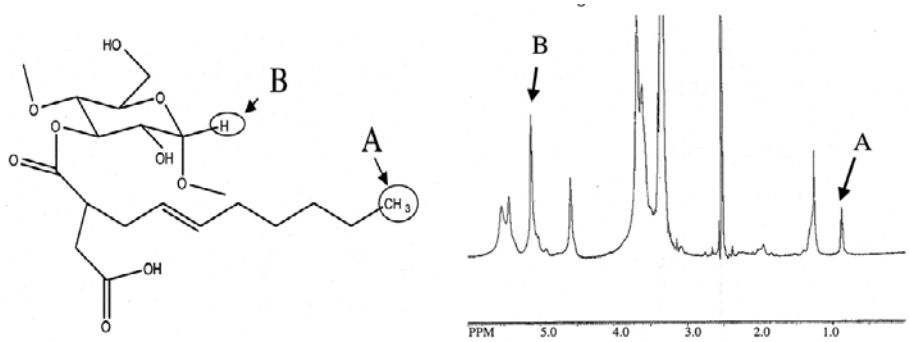
Determination of Composition and Structure of OS Starches

Determination of DS

DS is a measurement of the average number of hydroxyl groups on an AGU (Newton & Peckham 1950) and it is calculated by alkaline titration (Mattisson & Legendre 1952). In a titration method, the OS starch was hydrolyzed by HCl and the acid form of OS groups was titrated by NaOH. DS was calculated from the titration volume of NaOH. In addition, OS starch can be hydrolyzed by NaOH solution and the excess alkali was back titrated by HCl. The OSA content was calculated from the titration volume of HCl.

Recently, ^{1}H-NMR was also used to determine DS (Shih & Daigle 2003, Wang et al., 1997). OS starch was dissolved in D_2O (10% solids) and placed in a boiling water bath for 20min. Then the product was freeze-dried and the residue (80mg) was dissolved in 5ml DMSO-d_6 for NMR experiment (Nilsson & Bergenstahl 2007). Figure 1.5 shows a ^{1}H-NMR spectrum of an OS starch product (Shih & Daigle 2003). Peak A at 0.80–89 ppm was assigned to the OSA methyl protons and it was used to measure the presence of the OS group. Peak B at 5.10–5.26 ppm was from the starch equatorial proton, which was used as the standard reference (Shih & Daigle 2003). Similar research was done on starch succinates (Wang et al., 1997). The signal at 2.5-2.7 ppm was from succinic anhydride. The equatorial proton of starch (5.4ppm) was used as an internal standard. The results of NMR were consistent with titration method.

Figure 1.5 The Structure of OS Starch and a ^{1}H-NMR Spectrum of the OS Starch Derivative (DS=0.070)



(Reprinted from Shih & Daigle 2003)

Determination of Total and Free OS Content

Few studies emphasized the total and free (unreacted) OS content. Free OS groups may remain in the product after reaction. Gas chromatography (GC)-mass spectrometry (MS) was used to quantify contents of total and free OSA of commercial OS starches (Park & Goins 1995). For free OS content, starch was removed from aqueous suspension by precipitation with methanol and the acidified supernatant was extracted. For total OS content, esterified OS starch was hydrolyzed first in an alkaline solution and was extracted by acidification (pH <2). The analysis of five batches of lipophilic modified starch showed that most OS group was in the esterified form represented by low levels of free OS (0.15-0.24% w/w) and the total OS level (2.10-2.41% w/w) was less than the regulatory limit of 3%. Although this approach was able to characterize the OSA content in the starch sample, the process was complicated and time consuming. A fast and efficient method to calculate free OS content needs to be developed.

Distribution of OS groups on Starch Granules and Starch Molecules

It has been reported that OSA modification did not change the crystalline structure of starch (Song et al., 2006; Shogren 2000; He et al., 2006; Chi et al., 2007). X-ray powder diffraction was used to investigate OS starch with DS of 0.11 and 0.08 as well as unmodified WM starch. All the spectra showed the native A-type crystalline pattern, indicating that reaction of WM starches with OSA in aqueous suspension did not change the crystalline structure of starch up to the DS of 0.11 (Song et al., 2006). It has been found that chemical substitutions mainly present in the amorphous domains (Van der Burgt et al., 1999). Because amylose is located in the amorphous domains and therefore is better accessible to esterification (He et al., 2006). Similarly, the DDSA modification did not change the crystalline structure of corn starch (Chi et al., 2007).

It has been suggested that most of the starch granules were accessible to and reactive with OS groups in a slurry system (Shogren 2000). Methods such as light micrographs, backscattered electron imaging and anion-exchange chromatograph have been used to analyze OSA modified WM starch and native WM starch. Shogren (2000) found that OSA groups were uniformly distributed on a cross section of starch granules, and most of the starch granules could be reacted with OSA. The substitution of OS groups was heterogeneous at the branch level, and the concentration of OSA groups on the immediate surface of the OS starch granules was about 3–4

times than that of the bulk (Shogren, 2000). OS starch was analyzed by scanning electron microscopy (SEM) (Song et al., 2006). Compared to native starch, OS starches showed pores on the surfaces of starch granules, suggesting that reaction may first take place on the surface and form pores there.

Physical and Chemical Properties of OS starch

Pasting and Thermal Properties

Physical properties such as pasting and thermal properties are important for the starch applications in industry. The pasting property of OS starch was studied by Rapid Visco-Analyzer (RVA) and the viscosity of OSA modified rice, tapioca, wheat, potato and corn starch has been reported (Bao et al., 2003; Song et al., 2006). OSA modified starch has significantly higher viscosity than native starch, regardless of the botanical sources. As OSA content increased, the paste viscosity increased (Bao et al., 2003; Song et al., 2006). Even when the DP of OSA modified starch was reduced to the half by acid treatment, the paste viscosity was still higher than acid treated native starch (Thirathumthavorn & Charoenrein 2006). The amylose content of rice starch has negative effect on major RVA profiles, such as peak viscosity and hot paste viscosity (He et al., 2006).

The gelatinization and retrogradation properties of OS starch were evaluated by differential scanning calorimeters (DSC) (Bao et al., 2003; Bhosale & Singhal, 2007, Thirathumthavorn & Charoenrein 2006). The gelatinization temperature of OS starch depended on the plant origin and DS. Rice, wheat and potato starches were modified with different weight percentages of OSA (Bao et al., 2003). OS rice starch had significantly lower gelatinization temperature even at very low DS (0.018) and the enthalpy required for gelatinization was lower than native rice starch. For OS wheat starch, gelatinization temperature was higher than native wheat starch when DS was 0.0225. Increasing DS to 0.043 did not significantly affect gelatinization temperature. For OS potato starch, gelatinization temperature was higher than that of native starch even DS was as high as 0.0397. However, the enthalpy for OS starch was lower than that for native starch (Bao et al., 2003). For WM starch, the range of gelatinization decreased with an increase in the concentration of OSA used for modification. Amaranth starch did not show particular trends in gelatinization temperature range as DS reached 0.0203 (Bhosale & Singhal 2007). The thermal properties of acid treated OS rice and tapioca starch were studied.

DS of OS rice and tapioca starch was 0.0137 and 0.0128, respectively. OSA modification decreased the onset temperature of both rice starch and tapioca starch, but the enthalpy and conclusion temperature did not decrease significantly for tapioca starch (Thirathumthavorn & Charoenrein 2006). The thermal properties depended on the degree of perfection of crystallites in the starch granules. Gelatinization enthalpies reflected the degree of crystallinity (Eliasson & Gudmunsson 1996). Because the hydrophobic groups of OSA reagent weakened the hydrogen bonding, as well as enhanced the structure flexibility, gelatinization temperature and enthalpy decreased (Bao 2003).

Nutritional Properties

OSA modification on starch effectively increased slowly digested starch (SDS) and resistant starch (RS) (Han & BeMiller, 2007). It has been reported that OS starches had a high percentage of RS (waxy corn, 22%; normal corn, 29%; potato, 33%; and tapioca, 28%) and a high content of SDS (normal corn, 25%; waxy corn, 36%). Dry heating (130 °C) could increase the SDS content and decrease RS content of OS waxy corn starch. The method of combining esterification with OSA and dry heating was suggested to effectively produce SDS from various kinds of starches. SDS can be produced from waxy corn starch, normal corn starch, tapioca starch and potato starch (Han & BeMiller 2007). OS starch was also human tested to compare the glycemic response with glucose (Wolf et al., 2001). It has been concluded that starch substitution with OSA attenuated the postprandial glycemic excursion compared to an equivalent glucose challenge and was well tolerated by fasting healthy adult subjects.

Because of its lower relative glycemic response, OS starch may serve as a carbohydrate source in a medical nutritional product developed for people with diabetes. An acute challenge of 25 g of OSA modified starch was well tolerated (i.e., no clinically significant gastrointestinal discomfort) by fasting healthy adults. The nutritional use of OSA-substituted starch should attenuate the postprandial glycemic response and may decrease the caloric density of food containing it (Wolf et al., 2001).

Emulsion Properties

OS starch functions as an effective emulsifier because it is able to absorb at the interface of water and oil, and cover the oil droplet rapidly to prevent recoalescence of the newly formed droplets (Nilsson & Bergenstahl 2007). It has been found that the presence of a hydrophobic

group in the OS starch molecule allows it to emulsify oil phases due to hydrophobic-hydrophobic interactions. The glucose parts of OS starch bind to the water molecules, while the lipophilic octenyl succinic parts bind to the oil molecules. In this way, complete separation of the oil and water phases is prevented (Murphy, 2000). The emulsification activity was not improved as DS of the OS starch increased. But the sample from organic media showed better emulsification activity at higher concentration level than the ones from aqueous media (Viswanathan 1999). OS starch can generate very high surface load (10mg/m^2). It was explained as an interaction between kinetic factors during the formation of the emulsion and the physical chemical properties of the hydrophobically modified starch, such as DS, molar mass and radius (Nilsson, & Bergenstahl 2007).

Industrial Applications

OS starch can be used to produce oil-in-water emulsions as the sole emulsifier. The applications include beverage emulsions, flavors, clouding agents, salad dressings, creams, fragrances, emulsion paints, latices, coatings and adhesives (Trubiano 1986).

OS starch with a DS of 0.03-0.07 can be used to absorb ions in water (Cu^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+}) (Kweon et al., 2001). The adsorption capacity of ionic starches reached an equilibrium state within 5-10 min for starch dispersion in metal solutions. OS starch can effectively bind Pb^{2+} compared to the other metal ions such as Cu^{2+}, Zn^{2+} (Kweon et al., 2001).

The long hydrophobic carbon chain of OSA, with its accessible carbonyl groups, enabled a strong plasticization effect on the tested polymers. Due to the excellent mechanical properties (e.g. a tough film structure with considerable flexibility) and low permeability of the plasticized films, OSA is proved to be an ideal plasticizer particularly for ethyl cellulose based coatings (Tarvainen, 2003).

Introduction of NMR Spectroscopy

NMR is a spectroscopic technique which depends on the magnetic properties of atomic nuclei (Abraham, Fisher & Loftus 1988). Certain nuclei such as ^{1}H or ^{13}C possess a property known as nuclear spin. When a magnetic field is applied, the nuclear moments orient themselves with only certain allowed orientations, as considered a quantum mechanical system. ^{1}H or ^{13}C are the nuclei of the most interest in the study of starch products.

Transitions between these orientations are accompanied by the absorption or emission of radio waves of the appropriate frequency, which called the NMR effect. As the energy difference is directly proportional to the value of the applied magnetic field, the NMR resonance frequency is thus proportional to the strength of the applied magnetic field, and the actual frequency in a given field being different for different nuclei. Due to the circulation of electrons, which also act as tiny magnets, about the nuclei, the actual magnetic fields experienced at the nucleus is less than the full external magnetic field. The degree of shielding depends on the distribution of electrons, and thus is a function of the chemical bonding to and charge of the atom. The dispersion of resonance frequencies for a given nucleus is referred to as the "chemical shift". The NMR spectrum is presented normally as a plot of signal intensity vs. chemical shift.

The starch profiles of ^{1}H-NMR and ^{13}C-NMR were studied by many researchers (Xu & Seib 1997; Gidley 1985). Simple starch products like panose and glucose were characterized by ^{1}H-NMR and ^{13}C-NMR for better resolving the structure of carbohydrate (Mclntyre et al., 1990). The signals were characterized, and ^{1}H-NMR with high sensitivity is possible to determine the degree of polymerization and the degree of branching (Mclntyre et al., 1990).

Introduction of FT-IR Microspectroscopy

Infrared microspectroscopy combines the fields of infrared spectroscopy, microscopy and computer science (Wetzel, 1999). It does more than perform microanalysis on small samples; it allows spatially resolved localized chemical analysis *in situ* from a small portion of the microscopic field, there relating localized chemical analysis to the morphology (histology) of the specimen. Samples can be analyzed directly in air, at room temperature and pressure, wet or dry, without destroying the sample. The FT-IR microspectroscopy can be used to identify and determine the molecular chemistry in forensic science, material science, art restoration and the biological sciences (Wetzel, 1999).

In 1994, an indium antimonide (InSb) focal-plane array near infrared camera was optically interfaced to a step-scan FT-IR microspectrometer. This produced the first dedicated imaging instrument. More recently, a liquid nitrogen cooled Mercury-Cadium-Telluride (MCT) detector was coupled to a step-scan microspectrometer to provide the means of simultaneously obtaining spectra of 4,096 individual points (pixels). The advantage is that data from 4,096

pixels produces a detail image for interpretation and enough spectra for statistical application (Wetzel 1995).

The pioneering investigation of carbohydrates with classical infrared spectroscopy dates back to the fifties. Early progress of the techniques in the carbohydrate field was summarized by Mathlouthi and Koenig (1986) elucidating the structures of mono and oligosaccharides.

FT-IR spectroscopy was used to study the succination of starch (Fang et al., 2004; Song et al., 2006; Chi et al., 2007). The method of preparing starch sample was described by Fang et al. (2004). Native and modified starches were ground to powder and dried at 100°C for 24h. The fine powder sample was then mixed with dry potassium bromide (KBr) with a 1:100 ratio in an oscillating stainless steel capsule containing a stainless steel ball. The finely ground mixture was then transferred to a pellet presser to produce a 8.5mm disk and analyzed by FT-IR spectroscopy (Fang et al., 2004).

Structure of starch succinate was characterized by FT-IR spectroscopy (Kacurakova et al., 1998; Goheen & Wool 1991; Fang et al., 2004). The absorption bands at 1155 cm^{-1}, 1080 cm^{-1}, 1021 cm^{-1} and 930 cm^{-1} were attributed to C-O stretching vibration of native starch (Goheen & Wool 1991). The characteristic band at 1640 cm^{-1} from H-O-H deformation related to the tightly bound water in the starch (Kacurakova et al., 1998). The band at 2928cm^{-1} was from the C-H stretching vibration and the broad band at 3390cm^{-1} was due to hydroxyl groups. After OSA modification, a band at 1724cm^{-1} appeared and that band attributed to the C=O stretch of carbonyl group from OSA or DDSA. Another band at 1572cm^{-1} was attributed to asymmetric stretching vibration of carboxylate $RCOO^-$. Additionally, the C-H stretching vibration centered on 2926 cm^{-1} was increased in intensity from the CH contribute to the carbon chain of the ester (Fang et al., 2004). From the spectrum evidence, it was concluded that OSA or DDSA had been reacted into the starch backbone. The spatial resolution available with modern FT-IR microspectroscopy enables the chemical microstructure to be revealed. At each location for which a specimen is collected the relation population of these bands is determined. Localization of the modified sites is the ultimate goal.

Objectives

Based on literature review, it is not clear how OSA reactes with different physical forms of starch, and how the structure of OSA modified starches relate to their emulsion properties. The objectives of this study were to 1) study the reaction condition for preparing high DS OS starches from granular starch and non-granular starch. Reaction parameters such as OSA concentration, physical forms of starch, OSA addition rates, pH, and effect of Na_2SO_4 were examined. 2) determine the structure of OS starches and the location of OS group on AGUs of starch. 3) investigate the reaction homogeneity of OSA modification among granules and distribution of OS group in each single granule. Our ultimate goal is to establish the structural and functional relation of OSA modified starches.

CHAPTER 2 - Study the Reaction of OSA Modification

Materials and Methods

Materials

Octenyl succinic anhydride (OSA) was obtained from Cambrex Company (GBC Pasadena, TX). Waxy maize (WM) starch (Amoica TF) was a gift from National Starch and Chemical Company (Bridgewater, NJ). Maltodextrin (MALTRIN M100) was obtained by Grain Processing Corporation (Muscatine, IA). α-amylase (Liquozyme SC DS) was from Novozymes (Franklinton, NC). Low-e microscope slide was purchased from Tienta Sciences (Indianapolis, IN). The other chemicals used in the study were analytical grade.

Methods

Preparation of OS Waxy Maize Starch and OS Microporous Waxy Maize Starch

WM starch (100 g, dry weight) was suspended in 150mL water at room temperature, and the slurry was mixed homogeneously by an overhead stirrer. The pH of the slurry was adjusted to 7.0 with 3% (w/w) NaOH solution. 3% to 50% (based on starch weight) OSA was added by a burette. pH was controlled at 7.5, 8.5 or 9.5 by a pH controller (Model 501-3400, Barnant Co.). After pH was stable for 30 min and no further consumption of NaOH, reaction was ceased by adjusting pH to 6.0 with 1.0 M HCl. Starch was recovered by filtration, and washed by 300 mL of water, dried for 48 h, and ground by an analytical mill (A-10, Tekmar). Then 10 g of starch was washed by methanol (40 mL) to remove free OS groups. For comparison, reaction without pH control (pH~3.0) was also carried out. NaOH was not added to the slurry, and other procedures were made the same way as described above.

The method of adding OSA reagent was studied. 3% OSA based on starch weight was added by pouring within 30 sec or dropping wise for 2 h. pH was maintained at 7.5. The other procedures were made the same way as described above.

WM starch was treated in the acid condition before OSA modification (Billmers et al., 1997). WM starch (100 g) was mixed with water (150 mL) by a Waring blender (Model 31BL92, Dynamics Corporation of America) under a low speed for about one minute. pH was adjusted to

2.0 by adding 0.1 M HCl. OSA (3.0 g) was added to the slurry and mixed with starch slurry at a high speed for one minute. Then the pH was brought up to 7.5 and maintained by adding 3% (w/w) NaOH during the reaction. After the pH was stable for 30 min and no further consumption of NaOH, reaction was ceased by adjusting the pH to 6.0 with 1.0 M HCl. Starch was recovered by filtration, and washed by 300 mL of water, dried for 48 h, and ground by an analytical mill (A-10, Tekmar).

Microporous starch was prepared by Yijun Sang in the Department of Grain Science and Industry at Kansas State University. WM starch (35 g) was suspended in 65 mL acetate buffer (0.01 M) including $CaCl_2$ (0.0005 M) at pH 5.0. Spirizyme (Novozyme, NC) (30 µL) and Liquozyme (2.8 µL) were added to the slurry. The starch slurry was heated in a 45 ºC water bath for 120 min. The residue was filtered, washed by water and air dried.

Preparation of OS Maltodextrin

Maltodextrin (MALTRIN M100) **(**100 g dry weight) was dissolved in water to prepare a solution of 30%, 40% or 50% slurry solids. The pH of the solution was adjusted to 7.5, 8.5, or 9.5 using 3% (w/w) NaOH. 3% or 50% OSA based on the dry weight of maltodextrin was added in the slurry in 30 seconds. pH was maintained by a pH controller (Model 501-3400, Barnant Co.) during reaction with 3% (w/w) NaOH, and reaction was terminated after pH was stable for 30 min. The pH of the solution was adjusted to 6.0 with 1.0M HCl. The reacted starch solution was frozen in a dry ice-acetone bath and freeze-dried. Recovered OSA maltodextrin was washed by four-fold volume methanol, air dried, and ground by an analytical mill.

Titration

OS starch (5.00 g dry weight) was suspended in 20.0 mL of 0.100 M HCl, and stirred for 30 min. The suspension was filtered through a piece of No. 2 filter paper (Whatman International Ltd.), and the residue was washed with water until no Cl^- could be detected by 0.1 M $AgNO_3$ solution. Starch was re-suspended in 300 mL water, and heated in a boiling water bath for 20 min. The starch solution was titrated with 0.100 M NaOH solution, using phenolphthalein as an indicator. Native WM starch was titrated as a control. % OSA, DS and RE were calculated from following equations.

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

Where % OSA is the percentage weight 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.

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

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

Where %OSA added to the starch was 3%

Nuclear Magnetic Resonance (NMR) Spectroscopy

OS starch (2-3 g), sodium acetate (0.3-0.6 g) and α-amylase (Liquozyme SC DS) (10 μL) were mixed in 30 mL water. The slurry was heated in 85 ºC water bath with shaking for 2 h, and then placed in a boiling water bath for 30 min. After cooling to room temperature, the α-amylase hydrolyzed starch residue was recovered by freeze-drying. In order to remove the interference of water, 0.2 g residue was dissolved in 1 mL D_2O, freeze dried and repeated once. The residue (0.05 g) was dissolved in D_2O (0.50 mL) for NMR experiment.

The OSA maltodextrin sample for NMR was prepared by dissolving OS maltodextrin (0.2-0.3 g) in D_2O (1 mL), freeze dried. The residue (0.05 g) was dissolved in D_2O (0.50 mL) for NMR experiment.

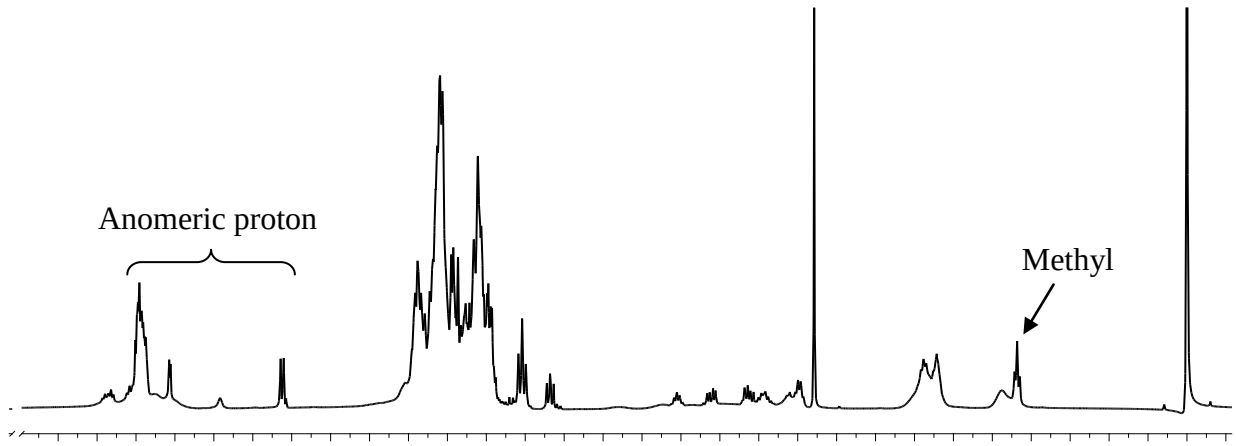
The NMR spectra were recorded on a 11.75 Tesla Varian NMR system spectrometer at 25 ºC (Biomolecular NMR facility Kansas State University). The NMR spectrometer is equipped with a 3 mm diameter triple resonance inverse detection pulse field gradient probe operating at 499.848 MHz for ^1H. For ^1H, spectra presaturation technique was used when suppression of the water signal was necessary. The spectra are the accumulation of 128 individual scans and sodium 3-(trimethylsulyl) - propionate-2,2,3-d_4 (TMS) was used as a reference (0 ppm). Chemical shifts were reported in ppm.

DS was calculated by the equation below.

$$DS=\frac{I_{methyl} \div 3}{I_{anomeric\ \ proton}}$$

I_{methyl} is integrated signals of the NMR peak from methyl group

$I_{anomeric\ proton}$ is integrated signals of the NMR peaks from anomeric proton



Light Microscopy

Starch slurry (1% slurry solids) was dropped on a microscope slide and covered by a microscope cover glass (Φ=18 mm, Fisher Sci.). Starch granules were observed by an optical microscope with a digital camera (Model BX51, Olympus) under normal visible light and polarized light.

Statistical Analysis

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

Results and Discussion

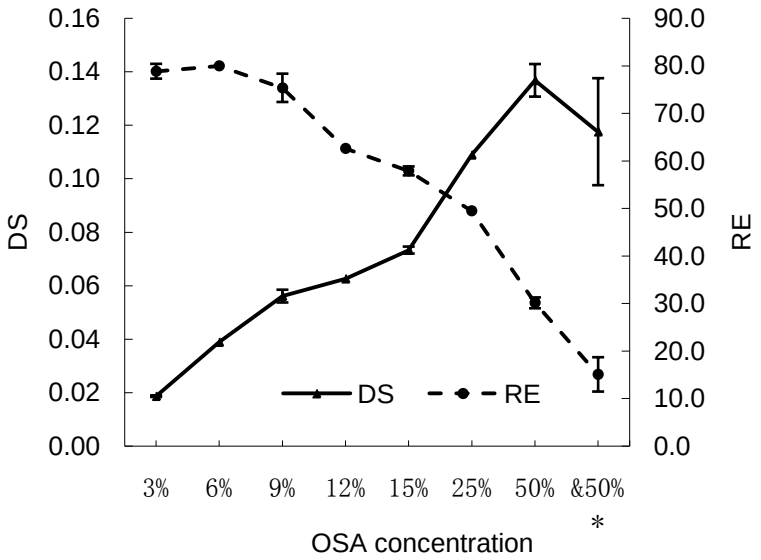
OSA Modification on Waxy Maize Starch

Effect of OSA to Starch Ratio

OSA reaction was carried out with different OSA /starch ratios. The starch concentration was 40% and pH was controlled at 7.5. As OSA increased from 3% to 50% based on the weight of starch, DS increased from 0.019 to 0.14. However, RE decreased significantly from 80% to 28% (Figure 2.1). OS starch with DS of 0.14 was redispersed in water and reacted second time with 50% OSA, but DS did not increase. These results indicated that only a limited amount of OSA can be reacted with WM starch, and the maximum DS of OSA modified WM starch was 0.12. It has been suggested that OS groups occurred in the amorphous region of starch rather than crystalline region (Song et al., 2006). The maximum DS in WM starch was restricted. On

the other hand, the hydroxyl groups on AGUs in starch may have different chemical activities. The location of OS groups on AGUs in starch will be discussed in Chapter 3.

Figure 2.1 Effect of OSA Concentration on Degree of Substitution and Reaction Efficiency of OSA Modification on WM Starch. Reaction pH was 7.5, starch concentration was 40%.



*OS starch (DS=0.14) reacted with 50% OSA (w/w)

Effect of Sodium Sulfate on OS Starch Swelling

Starch granules started swelling when the weight percentage of OSA by starch was higher than 9%. 15% OSA modified starch granules of different times were viewed under an optical microscopy. Figure 2.2 shows that starch granules started swelling and losing their Maltese cross at reaction time of 2 h. Significant amount of swelled starch granules were observed as reaction time reached 4 h. As reaction time increased, the number of swelled granules increased. Disappearance of Maltese cross of starch granules is an evidence of losing molecular order. It has been suggested that by introducing bulk groups into the backbone, the structural flexibility of starch was enhanced, and perfection of crystallites was reduced by OSA modification (Lawal, 2004; Miller et al., 1991; Eliasson & Gudmunsson, 1996). Our results agreed with these suggestions.

In the industry production of starch esters, it is preferred to use simple equipment and water as the only solvent. So that maintaining the insolubility of the starch granules during the chemical modification and purification is very important (Tessler & Billmer 1996). In order to prevent granules from swelling, sodium sulfate (Na_2SO_4) was added to the starch slurry. Figure

2.3 presents the effect of Na_2SO_4 on the 15% OSA modification of a 4 h reaction time. Adding 5% and 15% Na_2SO_4 (based on starch weight) inhibited starch granules from swelling, and did not affect the reaction efficiency (Figure 2.4). Since using less Na_2SO_4 would be more environmentally friendly, 5% Na_2SO_4 is preferred for the future processes.

Figure 2.2 Photomicrographs of WM Starch Granules Reacted with 15% OSA without Na_2SO_4. Reaction time was 1h (a), 2h (b), 4h (c). Reaction pH was 7.5, starch concentration was 40%.

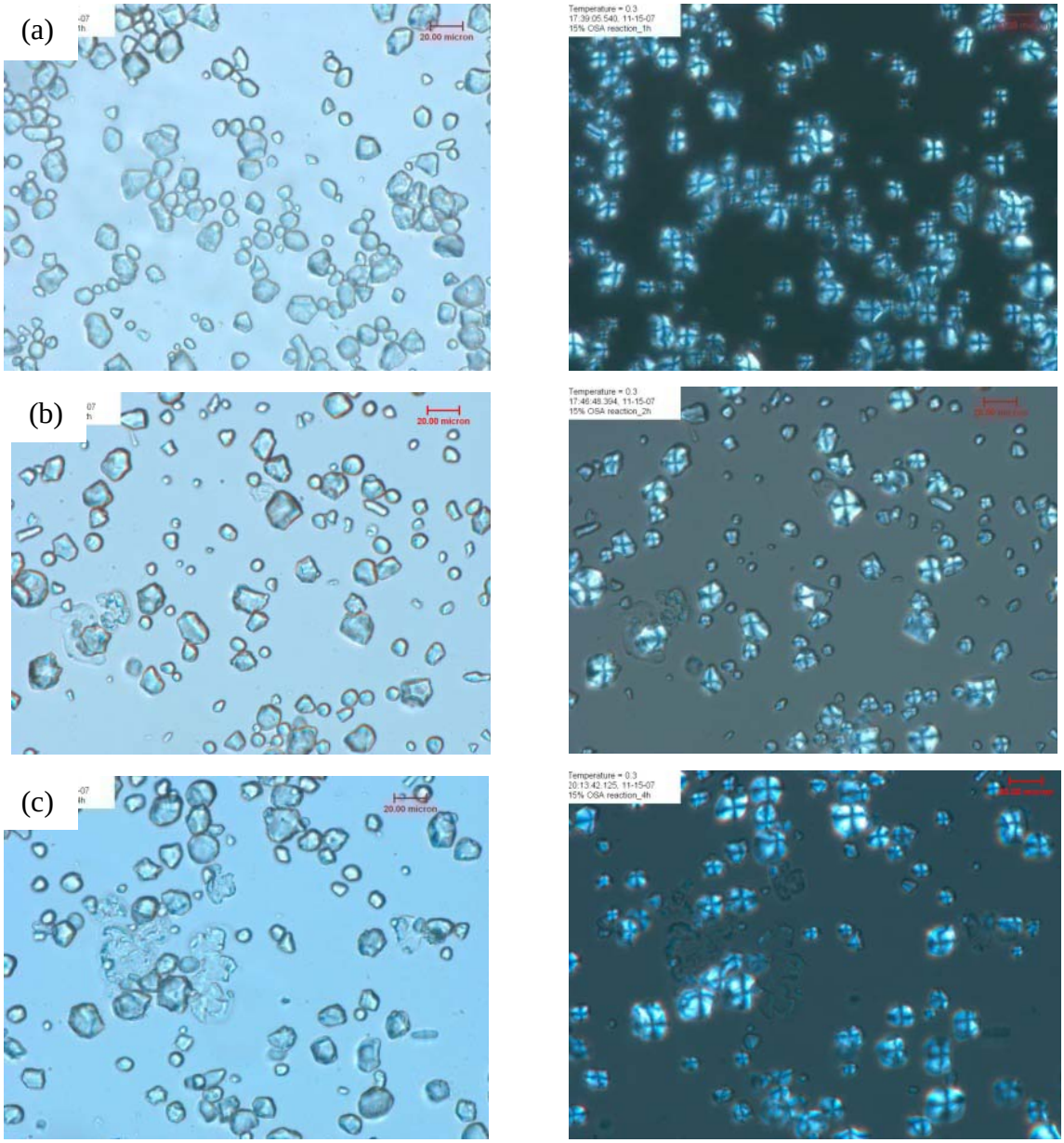


Figure 2.3 Photomicrographs of WM Starch Granules Reacted with 15% OSA with Na_2SO_4. Reaction time was 4h. (a) 5% Na_2SO_4, (b) 15% Na_2SO_4. Reaction pH was 7.5, starch concentration was 40%.

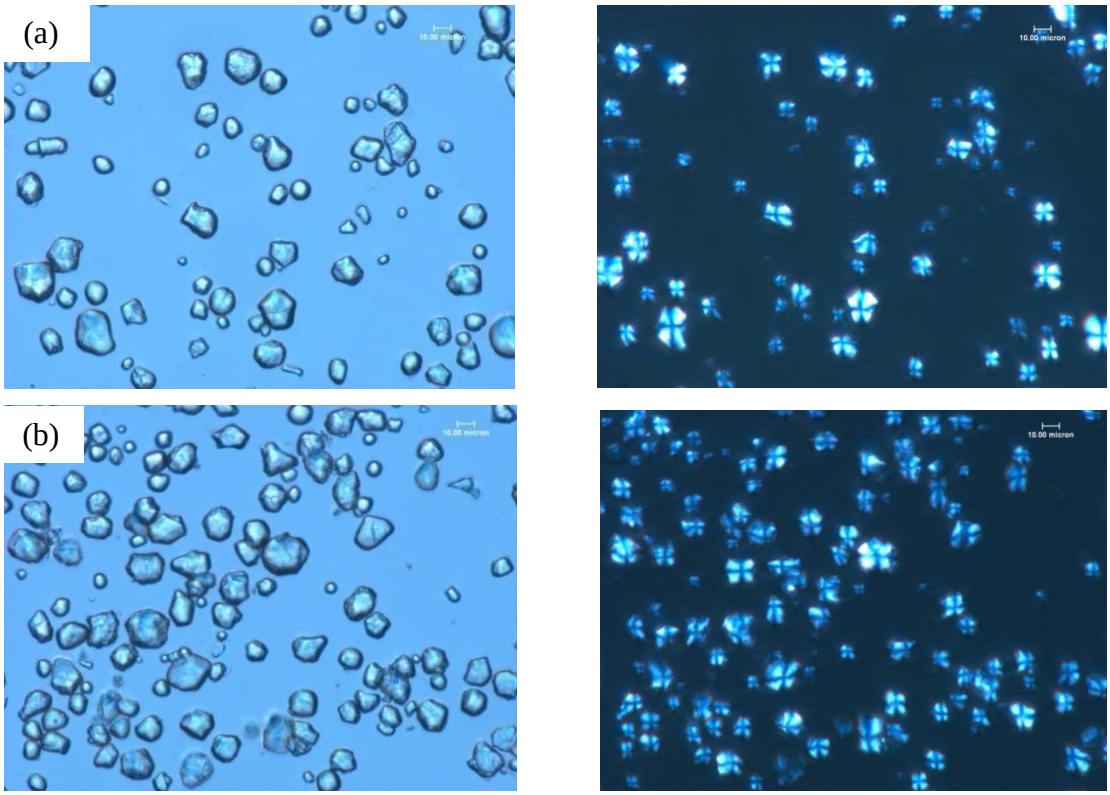
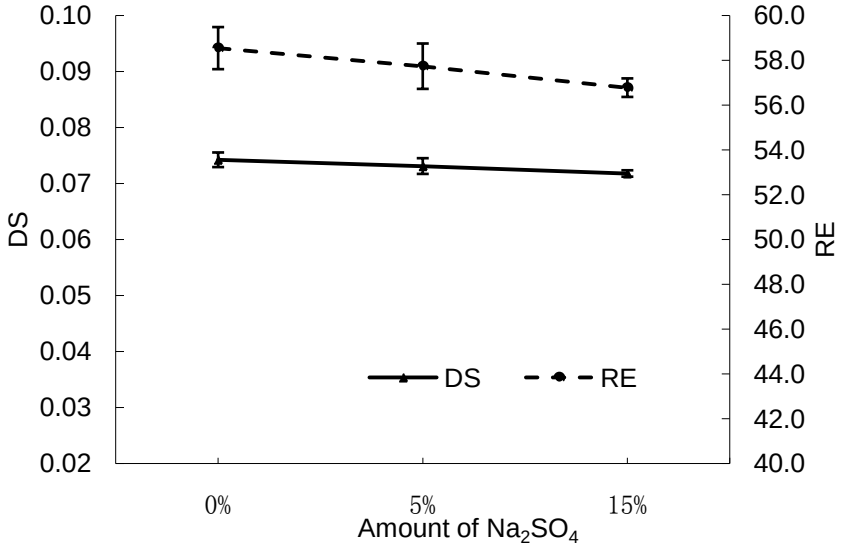


Figure 2.4 Effect of Amount of Na_2SO_4 on Degree of Substitution and Reaction Efficiency of 15% OSA Modification on WM Starch

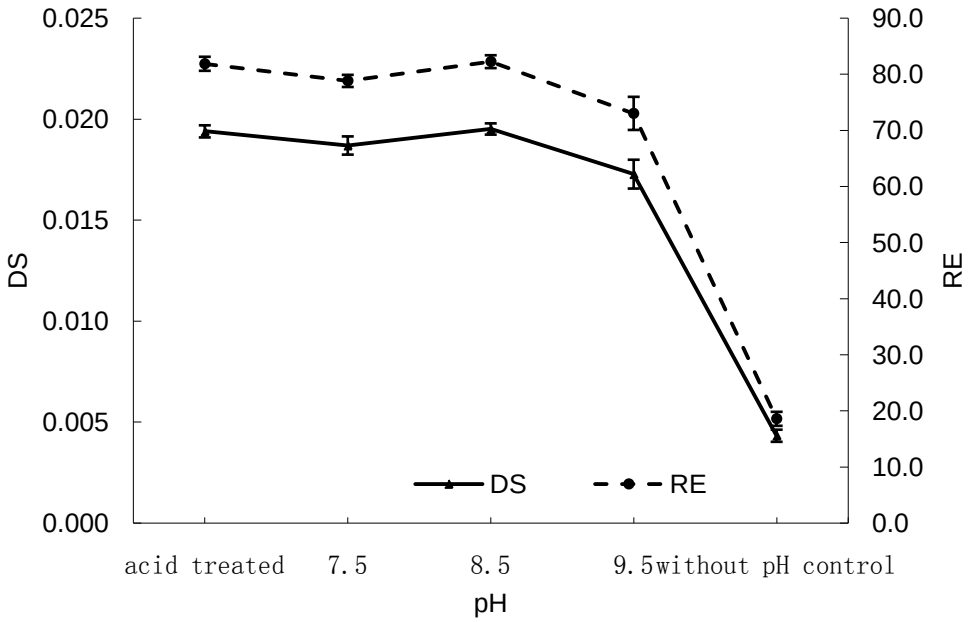


Effect of Reaction pH

OSA modification was compared in both acid and alkaline conditions, and the results were summarized in Figure 2.5. In the acid condition (without pH control), DS was 0.005 and RE was 18%. DS and RE increased to 0.019 and 80%, respectively, when pH was controlled at 7.5, Similarly, DS and RE reached 0.020 and 82% when pH was 8.5. However, DS and RE decreased as pH increased to 9.5 (Figure 2.5). These results indicated the optimum pH range for OSA modification of WM starch was from 7.5 to 8.5 for preparing starch esters. It has been known that starch granule is held together by hydrogen bonding, high alkalinity could disrupt the hydrogen bonding which may cause the swelling of starch granules (Tessler & Billmer, 1996). Therefore, low alkalinity was preferred.

A patent had been filed on improving OSA reaction efficiency by sufficiently mixing OSA with starch in acid condition (Billmers et al., 1997). However, DS and RE were not significantly improved when we repeated the experiment as described.

Figure 2.5 Effect of pH on Degree of Substitution and Reaction Efficiency of 3% OSA Modification of WM Starch. OSA/starch ratio was 3% and starch concentration was 40%.

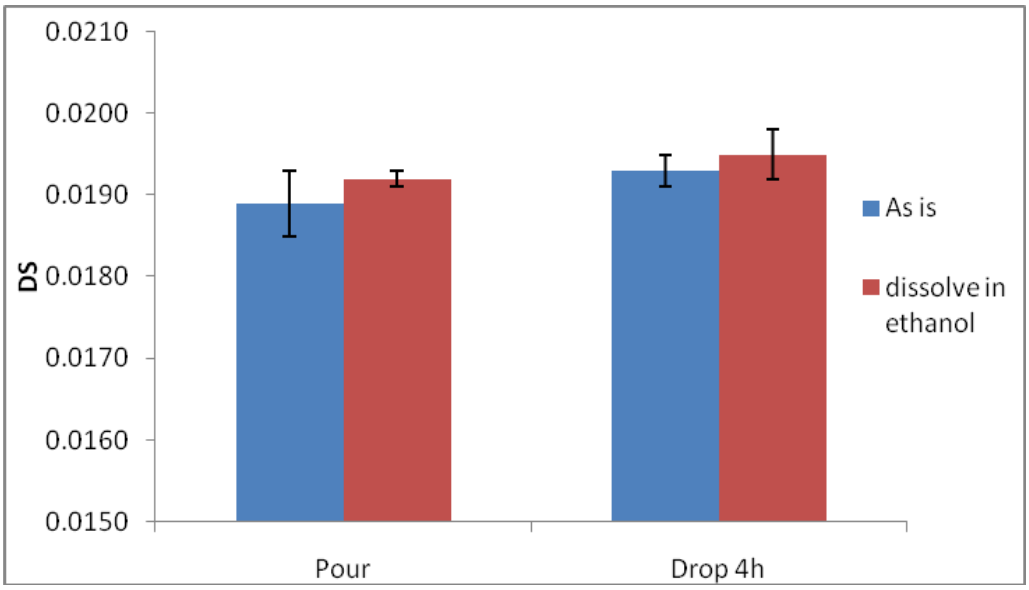


Effect of Addition Rate and Pre-dissolving of OSA in Ethanol

OSA reagent as received or dissolved in 20 mL ethanol was poured or added drop wise into a starch slurry. It was thought that slowly adding OSA and ethanol-dissolved OSA may improve the mixing of OSA in starch slurry, thus increasing the OSA and starch interaction.

Results showed that slowly adding OSA and ethanol-dissolved OSA did not significantly improve the DS (Figure 2.6). Similarly, the addition rate of OSA did not seem to affect the DS.

Figure 2.6 Effect of OSA Addition Rate and Pre-dissolving of OSA in Ethanol on Degree of Substitution of OSA modified WM Starch. OSA Concentration was 3%, Reaction pH at 7.5 and Starch Concentration of 40%.



Bound and Free OS Content of OS Starch Products

Total and free OS content of OSA modified WM starch were measured by titration and ^{1}H-NMR methods. Since OS groups are soluble in methanol, free OS groups can be removed from OS starch by methanol washing. The DS of this starch was from the bound OS groups. Free OS content can be calculated by substracting the total OS content by bound OS content.

Titration and ^{1}H-NMR methods were compared by analyzing total OSA of 3% and 15% OSA modified starches. Results in Table 2.1 show that the two methods gave very consistent resutls, indicating that DS of OS starch can be accurately measured by both methods.

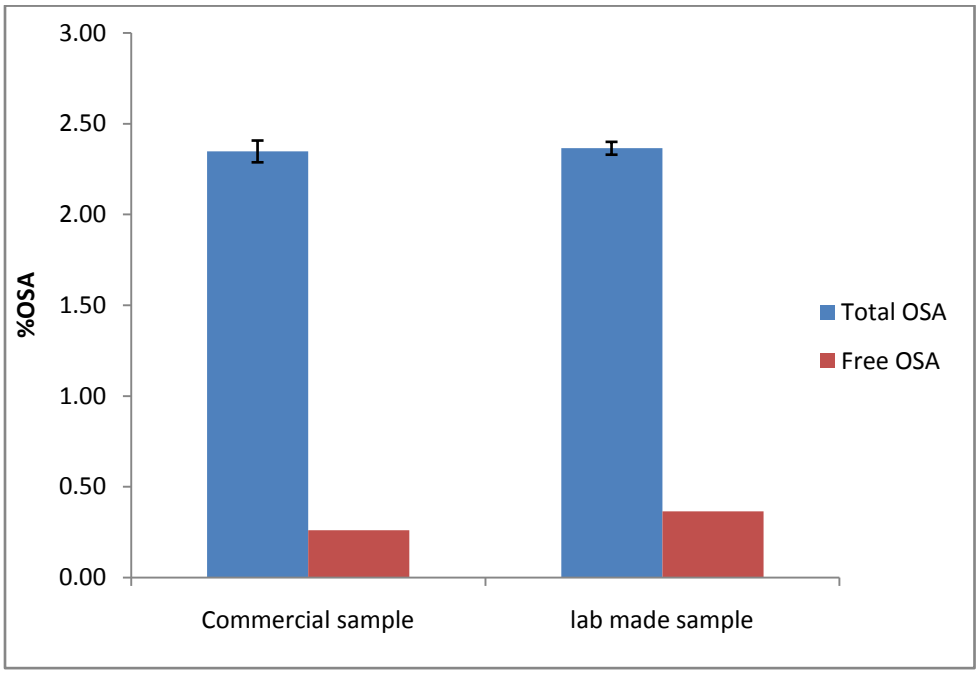
Table 2.1 Total OS Content of OS Starch Calculated by Titration and ^{1}H-NMR Methods

Starch	Bound OSA content (DS)	
	Titration	^{1}H-NMR
3% OSA	0.0187±0.001	0.0187±0.002
15% OSA	0.0733±0.001	0.0775±0.002

Total and free OS content of lab made OSA modified WM starch (DS=0.019) and a commercial sample were compared (Figure 2.7). The lab made OS starch had the same amount of bound OSA as the commercial sample. However, the free OSA content was slightly higher

than the commercial sample, which is probably due to the inefficient washing and purifying in the lab process.

Figure 2.7 Total OSA and Free OS Content of Commercial and Lab Made Products



Free OS content of 15% OSA modified starch was analyzed by ^{1}H-NMR. Table 2.2 shows that the total %OSA by starch weight of 15% OSA modified starch was 9.25%. The free %OSA based on the weight of starch was 0.57% .

Table 2.2 Total and Free OSA Content of 15% OSA Modified Starch

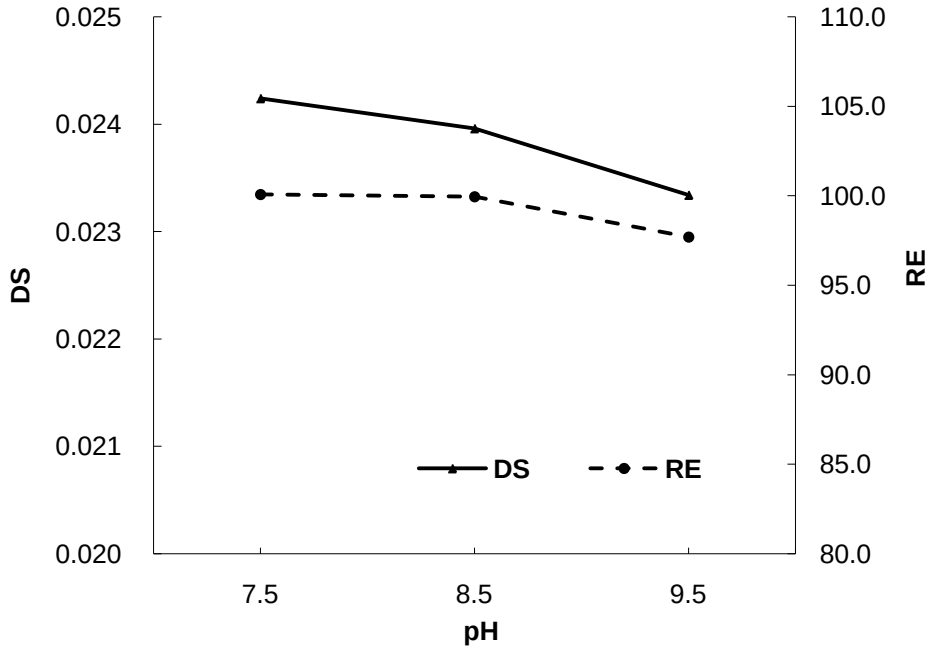
OS starch product	OS content	
	DS	**%OSA**
15% OS starch washed by methanol	0.072	8.68
15% OS starch without washing by methanol	0.079	9.25
Free OS	0.007	0.57

OSA Modification on Soluble Maltodextrin

Effect of pH

OSA modification on soluble maltodextrin was carried out under different pH values. Figure 2.8 shows that, RE reached 100% when pH was controlled at 7.5 and 8.5. RE slightly decreased when pH was 9.5. This indicates that pH at 7.5 to 8.5 was preferred for OSA modification on maltodextrin.

Figure 2.8 Effect of pH on Degree of Substitution and Reaction Efficiency of 3% OSA Modification on Maltodextrin. OSA concentration was 3%, maltodextrin concentration was 40%.



Effect of Slurry Solids

The effect of maltodextrin slurry solids on OSA modification was investigated. Maltodextrin was dissolved in water to prepare a solution with 30%, 40% and 50% slurry solids. Figure 2.9 shows that DS increased as the slurry solids increased, and RE was 100% when the maltodextrin slurry solids was 40% or 50%, Therefore,, 40% to 50% maltodextrin slurry solids was preferred for the modification.

Figure 2.9 Effect of Maltodextrin Slurry Solids on Degree of Substitution and Reaction Efficiency of OSA Modification. OSA Concentration of 3%, pH at 8.5

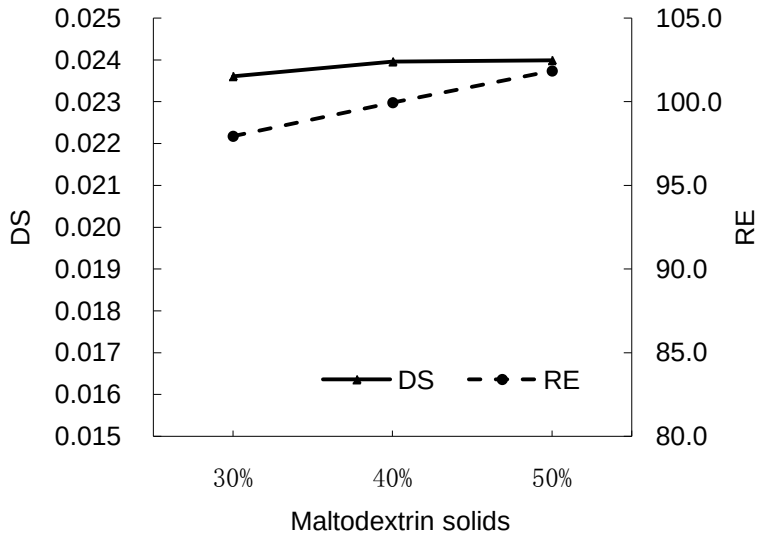


Figure 2.10 compares the DS and RE of OSA reaction with WM starch, microporous WM starch and maltodextrin. The reaction occured in 40% slurry solids and 3% OSA at pH of 7.5 for 2.5 h. The results show that OS maltodextrin had the highest DS and RE among the three products. OSA reagent completely reacted with maltodextrin (RE = 100%) and DS was 0.024. For WM starch and microporous WM starch, the RE was 74% and 70%, and DS was 0.019 and 0.016, respectively. These results indicate that modified WM starch had slightly higher OSA content than modified microporous starch.

WM starch and maltodextrin were also reacted with 50% OSA. Figure 2.11 shows that the OS maltodextrin had significantly higher DS and RE than OSA modified WM starch. DS of OSA modified WM starch was 0.14, which was the highest we could produce from the slurry method. In contrast, DS of OS maltodextrin was 0.27 and RE was 59% under the same reaction condition. However, 0.27 may not be the maximum DS for maltodextrin.

Starch granules have amorphous and crystalline regions. It has been reported that chemical reactions mostly occured in the amorphous regions and the OSA content on the surface of OS starch granules was 3 to 4 time higher than that of the bulk (Shogren et al., 2000). Reaction may first start on the surface and then reach the inner starch granules (Song et al., 2006). For microporous starch, starch was digested by enzyme to create pin holes in the starch granules (Figure 2.12). The holes in starch granules did not improve the DS and RE. For

maltodextrin, RE reached 100% because of its non-crystalline structure in which all the starch molecules were soluble and available for reaction.

Figure 2.10 **Degree of Substitution and Reaction Efficiency of OSA Modification with WM Starch, Microporous WM Starch and Maltodextrin. pH at 7.5, OSA/starch Ratio of 3% and Starch Slurry Solids of 40%.**

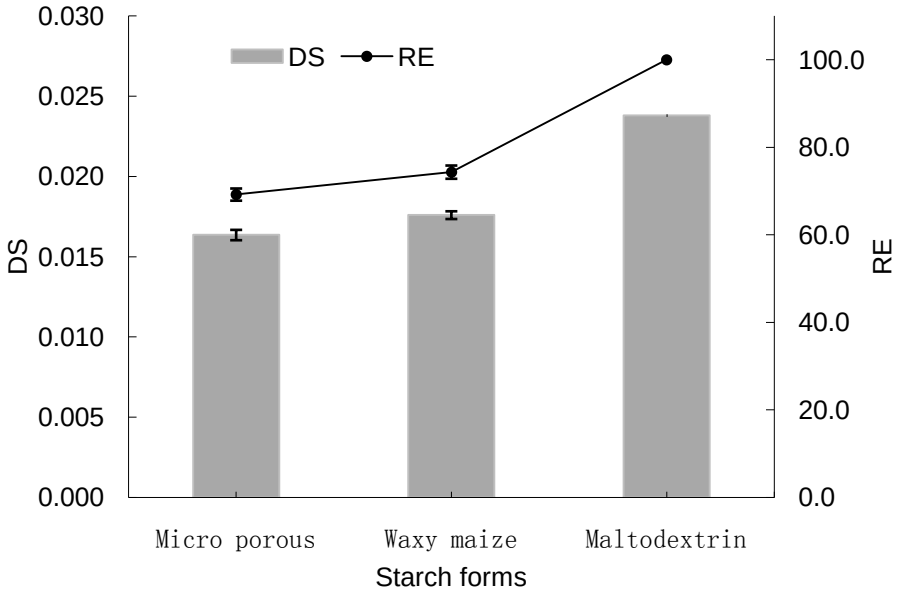


Figure 2.11 **Degree of Substitution of OS Starch and Maltodextrin. pH at 7.5, OSA/starch Ratio of 50% and Slurry Solids of 40%**

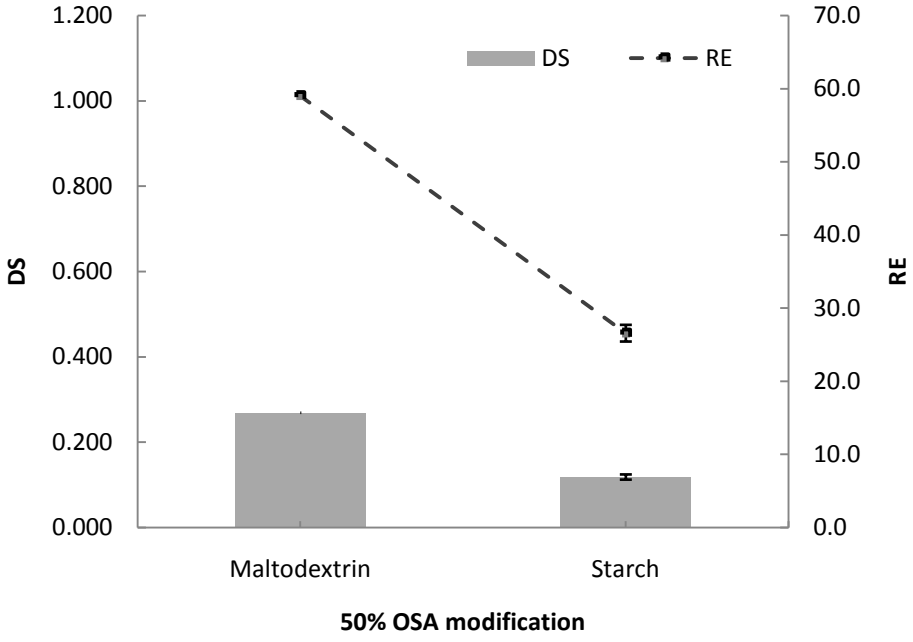


Figure 2.12 SEM Images of (a) WM Starch and (b) Microporous WM Starch

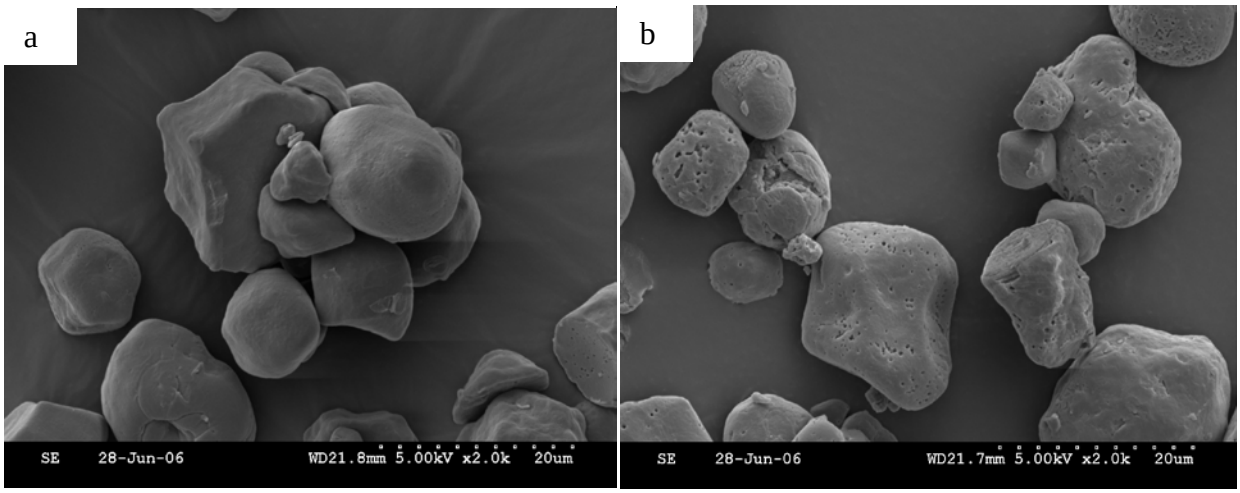
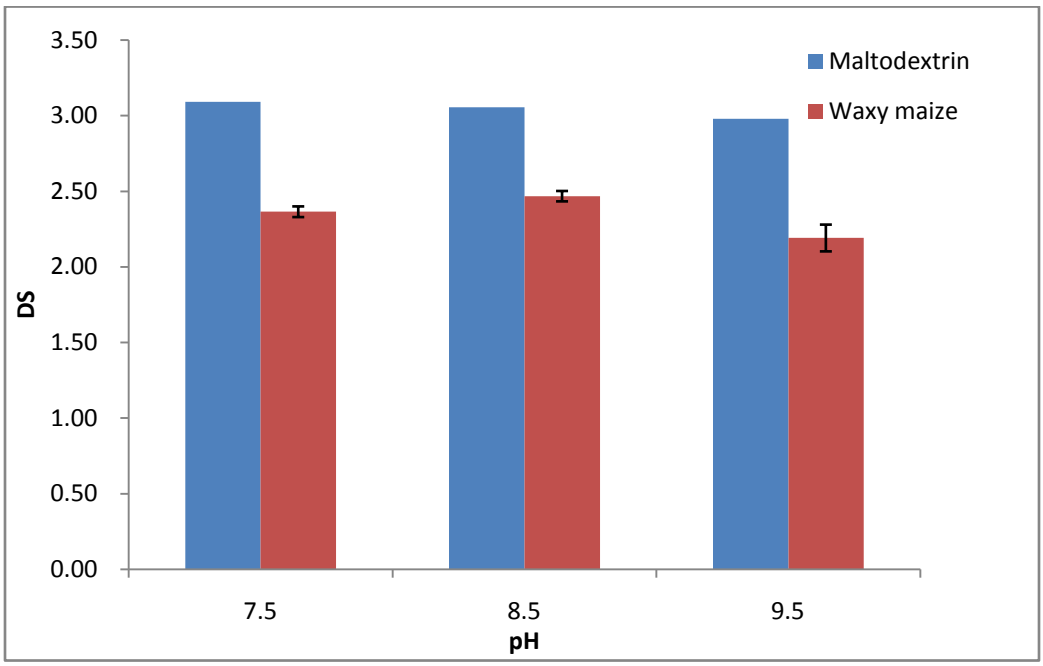


Figure 2.13 shows the effect of pH on the DS and RE of OSA modification with WM starch and maltodextrin. The optimum pH for OSA modification on WM starch and maltodextrin was from 7.5 to 8.5. Higher pH at 9.5 was not preferred for the reaction.

Figure 2.13 **Degree of Substitution of OSA Modification with WM Starch and Maltodextrin at different pH. OSA/starch Ratio of 3% and Slurry Solids of 40%**



CHAPTER 3 - Structure of OS Waxy Maize Starch and Maltodextrin

Material and Methods

In this chapter, the structures of OSA modified WM starches (DS = 0.019, 0.051, 0.073 and 0.12) and OSA modified maltodextrin(DS = 0.024 and 0.27) were investigated by NMR spectroscopy. The NMR spectra were recorded on a 11.75 Tesla Varian NMR spectrometer at 25 ºC. The NMR spectrometer was equipped with a 3 mm diameter triple resonance inverse detection pulse field gradient probe operating at 499.95 MHz for ^{1}H. For ^{1}H spectra, presaturation technique was used when suppression of the water signal was necessary. The spectra were an accumulation of 128 individual scans. For ^{13}C experiment, the spectrometer was operated at 125.68 MHz. Spectra were collected with an accumulation of 6000 scans. Distortionless enhancement by polarization transfer (DEPT) experiments were performed to assist the interpretation of the ^{13}C spectra. The pulse sequence used was a part of "Bio-pack" provided by the vendor Varian. Heteronuclear Single Quantum Coherance (HSQC) ^{1}H-^{13}C 2D experiments were also conducted using the pulse sequence included in "Bio-pack", with 512 transients and 16 scan per transient. Sodium 3-(trimethylsulyl) - propionate-2,2,3-d_4 (TMS) was used as a reference (0 ppm). Chemical shifts were reported in ppm.

Results and Discussion

Structure of OS Waxy Maize Starch and Maltodextrin

OSA modified WM starches and native WM starch were digested by α-amylase. The α-limit dextrin of the starch was analyzed by ^{13}C-NMR (Figure 3.1). In agreement with the literature data (Mclntyre et al.,1990), the C-6 of starch AGU was assigned to the signal at 63.29 ppm. The multiplets from 70.0 ppm to 80.0 ppm were from C-2, C-3, C-4, and C-5. The resonance at 71.99 ppm was assigned to C-4 at non-reducing end, the other signals from 70.0ppm to 80.0ppm were C-2, 3, 5. Because of α and β forms of reducing ends, C- 2, 3 and 5 positions showed additional signals. They overlapped with each other, and were not well resolved in 1-D NMR spectra. The carbons of α- and β-forms of reducing ends were well

resolved, and gave signals at 98.59 ppm and 94.54 ppm, respectively. Signal at 102.64 ppm was from internal anomeric carbon.

The 15% OSA modified WM starch showed several additional signals that were clearly resolved. With the assist of DEPT experiment (Figure 3.2), the resonances were assigned. As shown in Figuture 3.2, the signal at16.26 ppm was the carbon with three protons attached to it. So it was from the methyl carbon of OS group. Signals from 24.63 ppm to 42.81 ppm excluding the sodium acetate (25.93 ppm) were from the carbons with two protons attached to them. They were assignied to the methylene carbons of the OS side chain. The carbons linked by double bond had one proton attached to them, and they appeared at 130.21 ppm and 136.58 ppm. The resonances at 186.27 ppm and 188.19 ppm in Figure 3.1 were not shown in Figure 3.2. They were the carbons withouot proton, and they should from the carbonyl of OS group. All the additional signals comapred with native starch were from OS group, providing the evidence of OSA modification on WM starch.

Figure 3.1 Typical ^{13}C-NMR Spectra of the α-limit Dextrin of (a) Native WM Starch OSA Modified WM Starch, (b) DS=0.019, (c) DS=0.056, (d) DS=0.073 with Added Sodium Acetate.

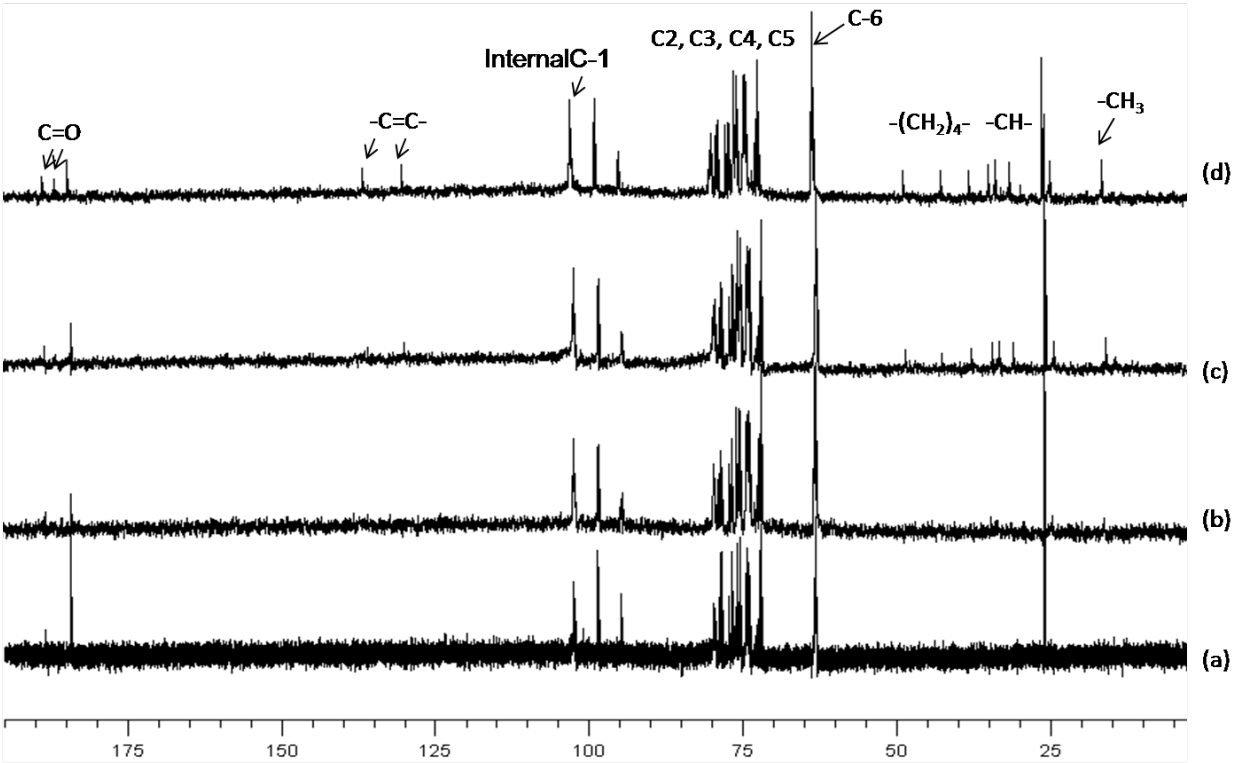


Figure 3.2 DEPT Spectra of OSA Modified WM Starch (DS=0.073) without Methanol Washing

Typical ^{1}H-NMR spectra of α-amylase digested OSA modified WM starch and native starch are shown in Figure 3.3. The signals from native WM starch were assigned based on the work of Gidley (1985). The signal at 1.91ppm was from methyl proton of added sodium acetate. The methane protons of AGUs excluding the aromatic proton gave signals from 3.20 ppm to 4.10 ppm. The peaks in this region were complex. It has been suggested that the signal at 3.43 ppm was the proton attached to C-4 of the non-reducing end, and the multiplet at 3.27 ppm was assigned to H-2 of β-reducing end (Mclntyre et al., 1990). The anomeric region in a ^{1}H-NMR spectrum of starch has been studied by Gidley (1985). The α- and β-forms of the reducing unit gave signals at 4.56 ppm and 5.16 ppm respectively. The singlet at 4.90 ppm was assigned to the anomeric proton at α-1,6 linkage and signal at 5.38 ppm was from the anomeric proton of internal α-1,4 linkage of glucosidic bond.

Compared to the native starch, the OSA modified WM starch had several additional signals (Figure 3.3). The intensities of the signals increased as DS increased. These signals were identified with the assist of ^{13}C-NMR and HSQC spectra (Figure 3.4). It is shown that, the peak at 0.87 ppm in ^{1}H-NMR was corresponding to the signal at 16.26 ppm in ^{13}C-NMR spectrum, which was due to the methyl protons of OS group. The peaks from 1.28 ppm to 2.62 ppm were from the methylene and methine protons of the side chain of OS group. There was a multiplet at

5.54 ppm, and a shoulder with multiple peaks around 5.4 ppm. These protons were attached to the carbons showing signals at 130.21 ppm and 136.58 ppm, and were from the protons of the double bond in OS group. The intensity of methyl group (0.87 ppm) was used to calculate the DS.

Figure 3.3 ^{1}H-NMR Spectra of the α-limit Dextrin of (a) Native WM starch and OSA Modified WM Starch, (b) DS=0.019, (c) DS=0.056, (d) DS=0.073 with Added Sodium Acetate

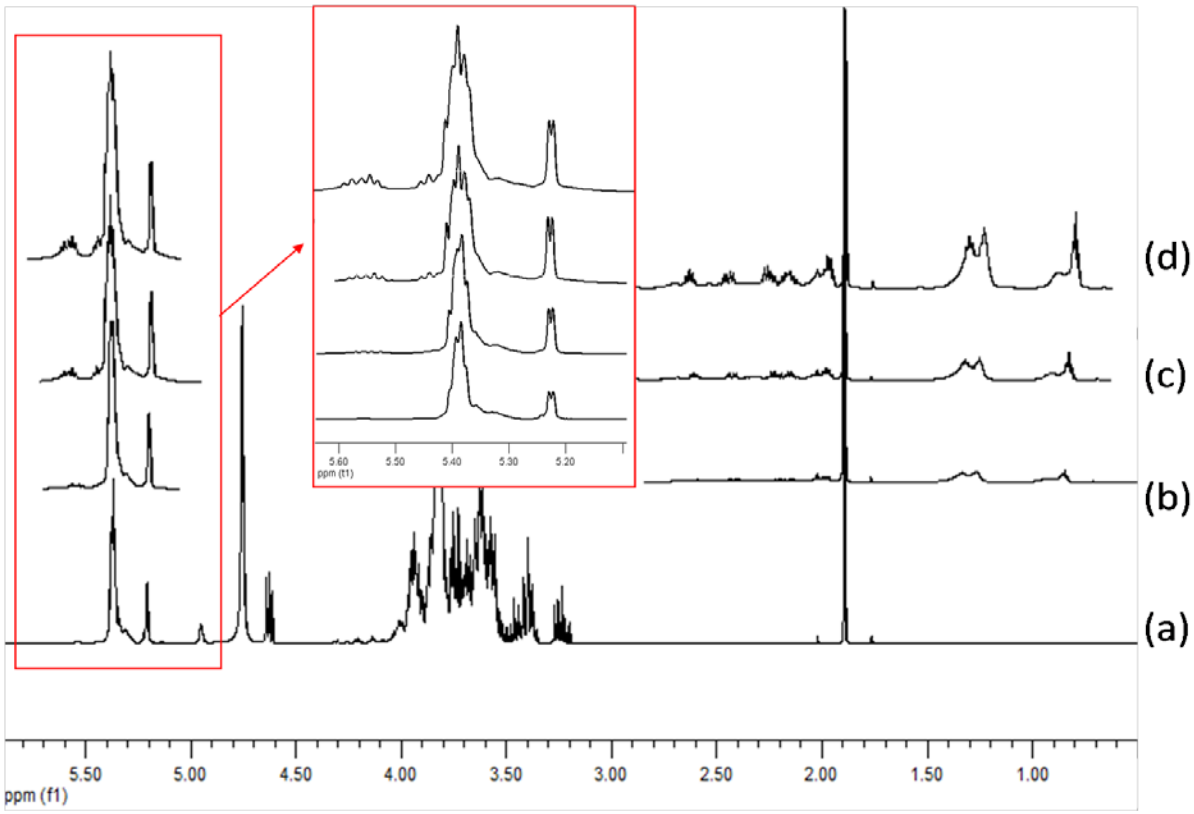
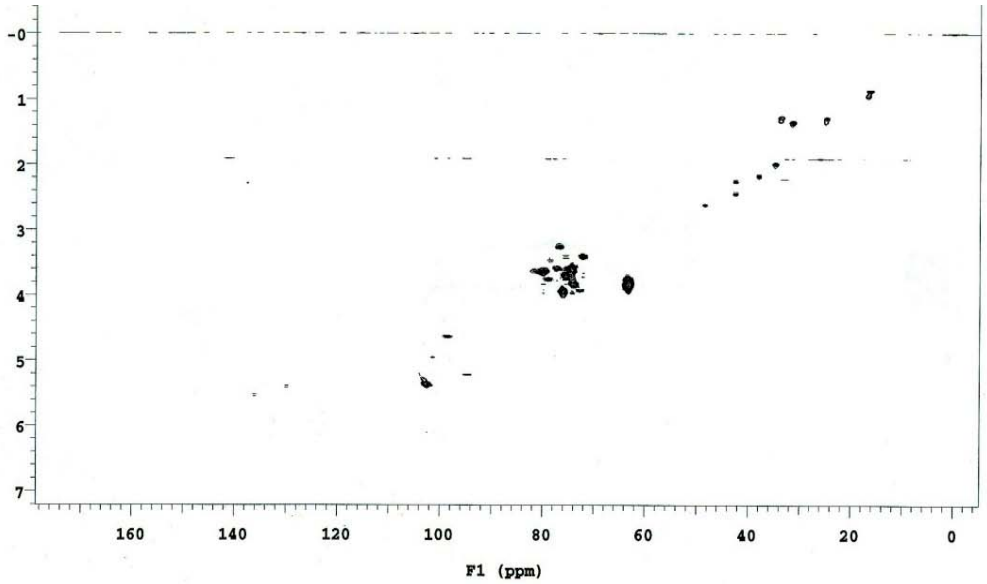


Figure 3.4 HSQC Spectrum of OSA Modified WM Starch (DS=0.073)



Maltodextrin before and after 3% OSA modification were analyzed by ^{13}C-NMR and ^{1}H-NMR (Figure 3.5 and Figure 3.6). The signals were similarly assigned as OSA modified WM starch. In the ^{13}C-NMR spectra (Figure 3.5), the signals from 94.0 ppm to 102.5 ppm were given by anomeric C-1. The singlet at 102.34 ppm was assigned to internal C-1. The signals at 98.49 ppm and 94.64 ppm were from the β-and α-forms of C-1 at reducing end, respectively. Due to the low DS of the OS maltodextrin, the signals from the OS group were hard to be detected.

Figure 3.6 shows ^{1}H NMR spectra of maltodextrin before and after 3% OSA modification. Signals from 4.5 ppm to 5.4 ppm were from anomeric proton, except the water peak at 4.67 ppm. The signal at 4.64 ppm was assigned to H-1 of β-form reducing end, whereas the singlet at 5.23 ppm was from α-form reducing end. The small peak at 4.96 ppm was the H-1 at α-1,6 branching point, and H-1 at α-1,4 internal linkage was characterized at 5.37 ppm. The signals for protons of H-2, H-3 and H-5 were overlapping with each other at the region of 3.19 ppm to 4.09 ppm. In agreement with the literature (Mclntyre et al 1990), the signal at 3.95 ppm was from H-3 of internal, and the peak at 3.84 ppm was the overlapping of internal H-3 and H-5, while the signal at 3.64 ppm was assigned to the combine of H-2 and H-4 internal. The resolved signal at 3.43 ppm was from H-4 internal and the multiplet at 3.27 ppm was assigned to H-2 of β-form reducing end. After OSA modification, the addition signals appeared in the 0.80 ppm to 3.00 ppm region and a shoulder attached to α-1,4 internal linkage signal. The shoulder at 5.56 ppm

was from protons attach to C=C of OS side chain. The doublet at 0.95 ppm was the methyl from OSA and it was used to calculate DS of OS maltodextrin.

Figure 3.5 ^{13}C-NMR Spectra of (a) Maltodextrin and (b) OS Maltodextrin (DS=0.024)

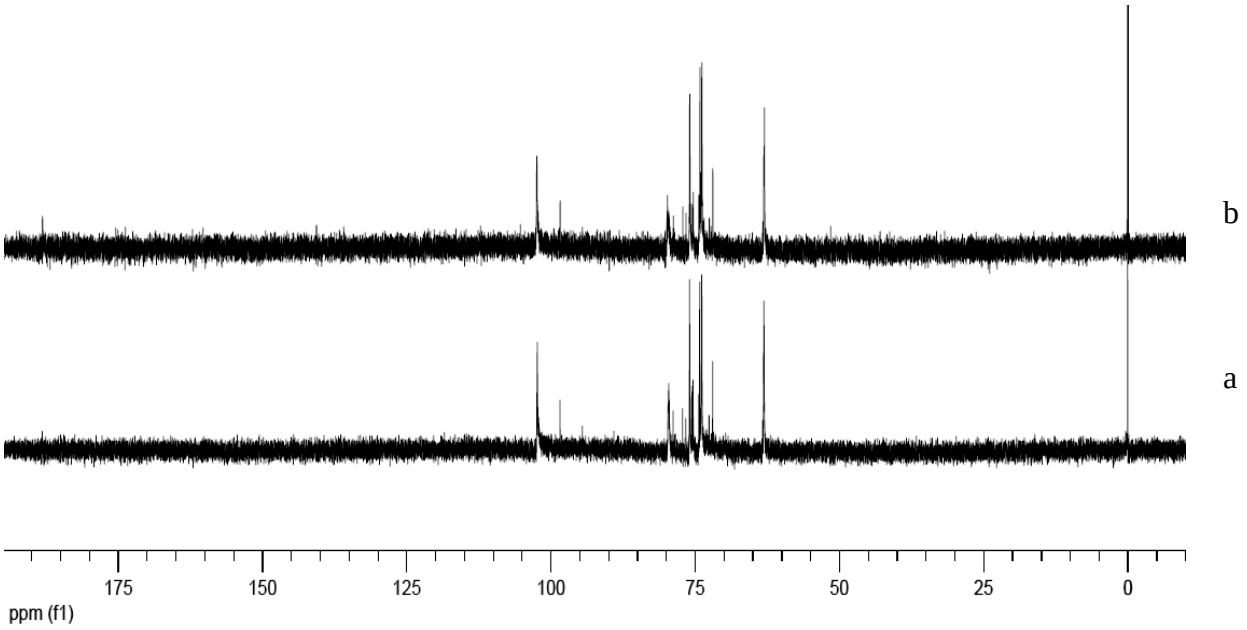
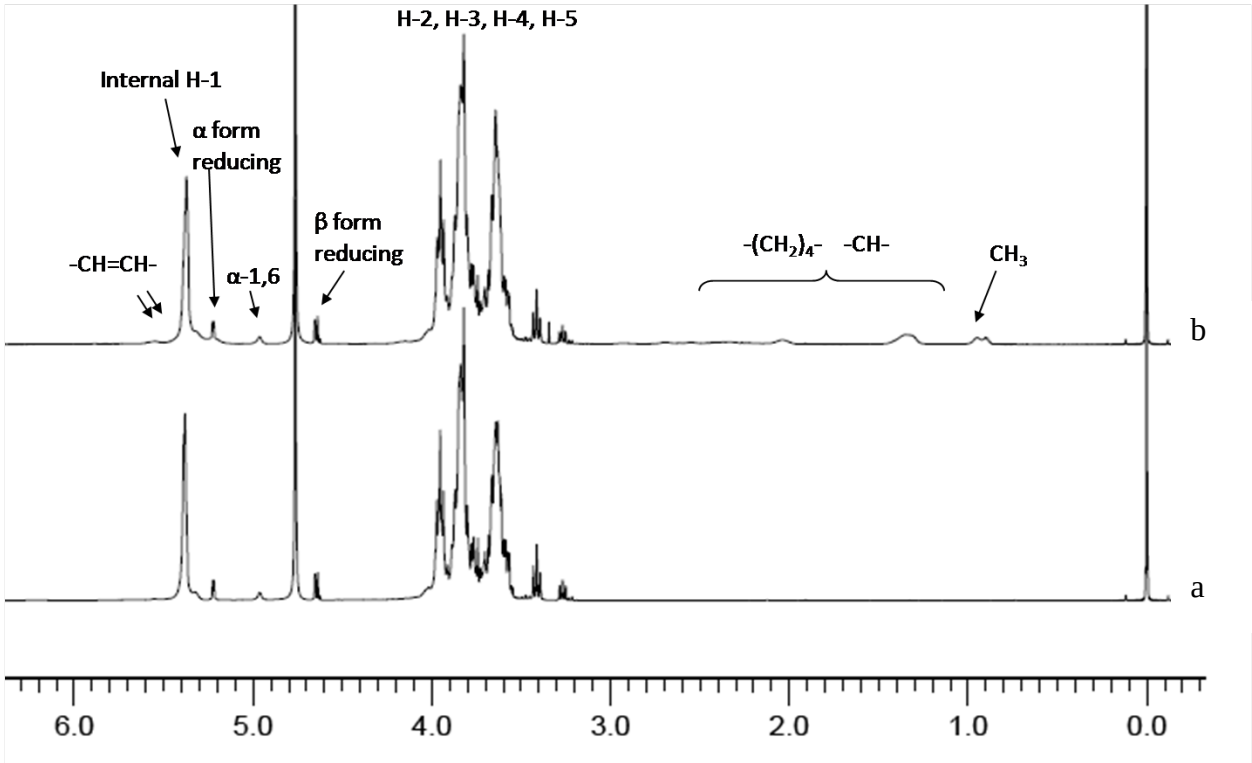


Figure 3.6 ^{1}H-NMR Spectra of Maltodextrin (a) and OS Maltodextrin (DS=0.024) (b)



The Substitution Position of OS Group on Waxy Maize Starch and Maltodextrin

In the AGUs of a starch molecule, there are three hydroxyl groups available for chemical modification. They are located at C-2, C-3 and C-6 positions. Hydroxyl groups at C-2 and C-6 are more active than C-3 because of their steric property. Substitution with the hydroxyl at C-2 position can change the chemical shift of internal C-1, whereas, substitution at O-6 can change the chemical shift of C-6.

^{13}C-NMR spectra of OSA modified WM starches were analyzed to study the substitution position of OS groups on starch molecules. Resonances of internal C-1 at 102.42 ppm and C-6 at 62.79 ppm were expanded for better resolution. Figure 3.7 shows that the internal C-1 of OSA modified WM starch had an additional shoulder at 102.25 ppm compared to native WM starch. The shoulder became more resolved as DS increased from 0.019 to 0.073, suggesting the substitution at O-2 position increased. However, the peak of C-6 did not show addition peak until DS of the OSA modified WM starch reached 0.073. These results suggested that the OS groups mostly substituted at O-2 position and started substituting at O-6 position when DS was over 0.073.

Substitutional position of OS groups on maltodextrin was studied by ^{13}C-NMR spectra of maltodextrin and OS maltodextrin (DS=0.024). Figure 3.8 shows expanded peaks at 100-104 ppm and 62-64 ppm and there were no additional signals revealed at both peaks. The absences of additional signals at both peaks might due to the incomplete dissolution of maltodextrin in water. A better resolved spectrum may be obtained after modifying the method of preparing OS maltodextrin samples for NMR experiment.

Figure 3.7 Expanded ^{13}C-NMR Spectra (100-104 ppm and 62-64 ppm) of (a) Native WM Starch, OSA Modified WM Starch with (b) DS=0.019, (c) DS=0.056 and (d) DS=0.073

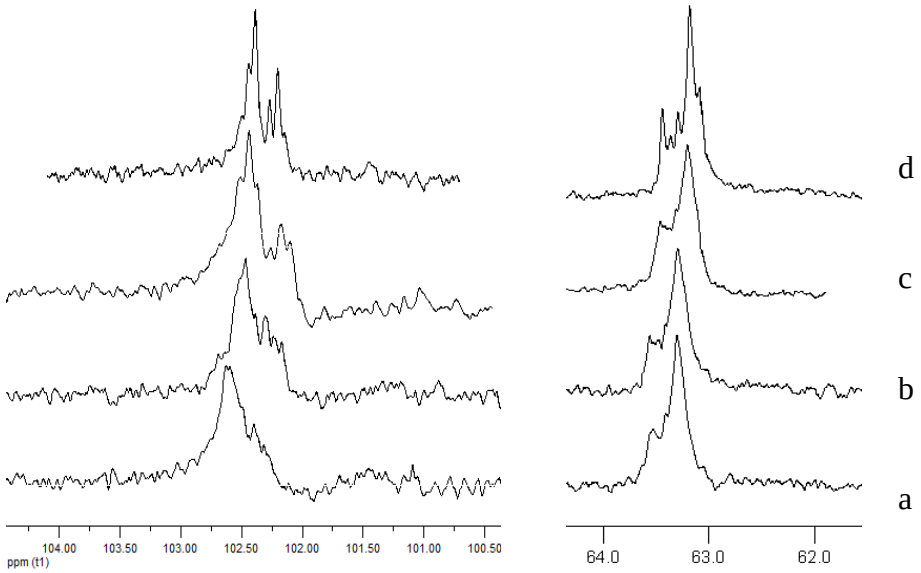
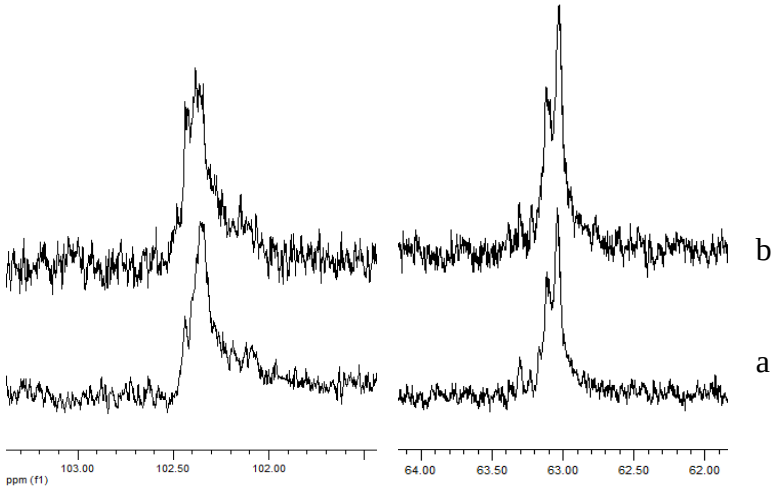


Figure 3.8 Expanded ^{13}C-NMR Spectra (100-104 ppm and 62-64 ppm) of (a) Maltodextrin and (b) OS Maltodextrin (DS=0.024)



Detect Unreacted OS Group of OS Waxy Maize Starch

15% OSA modified WM starch was washed by four-fold volume of methanol. Sample before and after methanol washing were analyzed by ^{1}H- and ^{13}C-NMR (Figure 3.9). It is shown that the integrated area of methyl peak (at 0.87 ppm) of OSA modified WM starch with

unreacted OS was slightly larger than that of OSA modified WM starch without un-reacted OS. However, the overall spectra profile was not changed.

^{13}C-NMR spectra in Figure 3.10 shows that OSA modified WM starch without free OS group had two additional peaks at 186.27 ppm and 188.19 ppm. They were assigned to the carbonyl from the unreacted OS groups.

Figure 3.9 ^{1}H-NMR Spectra of (a) 15% OSA Modified Starch with Unreacted OSA and (b) 15% OSA Modified Starch without Unreacted OSA

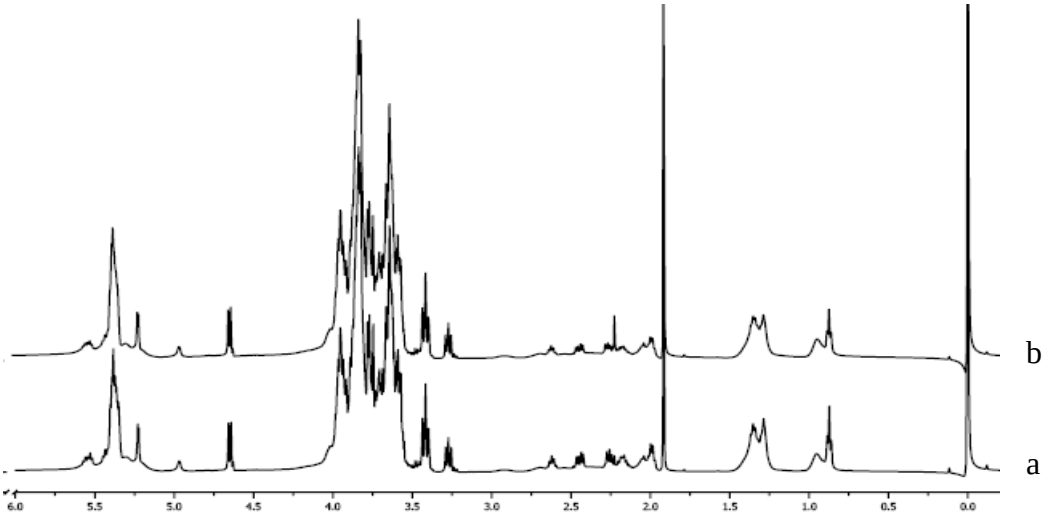
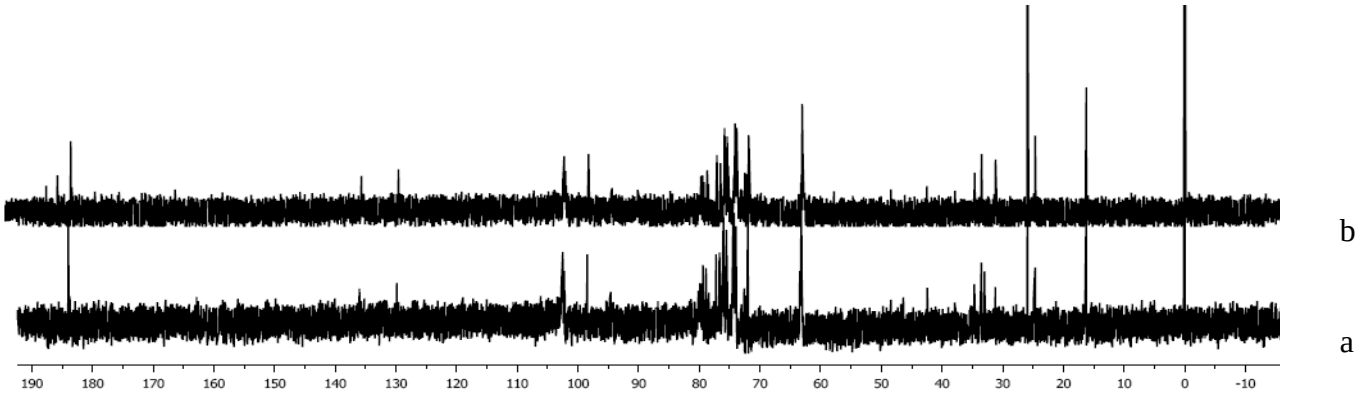


Figure 3.10 ^{13}C-NMR Spectra of (a) 15% OSA Modified Starch with Unreacted OSA and (b) 15% OSA Modified Starch without Unreacted OSA



CHAPTER 4 - Determination of Heterogeneity of OS Waxy Maize Starch by FT-IR Microspectroscopy

Materials and Methods

Waxy maize (WM) starch (Amoica TF) was obtained from National Starch and Chemical Company (Bridgewater, NJ). Laboratory prepared specimen of OSA modified WM starches had different DS including 0.018, 0.056, 0.073 and 0.11. A starch blend with a DS of 0.019 was prepared by mixing 3.00 g 15% OSA modified starch (DS=0.073) and 7.00 g native WM starch. 1 wt.% starch slurry was prepared by suspending 0.1 g of starch into 10 mL water. Aqueous starch slurry was droped on a reflecting microscope slide (Low-e, Indianapolis, IN) and dried in oven at 105 ºC for 2 h. After the excess water was removed, dried starch granules were flattened under the microscope by a stainless steel roller until they became transparent.

FT-IR Microspectroscopy

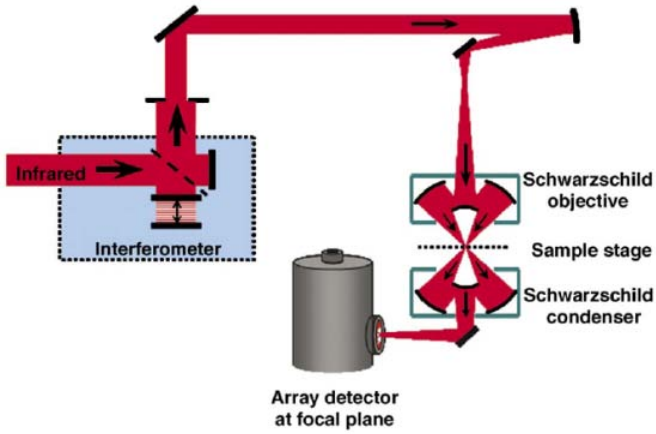
FT-IR spectra were acquired using a Perkin-Elmer® (Shelton, CT, USA) Spectrum™ Spotlight™ 300 imaging system. The instrument was equipped with a mercury cadmium telluride focal plane array (FPA) detector (16 elements) and a microprocessor controlled stage with software that enabled automated data acquisition.

The visible microscopy light video image of the sample was magnified. Multiple video images were viewed as a mosaic to produce a wide view. As the sample on the motorized stage moves, a series of spots on the sample were analyzed. Manual focus was maintained automatically during the experiments. Both infrared radiation and visible light were sent to the remote aperture by the sample cassegrain mirror lens (Koc 2007).

Data were collected from one point at a time with a single liquid nitrogen cooled MCT detector from the spot size determined by an imaging plane mask. The instrument designed for imaging was also operated under image mode with a 16 element linear MCT array that records spectra simultaneously in parallel. In image mode, an area of interest was selected to produce chemical images. Individual spectra were extracted from the points in the image for further

examination. Depending on the illumination optics, either a 25 μm × 25 μm or a 6.25 μm × 6.25 μm area of specimen on the stage was projected to each detector element.

Figure 4.1 Optical Schematic of Perkin Elmer® Spotlight™ (Shelton, CT) Microscope-spectrometer



Under the single point mode operator, each spectrum was acquired in reflection absorption mode at a spectral resolution of 8 cm^{-1} with 128 scans coadded. The image plane mask produced a 15 μm × 15 μm spot on the stage and the spectral range selected was 4000-800cm^{-1}. Each spectrum was referenced to a background spectrum acquired from a blank region of the infrared reflecting microscope slide. Water vapor and CO_2 contributions were removed by ratioing the sample spectrum to the background.

A microspectropic image of the specimen was obtained from an area of 100 μm × 150 μm using the linear "pushbroom" array of focal plane array FT-IR microspectrometer. The sample was mapped in the optical reflection mode but with a reflection absorption mechanism with infrared reflecting glass slide. A spectral resolution of 8 cm^{-1} was used with 128 scans coadded. The system enabled 4096 spectra to be acquired from a 100 μm × 150 μm region in approximately 20 min.

Data Processing and Analysis

The spectra were processed using the Spectrum (Version 5.3.1, PerkinElmer, Inc) software. Band ratios were calculated showing the relative carbonyl concentration. In order to account for thickness changes within the specimen, the baseline corrected integrated peak area of carbonyl band at 1723 cm^{-1} was ratioed to the carbohydrate area from 1150 cm^{-1} to 1017 cm^{-1}.

$$\text{Band ratio}=\frac{Area\ of\ carbonyl\ \ (1723\ cm^{-1})}{Area\ of\ carbo\ hydrate\ \ (1150\ to\ 1017\ cm^{-1})}\times 100$$

The image data were processed using the Spotlight (Version 1.1.0 B 38, PerkinElmer, Inc) software. Functional group images were procedued to show carbonyl concentration, which referred as chemical maps. Carbonyl concentration was determined from the integrated area of the carbonyl band at 1723 cm^{-1}. Chemical maps were showed using the false color scheme in spotlight, with red indicating the strongest absorbance and blue the lowest.

Mammalian or plant tissue specimens are routinely prepared as microtomed frozen section 1-8 µm thick for transmission measurement. In this experimental work the WM starch granule has a spherical shape with average diameter of 15 µm. Under a light microscope, individual starch granules appeared opaque in contrast to the plain glass microscope slide (Figure 4.2). One single granule as pointed in the photomicrograph was scanned by FT-IR microspectroscopy. However, the spectrum did not resemble starch (Figure 4.3). This can be explained by light loss from the lensing effect generated by the spherical starch granules. The residual signal detected by the instrument was too weak, and it was not able to show the chemical composition of the starch granules.

In order to reduce the lensing effect and sample thickness, starch granules were flatted by a stainless steel roller under a low power microscope. After flattening, the diameter of the starch granule increased to 50 µm and it became transparent under a light microscope (Figure 4.4). The spectrum from the flattened granule was recognized as starch with well resolved sharp bands (Figure 4.5). Spectra of flattened and unflattened starch granules were compared in Figure 4.6. The flattened granules were not completely destroyed and maintained their granule shape, so that they could be identified from each other and analyzed individually.

Figure 4.2 Photomicrograph of Unflattened Granules of OSA Modified WM starch (DS=0.073)

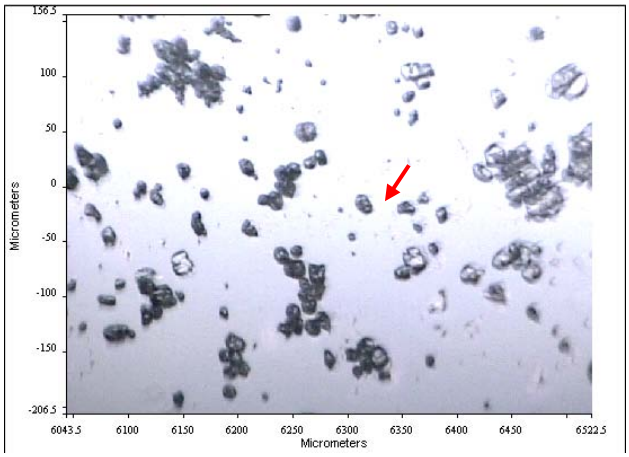


Figure 4.3 Spectrum of a single Unflattened OSA Modified WM starch (DS=0.073)

Figure 4.4 Photomicrograph of Flattened Granules of OSA Modified WM Starch (DS=0.073)

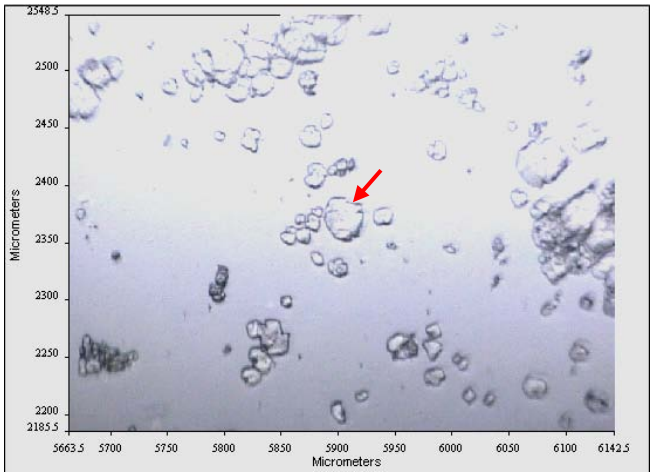


Figure 4.5 Spectrum of a single Flattened Granule of OSA Modified WM Starch (DS=0.073)

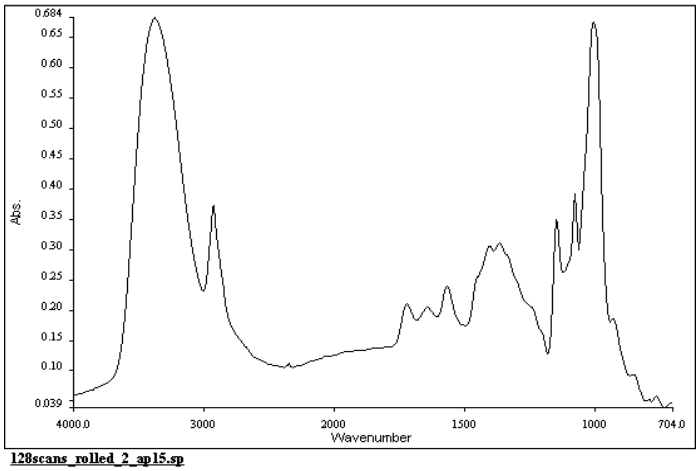
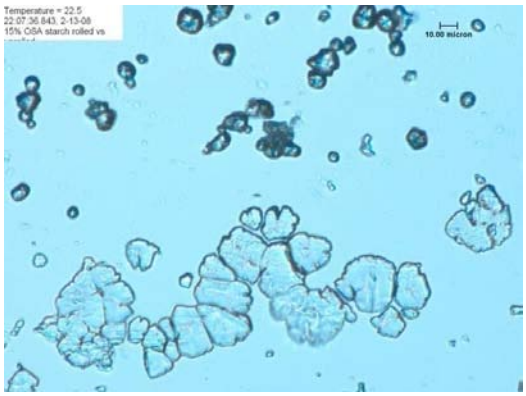


Figure 4.6 Photomicrograph of OSA Modified WM Starch (DS=0.073) Unflattened (Top) and Flattened (Bottom) Granules



The aperture size of the instrument was selected from spectra of flattened starch granules with a resolution of 8 cm^{-1} and 128 scans coadded using different aperture sizes. A flattened granule of OSA modified WM starch (DS=0.073) was scanned under aperture sizes of 6 µm × 6 µm, 10 µm × 10 µm, 15 µm × 15 µm and 50 µm × 50 µm, respectively. Figure 4.7 and Figure 4.8 shows that the spectra scanned with aperture sizes of 15 µm × 15 µm and 50 µm × 50 µm gave smoother spectra with the stated resolution and coadded scans than that of 6 µm ×6 µm and 10 µm × 10 µm. As a result, the aperture size of 15 µm × 15 µm was used to be respresentation of single granules when centered and produced very good spectra in less than 2 min without excessive coaddition of scans.

In one field of view, typically there were 30 to 40 starch granules. Transparent granules which still kept their granule received a preliminary test of 10 scans. Only those granules with clear spectrum and a smooth baseline were selected to be scanned 128 times for recording their spectra for further analysis.

Figure 4.7 Spectra of OSA Modified WM Starch (DS=0.073) Scanned with Aperture Size of 15 µm × 15 µm and 50 µm × 50 µm

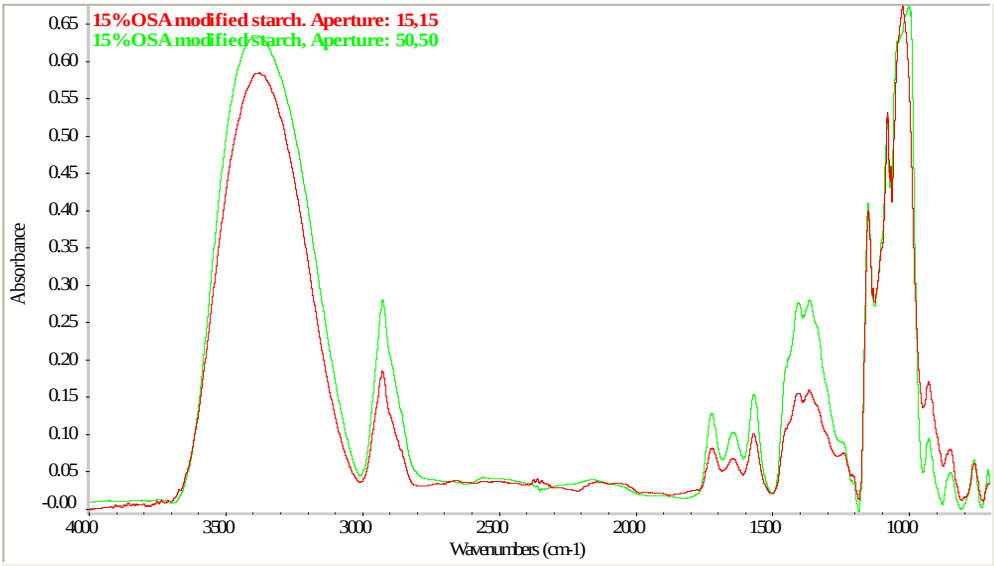
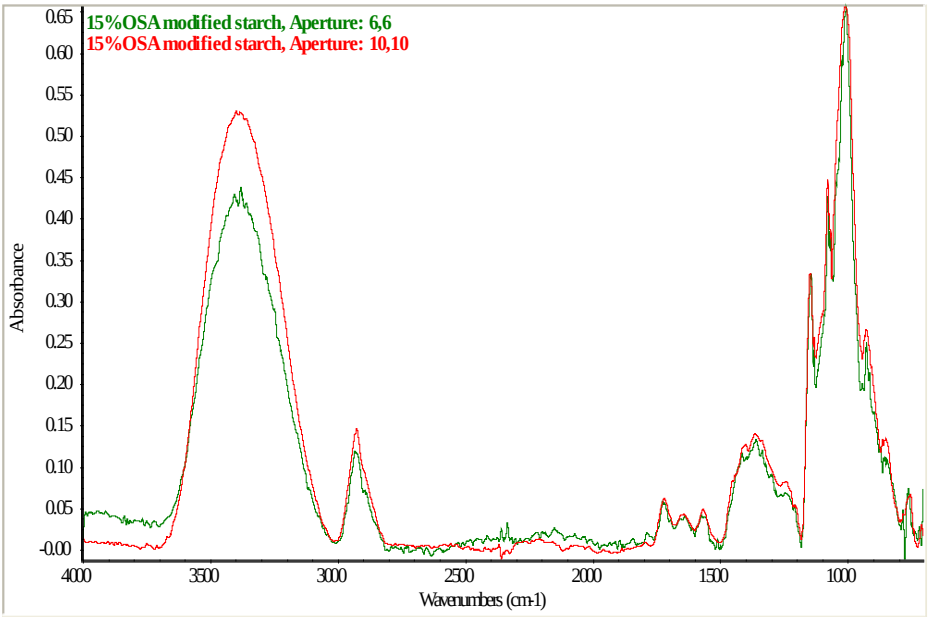


Figure 4.8 Spectra of OSA Modified WM Starch (DS=0.073) Scanned with Aperture Size of 6 µm × 6 µm and 10 µm × 10 µm

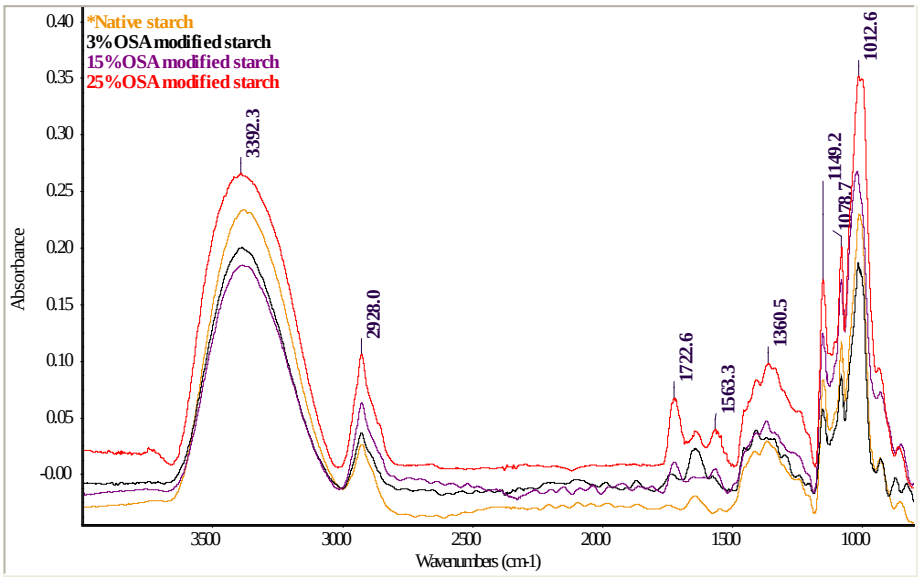


Characterization of FT-IR Spectra of OS Waxy Maize Starch

Native starch and OSA modified starches were characterized by FT-IR microspcetroscopy. The peak at 3392 cm^{-1} was assigned to hydroxyl group from modified starch. A characteristic peak occurred at 2928 cm^{-1} was due to CH_2 stretching vibration. Compared to

native WM starch, modified starch had two new bands at 1723 cm^{-1} and 1563 cm^{-1}. The band occurring at 1723 cm^{-1} originated from carbonyl group as an evidence of formation of an ester group. The second band was ascribed to carboxylate group. The H-O-H deformation band at 1641 cm^{-1} was presumably due to the residual bound water. Peaks at 1149 cm^{-1}, 1078 cm^{-1} and 1012 cm^{-1} were all assigned to C–O stretching vibration from starch. Note from the spectra (Figure 4.9) that the ratio of the 1723 cm^{-1} peak height to that of 1078 cm^{-1} increased as the OSA concentration increased.

Figure 4.9 FT-IR Spectra and Peak Assignment of OSA Modified WM Starches and Native WM Starch



Homogeneity of OS Waxy Maize Starches via a Census of Individual Starch Granules

Native Waxy Maize Starch

The band ratio uniformity distribution of native WM starch is shown in Figure 4.10. The entire population of 110 native starch granules showed a band ratio below 1, and 66% of them were in the range of 0.1-0.2. In addition, 90% granules showed the ratio below 0.6. Granules with band ratio below 1 were regarded as native starch (Figure 4.11). Uncertainty in both peak areas being ratioed may be responsible for apparent aberrant values for a few of the granules.

Figure 4.10 Band Ratio Distribution of Native WM Starch

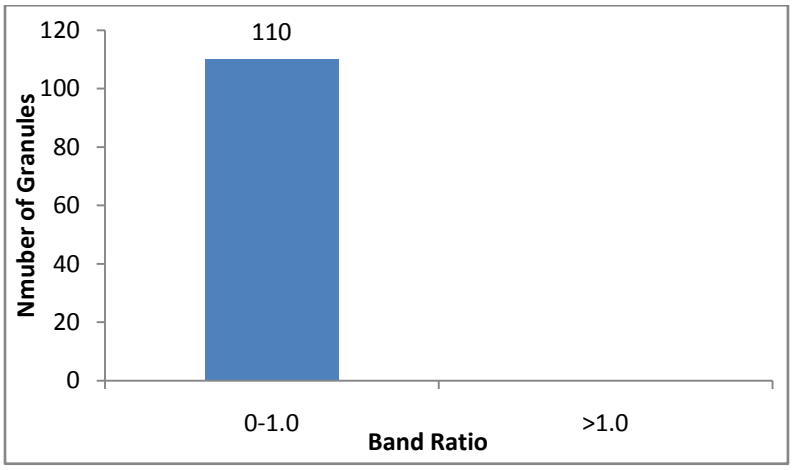
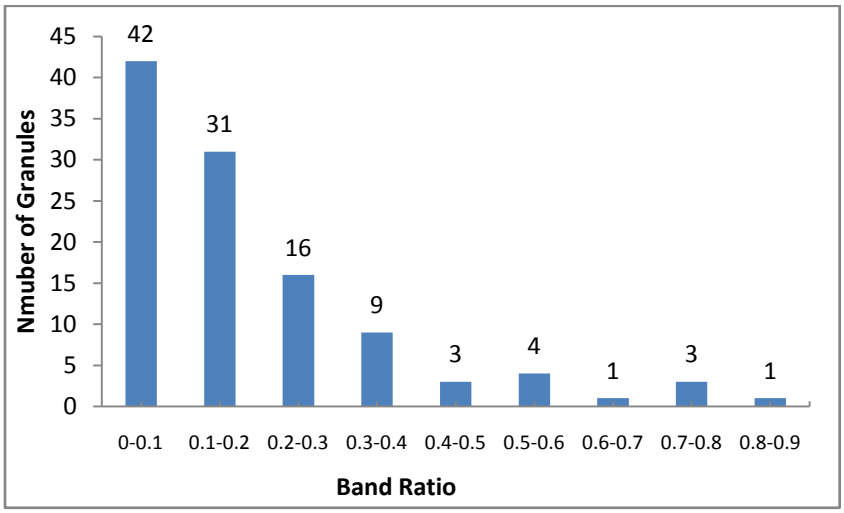


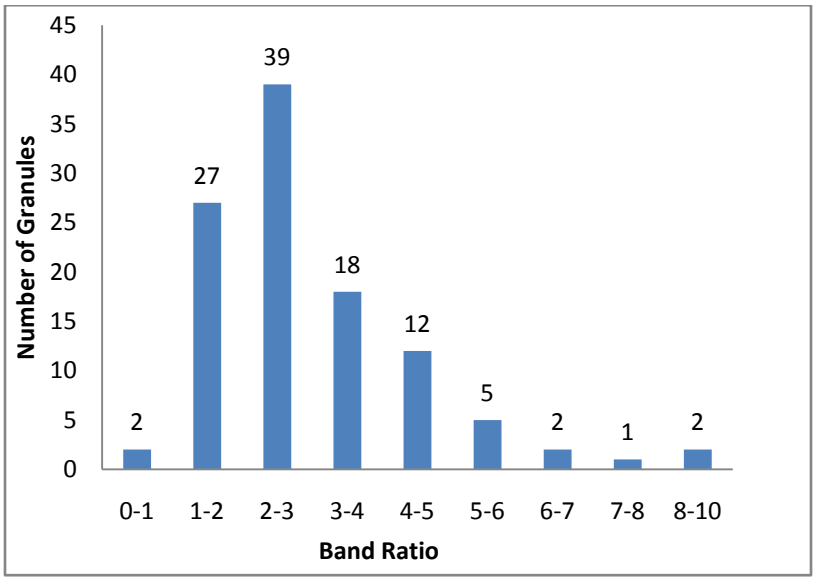
Figure 4.11 Band Ratio (<1.0) Distribution of Native WM Starch



15% OSA Modified Starch

One hundred and eight individual starch granules of 15% OSA modified starch (DS=0.073) were analyzed by FT-IR microspectroscopy. Spectra from those granules gave a high signal to noise ratio, and the absorbance of each functional group was within the acceptable limit. The band ratio value was divided into nine categories (Figure 4.12). The results showed that 99% of the individual starch granules had a carbonyl to carbohydrate band ratio higher than 1, and 89% starch granules had band ratios from 1-5. These results indicate that 99% of OSA modified WM starch granules were highly enough modified by OSA to be detected and classified as being modified; however the OSA content of individual starch granules varied.

Figure 4.12 Band Ratio Distribution of OSA Modified WM Starch (DS=0.073)



3% OSA Modified Starch

When the lowest modification level OSA modified WM starch granules (DS=0.019) were analyzed by FT-IR microspectroscopy, 93% granules showed an absorption ratio below 1 and the rest of the granules had absorption ratios from 1-3 (Figure 4.13). Compared with native starch, OSA modified WM starch had fewer granules with the absorption ratio at 0-0.2 but more in the range of 0.3-0.5 (Figure 4.14). These results indicated that some starch granules were reacted with OS groups. However, as anticipated, the degree of substitution of each granule was very low. Also from these analytical results, the distribution of OS group in 3% OSA modified starch was apparently nonuniform .

Figure 4.13 Band Ratio Distribution of OSA Modified WM Starch (DS=0.019)

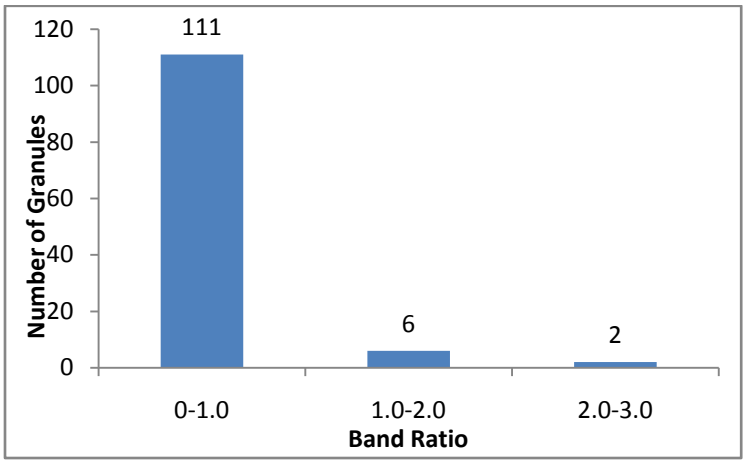
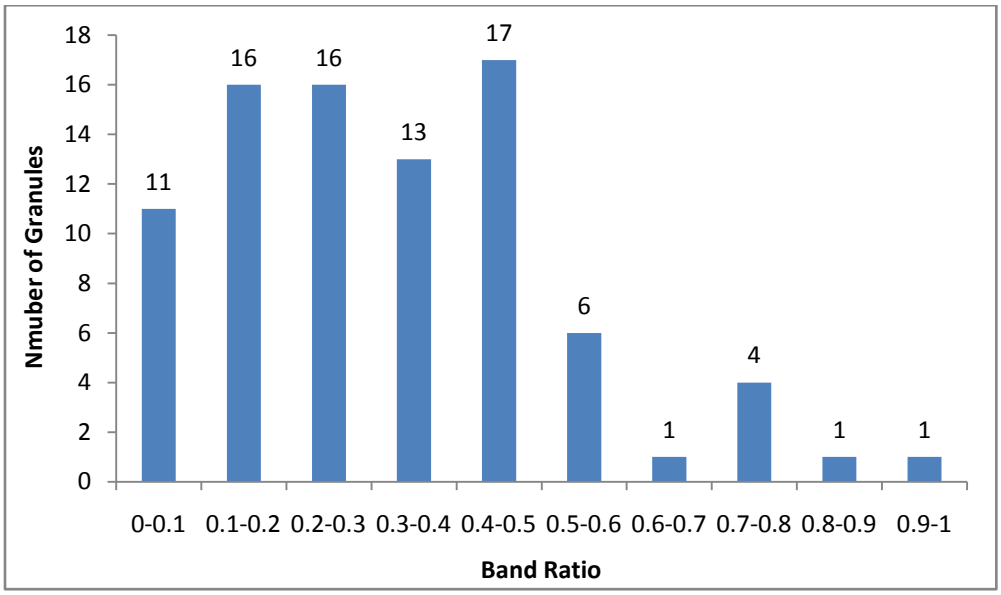


Figure 4.14 Band Ratio (<1.0) Distribution of OSA Modified WM Starch (DS=0.019)



Single Granule Census of OS Waxy Maize Starch and Native Starch Blends

A blend of OSA modified WM starch (DS=0.019) was analyzed by FT-IR microspectroscopy. Among the 112 granules examined, 30% starch granules had band ratios higher than 1 and 70% starch granules had band ratios from 2-10 (Figure 4.15). When the starch granules with band ratio lower than 1 were further analyzed (Figure 4.16), it is as observed that the shape of the distribution was similar to that of native starch. The experimental census of 112 individual granules matched the calculated formulation used to produce the synthetic mixture that simulates industrial formulations. The results of this novel single granule analysis suggest that FT-IR microspectroscopy is a useful tool to detect heterogeneity of starch blends containing OSA modified WM starch and native starch. Any deviation for heterogeneity of the highly modified blending starch may also be assessed by this mean.

Figure 4.15 Band Ratio Distribution of the Starch Blend

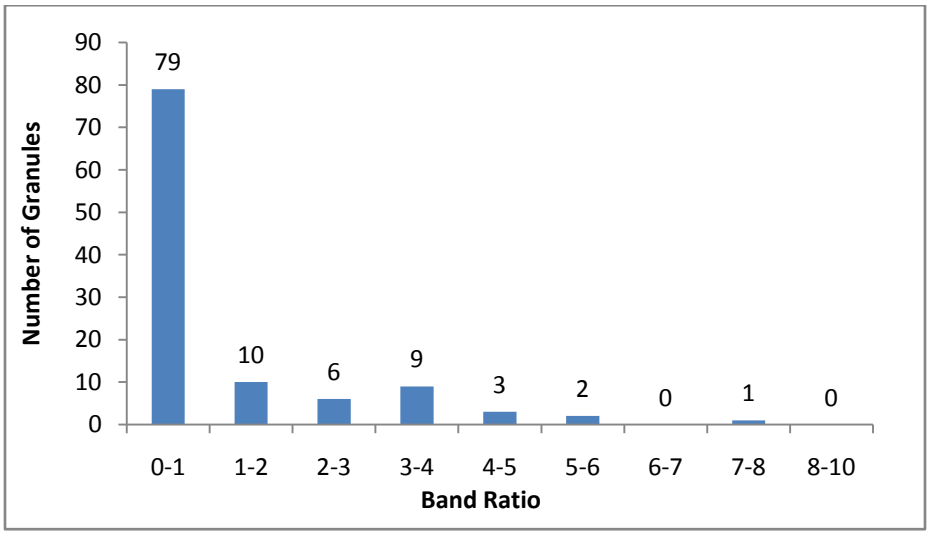
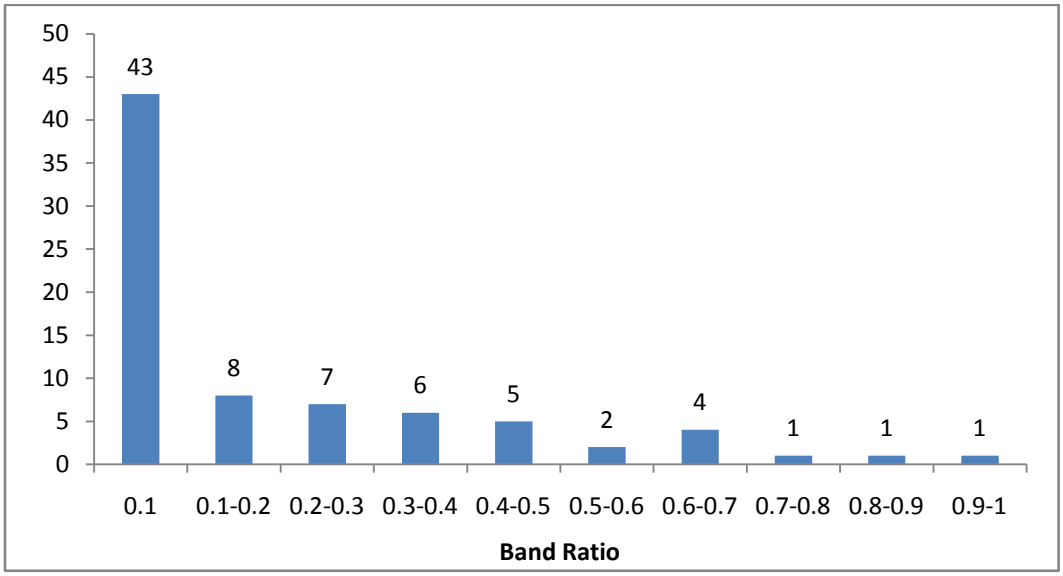


Figure 4.16 Band Ratio (<1) Distribution of the Starch Blend



Determination of Distribution of OS Group by Mapping

A bulk area of OSA modified WM starch granules (DS=0.11) indicated in Figure 4.17 was mapped by FT-IR microspectroscopy. From the chemical map (Figure 4.18) of carbonyl band at 1723 cm^{-1} that was produced. It is noted that the carbonyl absorption was uniformly distributed through the specimen. This revealed the starch granules were uniformly reacted with OS groups. However, the quantitative information was affected by the sample thickness because bands for comparison were not available with this thick specimen.

Figure 4.17 Photomicrograph of OSA modified WM Starch (DS=0. 11)

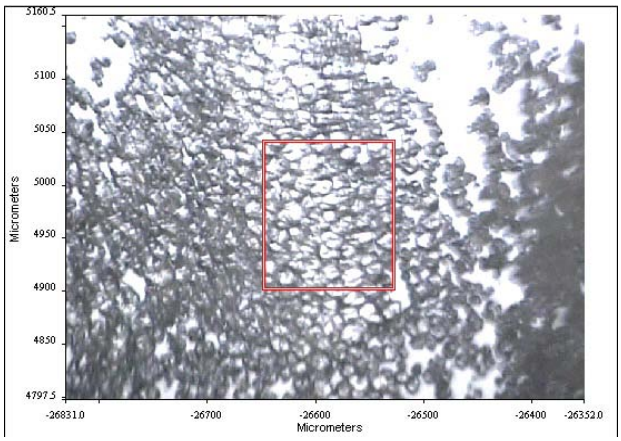
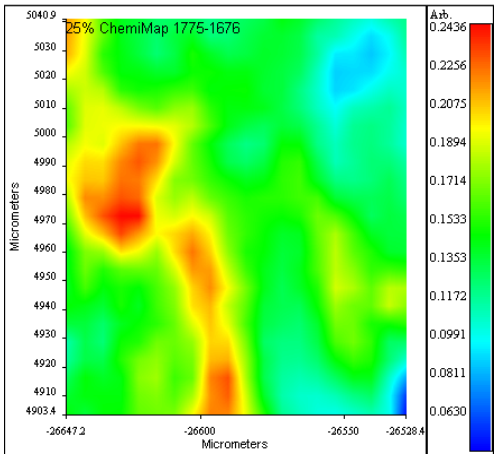


Figure 4.18 A Single Band (1723 cm^{-1}) Chemical Map of OSA Modified WM Starch (DS=0. 11)



A specimen of OSA modified starch with DS of 0.056 was also mapped (Figure 4.19). In the selected area of the specimen, carbonyl absorption was uniformly distributed (Figure 4.20). This result suggests that the majority of starch granules were reacted with OS groups, although the OS content differed from granule to granule.

Figure 4.19 Photomicrograph of OSA Modified WM Starch (DS=0.056)

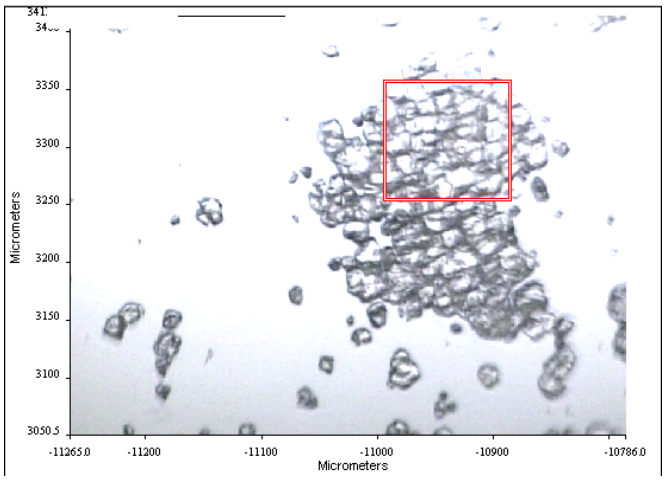
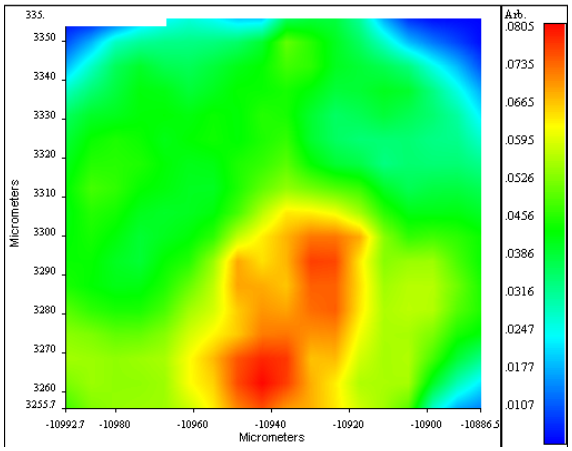
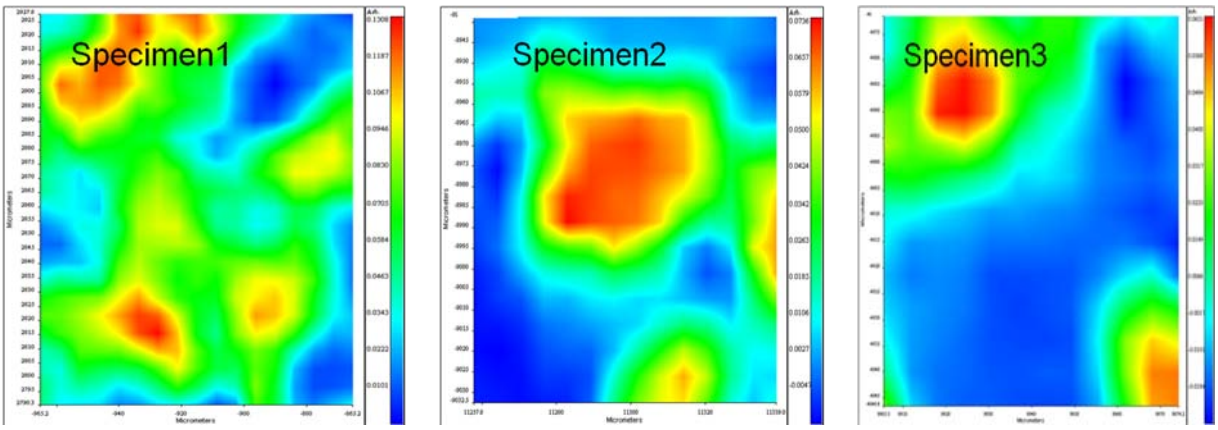


Figure 4.20 A Single Band Chemical Map (1723 cm^{-1}) of OSA Modified WM Starch (DS=0.056)



A precision blend of OSA modified WM starch (DS=0. 11) and native starch were mapped using the FT-IR imaging method. Some spots having very low carbonyl absorption (0-0.01) are shown in blue (Figure 4.21). These spots were assumed to be native starch. The FT-IR microspectropic techniques used were able to detect the native starch, and they also differentiated starch blends from uniformly modified starch lots. The time of mapping a 100µm×200µm size specimen was 20 min or less which was much shorter than required for analyzing individual starch granule one by one. The mapping procedure is an accurate and effective method to detect the two components and distribution within a starch blends.

Figure 4.21 Single Band Chemical Maps (1723 cm^{-1}) of the Starch Blend



A single starch granule of OSA modified starch with DS of 0.056 was mapped. The granule was flattened and the granule size was about 30µm×50µm (Figure 4.22). A chemical map was produced by ratioing the carbonyl peak to the carbohydrate peak (Figure 4.23), which eliminated the effect of the thickness variation within the sample. The spatial resolution used did not permit pinpoint single modification sites. However, the result showed that the distribution of carbonyl absorption was not uniform within a starch granule. The center of the granule showed higher carbonyl absorption than the out layer, indicating the center of the starch granule had higher OS content than.

Figure 4.22 Photomicrograph of a OSA Modified Starch Granule (DS=0.056)

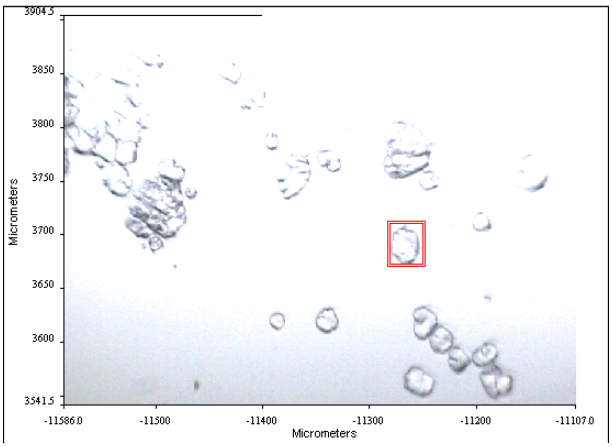
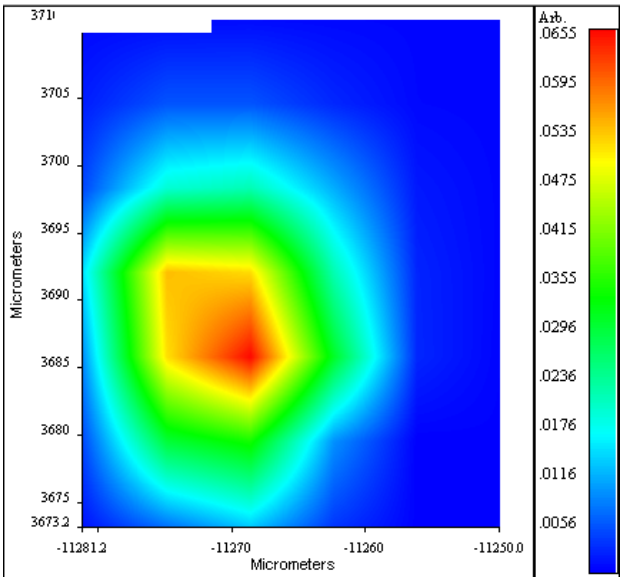


Figure 4.23 A Band Ratio Chemical Map (carbonyl to carbohydrate) of a Starch Granule of OSA Modified WM Starch (DS=0.056)



CHAPTER 5 - Conclusions

OSA modification was affected by pH, starch slurry solids, OSA concentration and physical form of starch. The optimum reaction condition for OS modification of WM starch was 40% slurry solids, pH 7.5 to 8.5. When 3% OSA (based on the weight of starch) was used, DS of OSA modified WM starch was 0.019. As OSA concentration increased to 50%, DS increased but RE decreased. The highest DS of OSA modified WM granular starch was 0.14. When OSA/starch ratio reached 9%, starch granules started swelling and losing their maltese cross. Addition of 5% sodium sulfate to the starch slurry before adding OSA effectively prevented starch granules from swelling. Parameters such as OSA addition rate, adjusting pH of the starch slurry to acid condition before reaction, and dissolving OSA in ethanol did not affect the RE. The optimum reaction condition for maltodextrin was similar with that for WM starch. However, for the 3% OSA, DS of the OS maltodextrin was 0.027 and RE was 100%. The 3% OSA modified microporous starch and WM starch had lower DS and RE compared to maltodextrin.

The structure of OS starches was characterized by ^{1}H-NMR, ^{13}C-NMR and 2-D NMR. The resonances from 1.28 ppm to 2.62 ppm were signals of methylene and methine protons of OS group. The multiplet at 5.54 ppm and the multiple shoulder on the α-1,4 internal were from the proton of double bond of OSA. ^{13}C-NMR suggested that the OS group mostly substituted at O-2 position, and it started substituting at O-6 when DS reached 0.073.

Unreacted OS group was characterized by ^{13}C-NMR. The signals at 186.27 ppm and 188.19 ppm were from unreacted OS group. The free OS content of 15% OSA modified starch was 0.57% by the weight of starch.

The homogeneity of OSA modification on WM starch was investigated by FT-IR microspectroscopy. For the 15% OSA modification, most starch granules were reacted with OS groups, although the OS content varied. For the 3% OSA modification, majority starch granules did not show the carbonyl absorption, indicating the reaction was not uniform. FT-IR was also useful to detect native starch in a blend of OS starch (DS 0.075) and native starch (3:7 w/w). The mapping procedure was an accurate and effective method to detect native starch and OS starch and their distribution within a starch blends

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