Preparation, characterization, and applications of highly substituted starch derivatives

by

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B.S., North Dakota State University, 2011
M.S., Kansas State University, 2013

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
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Abstract

Starch esterification and etherification have been known for decades and are of interests to academic and industrial researchers. While lightly substituted starches, primarily used in food applications, have been extensively studied, highly substituted starches could find many industrial applications but have not been widely commercialized. In this dissertation, recent advances in preparation and properties of intermediately and highly substituted starches were reviewed. Experiments were conducted to: (1) develop methods of synthesizing highly substituted starch esters and ethers without using conventional organic solvents, (2) characterize the starch derivatives synthesized from solvent-free methods, and (3) plasticize the starch derivatives and explore their potential application as chewing gum bases.

Waxy, normal, and high-amylose maize starches were acetylated to degree of substitution (DS) 0.1-1.7 in aqueous medium. Reaction efficiency (RE) of acetylation was in the order of high-amylose>waxy>normal starch. Distribution of acetyl groups on anhydrous glucose monomer level was determined by ^{1}H-NMR after peracetylation (with deuterated acetic anhydride) or perpropionylation. Acetylation was greatly preferred in C2 position in all DS levels regardless of amylose/amylopectin ratio of the base starch. Selected starch acetates were pre-gelatinized, dried, and melted in excessive octenylsuccinic anhydride (OSA) at 160 °C, to produce octenylsuccinylated acetylated starch (OS-Ac-starch) at three different scale levels (10 mg, 5 g, and 60 g). The representative OS-Ac-starch achieved a combined DS up to 2.85 (1.71 acetyl and 1.14 octenylsuccinyl) and contained less than 0.2% unreacted OSA. RE of octenylsuccinylation was positively related to acetyl DS and was between 0.5 to 16.2%. Pregelatinization significantly improved RE of OSA when acetyl DS was below 0.88; however, was not helpful when initial acetyl DS was higher.

Triacetin was tested in OSA-starch acetate melting reaction as a diluent to reduce the usage of OSA. Addition of triacetin thinned the reaction melt and prevented acidity build-up during reaction. OS-Ac-starch produced in triacetin-added reaction achieved combined DS up to 2.55 and was structurally similar to starch ester synthesized in pyridine medium. RE of OSA was improved from 16.2 to 37.7% by triacetin addition. OS-Ac-starches had glass transition temperature (T_g) around 56 °C and were partially to fully soluble in organic solvents such as acetone and chloroform, suggesting their potential uses as water-resistant thermoplastic materials. Chewing gum base made from high-amylose starch ester had great stretch (360% elongation before breaking) and was superior to waxy and normal starch-based gums.

In another approach, high-amylose maize starch was hydroxypropylated in aqueous isopropanol with propylene oxide to various molar substitution (MS) levels (0.56-1.64) and further acetylated to a range of DS (0.09-1.97), by either reacted in anhydrous acetic anhydride (dry heat melting reaction) or aqueous acetylation, to prepare hydroxypropylated acetylated starches (HPAcS). T_g of HPAcS was synergistically lowered by hydroxypropylation (HP) and acetylation (Ac) and was between -11 to 110 °C. Solubility of HPAcS in water was greatly enhanced by hydroxypropylation but significantly reduced by acetylation. From varying substitution levels of HP and Ac, it was practical to prepare thermoplastic starch materials of different water resistance. Chewing gum bases formulated from water insoluble HPAcS withstood different stretching forces and showed no sign to break at 750% elongation.

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Chapter 1 - Preparation, characterization, and applications of intermediately to highly substituted starches, a review

Abstract

Starch can be esterified or etherified to yield products with altered physicochemical properties. While lightly substituted starches have been a focus for food applications, intermediately and highly substituted starch could find many industrial applications. Preparation, properties, and applications of starch esters and ethers with degree of substitution greater than 0.2 are reviewed. The latest developments of synthesis routes, influence of synthesis media (e.g. aqueous, non-aqueous, and solvent-free), catalysts, and reaction conditions are summarized. Characterization methods and physicochemical properties (e.g. thermal properties and solubility) of intermediately to highly substituted starches are discussed.

Introduction

Starch is an abundant glucose-based polymer synthesized by higher plants for energy storage. In recent trend of renewable material development, starch has received much attention for its potentials as industrial bioplastics; however, some shortcomings such as high hydroscopicity, sensitivity to enzymatic and acidic degradation, insolubility in organic solvents, and more importantly, high glass transition temperature (T_g) that limits its processibility, have hurdled native starch from becoming a widely used industrial polymer (BeMiller & Whistler, 2009). Chemical modifications, such as esterification and etherification, have been applied to starch for decades to overcome these shortcomings by grafting different functional groups to starch (Ačkar et al., 2015; Hjermstad, 2012). Comparing to food grade modified starches, which often lightly modified to degree of substitution (DS; substitution only on starch hydroxyl group)

or molar substitution (MS; substitution possible on both starch and substituent hydroxyl groups)< 0.2, industrial starches often require higher substitution levels (DS or MS > 0.3) for desired functionalities such as low T_g, enzyme resistance, and improved solubility in various solvents (Spychaj, Wilpiszewska, & Zdanowicz, 2013). As DS increases, solvent compatibility of starch esters may shift from water-soluble to organic-soluble depending on types of substituent group (Chi et al., 2008). The improved solubility in organic solvents, along with a decreased T_g, imparts starch esters enormous potential as water-resistant, thermoplastic, and biodegradable materials that find applications in many fields, e.g. packaging films, coatings, foams, etc., through casting, blowing, hot pressing, and extrusion (Shogren, 1996). Starch etherification also introduces substituent groups; however, these substituents such as hydroxypropyl, hydroxyethyl, or carboxylic groups, contain hydrophilic functional groups, and thus aqueous solubility of starch ethers is often enhanced as substitution level increases (Hjermstad, 2012; Tuschhoff, 1987).

Despite numerous advantages, commercial production of high DS starch materials has been limited due to several difficulties. The most challenging hurdle is lack of a suitable solvent that is low toxicity and cost-effective to overcome high viscosity due to disruption of granular structure while keeping starch effectively dispersed throughout synthesis (Aburto, Alric, & Borredon, 1999; Gilet et. al., 2018). Furthermore, highly modified starches tend form a viscous and aggregated mass in reaction media to impact efficiency in reagent penetration and byproduct removal. Recently, researchers have studied different pathways, reaction media, and pretreatments to enhance starch esters synthesis to make such process feasible in industrial scale. Unconventional methods for starch derivatization, mostly for making substituted starches with < DS 1, have been reviewed by Gilet et al. (2018). This review focuses recent advances in

synthesizing high DS starch, particularly on synthesis media and pathways of traditional

synthesis routes, and routes that feature "green chemistry" without using conventional starch

solvents such as pyridine, dimethyl sulfoxide (DMSO), toluene, dimethylformamide, dioxane

etc. Alternative synthesis methods with green chemistry are summarized and the information is

useful for assessing production feasibility of highly substituted starch.

Initial literature search was carried out using SciFinder® with keywords "starch ester"

and "starch ether" and proceeded with specific mainstream starch derivatives listed in Table 1.1

and illustrated in Fig 1.1. We reviewed the search results and selected the studies that

emphasized DS > 0.3 for this review. Some old, yet very valuable studies were also included in

the review. Carboxymethyl and hydroxyethyl starch were excluded from the scope, because there

are already recent reviews available (Hjermstad, 2012; Spychaj et al., 2013). Some extensively

studied concepts such as ionic liquids and enzyme -catalyzed acylation, and microwave and

ultrasound -assisted reactions fit the concept of "green chemistry" but have been thoroughly

reviewed (Alissandratos & Halling, 2012; Gilet et al., 2018; Yang, Qiao, Li, & Li, 2016).

Therefore, those areas are not covered in this review.

Table 1.1. Number of publications found by SciFinder® from year 2000 to 2018 by using different keywords.

Keywords	# of literature match/containing keywords	Most relevant reviews
Starch ester	121/1060	Ačkar et al., 2015; Hong, Zeng, Brennan, Brennan, & Han, 2016
Starch ether	70/816	Hjermstad, 2012
OSA modified starch	55/214	Altuna, Herrera, & Foresti, 2018; Sweedman, Tizzotti, Schäfer, & Gilbert, 2013
Octenyl succinic anhydride starch	36/237	
Hydrophobic starch	65/852	

Ionic liquid & starch	300	Gilet et al., 2018; Laus et al., 2005; Wilpiszewska & Spychaj, 2011; Yang et al., 2016
Starch fatty acid ester	25/1088	Ačkar et al., 2015
Acetylated starch	204/685	Golachowski et al., 2015
Enzyme & starch & esterification	67	Alissandratos & Halling, 2012; Van den Broek & Boeriu, 2013
Hydroxypropylated starch	113/702	Hjermstad, 2012
Carboxymethyl starch	852/1030	(Heinze, Liebert, Heinze, & Schwikal, 2004; Spychaj et al., 2013)
Hydroxyethyl starch	3494/4097	(Hjermstad, 2012; Westphal et al., 2009)
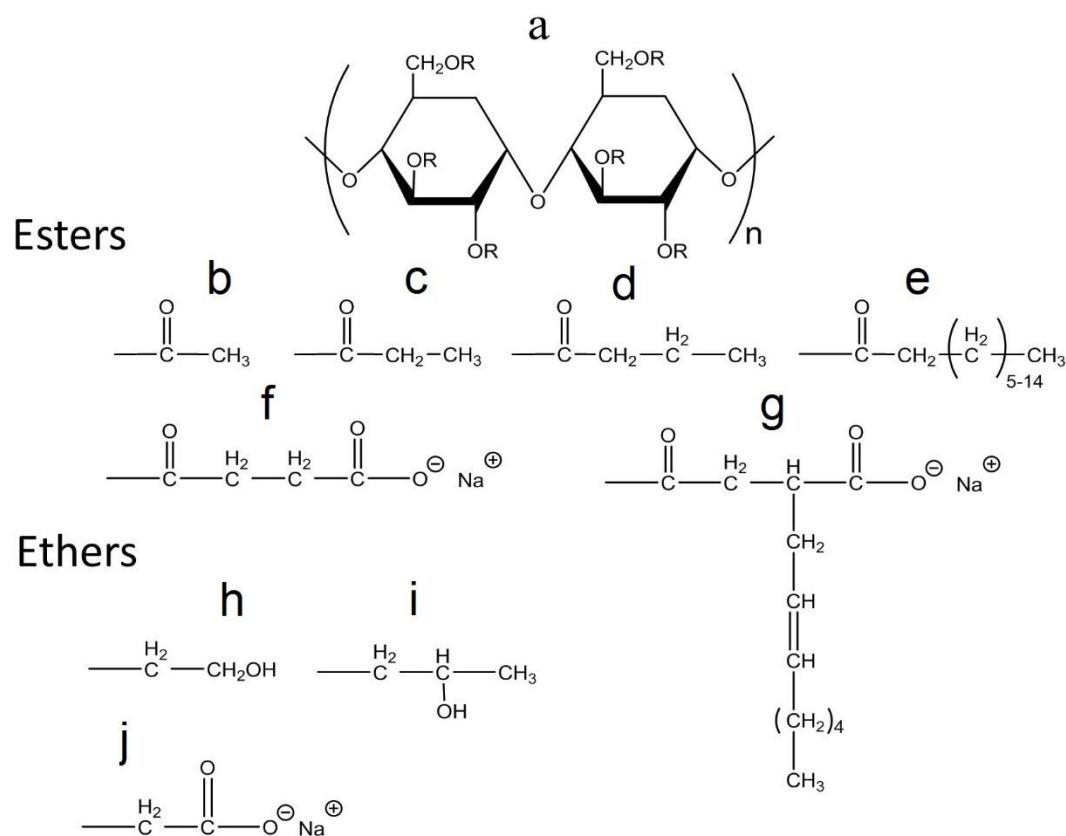
Figure 1.1. Structure of recurring glucose units with substituted groups (a); R represents substituent groups; representative substituents are: acetate (b), propionate (c), butyrate (d), long chain carboxylates (e), succinate (f), and octenylsuccinate (g); hydroxyethylate (h), hydroxypropylate (i), and carboxylate (j).

Synthesis of intermediately to highly substituted starch

Conventional methods

Synthesis of starch esters in aqueous medium

Starch acetate is considered the simplest starch ester and has been studied the most compared with more complicated acyl groups. Methods for starch acetylation are often applicable to propionylation and butylation with appropriate acyl donor. Aqueous synthesis is the most widely used starch esterification method. In such method, an acyl donor, e.g. acyl anhydride or chloride, is introduced to a starch aqueous medium that maintained at slight basic pH. It is known that the DS value of starch esters produced in aqueous medium is low, mainly because (1) starch slurry is diluted as reaction proceeds and (2) water and water-carried reagents have poor penetration to starch granule, unless the starch granule is disrupted or swelled (BeMiller & Whistler, 2009). Low DS starch derivatives typically retain granular structure and can be washed and dewatered readily (Ačkar et al., 2015; Golachowski et al., 2015). However, a swelled starch, which typically due to elevated temperature or localized alkali, is much more difficult, if not impractical, to dewater than granular starch (BeMiller & Whistler, 2009). According to Rutenberg & Solarek (1984), starch acetylated in aqueous medium can be concentrated by repeated filtration to achieve DS over 0.5.

Synthesis of starch esters in anhydrous medium

Acyl anhydride can be used in excessive amount to serve as a reactive solvent in adjunct with acid or alkali catalysts. Sulfuric acid (Zhang, Xie, Zhao, Liu, & Gao, 2009), acetic acid (Diop, Li, Xie, & Shi, 2011), methanesulphonic-acid (Chi et al., 2008) and p-toluene sulfonic acid (Han et al., 2013) and tartaric acid (Tupa et al., 2013) were catalysts reported produced high DS starch acetates. Alkali metal hydroxides are popular catalysts first reported by Mark &

Mehltretter (1972) in a facile anhydrous process that reacted starch with fourfold quantities of

acetic anhydride with 11% (w/w starch) NaOH at 123 °C. Under such conditions, the starch was

fully substituted (DS=3) and the DS can be conveniently adjusted by varying reaction time. This

method has been adopted by many researchers in producing high DS starch esters (Bello-Pérez,

Agama-Acevedo, Zamudio-Flores, Mendez-Montealvo, & Rodriguez-Ambriz, 2010; Shogren,

1996; Volkert, Lehmann, Greco, & Nejad, 2010; Xu & Hanna, 2005; Xu, Miladinov, & Hanna,

2004) and extended to starch propionate (Di Filippo, Tupa, Vázquez, & Foresti, 2016), butyrate

(Tupa et al., 2013) and acetate-maleate (Xu, Miladinov, & Hanna, 2005).

  To synthesize starch esters that possess longer alkyl chains (C_4-C_{18}), aqueous medium

strongly unfavored due to several reasons, (a) immiscibility of water and the oily reagent, (b)

poor penetration of the reagents into starch granule, and (c) high rate of side-reaction (Namazi,

Fathi, & Dadkhah, 2011). Ultimately, reaction stops from lack of dispersion because starch ester

separates from aqueous phase as DS increases. Conventional preparation of high DS starch ester

(long chains) often requires at least one organic solvent to facilitate starch ester dispersion

(Ačkar et al., 2015; Hong et al., 2016; Lower, 1996; Tessler & Billmers, 1996). Table 1.2

summaries common methods to achieve high DS for starch esters of various substituent chain

length. Eliminating the use of organic solvents remains a strong interest to the field.

Table 1.2. Conventional synthesis routes for high DS starch derivatives

Starch derivative	Starch (g) d.b.	Reagent, Mole ratio to starch	Solvent (mL)	Catalysts	Conditions	Wash solvent	DS	Reference
Acetate	30	Acetic anhydride, 4:1	Acetic anhydride	10.2 g 50% aqueous NaOH	123 °C, 4h	Water	2.59	Xu et al., 2004
	10	Acetic anhydride, 4.7:1	10 mL Acetic acid	0.38 mL H_2SO_4	70 °C, 3h	Water	2.67	Zhang et al., 2009
Succinate	10	Succinic anhydride, 4:1	60% pyridine	None	115 °C, 10 min	Ethanol	3	Bhandari & Singhal, 2002

Propionate	25.8	Propionic anhydride 7:1	300 mL pyridine	Pyridine	95 °C, 4h	Methanol	2.61	Garg & Jana, 2011a, 2011b; Zhu et al., 2017
Medium chain esters (C4-C6)	16.2	Acid chloride, 4.5:1	200 mL Dioxane & 200 mL toluene	50 mL Pyridine	100 °C, 6h	Ethanol & water	>2.80	Sagar & Merrill, 1995
Long chain esters (C8-C18)	0.5	Vinyl esters 3-5:1	5 mL DMSO	Various basic salts	110 °C, 24h	Methanol	>2.90	Junistia et al., 2009
	2	Acyl chlorides, 18:1	15 mL pyridine	Pyridine	105 °C, 3h	Ethanol	2.7	Aburto, Alric, Thiebaud, et al., 1999; Thiebaud et al., 1997
Octenylsuccinate	20	OSA, 3.6:1	80 mL Pyridine	Pyridine	97 °C, 2.7h	70% ethanol & water	1.35	Gu et al., 2015; Li et al., 2012; Wang et al., 2011
Hydroxypropylate	10	Propylene oxide, 2:1	40 mL water	5% NaOH	40 °C, 24h	water	0.66	Aminian, Nafchi, Bolandi, & Alias, 2013; Hjermstad, 2012

Changes to conventional methods

Synthesis of high DS starch short chain carboxylates in aqueous slurry

Water is the most preferred medium in starch modification for its lowest cost and environmental impact. When synthesized in water, DS of starch ester, e.g. starch acetate, typically do not raise beyond 0.2 (BeMiller & Whistler, 2009; Golachowski et al., 2015). A one-step in aqueous synthesis for high DS starch acetate was disclosed by Billmers and Tessler, (1994). In a such synthesis, high-amylose starch reacted with 200% (starch dry wt.) acyl anhydrides, catalyzed by 25% NaOH at pH 8, reached DS 1.71 and 1.57 for acetate and propionate substituent, respectively. Billmers and Tessler (1994) have also extended acetylation to waxy and normal starches, flours, hydrolyzed starch and several dextrins. Luo and Shi (2012) characterized starch acetates synthesized Billmers and Tessler (1994) method and reported high-amylose starch reached highest DS (1.29) value comparing to waxy (1.08) and normal (0.71) starches. Luo & Shi (2012) noted that starch acetate experienced "swelling and shrinking",

reflected by reaction slurry settling volume, during acetic anhydride addition. Regardless of starch amylose content, starch swelling peaked at 33% acetic anhydride (starch wt.) addition. They attributed the swelling to granule surface gelatinization at DS about 0.3. Such swelling was limited when DS was higher as starch become more hydrophobic and tends to settle out from the slurry. Pu et al. (2011) reported a starch acetate of DS 2.93 synthesized in aqueous medium with 400% acetic anhydride usage. Pu et al. (2011) carried out the synthesis at 80 °C to allow full contact of starch and reagent; however, a specific mechanical mixer must be employed to overcome anticipated high viscosity. Other variations of the aqueous slurry method includes using excessive reagent to achieve DS 0.45 starch succinate (Zhu et al., 2001), DS 0.29 starch heptanate (Fang, Fowler, Sayers, & Williams, 2004), and DS 0.51, 0.30, and 0.15 for starch octanate, laurate, and palmitate, respectively, with alkali gelatinized starch (Fathi & Namazi, 2014; Namazi & Dadkhah, 2010). Approaches to enhance starch-reagent contact, e.g. pre-gelatinization of starch (Hassan Namazi & Dadkhah, 2010; Pu et al., 2011; Thitisomboon, Opaprakasit, Jaikaew, & Boonyarattanakalin, 2018), using emulsified reagent (Chi et al., 2007), microwave and ultrasonic treatments are effective to higher DS for some substituents but less prominent for long chain esters (Gilet et al., 2018).

Synthesis of highly substituted starch ether in aqueous slurry

Starch ether, on the other hand, can be synthesized to high substitution in water relatively easily compared to starch esters, due to high compatibility of water and reagent as well as water and starch ether. For instance, hydroxypropylated starch can be prepared in water and achieve high substitution levels as long as sufficient reagent is supplied. Owning to the excellent solubility of propylene oxide in water, hydroxypropylated starch can be synthesized in water up to DS 1.4 (Jyothi, Sreekumar, Moorthy, & Sajeev, 2010). However, starch recovery at such a

high DS is problematic due to excessive swelling. Dialysis, alcohol or acetone washing must be employed (Han & BeMiller, 2005; Hjermstad, 2012).

Development in pyridine-free synthesis routes

In laboratory synthesis of starch esters, pyridine has been long used as a multi-purpose reagent to swell the starch granule, catalyst the esterification, and protect starch backbone by neutralizing liberated acid (Aburto, Alric, Thiebaud, et al., 1999; Thiebaud et al., 1997; Whistler, 1945). Pyridine involved starch esters has little industrial importance because it is a highly toxic, expensive, odor-intense, and polluting chemical. However, variations of pyridine media are still routinely used nowadays in lab-scale synthesis to obtain full substitution for a wide array of substituents and to minimize starch degradation. Typical variations including using a diluent such as DMSO (Biswas, Shogren, Kim, & Willett, 2006; Rosu et al., 2013), ethyl acetate (Sun et al., 2016), dimethylformamide (DMF) (Lukasiewicz & Kowalski, 2012; Rajan, Sudha, & Abraham, 2008), toluene (Zhang, Macquarrie, Clark, & Matharu, 2014), N,N-dimethylacetamide (Vanmarcke et al., 2017), trifluoroacetic anhydride (Yang & Montgomery, 2006, 2008) to correct viscosity and reduce pyridine usage. Ideal diluent should be compatible with reagent yet inert, non-flammable, non-toxic, and recyclable. However, the reagents mentioned above may still impose toxicity and reactivity. In more recent studies, DMSO in adjunct with basic salts (Dias et al., 2013; Junistia et al., 2008, 2009; Winkler et al., 2014; Winkler et al., 2013) and lipase enzymes (Alissandratos & Halling, 2012; Söyler & Meier, 2017) have been used; removing DMSO from final starch is still industrial uneconomic due to its high hydroscopicity (Schmitz, Dona, Castignolles, Gilbert, & Gaborieau, 2009). Ionic liquids media has become popular in replacing pyridine and DMSO recently as they are excellent solutions for starch over a wide DS range (Yang et al., 2016). Particularly, enzyme catalyzed reactions in ionic liquids are

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thought to be true green methods; however, low enzyme activity in ionic liquids, cost to recycle ionic liquid, as well as possible toxicity in waste water, remained unresolved (Adak & Banerjee, 2016; Alissandratos & Halling, 2012; Gilet et al., 2018; Laus et al., 2005; Wilpiszewska & Spychaj, 2011).

Solvent-free synthesis

Oftentimes, esterification reagents are added in great excess to ensure high DS and the reagent themselves can as well serve solubilizing/plasticizing purpose along the reaction. The term "solvent free" implies to the reactions utilize zero solvent in addition to the reactive solvents. Strictly speaking, conventional methods used for starch acetylation i.e. heating starch and acetic anhydride with a very limit amount of water, is the simplest form of a solvent-free reaction (Mark & Mehltretter, 1972; Volkert et al., 2010). In such reaction, starch swelled and acetylated initially in alkali water and the partially acetylated starch solubilized into acetic anhydride, allowing the reaction to continue. Many starch esters, e.g. acetate (Biswas et al., 2008; Shogren, 2003; Tupa et al., 2013), propionate (Di Filippo et al., 2016), succinate (Koroskenyi & McCarthy, 2002; Wang, Shogren, & Willett, 1997), phosphates (Passauer, Bender, & Fischer, 2010), malate (Zuo et al., 2013), and citrate (Kapelko-Żeberska, Zięba, Pietrzak, & Gryszkin, 2016) have been synthesized at high DS in a pressurized reactor, microwave, extrusion, or open roasting set-ups. Tupa, Ávila Ramírez, Vázquez, & Foresti (2015) reported a solvent-free synthesis of starch acetate that utilized starch's own moisture without adding water. They achieved near full substitution at 130 °C, 1.85 g catalyst/g starch, 6.5 g acetic anhydride/g starch. Chang, Qian, Anderson, & Ma (2012) claimed destroying starch crystallinity by pre-gelatinization and freeze dry was an effective pre-treatment for starch succinylation. In their study, succinic anhydride (1.5 mol per mol AUG) was diffused into a porous starch matrix

and reacted at 110 °C for 4h to achieve DS 1.9. This system works robustly for short chain esters but not well for long chain esters, probably because the long chain imposes greater stearic hindrance and prohibits initial acylation. One practical route is to first derivatize starch with a short chain substituent to intermediate DS so when the reagent for secondary long chain is introduced, starch short chain ester can readily swell in it (Yang & Montgomery, 2006, 2008). Aburto, Alric, & Borredon (1999) carried out a solvent-free two-step synthesis for starch octanate. They first derivatized starch in formic acid (4.3 eq/AGU) to obtain DS 1.7 starch formate and dropwise added octanoyl chloride (6 eq/AGU) to the formylation reaction. The highest DS for octanate was 2.1 at 68% yield. Starch formate was readily dispersible in octanoyl chloride; however, they noted the starch degradation, from a low yield and high glucose in upper liquid, was severe in some conditions due to liberated HCl and lack of neutralizing base. Aburto, Hamaili, Mouysset-Baziard, et al., (1999) extended the optimal conditions to starches of different crops and their waxy cultivars. No significant difference was found in either formate or octanoate DS, probably because destruction of the granule and crystallinity in all starches thus the reaction was rather homogenous. Experiments with longer fatty acid showed a clear trend that DS diminished as chain length increase. Shogren, (2003) reported a starch acetate (DS 0.34) octenylsuccinate (DS 0.49) mixed ester from co-heating acetic anhydride, OSA, and acetic acid mixture to 180 °C for 20 min. However, the reaction was carried out in a pressure-resist DSC pan without agitation, discoloration and possible degradation were reported. Aburto, Alric, & Borredon, (2005) reported another solvent-free route for DS~0.6 starch laurate by first subjected starch to thermal aqueous pretreatment and then transesterified with emulsified lauric acid at 190 °C for 4h. Horchani, Chaâbouni, Gargouri, & Sayari, (2010) claimed a solvent-free route to prepare DS 2.86 starch oleic acid ester using CaCO3-immobilized lipase. However, the actual

media was water and their DS calculation method may be overestimated as pointed out by Alissandratos & Halling (2012).

Properties of highly substituted starch

DS determination

Titration methods

Titration is the most common method that is used for DS determination in literature. In a typical titration, acyl group is saponified by a known amount of alkali and the alkali consumption is determined by back-titrating with acid (Golachowski et al., 2015; Zhu et al., 2017). Although titration method is widely used, it has been acknowledged unsuitable for esters that have substituent side chain longer than butyrate. As pointed out by Alissandratos & Halling (2012), high-viscosity and insolubility of starch esters and fatty acids lead to heterogenicity of the titration media and often compromise a clear end-point.

NMR spectroscopy

NMR spectroscopy has been regarded as a powerful tool for DS determination featuring rapidness, greater accuracy, and small sample usage (Hong et al., 2016). Owning to the distinct chemical environment between starch ring protons and substituent protons, it is possible to obtain distinct starch backbone proton peaks, from 3.0 to 5.5 ppm, and below 2.1 ppm for methine and methylene groups in various substituents. DS of starch esters can be resolved from equation $DS = 7A/3B$, where A is the sum of end methyl proton signal areas and B is the sum of starch ring proton signals areas (Barrios, Giammanco, Contreras, Laredo, & López-Carrasquero, 2013; Chi et al., 2008; Muljana et al., 2017; Vanmarcke et al., 2017). NMR method has been noted the most accurate for DS above 2.0 for carboxylic esters, e.g. acetylated starch, where the starch ester can dissolve in deuterated chloroform. At below DS 2.0, starch ester could only

dissolve in DMSO-d_6. Spectra obtained from DMSO-d_6 often interfered by (1) large residual water peak at 3.3 ppm, and (2) broadening of starch ring proton and thus the accuracy of DS calculation was reduced. Alissandratos & Halling (2012) and Elomaa et al. (2004) attributed the signal broadening to DMSO's high hygroscopicity and viscosity; however, substituent at different AGU positions, i.e., 2,3,6-tri-, 2,3-di-, 2,6-di-, 3,6-di-, 2-mono-, 3-mono-, 6-mono-, and unacetylated AGUs, shielded ring protons to different extents thus caused the ring protons of same position to possess various chemical shift values which ultimately resulted in broadened peaks (Kono, 2013; Kono, Hashimoto, & Shimizu, 2015). To determine DS unambiguously, some studies further fully derivatize the starch esters with a secondary substituent, i.e. acetyl or propionyl, to resolve starch ring proton regions (Junistia et al., 2008, 2009; Liebert et al., 2011).

Other DS determination methods

Alternatively, elementary analysis was carried out for starch esters to compute the DS from elementary composition (Winkler et al., 2014; Winkler et al., 2013). FT-IR spectroscopy was also used to estimate DS of starch acetate from the ratio of emerged carbonyl signal (C=O) at 1743 cm^{-1} to starch CH$_2$ vibration (2929 cm^{-1}) versus acetate standards (Shogren, 2003). Elomaa et al., 2004, carried out TGA/FT-IR to quantify acetic acid released during heating of starch acetate and obtained a high correlation with titration method determined DS (R^2=0.985). Qiu, Bai, & Shi, (2012) analyzed DS of starch octenylsuccinate using HPLC to measure saponified octenylsuccinic acid, and the method was able to distinguish octenylsuccinic acid between covalently-bounded and unreacted free forms.

Compatibility of substituted starches and organic solvents

Solvents and starch esters

Native starches are cold-water insoluble as they occur in nature as tightly-packed granules through H-bond forming hydroxyls and are partially crystalline. However, starch is soluble in hot water, because upon heating, starch granules swell to form a paste in water (known as gelatinization). When a starch is highly substituted, starch-water compatibility is affected by the substituent and could become water-resistant or water-soluble depending on the nature of substituents. Solvent compatibility is a crucial property to consider in starch because it plays a key role determining the end-use of a starch. Table 1.3 summarized compatibility of substituted starch and common organic solvents. These solvents are generally recognized as evaporative, inert, and readily removable after being used as a processing aid, e.g. evaporate from a casted starch film. It is noteworthy that some solvents e.g. DMSO and dioxane, are not evaporative and they are mostly used during synthesis or characterization rather than in end product processing.

Plasticizers of starch derivatives

Plasticizers are additives of small molecules with high boiling points and low vapor pressures that increase plasticity and fluidity of a material (Han, 2014). For a starch-based material such as starch esters, a plasticizer is often incorporated to increase mobility of the polymer chains; thus, the starch may be manipulated during processing. Plasticizers compatible with starch esters includes glycerol, polyols, triacetin, caretriethyl citrate, dibutyl sebacate, 2-octenyl succinic anhydride, 2-tridodecen-1-yl succinic anhydride propylene glycol, triethyl citrate, and dibutyl phthalate (Mekonnen et al., 2013; Tarvainen et al., 2003; Vieira et al., 2011; Zhu et al., 2013). According to Bonacucina et al. (2006), 10-30% (starch wt) plasticizers in end-products (e.g. film, foam, or packaging materials) are typically used to reduce brittleness. Other researchers used triacetin as a plasticizer in high DS starch ester films and noted triacetin significantly soften the starch acetate film and lower storage tensile modulus (Zhu et al., 2013).

Tarvainen et al. (2003) reported that n-alkenyl succinic anhydrides, particularly 2-octenyl succinic anhydride can be a good plasticizer for potato starch acetate (DS 2.8). The film plasticized with 60% 2-octenyl succinic anhydride exhibited excellent elongation (12.8%) comparing <3% for other starch acetate films. When choosing a plasticizer for starch-based materials, it is helpful to consider using a non-polar, oil nature, e.g. triacetin, and triacylglycerols, to couple with highly substituted starch esters, and use plasticizers of some hydrophilic behaviors, e.g. propylene glycol, with intermediate or lightly substituted starch esters. In the case of water compatible starch ethers such as hydroxypropylated starch, glycerol and polyols are widely used (Han, 2014; Zhang et al., 2013; Zhu et al., 2013).

Table 1.3. Solvent compatibility of substituted starches.

Starch derivatives	DS*	Starch-solvent compatibility	Reference
Acetate, propionate, and butyrate	0-2.5	Solubility in water decreased as DS increased	Garg & Jana, 2011a; Shogren & Biswas, 2006
Acetate Propionate	2.0 acetyl, 1.6 propionyl	Partially soluble in petroleum ether at 1% solid	Di Filippo et al., 2016; Tupa et al., 2015
Acetate	2.5-3.0	Soluble in acetone and ethyl acetate at 1.7% solid, solubility inversely related to molecular weight	Lehmann & Volkert, 2011
Succinate	0.2-1.9	65% soluble in water (DS 0.2, 80 °C), moisture pickup decreased significantly as DS increased	Chang et al., 2012; Lawal, 2012
Maleate	0.35	Surface wetting significantly reduced as DS increased	Zuo et al., 2013
Carboxylates (C_{6-18})	>2	Insoluble in DMSO* and water, soluble and swellable at 10% solid in chloroform and tetrahydrofuran	Winkler et al., 2013
Carboxylates (C_{12-22})	0.1-0.6	Insoluble in water, DMSO, acetone, and methanol; swellable in chloroform and tetrahydrofuran	Barrios et al., 2013
Fatty acid esters (C_{18})	1.3	Soluble in tetrahydrofuran, chloroform, methylene chloride, toluene, xylene, benzene; swellable in water and DMSO	Söyler & Meier, 2017
Palmitate	> 1.3	Soluble in methylene chloride	Liebert et al., 2011
Hydroxypropylate	0.3-1.4	Soluble in water up to 25% solid	Jyothi et al., 2010

DS, degree of substitution; DMSO, dimethyl sulfoxide. Thermal properties of highly substituted starch

Thermal properties of high DS starch

      Glass transition

      Thermal behavior of a highly substituted starch follow typical "glass➔rubber➔melt➔degradation" path as temperature elevates (Liebert et al., 2011). Depending on nature of substituents, extent of substitution, and presence of plasticizers, temperatures for these thermal transitions vary. Glass transition temperature (T_g), melting temperature (T_m) and thermal decomposition temperature (T_d) are important characteristics and play key roles in thermal processing of starch. These temperatures are conveniently determined by differential scanning calorimetry (DSC), dynamic mechanical analyzer (DMA), and thermogravimetric analyzer (TGA). A summary of thermal transitions in highly substituted starch are presented in Table 1.4. In typical thermal processing (e.g. extrusion, hot press, and molding), processing temperature must be above T_g, is preferably above T_m, but well below T_d to ensure the starch softened, and therefore to be manipulated without degradation (Junistia et al., 2009; Shogren, 1996). Dry native starches have T_g (226 °C) well above typical thermal processing temperature (150 °C) and close to T_d (around 300 °C) (Shogren, 1996), and therefore, is not suitable for thermal processing unless large amount of external plasticizers such as water, glycerol, or polyols is present. Substituents provide intermolecular plasticization (Fringant, Rinaudo, Foray, & Bardet, 1998) and lower the T_g of a dry starch to manageable temperature for thermal processing. In pure short chain carboxylic starch esters, DS and chain length are the most determining factors for T_g. Shogren, (1996) reported that acetyl DS of 1.5-2.5 could effectively lower dry starch T_g to around 150 °C, granting a larger margin for processing tolerance. For bulkier substituents groups such as fatty acid esters, multiple thermal transitions often observed due to side chain melting, oftentimes lower than or overlapping with starch

backbone glass transition (Winkler et al., 2014). The side chain melting temperature mostly correlates with length of side chain and is less relevant to DS for long chain substituents. For example, both starch laurate of DS 1.6 and 2.3 had melting temperature between 4-5 °C (Junistia et al., 2009; Winkler et al., 2014). There is no study showing clear cutoff chain length for side chain melting peak to occur; however, C_{10} (Liebert et al., 2011) and C_{12} (Junistia et al., 2008; Vanmarcke et al., 2017; & Winkler et al., 2014) esters are some short fatty acid chains reported to exhibit distinguished side chain melting transition (Winkler et al., 2014). Frequently, highly substituted starch fatty acid esters give an apparent T_m for their "hot-gel" properties but it is indeed the interplay of side chain and backbone melting (Liebert et al., 2011).

Thermal degradation

Zhang et al. (2014) studied thermal degradation of starch esters with different DS by thermogravimetric analysis. Peak degradation temperature was elevated approximately 70 °C as DS increased from 0 to 2.5. The improved thermal stability is largely attributed to reduced number of –OH groups that undergo dehydration upon heating (Fang, Fowler, Tomkinson, & Hill, 2002; Xu et al., 2004). The starch ester substituent is eventually cleaved, but the cleavage temperature is higher than hydroxyl dehydration temperature (Barrios et al., 2013; Elomaa et al., 2004). It is noteworthy that a few studies reported starch esters were less heat stable than native starches (Di Filippo et al., 2016; Tupa et al., 2015; & Zuo et al., 2013). These samples were synthesized with "dry" or "solvent-free" media that lack of a countering alkali. Therefore, trace acid residue in the sample might have destabilized the starch ester during heating.

Table 1.4. Thermal transitions of common starch esters.

Starch derivatives	DS	Thermal transitions (°C)*	Degradation (°C)	Reference
Maleate	0.35		250-320	Zuo et al., 2013
Succinate	1.7		260-320	Chang et al., 2012

Acetate	2.9	171 (T_g)		Yang & Montgomery, 2008
Propionate	2.5		350-400	Garg & Jana, 2011b
Propionate	2.4-2.6		320-390	Zhu et al., 2017
C_6	3.0	69 (T_g)		Yang & Montgomery, 2008
C_6	2.4	68 (T_g)		
C_{12}	2.4	4 (T_m); 78 (T_g)	320-380	Winkler et al., 2014
C_{18}	2.2	26 (T_m); 100 (T_g)		
C_{16}	3.0	30-50 (T_m); 45-60 (T_g)		Liebert et al., 2011
C_8	2.8	40 (T_g)		
C_{12}	2.8	-40 (T_m); 40 (T_g)	300-350	Vanmarcke et al., 2017
C_{16}	2.8	10 (T_m); 40 (T_g)		
C_{12}	1.8	27 (T_m)	310-390	Junistia et al., 2009
C_{18}	1.5	21 (T_m)		
C_{18}	1.3	82-92 (T_g)	310-360	Söyler & Meier, 2017

* T_m, side chain melting temperature; T_g, starch backbone glass transition temperature.

Conclusions

Substituted starches with intermediate to high DS are environment-friendly biomaterials based on abundant and renewable resource. While high DS short chain starch carboxylic acid esters can be prepared in aqueous or anhydrous media without using organic solvents, mainstream methods for long chain esters still involve large amount of solvents, mainly pyridine analogs, to aid catalyzation and starch dispersion. A major challenge in the field is to discover a "true green" pathway that is feasible for large scale production that is cost effective and environmentally friendly. Areas that have been focused on including: (1) increasing reagent-starch combability and dispersity with emulsions, microwave, and ultrasonic treatments, (2) using less harmful solvents (e.g. DMSO, toluene, and ionic liquids), (3) using enzyme to transesterify acyl from plant oil to starch, and (4) "solvent-free" route that first derivative the starch with a less bulky substituent, e.g. formate and acetate, to make the starch compatible for

further reaction media. In author' opinion, the latter two directions fit the concept of "green chemistry", because exclusion of harmful solvents reduce overall cost for synthesis and purification.

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Chapter 2 - Position of acetyl groups on anhydroglucose unit in acetylated starches with intermediate degrees of substitution

Abstract

Waxy, normal, and high-amylose maize starches were acetylated to various degrees of substitution (DS) in aqueous medium at pH 8.0 and the substitution pattern of the acetyl group on anhydroglucose unit (AGU) was determined. A stepwise addition of acetic anhydride enhanced reaction efficiency and DS was in the order of high-amylose maize starch (DS 1.72) > waxy maize starch (DS 1.60) > normal maize starch (DS 1.03) when 240% (w/w) acetic anhydride was added. Starch acetates were further fully esterified to DS 3.0 using acetic anhydride-d6 or propionic anhydride to analyze position of acetyl groups on AGU by ^{1}H NMR. The hydroxyl group linked to C-2 in AGU was the preferred reaction site when the DS was lower than 0.14. At DS 1, the distribution of acetyl groups was ca. 44%, 32%, and 24% at C-2, C-3, and C-6 positions regardless of amylose content. Deuterated acetyl group was a preferred secondary substituent over propionyl group in subsequent 1H NMR analysis because it resulted a clean spectrum without interference from propionyl methyl protons.

Keywords

starch acetate, substituent distribution, ^{1}H NMR, peracetylation

Abbreviations

DS, degree of substitution; NMR, nuclear magnetic resonance; AGU, anhydroglucose unit; CA, cellulose acetate; SA, starch acetate; CDCl$_3$, chloroform-d; DMSO-d_6, dimethyl sulfoxide-d_6;

Introduction

Starch is an abundant and renewable natural polymer that is widely used in food and industrial applications. Chemical modifications are often used to improve physiochemical properties of starch for specific applications (BeMiller & Whistler, 2009; Wurzburg, 1986). Repeating glucose units in a starch molecule provide ample hydroxyl (–OH) groups, which are excellent sites for grafting various functional groups. Acetylation is one of the modifications by reacting vinyl acetate or acetic anhydride with starch (Wurzburg, 1986) to esterify a fraction of –OH groups with acetyl groups. Acetylated starch that has an acetyl content less than 2.5% (DS 0.097) is approved by the US Food and Drug Administration (FDA) as a food additive (FDA, 2017).

Intermedium- to high-DS (0.8-3.0) starch acetates (SA) become soluble in acetone and chloroform, have improved thermoplasticity (Wurzburg, 1986), resistant to enzymatic digestion (Bird, Brown, & Topping, 2006), and have potential applications in hot melt adhesive, biodegradable package materials, tablets, and encapsulation of bioactives (Billmers & Tessler, 1994; Korhonen, Kanerva, Vidgren, Urtti, & Ketolainen, 2004; Robert, García, Reyes, Chávez, & Santos, 2012; Tessler & Billmers, 1996). For preparation of medium- to high-DS SA, pyridine was often used to facilitate granule swelling and improve starch reactivity (Wurzburg, 1986). However, due to its toxicity and difficulty to be removed after reaction, pyridine is not widely used for large-scale production of SA. Mark and Mehltretter (1972) reported a pyridine-free method to produce starch acetate up to DS 3.0 using acetic anhydride as a reactive solvent; however, the reaction was done at 123 °C resulting granular structure destroyed and off-color (Xu, Miladinov, & Hanna, 2004). Billmers and Tessler (1994) disclosed a one-step process of

making medium-DS SA in aqueous media by using high levels of acetic anhydride and concentrated alkaline.

Properties of chemically modified starches, including acetylated starches, are dependent on total DS, pattern of reaction within the granule, and pattern of modification on individual starch molecules (Huber & BeMiller, 2009). At the universal level, the overall extent of acetylation or DS of an acetylated starch is affected by the sources of starch and may be determined by titration or NMR (Chi et al., 2008; Dicke, 2004; Elomaa et al., 2004; Luo & Shi, 2018; Mormann & Al-Higari, 2004; Shogren & Biswas, 2010). When reacted with acetic anhydride in water by the method of Billmers & Tessler (1994), high-amylose maize starch had a higher DS than waxy and normal maize starches (Luo & Shi, 2012). At the granular level, the uniformity of acetylation is dependent on reagent type (acetic anhydride vs. vinyl acetate) and granule size (Huang, Schols, Jin, Sulmann, & Voragen, 2007a; Huang, Schols, Jin, Sulmann, & Voragen, 2007b; Huang, Schols, Klaver, Jin, & Voragen, 2007). Vinyl acetate tends to penetrate deeper to the granule and produce clustered acetylation on starch polymer chains, whereas acetic anhydride is rather fast reacting on the surface of the starch granule (Huang, Schols, Klaver, Jin, & Voragen, 2007). At the molecular level, substitution patterns along starch chains are often obtained by treating modified starches with enzymes and analyzing the resulted hydrolyzed products (Bai, Kaufman, Wilson, & Shi, 2014; Mischnick & Momcilovic, 2010; Richardson & Gorton, 2003). Luo & Shi (2018) reported that catalyst concentration affected the substitution pattern along starch chains; 20% NaOH favored acetylation on external chain or away from branching points, whereas 3% NaOH favored substitution in inner regions. Bai, Kaufman, Wilson, & Shi (2014) reported that octenylsuccinic anhydride prefers to react near branching points and reducing ends in amylopectin.

At the molecular level, substitution positions on anhydrous glucose unit (AGU) also may affect properties of modified starches. Gas chromatography/mass spectrometry (Lee & Gray, 1995) and liquid chromatography, particularly high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Richardson & Gorton, 2003) have been used to determine substitution positions on AGU in cellulose and starch derivatives; however, those approaches have disadvantages such as laborious methylation, difficult to fully methylate starch, and lack of suitable standards (Richardson & Gorton, 2003). Nuclear magnetic resonance (NMR) such as ^{13}C NMR has been reported as a powerful tool for determining substitute position on AGU by providing the ratio of substituted carbon signal at a shifted ppm to the unsubstituted carbon (Kono, Hashimoto, & Shimizu, 2015). However, the resonance of C-2 to C-5 (85-70 ppm) of AGU monomer is overlapped and difficult to be quantified (Heins, Kulicke, Käuper, & Thielking, 1998). Furthermore, the resolution of ^{13}C NMR spectrum can be unsatisfactory because the analysis required a highly concentrated, non-viscous sample solution, but starch acetates with low and intermediate DS have poor solubility in NMR solvents, e.g. D_2O, chloroform-*d*, and DMSO-*d*$_6$ (Richardson & Gorton, 2003). Tezuka and Tsuchiya (1995) first reported a perpropionylation pretreatment on cellulose acetate to quantify partial DS at C-2, C-3, and C-6. The pretreatment substituted unreacted hydroxyl groups with propionyl groups, rendering the sample readily soluble in chloroform at a high concentration. The DS on individual C position was calculated from propionyl and acetyl C=O signal resolved at 174-169 ppm. However, ^{13}C spectrum requires several hours to achieve quantitative signal-to-noise ratio and it is not feasible when a large number of samples are to be analyzed (Kono, 2013). ^{1}H NMR can be an option to determine the substituent distribution much quicker if the methyl protons at different carbon locations can be resolved. When dissolved and analyzed in

DMSO-d_6, methyl protons appear as one large splitting peak (Chi et al., 2008) which is only useful for total DS determination. Goodlett et al. (1971) first reported an approach to peracetylate cellulose acetate (CA) with acetyl-d_3 chloride to make the CA soluble in $CDCl_3$. Because $CDCl_3$ is a much less-viscous solvent to DMSO-d_6, and the deuterated methyl causes no interference to the ordinary methyl signal, the ^{1}H NMR spectrum gives improved resolution and could be used to quantify the DS on individual carbon. A limited number of studies have been reported on the position of acetyl groups on AGU in acetylated starches and distribution patterns are affected by the reaction medium, type of reagent (e.g. vinyl acetate vs. acetic anhydride), and catalyst (Hampe & Heinze, 2014; Mormann & Al-Higari, 2004; Richardson, Andersson, Brinkmalm, & Wittgren, 2003; Shogren & Biswas, 2010). Dicke (2004) determined substitution pattern by carrying out perpropionylation followed by ^{1}H NMR on a series of starch acetates (synthesized in DMSO medium with various catalysts) and concluded that the acetylating reagent and type of catalysts were influential to substitution pattern. Vinyl acetate with 2% of neutral or alkaline salts was reactive at starch C-2 position exclusively, whereas acetic anhydride resulted in a more even substitution with some preference at C6 position. Unlike acetylation of starch in DMSO (Dicke, 2004), when potato starch was reacted with vinyl acetate in water in the presence of a base, no regioselective distribution of acetyl groups was observed; ca. 46% acetyl groups was on C2 and more than 50% acetyl substituents were on C3 and C6.

Preparation of starch acetates with intermediate DS using acetic anhydride in aqueous medium avoids toxic chemicals (Billmers & Tessler, 1994; Luo &Shi, 2012; Luo & Shi, 2018; Tessler & Billmers, 1996); however, the position of acetyl groups on AGU made by this approach has not been studied. To our knowledge, there is no report on if starches with different amylose contents would have the same or different acetyl distribution patterns. In this study,

maize starches of various amylose contents were acetylated in aqueous medium to up to 1.71 DS and the change of acetyl distribution on monomer level (AGU) was investigated as the reaction proceeded for the first time. A side-by-side comparison of peracetylation and perpropionylation pretreatments prior to ^{1}H NMR was carried out to evaluate their effectiveness of improving the spectrum resolution. The objectives of this study were to investigate (1) acetylation efficiency of SA produced from using a concentrated (10 N) NaOH solution with acetic anhydride added in one dose or stepwise manner, (2) perdeuterated acetylation verses perpropionylation as pretreatments for subsequent ^{1}H NMR analysis, and (3) distribution of acetyl groups in the starch acetate synthesized in aqueous medium.

Materials and methods

Materials

Waxy, normal, and high-amylose starches were obtained from Ingredion Incorporated (Bridgewater, NJ). Acetic anhydride and acetic anhydride-d_6 (98.5% purity, Cat. No: AC174670050) were purchased from Fisher Scientific (Waltham, MA). Propionic anhydride and (Cat. No: 240311) chloroform-d (Cat. No: 151858) were purchased from Sigma-Aldrich (St. Louis, MO).

Synthesis of SA of various DS

Waxy, normal, and high amylose maize starch (35 g each) were suspended in distilled water to form 30% solid slurries in a 500 mL three-neck-flask and stirred using an overhead mechanic stirrer in an icy water bath for 30 min. The actual temperature of starch slurry was 5 °C throughout the reaction. The pH of starch slurry was adjusted and maintained at 8.0 by a pH controller system (Eutech Instruments, Vernon Hills, IL) delivering drop-wise 10 N sodium hydroxide. After initial pH was stabilized for 30 minutes, 10, 85, 120, and 240% acetic

anhydride was introduced using a peristaltic pump at 1% acetic anhydride per min for total 10, 85, 120, and 240 minutes, respectively. The slurry was adjusted to pH 6.0 with 1N HCl, filtered, and the starch cake was washed three times with 500 mL distilled water, and dried in an air-forced oven at 40 °C overnight. The 240% acetic anhydride experiment was carried out with a stepwise procedure where the acetic anhydride was added at three doses of 120, 60, and 60%. The starch slurry was filtered and re-suspended into two parts of water in between each addition. The dried acetylated starch was ground by a food blender and passed through #60 mesh and stored in air tight containers. Control experiments were carried out using an equal weight of acetic acid instead of acetic anhydride and so that the starches were only subjected to 10 N NaOH treatment but not acetylated.

Titration to determine the DS of the SA

Titration method was used to determine the DS of the SA (Elomaa et al., 2004). Briefly, 0.5 grams of SA was mixed with 50 mL distilled water and the pH was adjusted to 7. Hydrolysis was done by adding 25 mL 0.5N NaOH to the sample and stir at room temperature for 24 h. HCl (0.5 N) was used to titrate the mixture to pH 7 and the consumption of HCl was used to calculate DS using the following equations.

$$\%Acetyl = \frac{(V_{Blank} - V_{AC}) \times 0.5 \times 0.043}{0.5} \times 100\% \qquad (1)$$

$$DS = \frac{162 \times \%Acetyl}{43 - 42 \times \%Acetyl} \qquad (2)$$

where V_{Blank} was the volume of HCl consumed in titrating unmodified starch and V_{AC} was the HCl volume consumed in titrating acetylated starch

Determination of reaction efficiency (RE)

RE% was calculated as DS × AGU (mol)/acetic anhydride (mol) × 100%.

Morphology of acetylated and unmodified starches

Starch (10 mg) was mixed with 1 mL glycerol/water (50/50) and stirred for five minutes. The slurry was observed under an optical microscope (Olympus BX51, Tokyo, Japan) using a 40× objective lens.

Full acetylation or propionylation of starch acetate

The peracetylation procedure was modified from Mormann & Al-Higari (2004). Starch acetates of various DS were dried in a vacuum oven at 80 °C overnight. Each dried starch acetate (100 mg) was mixed in 10 mL pyridine at 60 °C until fully dispersed. Acetic anhydride-d_6 (400 mg) was reacted for 48 h. The reacted product was washed in methanol (30 mL) for three times and dried in an air-forced oven at 60 °C overnight. The perpropionylation was carried out in similar fashion except that the reaction temperature was elevated to 80 °C and the reaction time was shortened to 24 h.

^{1}H NMR analysis

The percetylated and perpropionylated starch derivatives (10 mg, each) were dissolved in chloroform-d_1 (1 mL) and transferred into 3 mm NMR tubes. All NMR spectra were recorded at 298 K on a Varian 500 MHz NMR System (Varian Inc., Palo-Alto, CA) equipped with a triple-resonance, inverse-detection, pulse-field-gradient probe. The chemical shifts were calibrated against chloroform proton at 7.26 ppm. The NMR spectra were Fourier transformed and analyzed using ACD/NMR Processor (Advanced Chemistry Development, Inc., Toronto, Canada). Individual DS was calculated using the following equation (Chi et al., 2008):

$$DS_{acetyl} = \frac{7A}{3B}$$

$$DS_{propionyl} = \frac{7P}{3B}$$

where A was the area of acetyl's methyl protons between 1.90 to 2.27 ppm; P was the area of propionyl's methyl protons between 1.30-0.98 ppm; B was the sum of the seven glucose ring protons higher than 3.8 ppm.

Statistical analysis

NMR analysis and titration were replicated two times for each starch sample. The DS changes was analyzed by one-way analysis of variance (ANOVA). A probability of $P \le 0.05$ was considered significant. Statistical procedures were by SAS 9.3 (SAS Institute; Cary, NC).

Results and Discussion

Effect of ratio of acetic anhydride to starch on DS and RE

DS of SA increased as a function of the ratio of acetic anhydride to starch (Fig. 2.1). During 240 min reaction time, the DS change for all three starches exhibited hyperbolic curves that plateaued over 120% acetic anhydride based on the weight of starch. The increase in DS was faster at the beginning due to the ample amount of free hydroxyl groups, which depleted as reaction proceeded. At the beginning of acetylation, the slurry remarkably expanded, resembling an aerated foam that was approximately three times of liquid volume as the starches swelled. The swelling of starch was largely attributed to disruption of intermolecular bond by acetylation and was also noted by Billmers & Tessler (1994) and Luo & Shi (2012). Starch swelling as measured by a settling volume measurement peaked with an approximate 30 to 70% acetic anhydride addition (Luo & Shi, 2012). As more acetic anhydride was added, the reaction slurry thinned as the starches' hydrophobicity increased. High-amylose maize starch reached the highest DS among all three starches (Fig. 2.1), which could be explained by its low crystallinity in the granules (Luo & Shi, 2012); more amorphous regions provided more reaction space for acetic

anhydride. The DS for waxy starch was slightly less than the DS of high-amylose starch but greater than normal maize starch, which is probably due to more hydroxyl groups exposed in waxy maize starch (Luo & Shi, 2012), as the initial swelling volume of waxy maize starch was greater.

Reaction efficiency (RE) of acetylation decreased as more acetic anhydride was added (Fig. 2.1). The decrease in RE could be due to a combined effect of diminishing unreacted hydroxy group and dilution of starch content during the reaction. In this study, we designed a step-wise process determine the effect of re-concentrating the slurry on RE. The slurry was filtered and suspended in three parts (as to dry starch) of water between the additions of 120, 60, and 60% acetic anhydride. The acetic anhydride was divided in such proportion to aim ease of dewater and improve RE. Because after 120% acetic anhydride was added, the acetyl DS was high to constraint starch swelling so that the starch acetate could be readily filtered. Moreover, the DS increase was not evident after 120% acetic anhydride addition in continuous reaction. The step-wise procedure resulted in 5%, 4%, and 6% RE increase in waxy, normal, and high-amylose starch, respectively, and the DS was in the order of high-amylose maize starch (DS1.72) > waxy maize starch (DS 1.60) > normal maize starch (DS 1.03) when total 240% (w/w) acetic anhydride was added (Fig. 2.1). In a study reported by Pu et al. (2011), a higher DS (2.93) high-amylose maize starch acetate was synthesized in aqueous medium when the reaction was maintained at above gelatinization temperature (80 °C) and the acetic anhydride addition was 400% based on the weight of starch. However, in our preliminary trials, we found that reaction slurry greatly thickened at above gelatinization temperature and led to difficulties in temperature and pH control, and therefore we carried out acetylation at a lower temperature.
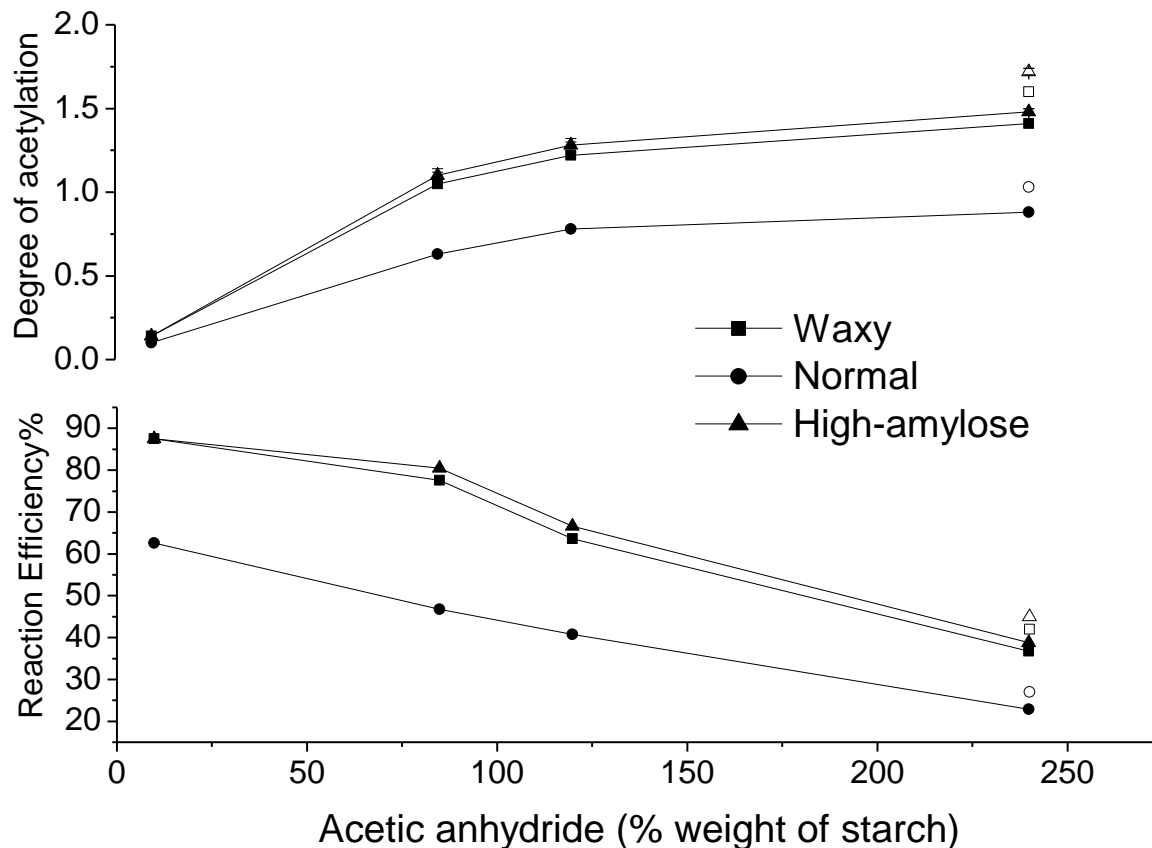
Figure 2.1. Reaction efficiency (RE) and degree of acetylation of starches as a function of acetic anhydride amount; hollow symbols represent the results of 240% acetic anhydride added stepwise in three portions.

Changes in morphology

Light microscopy was used to examine the starch morphologic change after acetylation (Fig. 2.2). After 10% acetic anhydride was added, edges of starch granules became rough and irregular. As DS increased to 1.0 and greater, loss of birefringence was observed (Fig. 2.2 b&c). This could be explained by the partial disruption of intermolecular H-bonds, which is the key force retaining starch granular structure. Previous studies reported that in when SA were synthesized at above 100 °C, granule structure was lost completely at DS 1.68 and above (Chi et al., 2008; Xu et al., 2004). Starch granular structure was retained largely in this study when

synthesized in aqueous media at 5 °C. In a controlled experiment where starch was only

subjected to acetic acid and countering alkaline, no noticeable change was observed comparing

to the untreated starch and therefore, the morphological changes were mostly attributed to

acetylation but not alkaline addition.
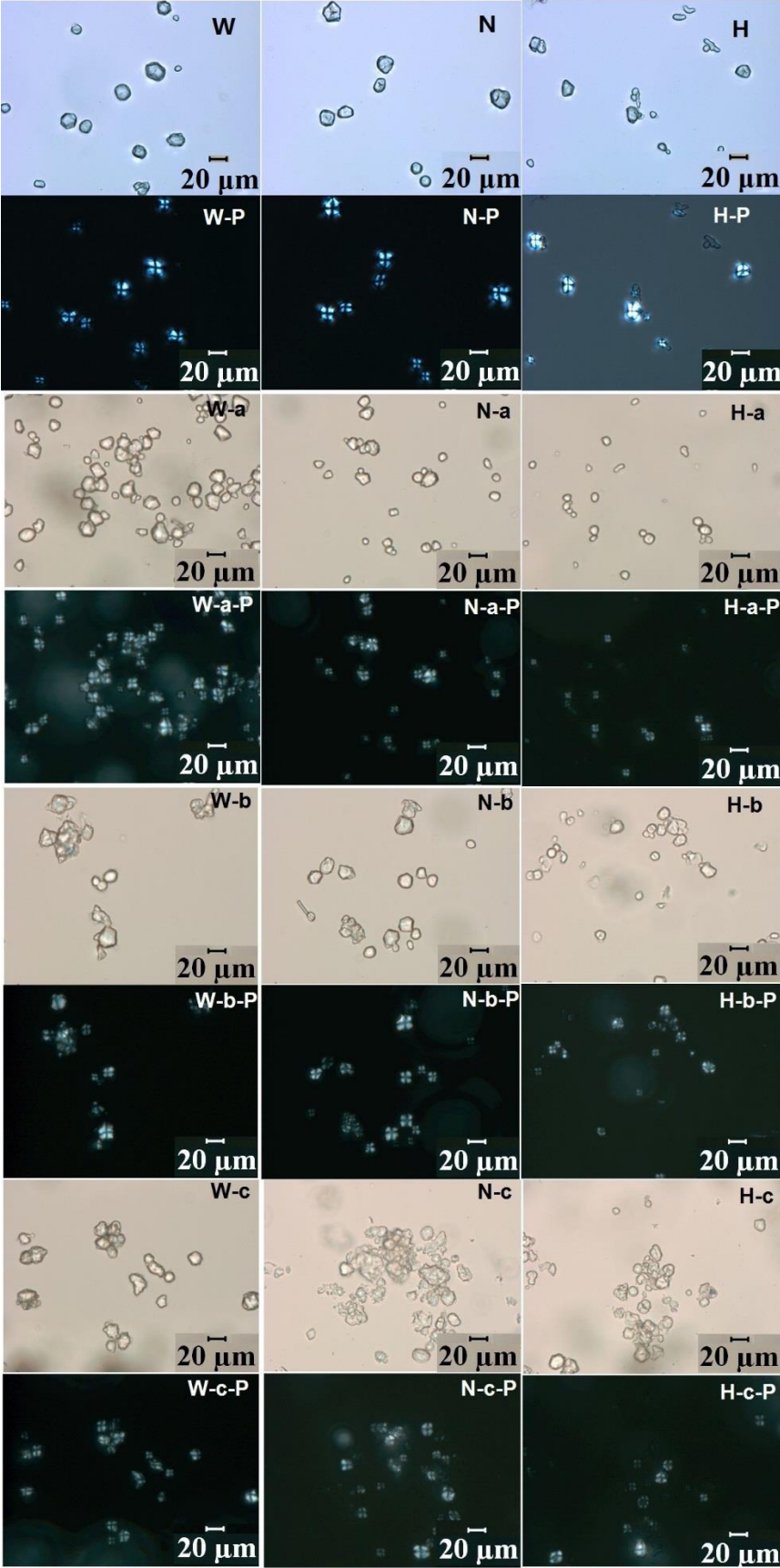
Figure 2.2. Light microscopic images of unmodified and acetylated waxy (W), normal (N), and high-amylose (H) maize starches prepared with 10 (a), 120 (b) and 240 (c)% acetic anhydride; "P" indicates that the samples were viewed under polarized light.

Peracetylation using acidic anhydride-d_6

Figure 2.3 shows the representative ^{1}H NMR spectra of SA in chloroform-d_1 after

peracetylation. To verify a full substitution was achieved, a parallel experiment using ordinary

acetic anhydride was carried out to ensure that the complete substitution was achieved. It should

be noted that three equally-sized acetyl peaks between 1.8 and 2.3ppm (DS 0.015) were observed

when native starch reacted only with acetic anhydride-d_6 (Fig. 2.3a). The three acetyl peaks were

presumably resulted from a trace amount (1.5%) of ordinary acetic anhydride in the acetic

anhydride-d_6 reagent. For a ^{1}H scans of partially substituted SA, broadening and shifting of the

ring protons signals has been observed (Chi et al., 2008; Kono et al., 2015). Because SA is a

mixture of eight types of AGUs (2,3,6-tri-, 2,3-di-, 2,6-di-, 3,6-di-, 2-mono-, 3-mono-, 6-mono-,

and unsubstituted) and the heterogeneity of substitute distribution caused the AGU ring proton

signals to shift downfield differently and peaks were not well resolved. However, peracetylation

allowed the AGU protons to be evenly shifted, resulting in well resolved spectra (Fig. 2.3).

Signals at 2.20, 2.03, and 1.99 ppm were identified as acetyl protons substituted at C-6, C-2, and

C-3, respectively (Goodlett et al., 1971; Mormann & Al-Higari, 2004). Adapting calculation of

resolution factor in a chromatogram (Equation 3), the resolution factor of C-2 and C-3 acetyl

signals was 1.2 (Fig. 2.3f), which was close to a baseline resolution 1.5 (Miller, 2005).

$$Resolution\ factor = (\delta 2 - \delta 3)/(0.5 \times (W2 + W3)) \tag{3}$$

Where $\delta 2$ and $\delta 3$ were chemical shifts value of C-2 and C-3 acetyl signal, and W2 and W3 were

the width of the C-2 and C-3 acetyl signal at half peak height in ppm.
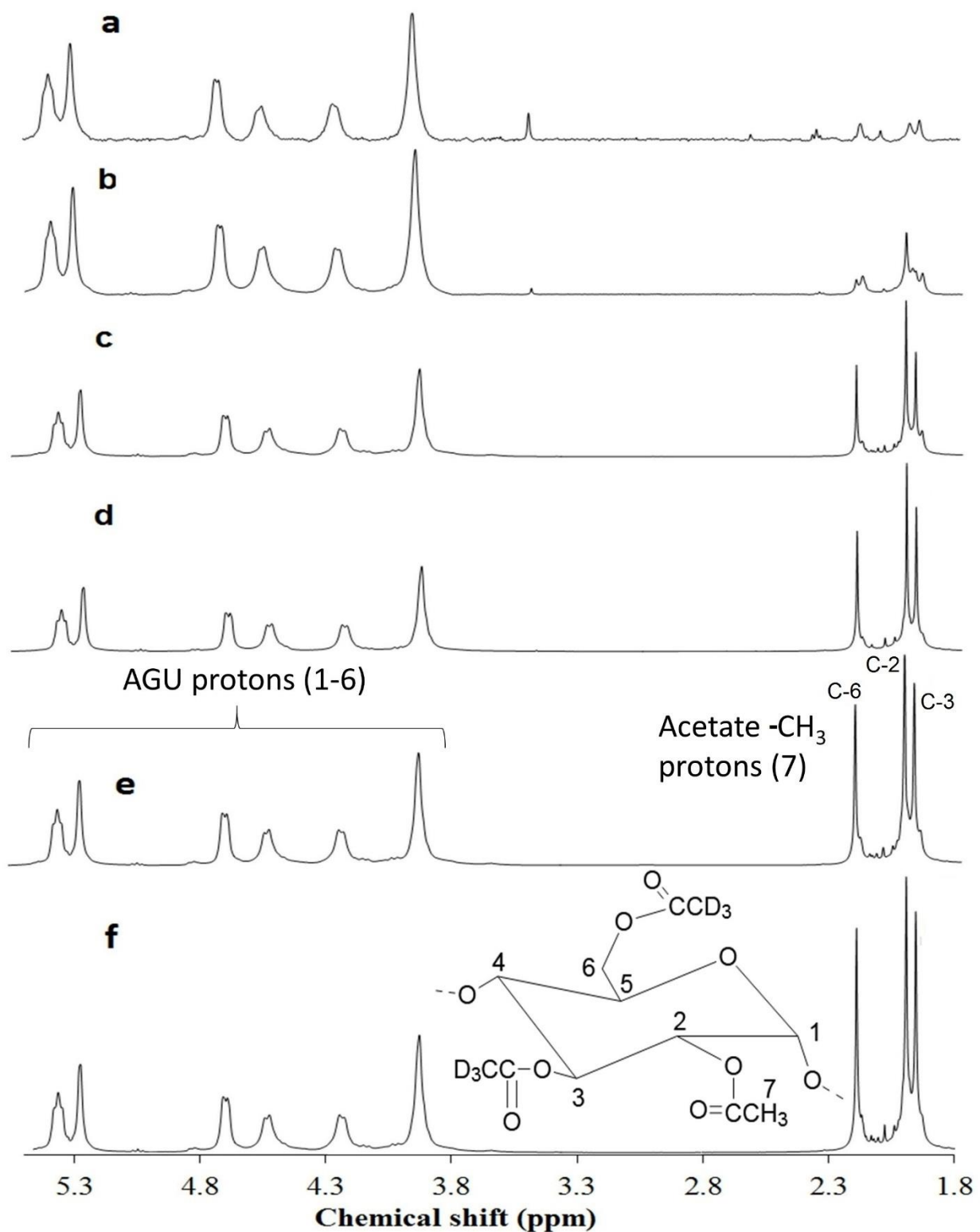
Figure 2.3. ¹H-NMR spectra of high amylose maize starch reacted with 0 (a), 10 (b), 85 (c), 120 (d), 240 (e), and 240% (stepwise) (f) acetic anhydride, and further fully acetylated to DS 3.0 with acetic anhydride-d_6. NMR signals of protons in anhydrous glucose units (AGU)

and protons of methyl in acetyl group attached to O-2, O-3, and O-6 on AGU are indicated in Fig 2.3e.

Perpropionylation using propionic anhydride

Propionylated starch showed proton signals from $-CH_3$ at 0.95-1.35 ppm and $-CH_2-$

signals at 2.65-2.10 ppm (Fig. 2.4a). The combined DS of acetyl and propoionly was

approximately 3.0 indicating the starch was fully substituted. However, the acetyl signal from C-

6 position was overlapped with $-CH_2-$ (Hampe & Heinze, 2014), making it impossible to

calculate its acetyl DS directly. To resolve that, we calculated the area attributed from the $-CH_2-$

in propionate group via the peak area of propionyl $-CH_3$, which was clearly resolved between

0.95-1.35 ppm (Fig. 2.4). The area from $-CH_2-$ was 2/3 of the peak area of propionyl $-CH_3$.

Therefore, the area from acetyl at C-6 was calculated from the total area between 2.1—2.65 ppm

minus 2/3 of propionyl $-CH_3$ peak area, and then acetyl DS at C-6 was calculated. C-2 and C-3

acetyl signals were broader than in the fully acetylated sample and the resolution factor was 0.8

(Fig. 2.4f), indicating a less than desired resolution than the peracetylated sample. Thus, acetyl

groups at C-2 and C-3 DS were calculated as a sum in starch acetate propionate. Our approach

allowed us to verify that total acetyl and propionyl DS was close to 3.0 and ensure that the full

derivation was achieved.
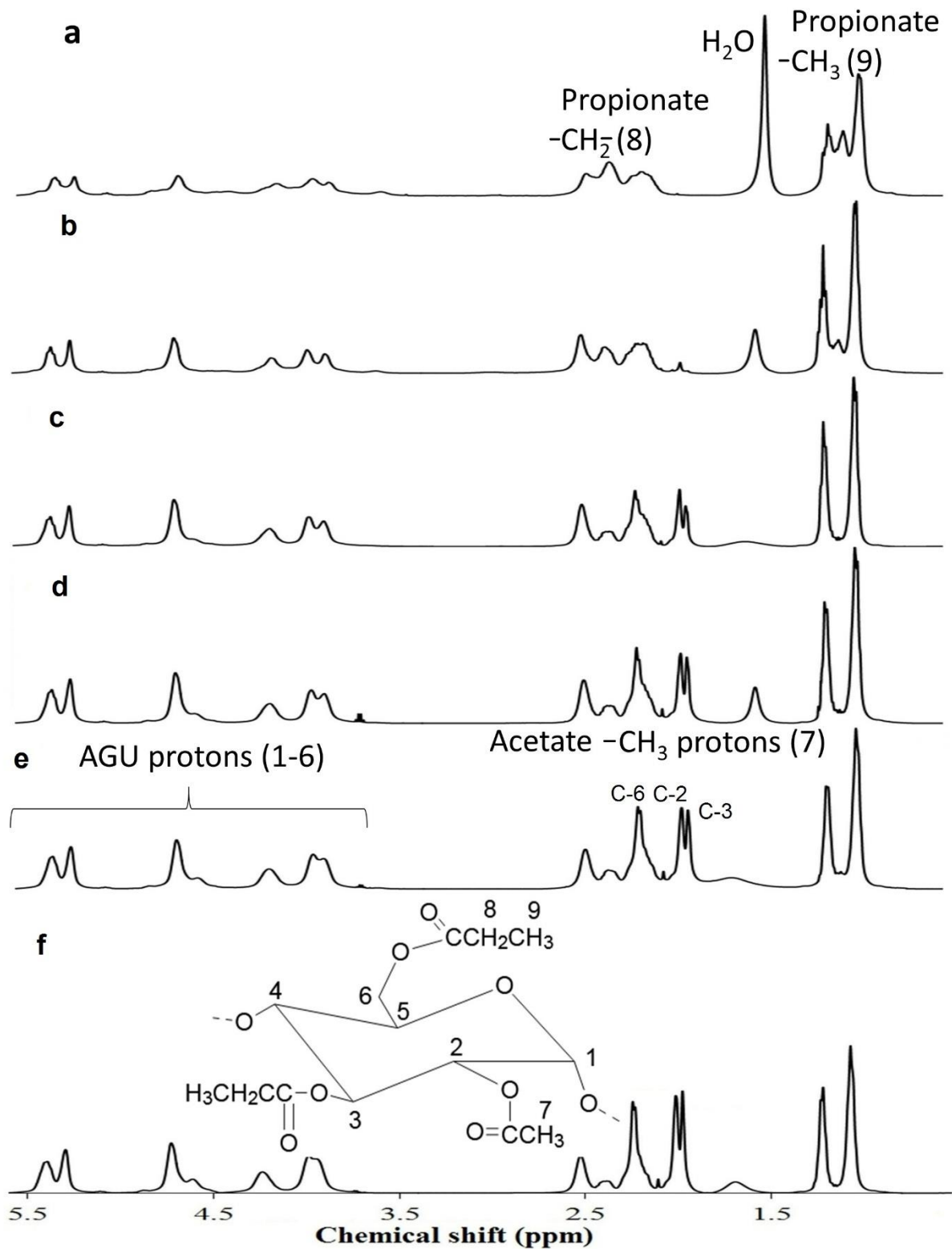
Figure 2.4. ¹H-NMR spectra of high amylose maize starch reacted with 0 (a), 10 (b), 85 (c), 120 (d), 240 (e), and 240% (stepwise) (f) acetic anhydride, and further fully propionylated

to DS 3.0 with propionic anhydride. Protons in anhydrous glucose units (AGU) and protons of acetyl methyl group attached to O-2, O-3, and O6 on AGU are indicated in Fig. 2.4e.

Substituted positions of SA

Position of acetyl groups on AGU

Tables 2.1 and 2.2 summarize the DS at each reaction site for waxy, normal, and high-amylose maize starches. After subtracting the acetyl peaks arisen from ordinary acetic anhydride in the deuterated acetic anhydride reagent, the spectrum of the starches reacted with 10% acetic anhydride (Fig. 2.3b) showed only substitution at C-2 position, indicating that there was a great reaction preference over other hydroxyl groups and acetylation started exclusively at 2-OH groups. At DS 0.10-0.15, regio-specific 2-*O*-acetylated starches were produced regardless amylose content (Table 2.1).

As the reaction proceeded, the overall acetyl DS increased (Table 2.1 and 2.2). At approximately DS 1.0, nearly half of acetyl group substituted at C-2 and a marginal preference of C3 over C6 was observed, regardless of amylose content of the base starch and the amount of acetic anhydride consumed. Mormann and Al-Higari (2004) reported a similar substitution pattern when they acetylated potato starch to DS 1 using vinyl acetate in water. Vinyl acetate is regarded as a slower reacting acetylation reagent and the same level of acetylation DS is achieved in longer reaction time but produce a more even substitution in granular level as compared to acetic anhydride (Huang, Schols, Jin, et al., 2007b; Huang, Schols, Klaver, et al., 2007). Our results suggest that at DS about 1, acetic anhydride and vinyl acetate produce similar position of acetyl groups at monomer level, which is a predominately 2-*O*-acetylated starch when prepared in water in the presence of a base. After more than 85% acetic anhydride (based on the weight of starch) was reacted, C3 and C6 had similar acetyl substitution (Table 2.2).

Relative reactivity of hydroxyl groups in carbohydrates has long been a subject of studies and reviews (Haines, 1976; Lawandi, Rocheleau, & Moitessier, 2016; Miljkovic, 2009, Sugihara, 1953). In an aldohexopyranose, the anomeric hydroxyl group (i.e. 1-OH) is most reactive (Damager et al, 2010). However, the level of reducing ends or anomeric hydroxyl groups in starch is very low. For AGU in starch, there are three hydroxyl groups that are linked to C-2, C-3 and C-6, and they all have an equatorial orientation. In general, the primary hydroxyl group (i.e. 6-OH) is more reactive than secondary hydroxyl groups due to steric factor (Lawandi, Rocheleau, & Moitessier, 2016; Miljkovic, 2009). However, higher reactivity is found at 2-OH in this study and other modified starches including methyl, hydroxylethyl, and hydroxypropyl starches (Richardson & Gorton, 2003). The higher reactivity of the 2-OH in modified starches has been noted when reactions use low alkali concentration (Richardson & Gorton, 2003). The enhanced reactivity of 2-OH is attributed to its greater acidity because of its proximity to the electron-withdrawing anomeric carbon, a carbonyl or a potential carbonyl group (Haines, 1976; Richardson & Gorton, 2003; Sugihara, 1953). In contrast, when higher alkali concentrations are used in making cellulose derivatives, 6-OH, the primary hydroxyl group, is the most reactive because it is sterically less hindered (Pitha, Trinadha Rao, Lindberg & Seffers, 1990; Richardson & Gorton, 2003). Under similar alkali concentrations, starch has a stronger preference for substitution at 2-OH than cellulose because starch and cellulose have α and β anomeric configurations, respectively. The differences in anomeric configuration may attribute to the lower acidity of the hydroxyl group on C-2 in cellulose (Richardson & Gorton, 2003).

It is remarkable that regioselective 2-*O*-acetyl starches were produced in water when 10% acetic anhydride was reacted in this study (Table 2.1). As the degree of acetylation increased, the availability of 2-OH groups decreased, and 3-OH and 6-OH were reacted when 85% acetic

anhydride was added (Table 2.1). The acetylation at 2-OH may have increased the reactivity of

the neighboring 3-OH groups.

Table 2.1. Distribution of acetyl group determined by peracetylation using acetic anhydride-d_6

Starch	Acetic Anhydride (%)	Acetyl C-2 DS	Acetyl C-3 DS	Acetyl C-6 DS	Total Acetyl DS
Waxy	10	0.13±0.01e	0d	0c	0.13±0.01e
	85	0.45±0.01d	0.29±0.01c	0.29±0.02b	1.00±0.04d
	120	0.50±0.01c	0.35±0b	0.28±0.01b	1.16±0.03c
	240	0.56±0.01b	0.45±0.01a	0.40±0.01a	1.42±0.02b
	240sw	0.65±0.00a	0.49±0.03a	0.46±0.02a	1.56±0.05a
Normal	10	0.10±0.00e	0d	0d	0.10±0.01e
	85	0.29±0.01d	0.21±0.01c	0.16±0c	0.65±0d
	120	0.33±0.01c	0.23±0bc	0.17±0c	0.74±0.01c
	240	0.41±0.01b	0.27±0.02ab	0.20±0.01b	0.86±0.02b
	240sw	0.47±0.01a	0.29±0a	0.25±0.01a	1.00±0.02a
High-amylose	10	0.15±0.01d	0d	0d	0.15±0.01e
	85	0.45±0.01c	0.30±0.02c	0.25±0c	1.02±0.02d
	120	0.53±0.01b	0.40±0.02b	0.31±0.01c	1.26±0.02c
	240	0.55±0b	0.44±0b	0.39±0.02b	1.38±0.01b
	240sw**	0.66±0.01a	0.53±0.01a	0.55±0.02a	1.70±0.04a

*Different letters indicate significant difference (n=2; $P<0.05$) between each starch type in each

column; **sw, 240% acetic anhydride was added stepwise in three portions.

Table 2.2. Distribution of acetyl group in starch AGU determined by perpropionylation of partially acetylated starches subtracted for propionate NMR signals.

Starch	Acetic anhydride (%)	Acetyl at C-2&C-3	Acetyl at C-6	Total acetyl DS	Propionyl DS	Total DS
Waxy	10	0.13±0.01d	0e	0.13±0.01e	2.84±0.06a	2.97±0.04a

	85	0.74±0.01c	0.28±0.03d	1.02±0.04d	2.02±0.04b	3.04±0.08a
	120	0.78±0.01c	0.35±0.01c	1.13±0.02c	1.91±0.02b	3.04±0.03a
	240	0.92±0.01b	0.42±0.01b	1.34±0.02b	1.71±0.02c	3.05±0.02a
	240sw	0.98±0.01a	0.53±0.01a	1.51±0.02a	1.50±0.02d	3.01±0.01a
	10	0.11±0.01d	0d	0.11±0.01e	2.90±0a	3.01±0.01bc
	85	0.51±0.01c	0.15±0.01c	0.65±0d	2.44±0.01b	3.09±0.01a
Normal	120	0.60±0.02b	0.18±0.01b	0.78±0.01c	2.28±0c	3.07±0.01a
	240	0.64±0.01b	0.21±0.01b	0.85±0.02b	2.14±0d	2.99±0.01c
	240sw	0.74±0.02a	0.31±0.01a	1.05±0.03a	2.00±0.01e	3.05±0.02ab
	10	0.14±0.01d	0d	0.14±0.01e	2.84±0.01a	2.98±0.01b
	85	0.73±0.02c	0.28±0c	1.02±0.02d	2.12±0.04b	3.13±0.02a
High-amylose	120	0.83±0.02b	0.38±0.01b	1.21±0.04c	1.88±0.02c	3.09±0.05a
	240	0.89±0.01b	0.42±0.01b	1.31±0b	1.76±0.01d	3.07±0.01ab
	240sw**	1.06±0.01a	0.55±0.02a	1.61±0.03a	1.41±0.01e	3.02±0.02ab

*Different letters indicate significant difference (n=2; P<0.05) between each starch type in each column; **sw, 240% acetic anhydride was added stepwise in three portions.

Conclusions

Peracetylation using deuterated acetic anhydride was a preferred pretreatment over perpropionylation for determining the distribution of acetyl group by ^{1}H NMR, because separation of acetyl proton signals after peracetylation was clear and unambiguous. In the case of perpropionylation, methylene signal interfered with acetyl protons on O-6 position, and the resolution of O-2 and O-3 acetyl methyl resonance was poor. At low levels of esterification, C-2 hydroxyl group was most preferred over C-3-hydroxyl and the C-6-hydroxyl. The regioselectivity at C-2 was diminished as more acetic anhydride was used. Amylose content had no evident effect on the distribution of acetyl on AGU.

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Chapter 3 - Preparation of highly substituted starch octenylsuccinate using granular and pre-gelatinized acetylated high-amylose maize starch

Abstract

Starch acetate octenylsuccinate mixed esters were prepared by a two-stage solvent-free melting reaction. High-amylose maize starch was first acetylated in aqueous medium to degree of substitution (DS) between 0.53-1.71 and pre-gelatinized. Acetylated starches before and after pre-gelatinization were reacted in excessive octenylsuccinic anhydride (OSA) at 160 °C to form a reactive melt, followed by purification with ethanol. Combined DS of starch esters ranged from 0.97 to 2.85. Glass transition temperature of the starch esters were between 53 to 107 °C. Pre-gelatinization of starch acetate of DS 0.53 and 0.89 resulted in significantly higher DS in subsequent octenyl succinate reaction; However, pregelatinization only showed marginal improvement when starch acetate DS was 1.3 or 1.7. A scale-up synthesis using DS 1.33 starch acetate (60 g) demonstrated excellent feasibility of such method in large-scale preparation. Unbounded octenylsuccinic acid in was 0.4% by weight in final purified starch ester in the scale-up experiment.

Keywords

Starch ester, hydrophobic starch, solvent-free synthesis, thermoplastic starch, OSA-modified starch, starch acetate

Abbreviations

OS-Ac-starch, octenylsuccinylated acetylated starch; pregel, pre-gelatinized starch. DS, degree of substitution; RE, reaction efficiency; T_g, glass transition temperature; TFA,

trifluoroacetic acid; DMSO, dimethyl sulfoxide; TMS, tetramethylsilane; OSA, octenylsuccinic anhydride;

Introduction

Starch is an abundant, low cost, and renewable natural polymer that is produced by higher plants as a storage polysaccharide. Besides being an important component of human diets, starch can be used in a number of industrial applications such as adhesives for paper, oil drilling, and cosmetics (BeMiller & Whistler, 2009). Starch has been studied for as an alternative for plastics from 1960s (BeMiller & Whistler, 2009); however, the high hydrophilicity and brittleness hurdled starch from producing satisfactory products, even after high amount addition of plasticizers such as glycerol, water, and sugar alcohols (Talja, Helén, Roos, & Jouppila, 2007).

Esterification of starch has been reported as an effective method to overcome some limits of native starch materials through disrupting starch recrystallization and lowering glass transition temperature (T_g) (Shogren, 1996). Depending on the degree of substitution (DS) and type of substituent, water resistance of starch materials can be largely improved (Winkler, Vorwerg & Rihm, 2014). The esterification process refers to reacting starch with esterifying reagents, most commonly being alkyl anhydride and chloride, graft ester to starch hydroxyl groups. Ester substituents that have been studied include short chain carboxylates (Garg & Jana, 2011), succinate (Chang, Qian, Anderson, & Ma, 2012), alkenylsuccinate (Chi et al., 2007; Jeon, Lowell, & Gross, 1999), malate (Zuo et al., 2013), and long chain fatty acid esters (Gao, Luo, & Luo, 2012). Typically, ester substituents must bound at high DS to impart internal plasticization and deliver functional properties such as water resistance and thermoplasticity (Fringant et al., 1998). While synthesis of low DS starch esters can be carried out by mixing acid anhydride,

chloride, or vinyl esters in aqueous slurries of starch in presence of a base, high DS starch ester synthesis is much more challenging, particularly in the cases when the substituents contain long alkenyl chains. Namazi et al. (2011) esterified starch with C1-C16 acyl groups in aqueous medium and reported decreasing reaction efficiency (RE) and DS as chain length increased. Chi et al. (2007) reported DS of dodecenyl succinic anhydride modified starch in aqueous medium could not pass DS 0.026 even after pre-emulsifying the reagent. Major hurdles in high DS reactions are (1) lack of suitable solvent to solubilize starch and starch ester throughout reaction because starch's hydrophobicity undergoes substantial change during esterification, (2) removal of unreacted chemicals and byproducts, and (3) degradation of starch backbone. To overcome such challenges, approaches to improve starch-solvent-reagent compatibility have been studied and achieved some success. Use of organic media, e.g. pyridine (Aburto, Alric, & Borredon, 1999; Fringant et al., 1998), molten imidazole (Liebert et al., 2011; Neumann, Wiege, & Warwel, 2002) and dimethyl sulfoxide (Junistia et al., 2008; Henning Winkler et al., 2013) kept starch ester solubilized throughout reactions and have become choices to produce highly esterified starch in literatures. However, high cost and toxicity of those solvents, as well as purification difficulties, hurdled large-scale synthesis. More recently, use of ionic liquids, e.g. 1-butyl-3-methylimidazolium chloride (Biswas, Shogren, Stevenson, Willett, & Bhowmik, 2006) have become popular in starch esterification and achieved comparable DS to starch esters synthesized in organic media. In a review paper by Wilpiszewska and Spychaj (2011), ionic liquids have been claimed as "green solvent" in many ways such as low vapor pressure, non-flammability, and recyclability; however, large-scale synthesis is still not feasible because ionic liquids are uneconomic and difficult to recycle, and pollution in water ways (Laus et al., 2005).

While all above studies aimed to increase starch solubility in reaction, a few studies carried out solvent-free synthesis and also achieved high DS (Aburto, Alric, & Borredon, 1999; Aburto et al., 2005; Shogren, 2003). Aburto, Alric, et al. (1999) first reported an approach utilizing starch-bound formic acid ester as an intermolecular plasticizer and achieved high DS in various starch fatty acid ester synthesis. In their work, starch was first reacted with formic acid to about DS 1.0 to impart a good compatibility in fatty acid chloride, and then heated with fatty acid chloride in absence of other chemicals to obtain high DS formated fatty acid starch ester. However, liberated HCl was reported as a major issue with this reaction setting, because HCl rapidly degraded the starch and caused low yield and discoloration (Aburto, Alric, & Borredon, 1999).

To resolve the acid degradation, using a different acyl donor e.g. octenylsuccinic anhydride (OSA), could be a choice because OSA release water as byproduct, which is clearly advantageous to acyl chlorides that release HCl. While low DS OSA modified starch is chiefly synthesized in aqueous media (Altuna et al., 2018; Sweedman et al., 2013), solvent-free high DS octenylsuccinic starch synthesis has only been reported by Shogren (2003) in milligram levels by heating starch, acetic acid, and OSA at 1/1/1 at 180 °C in differential scanning colorimetry (DSC) pans. In this paper, we propose a novel synthesis pathway to first acetylate the starch in water and followed by a melting reaction of dry starch acetate in excessive OSA to foster a high level of octenylsuccinylation (OS) (Fig 3.1). Furthermore, we pre-gelatinized the acetylated starch to investigate the effect of pre-gelatinization to subsequent OSA-starch reaction.
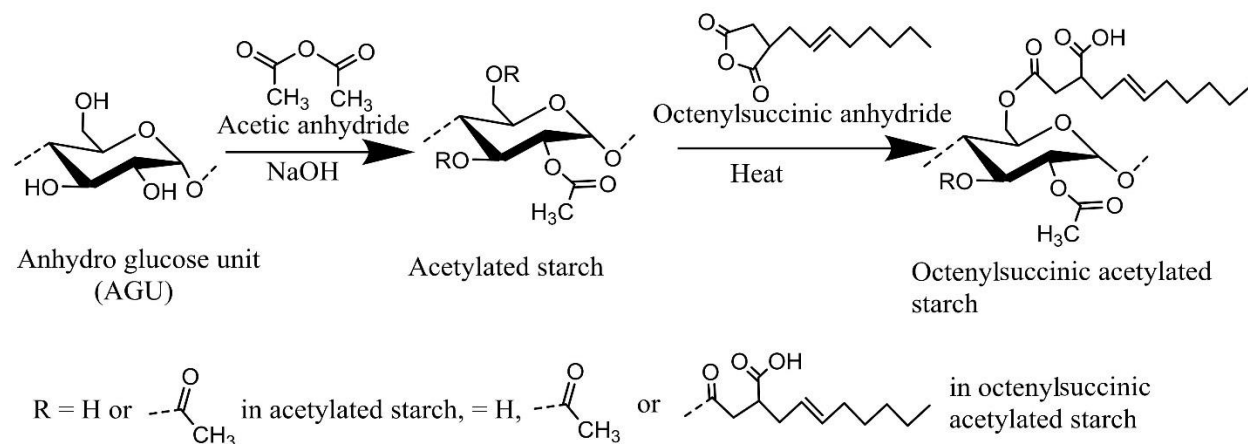
Figure 3.1. Solvent free two-stage starch mixed ester synthesis involving acetylation of starch in aqueous medium and subsequent octenylsuccinylation by dry heating starch acetate in octenylsuccinic anhydride.

Materials and methods

Materials

High-amylose starches (HYLON™ VII) was obtained from Ingredion Incorporated (Bridgewater, NJ). Acetic anhydride, sodium hydroxide, acetonitrile, trifluoroacetic acid (TFA), dimethyl sulfoxide (DMSO) and ethanol were purchased from Fisher Scientific (Waltham, MA). OSA was kindly provided by Gulf Bayport Chemicals (Pasadena, TX). Deuterium oxide, DMSO-d_6 and tetramethylsilane (TMS) were purchased from Sigma-Aldrich (St. Louis, MO).

Starch acetylation

High-amylose maize starch (200 g, 11% moisture) was slurried in distilled water to form a 30% solid slurry in a 4-L bucket. The bucket was jacketed in a polystyrene foam cooler containing ice water and the slurry was stirred by an overhead mixer for 30 mins. pH of starch slurry was adjusted and maintained at 8.0 ± 0.1 by a pH controller (Eutech Instruments, Vernon Hills, Illinois) coupled to a tubing pump (Masterflex, Gelsenkirchen, Germany) delivering 10 N sodium hydroxide. Acetic anhydride (50, 100, 150, and 200% to weight of dry starch) was introduced using a peristaltic pump at about 1% acetic anhydride per min. After the designated

amount of acetic anhydride was added and the pH was stable at 8.0 for 10 minutes, the slurry was adjusted to pH 6.0 using 3 N HCl and recovered by vacuum filtration. Each starch was washed three times with 1.5 L distilled water and dried in a vacuum oven at 40 °C overnight. Dried starch acetates were ground to pass a #60 mesh sieve and stored in an air tight container. DS of acetylation was determined using ^{1}H NMR (Appendix A).

Pre-gelatinization of starch

Unmodified or acetylated high-amylose maize starch (10 g each, dry weight) was suspended in distilled water to make a 5% solid slurry. Each slurry was heated directly on a heating plate and boiled for 10 min to precook the starch. The precooked starch slurry was transferred to a 250-mL hydrothermal reactor (HydrionScientific, Shanghai, China) and heated in an oven at until the slurry had reached 130 °C for 20 mins (oven setting 140 °C; time for reactor content to reach 130 °C pre-determined by heating mineral oil). At the end of the pre-gelatinization process, the slurry was smeared directly on a glass slide and viewed by a light microscope to ensure Maltese cross disappeared and freeze-dried to obtain pre-gelatinized starch (pregel).

Synthesis and purification of octenylsuccinic starch acetate (OS-Ac-starch) (Fig 3.2)

OS-Ac-starch synthesized by dry heating

Acetylated starch was dried in a vacuum oven at 80 °C for 2 h and weighed (5 g) into a glass bottle (ACE Glass Incorporated, Vineland, NJ). OSA (20 g) was then added into the bottle and the mixture was stirred by a magnetic stirring bar for 1 h. An aliquant of the mixture (20 μL) was transferred to a differential scanning calorimeter (DSC) pan and analyzed by DSC (see section 2.9.2). The rest of the mixture was heated in an air-forced oven pre-heated to 160 °C. During the first hour, the mixture was swirled manually every 5 min to prevent settling of the starch. Once

the starch formed a paste, the bottle was left undisturbed for a total heating time of 4 h at 160 °C.

Pure ethanol (50 mL) was added after the mixture was cooled to approximately 70 °C. The bottle

was capped and shook on an oscillating shaker (Eberbach Corp. Ann Arbor, MI) vigorously for 2

h to disperse the hot melt. One volume of water (50 mL) was added to precipitate the starch.

Flocculent precipitation was collected by a generic kitchen strainer and subjected to another

ethanol wash for a total of 4 times. The resulting octenylsuccinylated acetylated starch (OS-Ac-

starch), which was a white mass resembling a wheat flour dough, was soaked in 500 mL water

overnight and dried in a vacuum oven at 60 °C overnight. In the cases of using unmodified starch

and unmodified pre-gel as starting materials, simple ethanol wash and filtration was sufficient to

recover the starch because the unmodified starches were clearly powdery and settled out from

ethanol after reaction. DS of octenylsuccinylation was determined using HPLC (Appendix D)
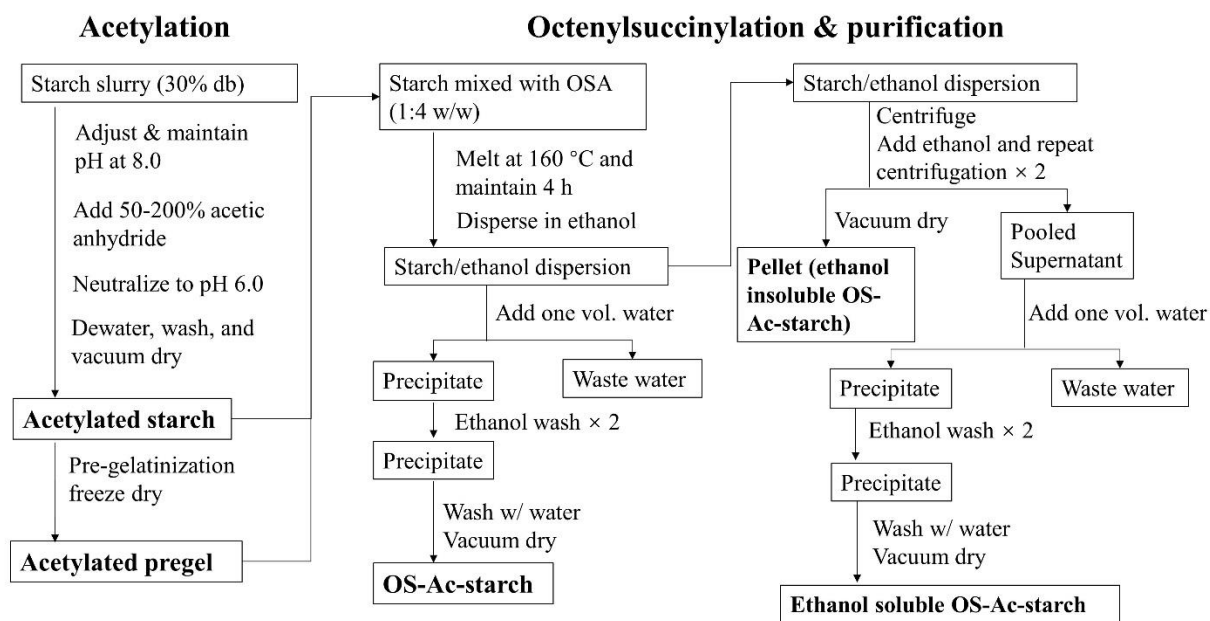
**Figure 3.2. Synthesis diagram of octenylsuccinylated acetylated starch (OS-Ac-starch)
using granular and pre-gelatinized (pregel) acetylated starch; methods of purification were
tested in a separate sample set.**

       Fractionation of OS-Ac-starch

Aiming to increase starch ester recovery yield, a separate set of experiment was carried out to collect OS-Ac-starch in two fractions. OSA (40 g) and starch acetates (10 g) was reacted similarly as shown in Fig 3.2. Cooled melt was then dispersed in 100 mL pure ethanol and centrifuged at $7500 \times g$ for 20 min. The pellet was subjected to ethanol wash for a total of three times and denoted as "ethanol-insoluble" fraction. Pooled supernatant was purified by water precipitation and denoted as "ethanol-soluble" fraction (Fig 3.2).

Scale-up of OS-Ac-starch synthesis

To determine feasibility of scale-up, a reaction of 60 g starch acetate (DS 1.33) and 240 mL OSA was carried out in a 950-mL bottle (ACE Glass Incorporated, Vineland, NJ). Synthesis was the same as in section 2.4.1 except that the purification was carried out in a 2.5 L glass jar and with mechanical stirrer.

Microscopic examination

Starch materials were examined under a light microscope (Olympus BX51, Tokyo, Japan) equipped with polarization lens. Starch slurry after pre-gelatinization was examined directly to ensure that the Maltese cross was lost for all starch granules. Dried starch acetates (10 mg) was vortexed in 1 mL mixture of glycerol and water (50:50, v/v) and observed. Starch-OSA melt made by DSC was dispersed in pure ethanol at 1:10 w/v.

X-ray diffraction (XRD)

Moisture of native, acetylated starch and pregels was uniformly dried to 5% moisture using a vacuum oven. The XRD measurements were carried out at 35 Kv and 20 Ma with a theta-compensating slit and a diffracted beam monochromator (PANalytical, Almelo, The Netherlands). The diffractograms were recorded between 2 and 35° (2θ). Crystallinity is

calculated by dividing the crystal peaks over amorphous area in OriginPro 8.5 software
(OriginLab Corporation, Northampton, MA).

Thermal properties of starch acetate and OS-Ac-starch

Discolor temperatures of OS-Ac-starch

Temperature that OS-Ac-starch began to discolor was determined visually in a
conventional capillary melting temperature apparatus (Barnstead Thermolyne, Dubuque, IA).

DSC analysis of starch esters

Gelatinization properties of starch acetates (granular and pregel) were measured by a
DSC-8000 (Perkin-Elmer, Norwalk, CT). Starch acetates (5 mg) and water (20 µL) were
weighed in a DSC pan and equilibrated overnight. Each pan was heated from 20 to 120 °C at 10
°C/min.

Glass transition of the OS-Ac-starches was determined at different moisture levels.
Rehydration of the samples was carried out in a humidity chamber due to their non-wetting,
hydrophobic natural. Vacuum dried OS-Ac-starches were placed in a 100% RH chamber at 25
°C for 1-7 days to rehydrate. Moisture absorption was determined by weight difference after 1, 2,
3, 5, and 7 days. The OS-Ac-starch at moisture level zero (dry), one (after 1 day) and two (after 7
days) were subjects to thermal analysis. OS-Ac-starch (Ca. 15 µg) in the DSC pan was heated
from 25 to 160 °C, quenched to -10 °C, and reheated from -10 to 160 °C at 10 °C/min. DSC data
was analyzed using Universal Analysis software (TA Instruments, New Castle, DE).

Statistical analysis

All starch ester synthesis was carried out in duplicates. NMR, HPLC, and DSC analysis
were average values of two analysis. DS, RE, and thermal property changes were analyzed by

one-way analysis of variance (ANOVA). A probability of $P \leq 0.05$ was considered significant. Statistical procedures were carried out by SAS 9.3 (SAS Institute; Cary, NC).

Results and discussion

Properties and characterization of starch acetate

DS determination by NMR

After acetylation, ^{1}H NMR spectra showed emerging methyl signals at 1.90-2.15 ppm, which intensified as the level of acetic anhydride increased (Appendix A). Four DS values were calculated using the acetyl peak area to starch proton signals. In the spectrum of native starch, H_2-H_6 protons signals showed in 3-4 ppm range and these signals broadened after acetylation. This could be explained by that carbonyl oxygen of the acetyl increased electron negativity of the neighboring protons (Kono, 2013). DS values of 50, 100, 150, and 200% acetic anhydride modified starch acetate were 0.53 ± 0.03, 0.89 ± 0.05, 1.33 ± 0.11, and 1.71 ± 0.08, respectively.

It was noteworthy that swelling of starch granules during the acetylation was first increased and then decreased as starch became more hydrophobic. Initial granule swelling rendered dewatering and drying very difficult. For example, the starch slurry was difficult to dewater when 50% acetic anhydride was added but can be readily filtered after 100% acetic anhydride was added. Because the use of concentrated alkali, localized pasting of starch must be avoided, by quick dispersion of alkali with sufficient agitation.

Pre-gelatinized starch acetate

Pre-gelatinization intended to destroy the remaining granular structure of starch acetate. Owning to higher amylose content, the starch had higher gelatinization temperature than normal maize starch (Luo & Shi, 2012). Therefore, an autoclaving process was necessary. Gelatinization

and freeze-drying did not alter the starch acetyl content, the DS of pre-gelatinized starch acetate were 0.53±0.02, 0.88±0.02, 1.30±0.04, and 1.71±0.01, respectively.

Crystallinity of starch acetate

X-ray diffractograms for granular and gelatinized starch acetate are shown in Appendix B. Crystallinity values are reported in Table 3.1. Type B crystal pattern was exhibited for unmodified starch, unmodified pre-gel, and starch acetate (DS 0.53) where crystallinity peaks at 5°, 15°, 17°, 20°, 22°, and 23° were observed (Cheetham & Tao, 1998). The crystallinity of unmodified starch was 15.7%, which was slightly lower than typical crystallinity (17.6% at 10% moisture) value reported for high-amylose maize starch (Cheetham & Tao, 1998). This is probably due to minimization of moisture content of the samples in the preparation of XRD analysis. Because starch acetates of DS 1.3 and 1.7 were difficult hydrate, all starches were dried to ~4% moisture to match the original moisture of DS 1.3 and 1.7 starch acetate (3-4%). Increase in acetyl DS resulted in a decreased crystallinity. This was expected as because acetyl group disrupt and reduce the formation of intermolecular H-bond, which is known to hold together starch semicrystal structure (Luo & Shi, 2012). Crystallinity was greatly reduced and disappeared in starch acetate DS > 0.53, which was in agreement with Shogren (1996). Crystallinity was reduced, but detectable, in unmodified pregels even the granular structure was destroyed. This reduced crystallinity was attributed to retrogradation of starch, particularly among amylose chains, occurred during freeze-drying. DSC demonstrated that after acetylation, the temperature required to gelatinize starch decrease significantly (Appendix C). Pre-gelatinized unmodified starch showed lower melting temperature, which were presumably from disruption of re-associated starch (Table 3.1). No melting peaks were detected in acetylated pregels.

Table 3.1. Gelatinization properties and crystallinity of unmodified and acetylated high-amylose maize starch as determined by DSC and X-ray diffraction.

Sample	Crystallinity %	To,°C[A]	Tp,°C[A]	Tc,°C[A]	ΔH J/g
High-amylose starch	15.7±0.08a	68.7±2.3a	90.3±1.0a	126.9±1.6a	13.0±0.1a
Pregel	6.9±0.03c	50.9±1.4b	60.1±1.3c	79.3±2.0c	6.1±0.3c
DS 0.53	11.6±0.05b	51.1±0.4b	69.1±1.8b	94.3±0.1b	7.7±0.1b
DS 0.89	0	49.2±0.4bc	64.4±1.1c	91.6±1.0b	6.0±0.1c
DS 1.33	0	48.9±1.6bc	62.2±0.4c	82.7±0.4c	4.4±0.3d
DS 1.71	0	44.2±0.4c	54.6±0.7d	73.7±0.4d	3.2±0.1e

[A] T_o, T_p, and T_c represent the onset, peak and conclusion temperature of the endothermic peaks; starch to water ratio was 1:3 w/w. Different letters indicate significant difference (n=2-3; $P<0.05$) between each starch type in each column;

Morphology of starch acetate before and after heated in OSA (by DSC)

Starch acetates were examined under normal and polarized light to document structural change during heating in OSA. Unmodified high-amylose maize starch contained a significant part of elongated granule that displayed skewed or no Maltese cross (Fig 3.3). According to Jiang et al. (2010), these elongated granules were mainly starchplasts that failed to separate and comprise more than 85% amylose. For starch granules that showed and retained distinct Maltese cross after acetylation, their amylopectin content was higher and probably more crystallized, rendering them more difficult to be acetylated. As DS increased, starch granules exhibited rough surfaces, aggregation, and irregular shapes. For starch acetates DS 0.89 and higher, loss of Maltese cross was observed. However, most of the granules still displayed, although less distinct, Maltese cross (Fig 3.3). Due to the low crystallinity in the high-amylose starch, acetic anhydride diffuses into the starch granule more easily (Luo & Shi, 2012). Therefore, it is reasonable to believe the reaction was rather heterogeneous and favored in those elongated, amylose-

dominated granules. A good way to test the heterogenicity of acetylation is by FT-IR

microspectroscopic census (Bai, Shi, & Wetzel, 2009). Regardless of acetyl DS, pre-

gelatinization effectively destroyed the granular structure and the starch acetate were flaky

particles after freeze-drying (Fig 3.3).
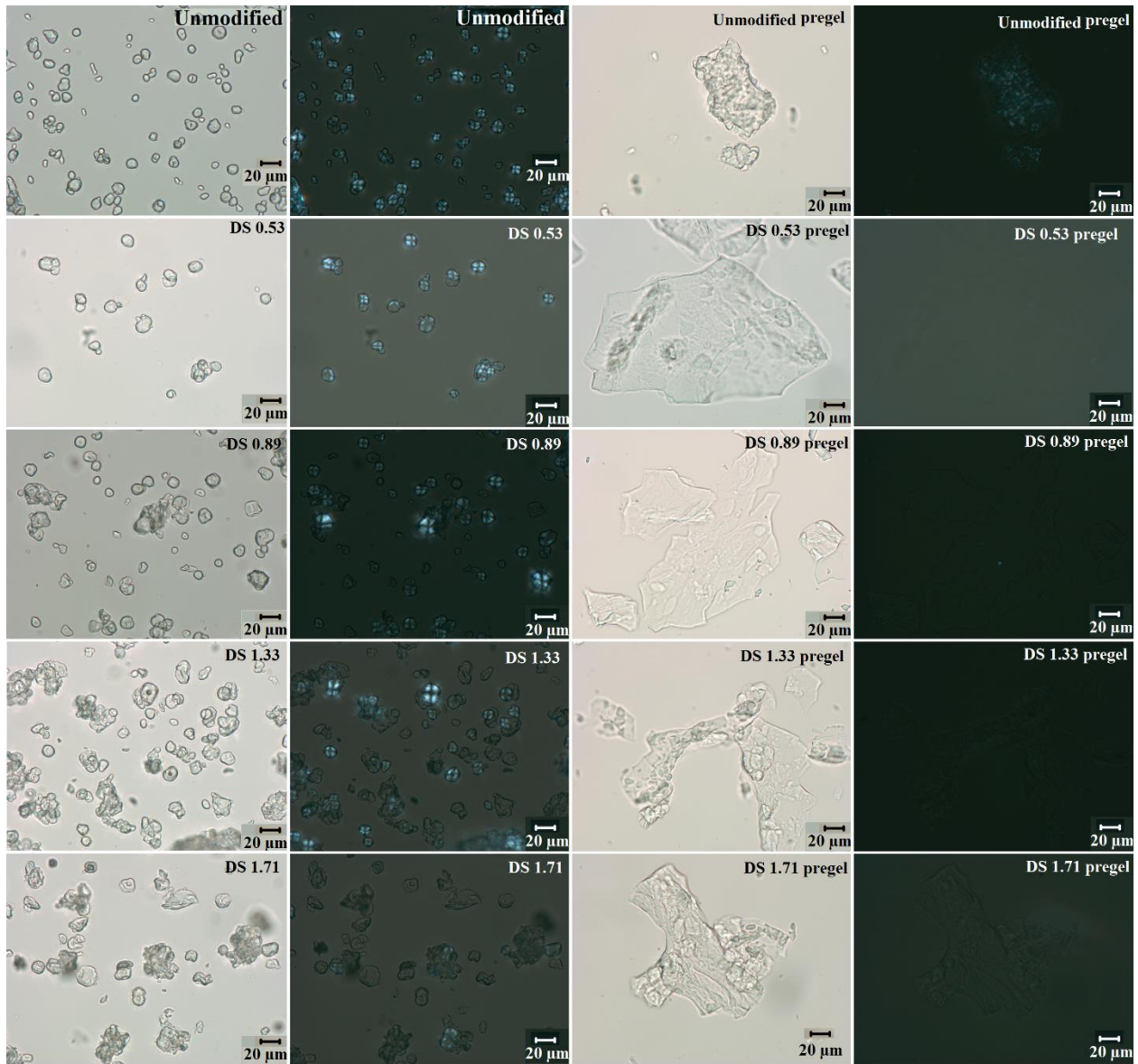
Figure 3.3. Microscopic images of granular and pre-gelatinized starch (pregel) acetates in 50% glycerol under normal (lighter background) and polarized light (darker background); DS, degree of substitution.

Granular starch acetate melted after heated in OSA. Number of remaining visible granules and Maltese cross clearly decreased with increased acetyl DS as demonstrated in Fig 3.4. The melting was probably due to increased starch-OSA compatibility brought by acetyl substituents. Above DS 1.33, the reaction mixtures appeared a homogenous transparent gel, and upon light microscopic examination, starch remnant was hardly found (Fig 3.4), whereas the reaction mixtures from the low DS starch acetate (0.53 and 0.89) were opaque gel with weeping OSA on top. After heating in OSA, those flakes of starch acetate pregels showed blur edges, which is a sign of partial solubilization. At DS above 1.30, the flakes fragmented and eventually melted completely (Fig 3.4). DSC thermograms revealed large exothermic events after ~125 °C indicating esterification reaction took place (Appendix C).
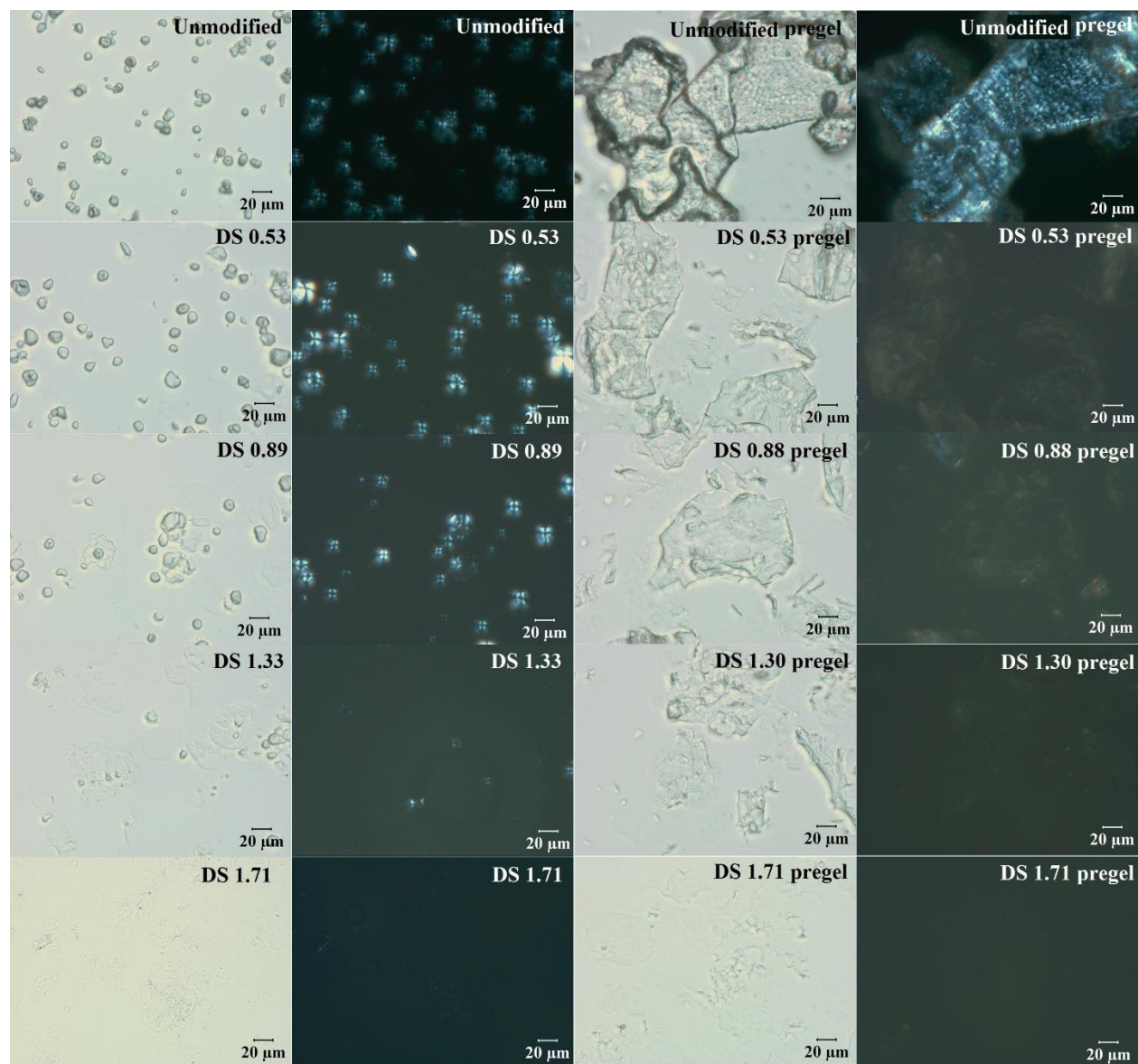
Figure 3.4. Microscopic images of unmodified and acetylated high-amylose maize starch (granular and pre-gelatinized) heated with octenylsuccinic anhydride (400% starch weight) from 25 to 160 °C in DSC pans; Images were taken in ethanol dispersion under normal light (bright background) and polarized light (dark background); DS in the images is the degree of substitution for the acetylated starches; "Pregel" in the images means that the starch was pre-gelatinized.

OS-Ac-starch synthesized with granular and pre-gelatinized acetylated starches

A 12-point calibration (0.032, 0.16, 0.32, 0.48, 0.64, 0.8, 0.96, 1.28, 1.6, 1.44, 1.92, and 2.4 µg per 10 µL injection) of OS acid was made from serial dilution of OSA. The standard curve (y=1010.8x; R^2=0.9998) was an excellent linear regression in the selected range of OS

acid concentration. Three OS acid isomers (1-OS acid, cis-2-OS acid, trans-2-OS acid) were eluted between 1.8-2.2 min, which was in agreement with (Qiu et al. 2012) (Appendix D).

Due to excessive OSA used as reactive solvent, unbound OS acid must be removed. In our preliminary studies, dispensability of reaction mixture was tested in methanol, ethanol, iso-propanol, chloroform, and hexane. Ethanol was the reagent of choice because the reaction melt was effectively dispersed, and the dispersion could be mixed with water to precipitate the hydrophobic starch esters. Three consecutive ethanol washes successfully removed unbound OS acid and the starch material showed firmer texture after each wash (Appendix E). As Table 3.2 shows, free OS acid value (%OS_{free}) were mostly between 0.1-0.4%, which are close to the limit (0.3%) set by Joint FAO/WHO Expert Committee on Food Additives (JECFA) of United Nation for food-grade OSA-starch. Bound OS acid (%OS_{bound}) from each OS-Ac-starch was converted to DS (Table 3.2). DS of OS modification ranged between 0.44-1.25 and showed positive relation to DS of acetylation (Table 3.2), this is because acetylation had (1) disrupted the H-bonds and freed more reaction sites, and (2) increased hydrophobicity of starch to allow melting taking place in OSA. Without acetylation, pre-gelatinization did not render the starch more reactive to OSA. At acetyl DS 0.53 and 0.89, pregelatinization resulted in significantly higher level of OS substitution compared to using granular starch acetate (sample 3-6) but the effectiveness of pregelatinization diminished at DS 1.33 and above (sample 7-10). The improved DS in sample 3-6 might due to the complete destruction of granular structure and more exposure of hydroxyl group for reaction. However, as acetylation DS increased, starch granular structure, as well as crystallinity, have already been greatly weakened; therefore, pre-gelatinization may have not provided significant improvement to octenylsuccinylation. Moreover, the limited

availability of hydroxyl group for octenylsuccinylation in highly substituted acetylated starch might have further reduced the effectiveness of pre-gelatinization.

A scale-up from 5 g to 60 g was carried out using granular acetylated starch. Same level of DS was achieved in both experiments (sample 7 and 11). The %RE was slightly lower in the scale-up experiment, which was probably due to the lost material that clung to utensils during purification.

Table 3.2. Octenylsuccinylated acetylated starch (OS-Ac-starch) synthesized by dry heating granular and pre-gelatinized (pregel) acetylated high-amylose maize starch in 400% starch weight of octenylsuccinic anhydride (OSA); DS, degree of substitution.

Sample #	Acetyl DS	OS-Ac-starch yield (g)	OS DS	%OSbound	%OSfree	Acetylated starch % recovery*	%Reaction efficiency
1	Unmodified	4.76±0.06	-	-	-	-	-
2	Unmodified (pregel)	4.74±0.04	0.02±0	2.07±0.09e	-	98	0.49±0.02e
3	0.53	0.32±0.02	0.44±0.01d	33.26±1.32d	0.3±0cd	67	0.53±0.02e
4	0.53 (pregel)	0.32±0.01	0.62±0.03c	41.10±1.45c	0.2±0de	59	0.66±0.02e
5	0.89	3.2±0.02	1.08±0.06b	53.25±0.43ab	0.9±0.1a	47	8.52±0.07d
6	0.88 (pregel)	3.51±0.02	1.41±0.01a	59.77±0.30a	0.2±0cde	40	10.49±0.05c
7	1.33	5.76±0.06	1.19±0.01ab	53.30±1.51ab	0.6±0b	47	15.35±0.43ab
8	1.30 (pregel)	5.81±0.09	1.23±0.01ab	54.19±1.86ab	0.2±0de	46	15.74±0.54a
9	1.71	6.14±0.05	1.25±0.04ab	52.19±1.33ab	0.1±0e	48	16.24±0.41a
10	1.71 (pregel)	6.21±0.08	1.14±0.01b	50.57±0.90ab	0.2±0de	49	15.70±0.28a
11	1.33 scale-up	64.29±1.2	1.20±0.09ab	53.68±1.81ab	0.4±0c	46	14.38±0.49b

* Weight fraction of acetylated starch in OS-Ac-starch divided by initial acetylated starch weight (5 g for sample 1-10, 60 g for sample 11). *Different letters indicate significant difference (n=2-4; $P < 0.05$) between each starch type in each column; each sample was synthesized in duplicate using the same starch acetate composite.

Ethanol-soluble and ethanol-insoluble fractions of OS-Ac-starches

Yield of OS-Ac-starch was lower our expectation as they translate to only about 46-67% of initial acetylated starch weight (Table 3.2). Therefore, we suspect that there is an ethanol-insoluble fraction washed away, as we only collected the material solubilized in ethanol and then precipitated by water. In another set of experiments, we collected the "washed-way" fraction by centrifuging starch-ethanol dispersion during the first wash cycle and recovered OS-Ac-starches in two fractions (Table 3.3). Ethanol-soluble fraction showed similar DS as to the OS-Ac-starch sample 3-10 in Table 3.2, while the ethanol-insoluble fraction was only substituted at DS 0.24-0.64, regardless of whether pre-gelatinized. By collecting the ethanol insoluble fraction, acetylated starch recuperation recovery increased from about 45% to 75%, proving the loss of recovery yield was due to wash away of ethanol-insoluble fraction. Considering together with microscopic analysis in Fig 3.3, it is reasonable to conclude the octenylsuccinylation was heterogenous and unfavored in those, possibly under-acetylated, starch materials.

Table 3.3. Octenylsuccinylated acetylated starch fractioned based on ethanol solubility.

Acetyl DS	Ethanol soluble fraction			Ethanol-insoluble fraction			Acetylated starch % Recovery*	%RE
	Yield	%OS$_{bound}$	DS	Yield	%OS$_{bound}$	DS		
DS 0.53	0.81±0.04d	35.11±0.50e	0.46±0.01e	8.98±0.45a	22.50±0.38d	0.24±0.01d	75	5.5±0.1d
0.53 (pregel)	0.77±0.01d	41.25±0.64d	0.62±0.02d	8.87±0.06ab	21.93±0.97d	0.25±0.01d	74	5.7±0.1d
DS 0.89	5.76±0.47c	56.30±0.33b	1.22±0.03bc	7.91±0.17abc	33.18±0.19c	0.44±0c	80	14.6±0.1c
0.88 (pregel)	6.03±0.13c	61.98±0.29a	1.54±0.02a	7.69±0.09c	34.97±1.14bc	0.51±0.03b	73	16.1±0.2b
DS 1.33	10.11±0.33b	53.93±1.49bc	1.14±0.07c	4.88±0.41d	38.26±0.02a	0.61±0a	78	17.8±0.2a
1.30 (pregel)	10.36±0.22b	55.40±0.23b	1.29±0.01b	4.69±0.10d	37.19±0.18ab	0.62±0a	76	18.7±0.2a
DS 1.71	12.03±0.20a	51.59±0.53c	1.17±0.02c	2.85±0.23e	36.30±0.80ab	0.61±0.02a	77	17.9±0.2a
1.71 (pregel)	11.84±0.08a	52.41±0.33c	1.23±0.02bc	2.79±0.01e	36.42±0.31ab	0.64±0.01a	74	18.1±0.2a

*: Sum of weight fraction of acetylated starch weight both fractions divided by initial acetylated starch weight (10 g); unreacted OS% were 2.0-3.4% and 0.1%-0.2% in ethanol soluble and

ethanol insoluble fractions, respectively; different letters indicate significant difference (n=2; P<0.05) between each starch type in each column; each sample was synthesized in duplicate using the same starch acetate composite; detailed fractionation scheme was drawn in Fig 3.2; %RE, reaction efficiency of octenylsuccinic anhydride; DS, degree of substitution.

Moisture uptake of OS-Ac-starches

The moisture uptake of OS-Ac-starches was hyperbolic during seven-day hydration in a 100% RH chamber (Fig 3.5). For all starch esters, moisture content increased rapidly during the first two days and plateaued after the third day. The maximum water content ranged from 25.1% to 4.4% for OS-Ac-starch that has lowest (0.53 acetyl, 0.44 OS) to highest (1.71 acetyl, 1.24 OS) combined DS. As expected, the maximum moisture content for starch esters reversely correlated with their combined DS, because water is repelled by the non-polar ester substituents.
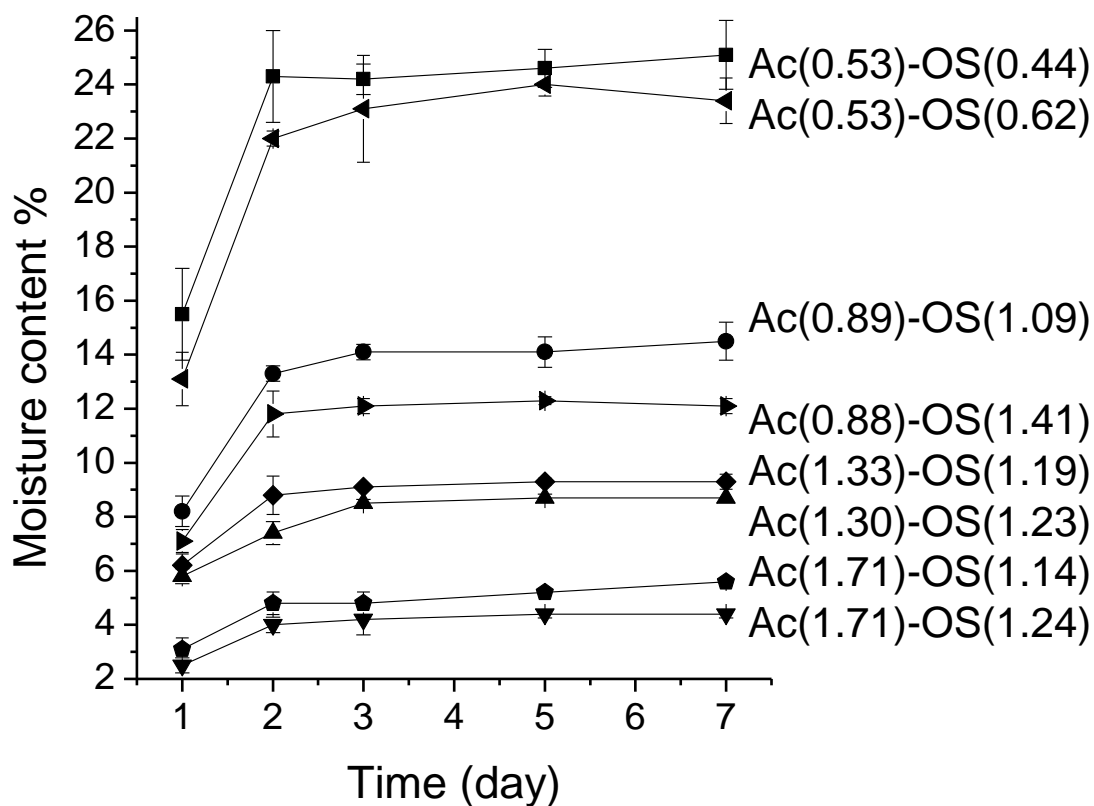
Figure 3.5. Moisture uptake of octenylsuccinylated acetylated starch esters in humidity chamber (100% relative humidity) up to seven days; Ac, acetate; OS, octenylsuccinate; the values in the parenthesis are the degree of substitution for Ac and OS groups.

Thermal properties of OS-Ac-starch

As expected, T_g of dry unsubstituted starches was not detected up to 120 °C (Table 3.4).

This could be explained by lack of molecular mobility from H-bond constraint. T_g of dry OS-Ac-

starch had T_g between 57.0-106.9 °C, which were significantly lower than unmodified high-

amylose maize starch that has T_g of 230 °C (Shogren, 1996). Ester substituents are small

moieties that provide intermolecular plasticization to reduce molecular friction and allow starch

molecules slide against each other more easily at lower temperatures. When heated to above

those temperatures (T_g), starch esters greatly soften as they undergo "glassy-to-rubbery"

transition. Extend of specific heat capacity (ΔCp) appeared to increase with DS of OS side chain.

According to Liebert et al. (2011), glass transition of starch ester is largely contributed by side chain re-alignment thus a high substitution level of long side chain should correspond to higher ΔCp. Discolor temperatures were determined for the OS-Ac-starch because it is an important factor to determine processing temperatures in applications. Temperature that OS-Ac-starch began to discolor was between 131-189 °C (Table 3.4). The cause of discoloration is probably due to acidity of OS group as because the OS groups were octenylsuccinic acid after the dry reaction. Therefore, it is reasonable to see a decrease in discolor temperature as OS substitution level increased. Differences between T_g and discolor temperature in certain OS-Ac-starch, i.e. Ac(1.33)-OS(1.20), was over one hundred Celsius degrees and should provide large freedom for temperature selection during thermal processing. Although there was no prior report on thermal properties of high DS OS-Ac-starch, our results showed high consistency with high DS starch fatty acid esters. According to Liebert et al., (2011), typical glass-to-rubber transition (T_g) of intermediate DS starch esters (chain length between C8-C18) were around 100 °C, where DS 2.5 and above could further lower the T_g below 50 °C. Winkler et al. (2014) reported T_g of starch hexanoate (DS 2.4) was around 70 °C.

Moisture appeared to be a good plasticizer in low DS OS-Ac-starch because of its good compatibility with large amount of residual hydroxyl groups. With 25% moisture increase, T_g of Ac(0.56)-OS(0.44) starch was evidently lowered by from 105 to 36 °C as shown in Fig 3.6. The decease of T_g because less pronounce as starch ester DS increased. T_g difference was less than ten Celsius degrees due to very low water adsorption (Appendix F). The low adsorption of water, together with minor T_g alteration after hydration could make high DS OS-Ac-starch ideal for water resistant materials. To alter the T_g of high DS starch esters, oil-like plasticizers, e.g. triacetin or polyene glycol, could be more effective than water.

Table 3.4. Thermal properties and discolor temperature of octenylsuccinylated acetylated starch determined by differential scanning calorimeter (2nd scan) and melting point apparatus; values in parenthesis indicate degree of substitution of acetylation and octenylsuccinylation.

Sample #	DS	Tgo,°C	Tgm,°C	Tge,°C	ΔCp (J/g°C)	Discolor (°C)
1	Unmodified	-	-	-	-	N/A
2	Unmodified (pregel)	-	-	-	-	N/A
3	Ac(0.53)-OS(0.44)	104.8±0.7a	106.9±1.0a	108.2±0.3a	0.199±0.01d	N/A
4	Ac(0.53)-OS(0.62)	101.7±0.6a	103.1±1.1a	105.1±1.2a	0.212±0.003d	N/A
5	Ac(0.89)-OS(1.08)	60.0±2.8b	71.51±2.3b	81.7±1.6b	0.286±0.004c	189±3a
6	Ac(0.88)-OS(1.41)	59.4±2.1b	64.0±2.6c	73.93±2.6c	0.341±0.004b	178±1b
7	Ac(1.33)-OS(1.19)	51.0±1.3c	61.0±2.8cd	67.9±1.3d	0.377±0.008a	165±3c
8	Ac(1.30)-OS(1.23)	52.1±1.5c	58.5±0.8cde	65.8±2.2d	0.387±0.003a	162±3c
9	Ac(1.71)-OS(1.25)	48.9±1.3c	54.9±1.8de	57.0±1.7e	0.382±0.008a	131±2d
10	Ac(1.71)-OS(1.14)	50.3±0.5c	53.1±1.3e	57.0±0e	0.376±0.006a	134±2d
11	Ac(1.33)-OS(1.20) scale-up	51.3±0.9c	59.5±0.6cde	65.2±0.1d	0.365±0.008ab	160±2c

different letters indicate significant difference (n=2; P<0.05) between each starch type in each column.
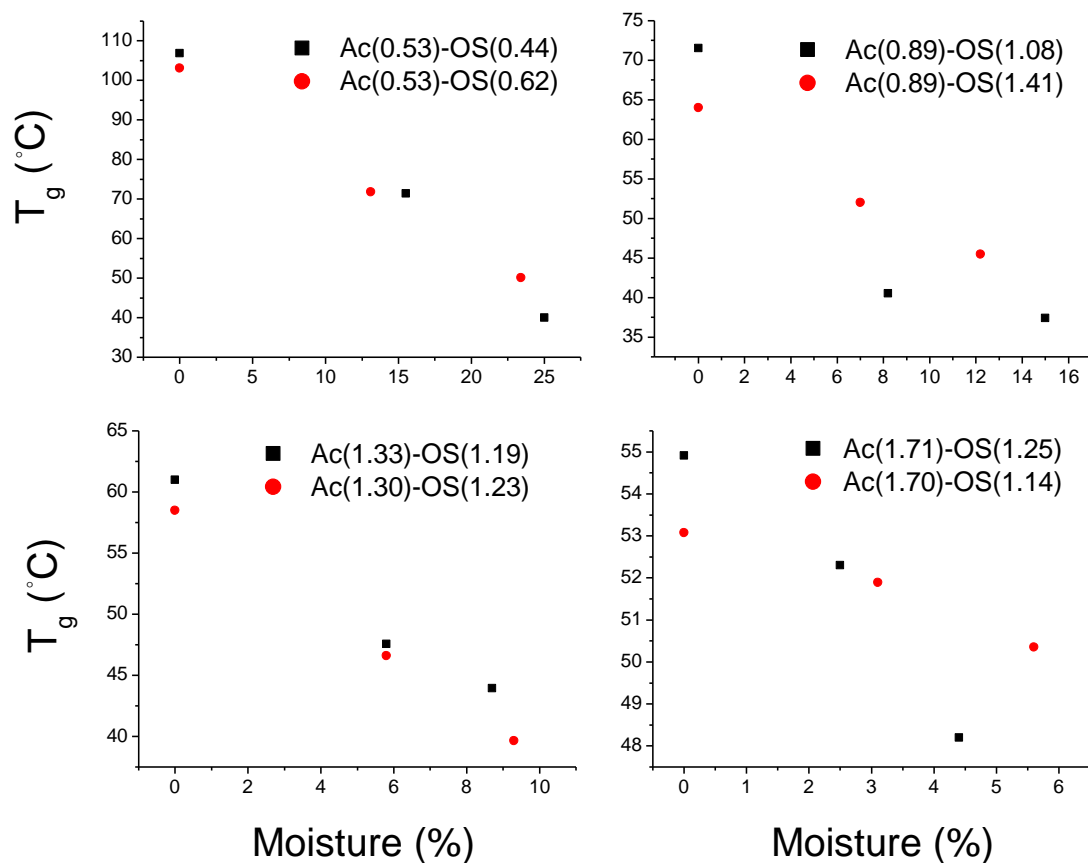
Figure 3.6. Glass transition (T_g, mid-point) temperature of octenylsuccinylated (OS) acetylated (Ac) starch at different moisture contents; values in parenthesis indicate degree of substitution of acetylation and octenylsuccinylation.

Conclusions

Acetylation to intermediate DS renders starch acetate meltable and reactive in OSA without aid of hazardous chemicals. Starch-OSA reaction was enhanced by higher acetyl DS. Acetylation (DS 1.3-1.7) resulted in higher OS substitution compared to acetylated starches of DS < 0.88, because the highly acetylated starches were very soluble/meltable in OSA and yet still had ample hydroxy groups for reaction. Pre-gelatinization of starch acetate significantly improved the bound OS content in the mixed ester when acetyl DS was below 0.88. Pre-gelatinization may be used when a high OS, low acetyl starch ester is desired. A scale-up trial

using 60 g starch acetate produced 64.29 g OS-Ac-starch esters with similar DS to 5 g batches. The mixed esters (acetyl and OS) had combined DS > 2.5, which was adequate to lower the glass transition temperature of the starch to below 60°C. Highly substituted starch esters were largely hydrophobic and showed great potentials as thermoplastic, water-resistant materials.

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Chapter 4 - Preparation, structure, and properties of highly substituted mixed esters from waxy, normal, and high-amylose maize starches

Abstract

Highly substituted acetylated octenylsuccinic starch esters were prepared using novel dry-heating method. Waxy, normal, and high amylose maize starches were acetylated to degree of substitution (DS) 0.85-1.31 and further reacted in mixture of octenylsuccinic anhydride and triacetin to produce octenylsuccinate (OS) substitution of DS 0.55-1.25. HPLC, FT-IR, and ^{1}H NMR revealed similar substitution signals to one produced from conventional pyridine-catalyzed method. The mixed starch esters were highly hydrophobic and showed upshift in thermograms at 56.4 °C, which possibly represented glass transition of OS side group. Plasticized starch mixed esters formed a gum mass resembled chewing gum base and showed excellent stretch (360% elongation). Stiffness (G') of the gums was in the chewable range at body temperature and underwent approximately 10-fold decrease when heated to 70 °C. Starch esters of high amylose maize starch produced by the novel dry-heating method have potentials as bioplastic materials.

Keywords

High DS starch, Hydrophobic starch, Solvent-free synthesis, Thermoplastic starch, Octenylsuccinic anhydride modified starch, Starch acetate

Abbreviations

AS, acetylated starch; OS-AC-starch, Octenylsuccinylated acetylated starch; DS, degree of substitution; RE, reaction efficiency; T_g, glass transition temperature; TFA, trifluoroacetic acid;

DMSO, dimethyl sulfoxide; OSA, octenylsuccinic anhydride; GPC, gel permeation chromatography; LVR, liner-viscoelastic region.

Introduction

Starch is an abundant renewable resource that is used in a board range of food and non-food industrial applications. Native starch has limited industrial uses because they are highly hydrophilic, susceptible to enzyme and acid breakdown, and require high amount of plasticizers when been processed to avoid thermal degradation because of their natural high glass transition temperature (T_g) (Kaseem, Hamad, & Deri, 2012; Vieira et al., 2011). End products of processed starch, often as films or foams, are brittle and vulnerable to moisture and gas permeation (Shogren, 1996). Starch derivatives, especially starch esters substituted with long alkyl side groups were known to have remarkable improved functional properties due to the hydrophobic nature and intermolecular plasticization effects offered by the substituents (Winkler, Vorwerg, & Rihm, 2014). Of all the characteristics of a starch ester, degree of substitution (DS) and substituent type are considered two most effective aspects to alter the overall properties of a starch. To render a starch ester thermoplastic and organic solvents compatible, the DS of such starch ester need to be above 2.0 in general (Winkler, Vorwerg, & Wetzel, 2013).

Despite many advantages and potentials reported in literatures, there are few commercial high DS starch esters at present. A major hurdle preventing large scale synthesis is use of solvents such as pyridine, imidazole, and ionic liquids which not only elevate the cost but also cause environment pollutions (Ačkar et al., 2015). Solvent-free synthesis of high DS starch ester has been reported as a "green" method. Aburto et al. (1999) first reported a solvent-free synthesis

of starch octanate (C_8) by first synthesizing starch formate and subsequent octanoyl chloride treatment. They claimed that DS 1.7 starch octanate would be produced by co-heating excessive octanoyl chloride and starch formate at 105 °C for 40 min. However, starch degradation was severe because of the liberated acid in various stages. Shogren (2003) synthesized a starch octenylsuccinate acetate mixed ester by co-heating starch, acetic acid, and octenylsuccinic anhydride (OSA) at 1:1:1 to 180 °C and obtained an ester that had DS 0.49 for octenylsuccinate and DS 0.34 for acetyl. In the previous chapter, we reported that starch acetylated to DS above 1.3 was highly reactive in OSA as because OSA alone can be both a highly effective plasticizer and acyl donor for starch acetate. Efforts were carried out to develop a true "green" synthesis of highly substituted starch ester using OSA as reactive solvent. However, in our previous work, excessive amount of OSA (400% of starch weight) was used to achieve the desired DS. Such high usage of OSA was due to several reasons: (1) to reduce viscosity of reaction mixture so that the reaction was stirrable, and (2) to prevent acid burn as we noted the starch quickly discolored when less OSA was used, possibly from high acid concentration in small reaction volume. The high amount of OSA imposed low reaction efficiency (around 18%), which, from cost perspective, must improve to favor industrial scale preparation Furthermore, excess use of OSA also leave large amount of unreacted reagent to remove. In this follow-up study, we aim to reduce the use of OSA by incorporating an inert diluent to the reaction system. The resulting starch esters was analyzed to evaluate (1) reaction efficiency change by adding a diluent, (2) thermal and rheological properties of starch esters with different amylose content in base starch, and (3) potential use of the materials as a chewing gum base.

Materials and methods

Materials

Waxy, normal, and high amylose maize starches were obtained from Ingredion Incorporated (Bridgewater, NJ). Acetic anhydride, sodium hydroxide, acetonitrile, trifluoroacetic acid (TFA), dimethyl sulfoxide (DMSO), pyridine, ethanol, chloroform, acetone, hexane, and calcium carbonate were purchased from Fisher Scientific (Waltham, MA). OSA was kindly provided by Gulf Bayport Chemicals (Pasadena, TX). Triacetin, deuterium oxide, and DMSO-d_6 and tetramethylsilane (TMS) was purchased from Sigma-Aldrich (St. Louis, MO). Acetylated monoglyceride (Acetem 70) was kindly provided by DuPont USA (Wilmington, DE). Pullulan standards were purchased from American Polymer Standards Corp (Mentor, OH).

Synthesis of starch esters

Synthesis of starch acetate

Waxy, normal, and high amylose starches were acetylated using the method of Luo & Shi, (2012) with some modifications: (1) Acetic anhydride (150% starch wt) was used across the board for every starch, and (2) 10 N aqueous NaOH was used as pH adjustor and catalyst (Fig 4.1).

Preliminary experiments

Swelling power of acetylated and octenylsuccinylated acetylated high-amylose maize starch (OS-Ac-starch; prepared by solvent-free synthesis from a previous study) were first tested in several potential diluents including acetylacetone, ethyl acetoacetate, ethylene glycol diacetate, propylene glycol diacetate, diethyl malonate, and triacetin (Appendix G).

Synthesis of OS-Ac-starch using triacetin and OSA

Triacetin and OSA (20 g each) were mixed with a magnetic stirring bar for 10 min in a 250-mL glass beaker. To this mixture, starch acetate (10 g) was added, and the mixing was continued at 25 °C for 1 h, and then heated in an oil bath at 150 °C. As soon as 150 °C was reached, the heating temperature was lowered to 120 °C and maintained at 120 °C for 2 h while been constantly stirred by a magnetic bar. The hot melt was then cooled to 50 °C. Pure ethanol (50 mL) was added and the content was stirred with an overhead mechanic stirrer to fully solubilize the hot melt which soon turned to a homogenous viscous solution. One volume of water (50 mL) was added as an antisolvent to precipitate the starch ester. The starch ester was collected by a 10-mesh sieve, reconstituted and precipitated for a total of three times using ethanol and water. The resulted gum, a white mass resembling a wheat flour dough, was briefly kneaded under running water and dried in a vacuum oven at 50 °C overnight. The starch material was then ground and passed through a 40-mesh sieve. An overall diagram to synthesis starch mixed esters is illustrated in Fig 4.1.

Acetylation

Starch (20g) slurried in 70 mL water

Dropwise add 30 g acetic anhydride, maintain pH 8.0 at 5 °C

Neutralize with 1N HCl and filter

Starch Cake

Repeat washing and filtration × 2

Freeze dry and mill to pass 40 mesh sieve

Acetylated starch

GPC

^{1}H NMR

FT-IR

Octenylsuccinylation

Acetylated starch (10 g) slurried in 20 g OSA and 20 g triacetin mixture

Heat to 150 °C and maintain at 120 °C for 2h

Add 100 mL ethanol, stir overnight

Starch/ethanol solution

Add 100 mL water

Precipitate   Waste water

Repeat ethanol washing × 2

Precipitate

Soak in 500 mL water overnight, vacuum dry

OS-Ac-Starch ester

^{1}H NMR

HPLC

DSC

FT-IR

Solvent compatibility

Rheological analysis

Figure 4.1. A flow chart of starch acetylation in aqueous medium and octenylsuccinylation using a novel solvent-free melting reaction.

Synthesis of OS-Ac-starch in pyridine

Octenylsuccinylation of AS was synthesized in pyridine to Wang et al., (2011). Briefly, high amylose maize starch acetate (DS 1.3) was dried in a vacuum oven at 80 °C for 2 hrs. Starch acetate (1 g) was dispersed in 10 mL pyridine at 90 °C. OSA (4 g) was added and the reaction proceed for 12 h at 90 °C while constantly stirred. Methanol (50%, 20 mL) was added to the mixture to precipitate starch derivatives. The precipitated starch was then dispersed in straight methanol (10 mL) and precipitated by adding 10 mL water for a total of four times. The resulted precipitate was then dissolved in 20 mL chloroform and shook with 20 mL 0.1 M H_2SO_4 in a separatory funnel to extract pyridinium salts into aqueous phase. Finally, the chloroform phase was collected and evaporated in a vacuum oven at 50 °C to yield an off-white starch curd which was then ground with mortar and pestle.

Characterization of starch mixed esters

DS of octenylsuccinate (OS) by HPLC

Bound and unbound OS contents were determined according to Qiu et al., (2012).

Reaction efficiency (RE) is calculated using Equation 1.

$$RE = {}^{\% \ bound \ OS \ \times \ Y}\!/\!_{OSA \ usage}. \tag{1}$$

Where Y is dry yield of OS-Ac-starch in grams, OSA usage is in grams.

Fourier-transform infrared spectroscopy (FT-IR) analysis

FT-IR spectra was collected in the region of 400-4000 cm^{-1} using a PerkinElmer Spectrum 100 FT-IR spectrometer (Waltham, MA). Pure starch acetate and OS-Ac-starch were scanned 32 times at 2 cm^{-1} resolution and absorption spectra were recorded.

^{1}H Nuclear magnetic resonance (^{1}H NMR)

OS-Ac-starches (30 mg for normal and high amylose starches, 20 mg for waxy starch) were dried at 80 °C in a vacuum oven for 2 hours, stirred in 1 mL deuterium oxide at room temperature overnight, freeze dried, and dissolved in 1 mL DMSO-d_6. The solution was analyzed by a Varian 400 MHz system (Varian Inc., Palo Alto, CA) at 25 °C. Sixteen scans were acquired for each sample and the relaxation time was 1.0 s. Chemical shift was calibrated to 0 ppm with TMS.

Differential scanning colorimetry (DSC) analysis

Ground OS-Ac-starches were dried in a vacuum oven at 50 °C overnight, removed to a desiccator for 24 hours and weighed (Ca. 10 µg) into a DSC pan. The sample pan was heated from room temperature to 120 °C at 10 °C/min to erase thermal history, quenched to 0 °C, and re-scanned to 250 °C at 10 °C/min. The DSC data was analyzed using Universal Analysis software (TA Instruments, New Castle, DE).

Molecular weight distribution by gel permeation chromatography (GPC)

Native starches and their acetylated counterparts (8 mg) were added into 2 mL DMSO and stirred overnight in 80 °C water bath, filtered through 0.45 micro filter and injected into a PL-GPC 220 instrument (Polymer Laboratories, Inc., Amherst, MA). Separation was performed on three Phenogel 10 μm columns arranged in order of 10^6 Å, 10^3 Å and 10^2 Å pore size (Phenomenex, Inc., Torrance, CA, USA), a guard column (Phenomenex, Inc., Torrance, CA), and recorded by a differential refractive index detector. Eluent system was DMSO containing LiBr (0.55% w/v) at a flow rate of 0.8 mL/min. Column oven temperature was controlled at 80 °C. Pullulan standards and maltose were used for molecular weight calibration.

Solubility of starch esters in organic solvents

Starch acetate and OS-Ac-starch (4% w/v) were stirred in chloroform, acetone, hexane, DMSO, and triacetin overnight. Opacity of each mixture was estimated against an aqueous 4% native starch slurry and recorded as soluble for transparent, partially soluble for reduced opacity, and insoluble for complete opaque.

Acid thinning for waxy maize starch

Waxy maize starch (100 g) was slurried in 200 ml water and treated with 2 g concentrated HCl (12 N) for 8, 12, and 16 h. Treated starch was recovered by filtration and dried. Alkali fluidity of acid treated starch was carried out to verify viscosity change. Briefly, starch (5 g dw) was stir in Ca. 9.4 ml water (minor adjusts for moisture compensation). NaOH (0.25 N, 85 ml) was added to the starch water and stirred for 3 min. The viscosity of alkali starch solution was tested with a Brookfield DV-II+Pro viscometer (Ametek Brookfield, Middleboro, MA) at 25 °C under shear rates 10-100 1/s. Molecular weight distribution was determined by GPC procedure.

Rheological analysis of starch esters

Rheological properties of OS-Ac-starch in organic solvents

OS-Ac-starch (1 g) was mixed with DMSO or triacetin at 1:1 (starch/solvent, w/w). The mixture was kept at ambient temperature overnight and the starch paste were briefly kneaded to ensure thorough cooperation. The starch paste was loaded on a set of 25 mm smooth surface parallel plate attached on an ATS rheometer (Canon Instrument Company, Bordentown, NJ) and pressed to 2 mm gap. Stress sweep (10^2-10^4 Pa) was carried out at 25 °C to find the linear-viscoelastic region (LVR), which was determined at 10% decrease of initial storage modulus (G'). Frequency sweep was carried out in from 1-50 Hz within the LVR.

Formulated starch ester as a chewing gum base

Formulation of starch chewing gum base was adapted from Liu, 2010. As shown in Table 4.1, plasticizers of choice (triacetin, hydrogenated soybean oil, and acetylated monoglyceride) at different amount, denoted as high, mid, and low triacetin formulas, were mixed in a 100-ml beaker and heated at 60 °C for one hour to form a one-phase plasticizer. The plasticizer mix was then added to starch ester calcium carbonate blend. The mixture was conditioned at 60 °C for 20 min and kneaded to a uniform dough. The dough rested at room temperature for 24 h before assessment. A commercial gum was obtained from water extraction of Trident Chewing Gum (washing and kneading the chewing gum in 40 °C water for 10 min).

Elongation of the gum base was determined by a TA.XT Texture-Analyzer (Texture Technologies Corp. and Stable Micro Systems, Ltd., Hamilton, MA) coupled to a double clamp geometry (TA-96). Starch gum base was rolled to 5 mm sheet using a rolling pin with 5 mm guide. Stripes (40 mm×20 mm) were cut from the gum sheet and clamped to the geometry with

each end clamping 10 mm of the gum stripe. The gum was stretched from 20 cm to 170 mm (150 mm travel distance) at 3.3 mm/s.

High-amylose based gum balls were slowly pressed to fit the gap (2 mm) of the geometry. Stress sweep was carried out from 10^2-10^4 Pa, frequency (1-50 Hz) and temperature sweeps (20-70 °C) were carried out using 500 Pa, which was determined in stress sweep in the LVR of all samples.

Table 4.1. Formulation of starch ester chewing gum base at different plasticizer levels; liquid plasticizers were first heated at 60 °C and mixed to form one phase and then blended with starch ester powder and calcium carbonate; commercial chewing gum was kneaded in water for 10 min to remove soluble materials.

Ingredients	Low triacetin	Mid triacetin	High triacetin	Commercial (Trident)
	g			
Commercial chewing gum curd (Trident)				10
OS-Ac-starch	5.6	5.0	4.5	
Triacetin	2.4	3.0	3.5	
Hydrogenated soybean oil	0.5	0.5	0.5	
Acetylated monoglyceride	0.5	0.5	0.5	
Calcium carbonate	1.0	1.0	1.0	

Statistical analysis

Swelling power of acetylated and OS-Ac-starch in different diluent (preliminary experiment) was carried out once. Starch octenylsuccinylation in pyridine and diluent of choice, triacetin, were carried out in duplicates and analyzed by NMR, HPLC, GPC, DSC, and rheometer for one time each sample. Acid thinning of waxy maize starch and subsequent esterification were carried out in duplicates. Esterification DS was analyzed by one-way analysis of variance (ANOVA). A probability of $P \leq 0.05$ was considered significant. Statistical procedures were carried out by SAS 9.3 (SAS Institute; Cary, NC).

Results and discussion

DS and yield

DS of acetylation was 1.20, 0.85, and 1.31 for waxy, normal, and high amylose maize starches reacted with 150% starch weight of acetic anhydride. The preparation AS presented no issues during filtration and milling. The starches recovered as free-flowing, fine, white powders. To further react AS in presence of a diluent, the diluent of choice must have good compatibility throughout the reaction and purification. Several key characters were considered for the diluent: (1) able to swell AS and OS-Ac-starch, (2) miscible with OSA and ethanol, (3) inert at reaction temperature (150 °C) and (4) having boiling point > 150 °C. Triacetin gave swelling power 4.6 and 7.3 for AS and OS-Ac-starch, which were the highest value among all diluents. Triacetin also had the high boiling point (259 °C) and flashing point (138 °C) and therefore, was chosen as the diluent for octenylsuccinylation.

DS of OS substituent was determined by HPLC and summarized in Table 4.2. Effect of different amylose content and acetyl DS played a role in determining the DS of starch esterification. Despite small difference of initial acetyl DS, high amylose starch acetate produced a noticeably thinner and stirrable slurry than waxy starch acetate in the OSA reaction, which could be the reason to a significant higher OS substitution. Normal starch acetate also formed a thin slurry with OSA and remained fluid throughout the reaction; however, did not achieve comparable DS to high-amylose starch, probably because the lower acetyl DS resulted poorer starch dispersibility in OSA. Taking consideration of recovered starch ester weight, overall RE of octenylsuccinylation was in the order of high amylose>waxy>normal starches. in another experiment, high-amylose AS was solubilized in pyridine, and reached full substitution with octenylsuccinate (combined DS 2.95). Synthesis of high DS starch octenylsuccinate in pyridine

has been studied by Li et al., 2012 & Wang et al., 2011 and the reported highest DS achieved was 1.35 using OSA/starch ratio of 4.6:1, heated at 97 °C for 2.7 h, translating to RE = 37.8% assuming all starch material was recovered. The result of our pyridine-free method was remarkable because the use of pyridine was completely avoided and the RE was even higher than a pyridine-involved reaction.

Table 4.2. OS-Ac-starch prepared from acetylated waxy, normal, and high amylose starch using novel solvent-free melting methods.

	Starch (g)	OSA (g)	Acetylation DS	OS DS	% Bound OS	Yield of OS-Ac-starch (g)	% RE
Waxy	10	20	1.20	0.83±0.04c	45.00±1.41c	14.45±0.1ab	32.50±0.37b
Normal	10	20	0.85	0.55±0.01d	36.74±0.40d	12.67±0.45b	23.26±0.58c
High amylose	10	20	1.30	1.25±0.03b	55.23±1.10b	13.71±0.21ab	37.69±0.57a
High amylose (pyridine)	1	4	1.30	1.65±0.05a	61.62±0.56a	2.31±0.06	35.44±1.39b

Unreacted OS% was 0.2% in pyridine catalyzed sample and was undetected in all other samples; different letters indicate significant difference (n=2-4; P<0.05) between each starch type in each column; each sample was synthesized in duplicate using the same starch acetate composite; %bound OS was determined by HPLC analysis; %RE was based on ratio of %bound OS in actual recovered starch esters to total OSA usage.

Molecular weight determination by GPC

Molecular weight distribution of native starches and AS was determined by GPC. It was as expected that Mw was in the order of high amylose (4.5×10^5) < normal (8.9×10^6) < waxy (1.9×10^7) corn starch (Fig 4.2a). It is noteworthy that the waxy and normal corn starch showed a heavier distribution in lower molecular weight range than normal values, which could indicate a slight degradation of the molecule during dissolution. Amylopectin portion of the high amylose starch (30% theoretically) did not distinguish from the amylose as a separated peak, it is

probably the right tail of the chromatogram overlapping with waxy starch. Acetylated waxy, normal, and high amylose starches had slightly larger molecular weight, possibly due to the grafting of acetyl groups, but retained the shape of their distribution curve to the native starches (Fig 4.2b). OS-Ac-starch was also analyzed through GPC however no signal was detected from reflective index detector. We suspected that octenylsuccinate side chain might have inferenced with the RI detector.
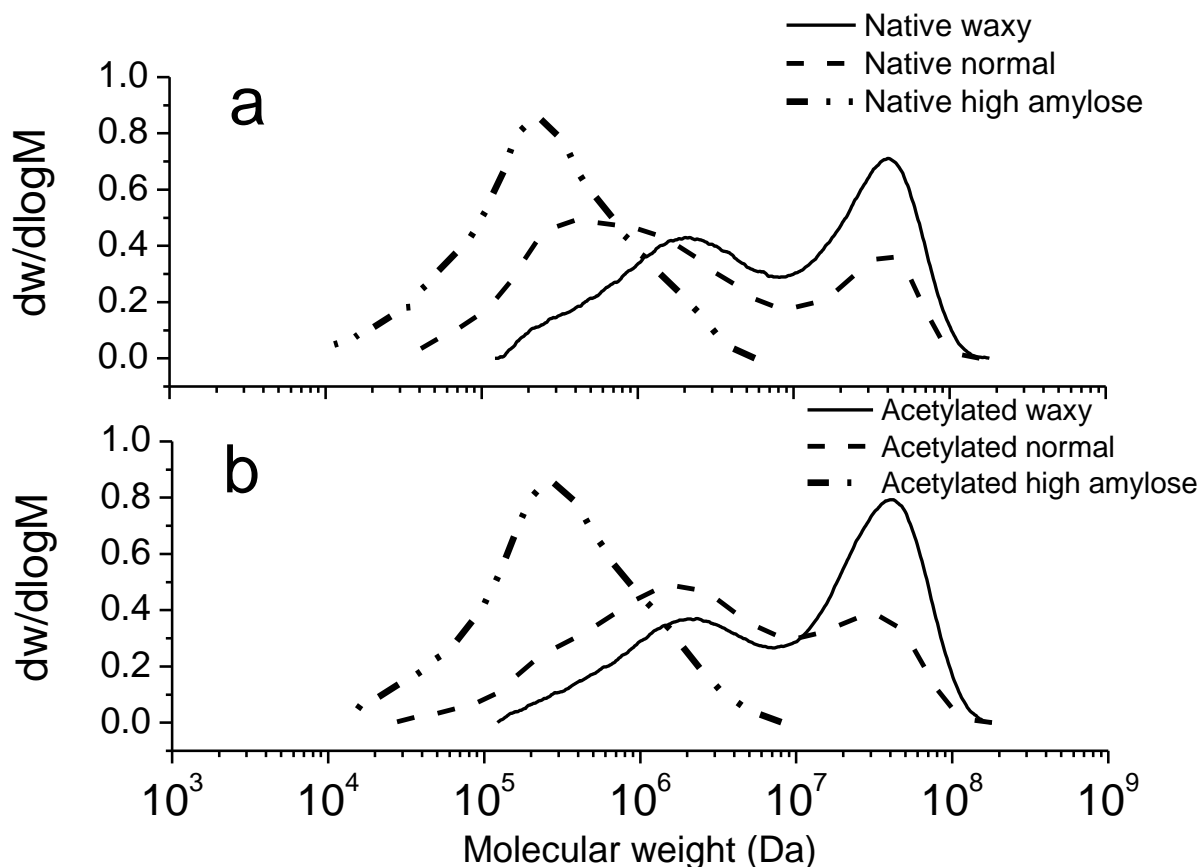
Figure 4.2. Molecular weight distribution of acetylated and unmodified base starches; molecular weight was estimated using pullulan standards.

FT-IR

As Fig 4.3a shows, highly substituted starches exhibited very little hydrogen bond stretch at 3000-3800 cm^{-1} which are typical for unmodified starch. The starch esters showed a cluster of bands from 2853 to 2953 cm^{-1} which represented the typical long chain hydrocarbon stretch

contributed by OS substituent. A distinct feature of a mixed ester, two distinct C=O symmetric stretching vibration bands, were clearly observed at 1736 and 1706 cm^{-1} (Jiang, Dai, Qin, Xiong, & Sun, 2016; Pu et al., 2011). Because OS moiety has a higher molecular mass than acetyl which translate to a lower stretching frequency, the OS carbonyl band was assigned to 1706 cm^{-1} and the acetyl carbonyl band was assigned to 1736 cm^{-1}. Intensity of two carbonyl stretch vibration bands also reflected the relative DS of each substituent and it was consistent with the DS determined by HPLC and NMR. Other major absorption bands appeared at 1364, 1218, and 1032 cm^{-1} were due to the CH_3 antisymmetric deformation, carbonyl C-O stretch, and C-O stretch of starch ring (Chi et al., 2008).

a

b

c
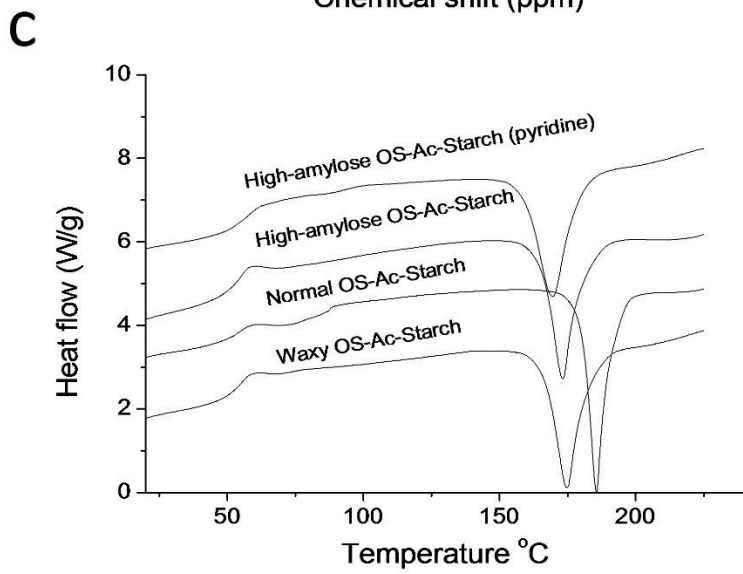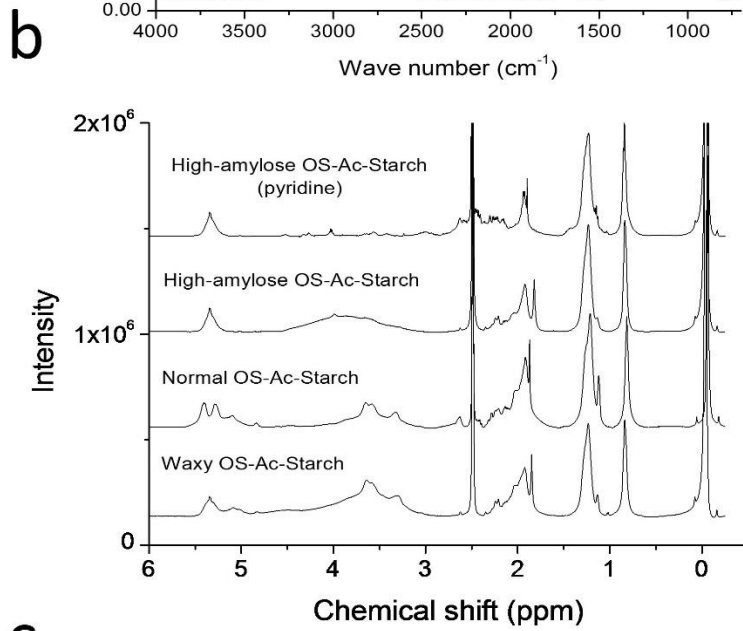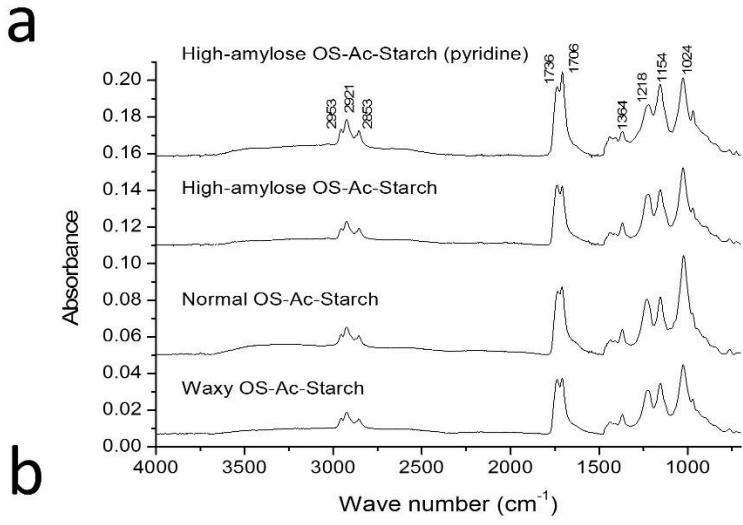
Figure 4.3. FT-IR spectra of OS-Ac-starch (a); ^{1}H-NMR spectra of OS-Ac-starch (b; starch was exchanged in deuterium oxide and solubilized in DMSO*d*-6); DSC thermogram of OS-Ac-starch ester (c; 2nd scan; pre-dried to minimize moisture).

^{1}H NMR

Based on the reports (Bai, Shi, Herrera, & Prakash, 2011; Chi et al., 2008; Sweedman et al., 2013), starch substituted with carboxylic acids gave strong methyl signal at lower ppm representing methyl and methylene resonance. As shown in Fig 4.3b, three major proton NMR peaks were clearly shown between 2.5-0.5 ppm. Acetyl methyl signal was found at 2.08 to 2.01 ppm, three methylene of OS substituent was identified as one broad peak at 1.45-1.05 ppm, and methyl of OS group was seen at 0.93-0.66 ppm. Starch ring protons signals of OS-Ac-starches were broadened and hindered the calculation to confirm the overall DS; however, ratio of acetyl methyl to OS methyl was ambiguously calculated as 0.8, 1.0, 1.5, and 1.6 for high-amylose (pyridine), high-amylose, waxy, and normal OS-Ac-starches, agreed with the DS ratio determined by HPLC as specified in Table 4.2. The broadening of starch ring protons NMR signal is common for intermediate DS starch esters. It has been reported by Kono, Hashimoto, & Shimizu (2015) due to uneven electron negativity drawing by heterogenous substituent distribution on anhydrous glucose unit.

DSC analysis

Upshifts in heat flow curve, which indicate typical glass transition, were observed for all OS-Ac-starches at around 55 °C. Considering the DS of acetylation typically do not greatly lower starch T_g, i.e. T_g between 165-185 °C for DS 1.6-2.5 AS (Shogren, 1996), these shifts should be attributed to OS substituent. Changes in DS of OS did not influence the T_g of all OS-Ac-starches, which seemed to disagree with Gordon-Taylor Equation that stated a higher fraction of low T_g plasticizer, e.g. OS substituent, shall lower the T_g of the polymer (Fringant et al., 1998). Therefore, it is reasonable to attribute this shift at 55 °C solely to T_g of OS substituent.

According to Liebert et al. (2011), highly substituted starch esters are expected to have much lower T_g than native starch and the extent of T_g decrease is mainly related to substituent chain length, DS, and presence of plasticizers. T_g of high DS OS-Ac-starch have never been reported. However, some insights can be provided by comparing it to starch esters that has close substituent chain length and DS. Fringant et al. (1998) reported starch acetate/caproate of DS 1.3/1.0 had T_g of 90 °C, while Yang and Montgomery (2008) reported starch acetate/octanoate of DS 1.66/1.03 and DS 1.91/0.67 to have onset T_g near 95 and 112 °C. According to Liebert et al. (2011), the DS of long chain substituent was responsible for the melting point of a starch ester and generally must achieve at least 2.5 for starch esters to melt at below 100 °C while acetyl had very little contribution in lowering the T_g, although acetylation was commonly practiced to elevate overall DS and water resistance. A major exothermic peak was observed at temperature 150-200 °C as showed in Fig 4.3c. Temperature of the exothermic peak appeared to negative relate to DS of octenylsuccinate. We suspect those peaks to be starch backbone degradation caused by OS substituent, because OS substituent presented in acid form (-COOH) and might cause cleavage of starch backbone at high temperature.

Solubility of OS-Ac-starch in organic solvents

Dissolution behavior of starch esters differs as DS changes in various solvents and the opacity of the so formed solution is a direct indication of solubility. The solvents listed in Table 4.3 covered a range of relative polarities and were representative in starch esters studies as either plasticizer, casting-solvent, diluent, or reaction medium. High-amylose OS-Ac-starch formed clear solution in chloroform, acetone, DMSO, and triacetin, indicating complete dissolution. Waxy and normal starch esters were partially soluble in the most solvents because of cloudiness in some solvents which might due to lower DS. In classic starch ester applications such as film

casting (Zhu et al., 2013), impregnation (Larotonda, Matsui, Sobral, & Laurindo, 2005) and coating (Han, 2014), high solubility in casting-solvents was always desired to allow complete molecular re-alignment for film formation. Therefore, only high amylose ester was suitable to cast to a film from solubility standpoint.

Table 4.3. Starch ester compatibility in different organic solvents.

Solvent	OS-Ac-starch		
	Waxy	Normal	High amylose
Chloroform	P	I	S
Acetone	P	P	S
Hexane	P	I	P
DMSO	S	S	S
Triacetin	P	P	S

S, soluble; P, partially soluble; I, insoluble.

Rheology properties of OS-Ac-starch

Triacetin and DMSO were chosen to mix with starch esters as they were non-volatile and widely used media for dissolving and plasticization of starch materials. Starch esters formed self-standing clear (DMSO) and opaque (triacetin) mass after 1:1 (w/w) mixing. Storage modulus (G'), the stored portion of applied force, was known to correlate with samples' solid-like behavior and apparent stiffness. G' of the starch ester mass at small oscillation stress was between 1 to 10 kPa, which was well on the high end for gels or pastes formed by starch, probably due to the high solid ratio (Rezler & Poliszko, 2010) (Fig 4.4a). Loss modulus (G''), also known as dissipated portion of applied stress due to samples' liquid-like character, was well below G' for normal and waxy starch ester mass and nearly equal to G' in high amylose mass, suggesting the normal and waxy ester were more solid-like than high amylose ester, which has

equal liquid- and solid-like behavior. Starch esters plasticized with DMSO was systematically lower in both modulus than with triacetin, indicating higher compatibility with DMSO to form less stiff starch mass. Linear viscoelastic region reveals a critical stress value that from which the sample structure starts to fail (Sang, Bean, Seib, Pedersen, & Shi, 2008). The LVR was for high amylose ester/triacetin was over 3 kPa, indicating a good elasticity for deformation recovery over a wide stress range. For frequency sweep, 500 Pa was chosen to accommodate the critical stress of waxy and normal starch ester masses. As Fig 4.4b shows, G' of starch ester masses was dependent of frequency, indicating the materials gained stiffness if the stress was applied at high frequency, e.g. rubbing, rolling, or chewing etc., and softened when stress was removed.

a

High amylose/DMSO

High amylose/triacetin

Normal/DMSO

Normal/triacetin

Waxy/DMSO

Waxy/triacetin

Stress (Pa)

b

High amylose/DMSO

High amylose/triacetin

Normal/DMSO

Normal/triacetin

Waxy/DMSO

Waxy/triacetin
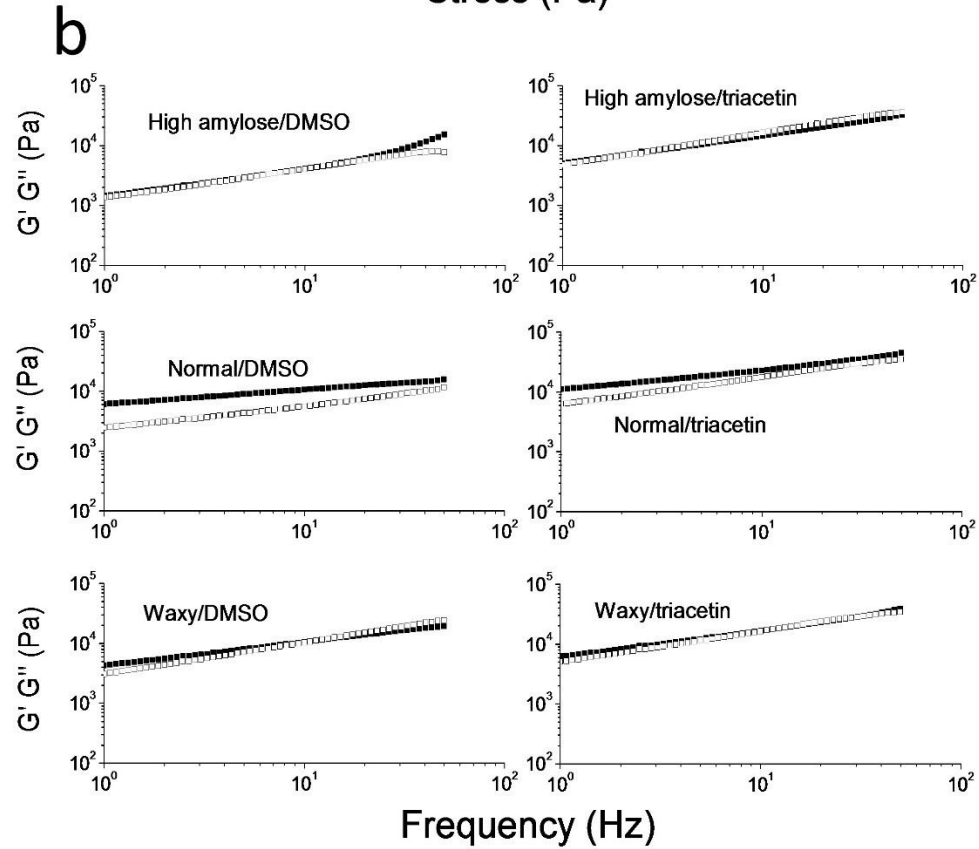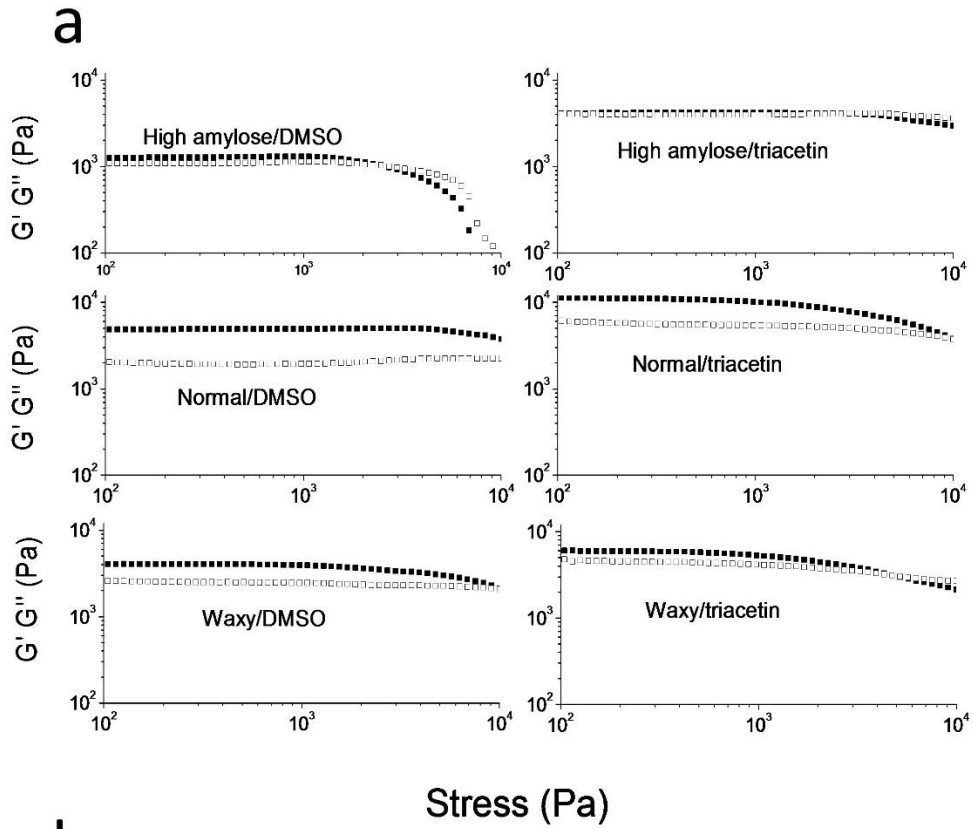
Frequency (Hz)

Figure 4.4. Stress sweep (a) and frequency sweep (b) of starch ester gels formed in DMSO and triacetin; tests carried out with 25 mm smooth surface parallel plate at 2 mm gap.

Chewing gum base made from OS-Ac-starch

Starch esters formed homogenous gum bases that resemble wheat flour dough after addition of a mixture of plasticizers. However, these gum bases exhibited distinct difference in extensibility when manipulated. Waxy and normal starch-based gums were very crumbling and easily broken apart when stretched while high amylose starch-based gum was clearly more extendable (Fig 4.5). In early studies, amylose has been reported to produce strong and elastic films similar to cellulose, owning to their long linear structure that favors inter-chain association (Lourdin, Valle, & Colonna, 1995; Shogren, 1996; Whistler & Hilbert, 1944). Yang and Montgomery (2008) also tested several starch ester films and concluded that amylose content was mainly responsible for film elongation, where higher amylopectin content resulted in brittle films that has significant low extensibility. Fig 4.5 demonstrated the differences in extensibility of starch esters that contain mainly amylopectin vs amylose. Waxy and normal starch esters were prone to break and clearly inferior to high amylose starch ester and therefore, only high amylose starch ester was selected for gum base rheology tests.
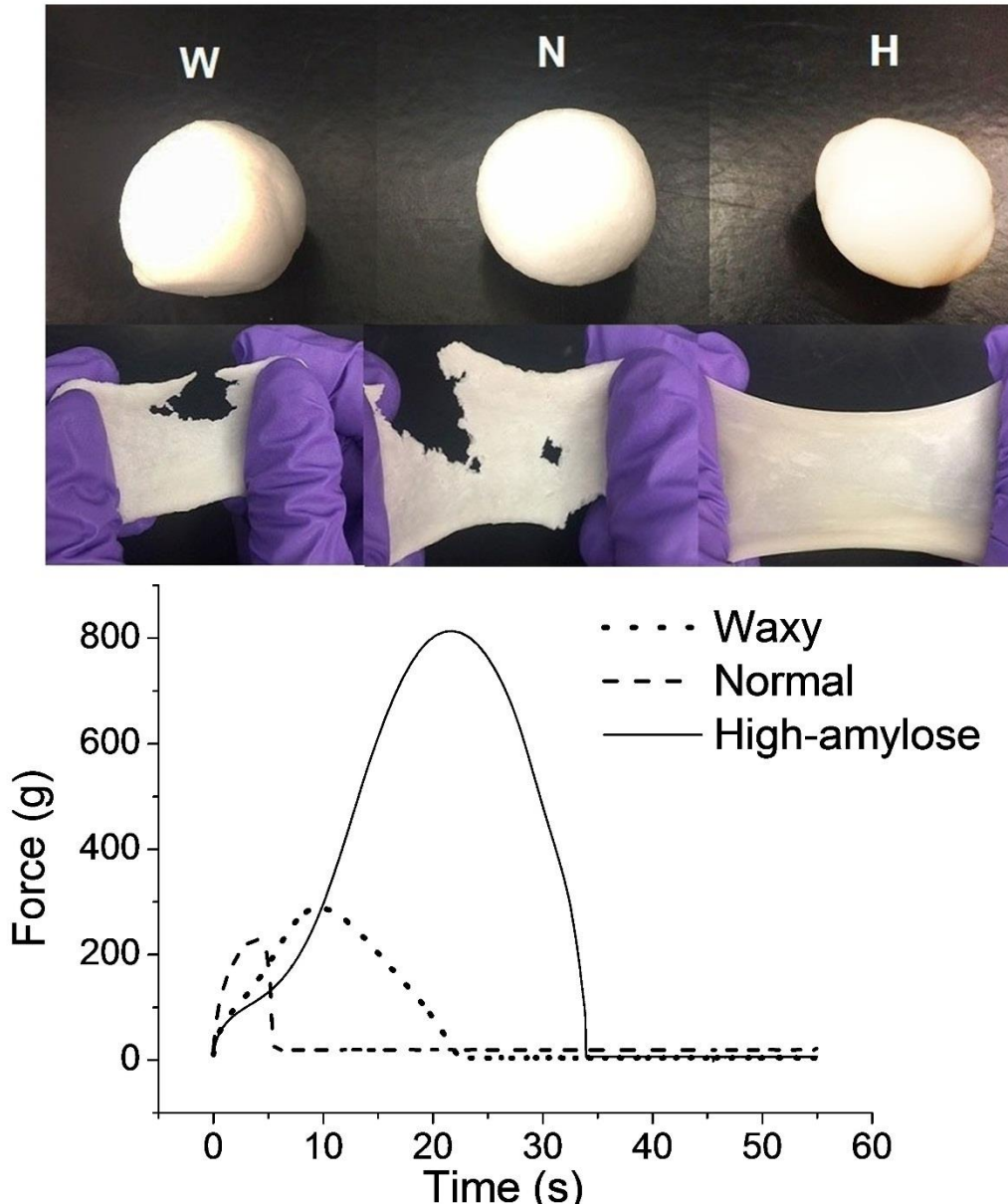
Figure 4.5. Formulated OS-Ac-starch to mimic starch-based chew gum rolled (first row) and stretched (second row); W, waxy; N, normal; H, high amylose starch; Elongation test was by texture profile analyzer; the gum curd was stretched from 20 mm to breakage at 3.3 mm/s; force and at break was 289, 228, and 813 g for normal, waxy, and high-amylose starch, respectively, corresponding to 31, 12, and 72 mm elongation.

Starch ester made from high amylose starch was compounded to chewing gum bases at three different triacetin levels and compared with commercial chewing gum. G' of the LVR was inversely related to the level of plasticization, which was expected because the plasticizer was mostly liquid and provided no stress storage (Fig 4.6a). As stress increased, the highly

plasticized starch showed slight decrease in G' from about 8 kPa oscillatory stress, where mid and low plasticized starch gum held G' constant through the tested range. Commercial chewing gum had a large initial G' (1 mPa) and was more sensitive than starch gums to applied stress as its G' decreased nearly two magnitudes during the stress sweep (Fig 4.6a). Translating to sensory descriptions, commercial gum stays as a solid mass and do not undergo deformation when sitting untouched, however softens greatly when forces are applied. Starch gums, on the other hand, can slowly deform when untouched, which is due to a much less initial G' at small stress. At above 5 kPa stress, both gums fall into the similar G' range, which was very desirable because chewing force is often associated with high stress (Martinetti et al., 2014). Frequency sweep showed that both starch and commercial gums were sensitive to frequency change as the G' and G'' increased steadily and the starch gums had a more liquid-like behavior as G''> G' (Fig 4.6b). It was also noteworthy that the plots of starch gums were smoother than the commercial gum, which is possible because starch gums were more adhesive while commercial gum encountered wall slipping against geometry surface.

a

b

c

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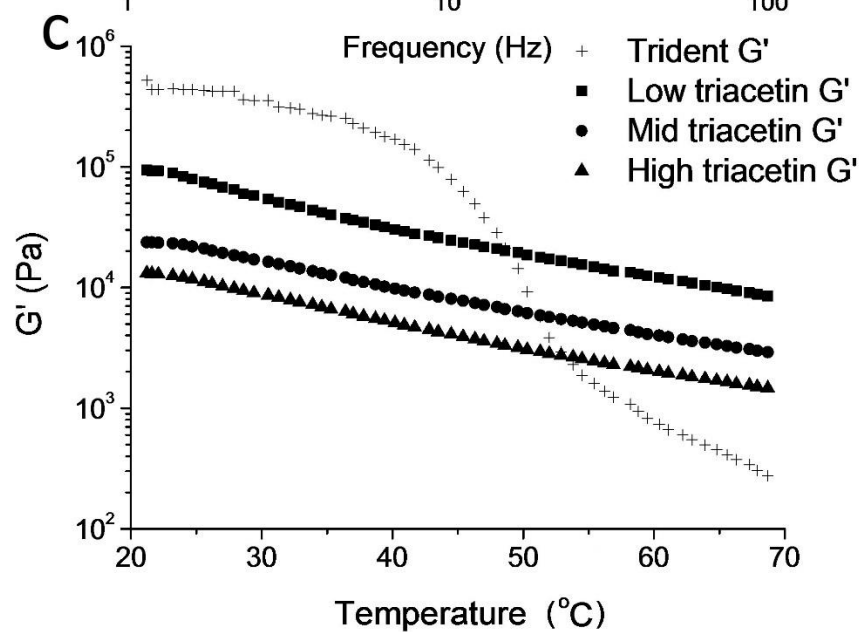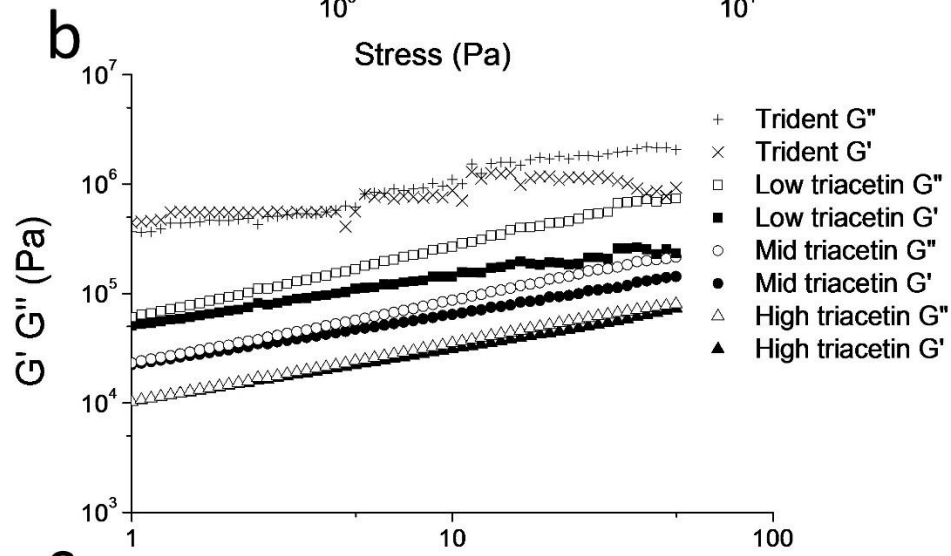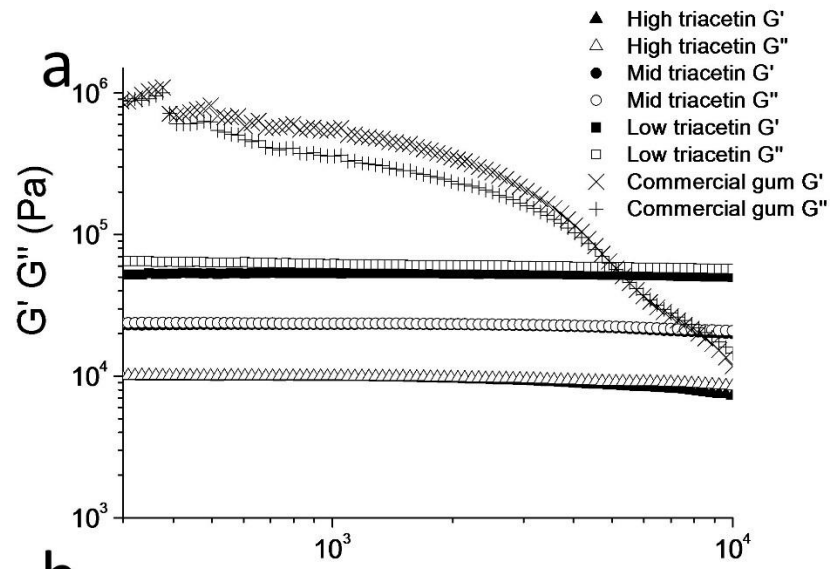
Figure 4.6. Stress sweep (a), frequency sweep (b), and temperature sweep (c) of high amylose starch-based gum; tests carried out with 25 mm smooth (starch gum) and serrated (Trident gum) surface parallel plates at 2 mm gap.

According to Fritz (2006), stiffness of chewing gum curd is highly dependent on temperature to satisfice good chew and ease to process. Phillips, Morgret, Xia, & Shen (2011) suggested that a chewing gum curd should produce a G' about 10^5 to 10^7 Pa at 25 to 37 °C upon chewing and rapidly reduce to less than 10^5 Pa at 60 °C. As showed in Fig 4.6c, the commercial chewing gum tested well fit in this G' range. Starch based gums had smaller G' at human body temperature (37 °C), which could translate to a softer texture when chewed. In addition, starch-based gums exhibited steady G' drop along the heating ramp and lacked an exponential G' decay occurred in commercial gum curd between 40-50 °C. However, according to Phillips, Morgret, Xia, & Shen (2011), a smaller temperature-dependent G' differential is strongly associated with ease of a gum to be removed from environmental surface, because the heating and cooling cycles are less likely to melt the gum and result an "interlocking" effect between gum and another surface.

 Effects of linear vs branched starch backbone structure

It was clear that high amylose-based gum gave the most stretch after plasticization (Fig 4.7), which could due to a couple of reasons, e.g. linear molecular conformation, smaller molecular weight, or higher DS, that particularly favors elongation. It is interesting to find out whether a starch ester that has branchy backbone will develop any stretch if the DS and molecular weight are the same to high amylose starch ester. To put this to an embodiment, we used an acid hydrolyzed waxy maize starch that has a similar molecular weight distribution as to high-amylose starch to make esterified samples.

a

b
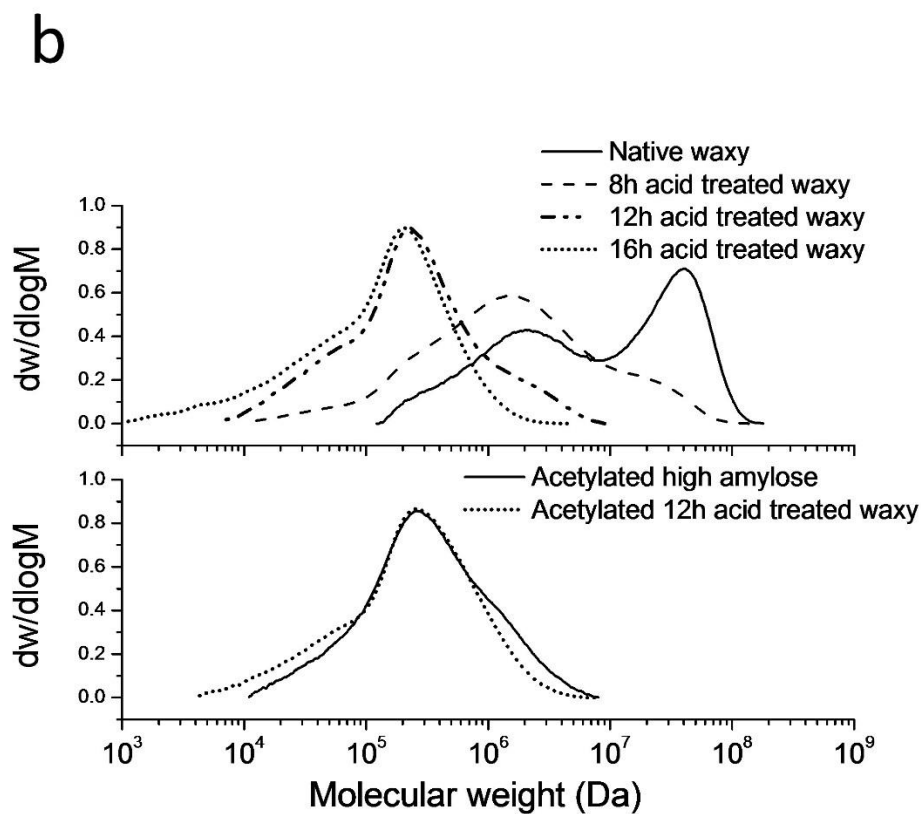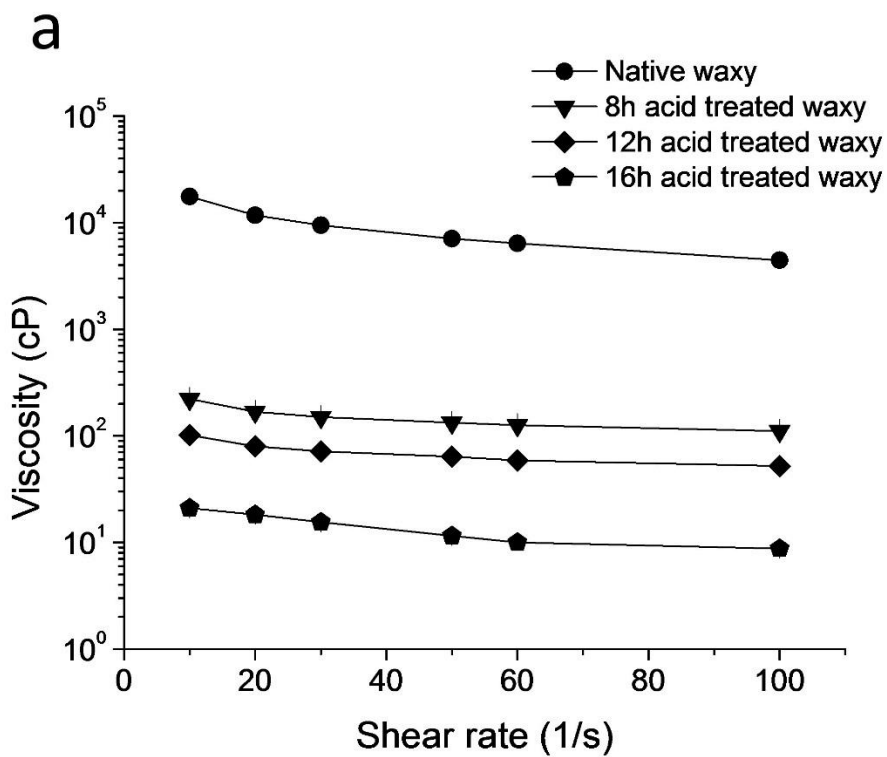
Figure 4.7. Alkali viscosity of native and acid treated waxy corn starch (5% solid) at 25 °C (a); molecular weight distribution of acid treated acetylated waxy corn starches (b).

Acid treatment greatly reduced molecular weight of waxy starch (Fig 4.7b), and reduction of molecular weight reflected on starch paste viscosity change (Fig 4.7a). Confirmed by GPC, acid treatment for 8, 12, and 16 h reduced Mw of waxy starch from 1.9×10^7 to 4.6×10^6, 4.7×10^5, and 2.2×10^5, respectively. The 12 h acid treated sample was chosen for esterification as it had similar Mw to high amylose starch (4.5×10^5). From preliminary trials, acid treated starch tended to have lower yield when recovered by filtration, which was possible due to higher solubility of low molecular weight fractions in aqueous media and wash water. Therefore, acetylated 12h acid treated waxy starch was recovered by dialysis instead of filtration. Acetyl DS and Mw of dialysis-recovered starch was 1.4 and 3.9×10^5 which was just slightly different than acetylated high amylose starch (1.3 and 5.5×10^5). Subsequent OS modification was carried out using OSA/triacetin medium and purified by dialysis against ethanol. The DS of octenylsuccinic group was 1.3, which was higher than the DS based on untreated waxy starch. The 12h acid treatment remarkably decreased the viscosity of OS reaction which was readily stirred by a conventional magnetic stir plate. Reduction of molecular weight enhanced reaction DS however created some difficulties in recovering the starch ester due to solubility change. A chewing gum base sample was formulated similarly using the "mid-triacetin" formula in Table 4.1. The gum base was not able to stay aggregated and broke easily when handled.

Conclusions

Acetylated waxy (DS 1.20), normal (DS 0.85), and high amylose (DS 1.30) starches were reacted with OSA at 120 °C in presence of triacetin as a diluent. The starch mixed ester had octenyl succinate DS of 0.83, 0.55, and 1.25 for waxy, normal, and high amylose starches,

respectively, as determined by HPLC. FT-IR and ^{1}H NMR analysis confirmed the successful grafting of both esters and their spectra patterns were same to a mixed ester synthesized in pyridine medium. DSC thermograms revealed the glass transition temperatures of those starches was 56 °C. High-amylose starch ester had higher solubility in acetone, chloroform, and hexane than waxy and normal starch esters, probably due to overall higher DS. All starch esters formed self-standing gel in DMSO and triacetin at 1:1 (w/w) ratio that had G' from 1 to 10 kPa in linear viscoelastic region and increase with stress frequency. When compounded with a mix of plasticizers to produce chewing gum base, normal and waxy starch gums were crumbling and ruptured easily while high amylose starch gum exhibited great extensibility. Temperature sweep (20-70 °C) revealed the G' of high amylose starch gum decreased approximately 10 folds (10^5-10^4 Pa) and had a potential as a chewing material. Acid thinning effectively reduced molecular weight of waxy starch to a range close to high amylose starch. Acid thinning was helpful to prepare higher DS starch ester by reducing octenylsuccinylation medium viscosity. However, the gum base formulated with acid thinned waxy starch was far inferior comparing to high amylose starch-based gum as it was extremely crumbling and lack of cohesiveness, possibly due to the branchy structure that was unfavored for molecular association. Future studies will include (1) exploring other inert diluents instead of triacetin for cost saving and reaction efficiency improvement, (2) investigate film forming properties of the starch esters knowing they were soluble in some volatile solvent, and (3) optimization of the chewing gum formula, e.g. addition of microcrystalline wax to harden and soften the gum curd upon temperature change and replacing triacetin with other low-cost plasticizers such as propylene glycol.

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Chapter 5 - Preparation and properties of highly substituted hydroxypropylated acetylated high-amylose maize starch

Abstract

High-amylose maize starch was reacted with various levels of propylene oxide in isopropanol-water mixture to obtain low (0.56), middle (1.18), and high (1.64) degree of molar substitution (MS) hydroxypropyl starches (HPS). The HPS were further acetylated with acetic anhydride to various extent (degree of substitution (DS) 0.09-1.97). Important physicochemical and thermal properties of HPS and acetylated hydroxypropylated starch (HPAcS) were determined. Hydroxypropylation alone at MS 1.18 and 1.64 rendered HPS completely cold water soluble where the HPAcS with high acetyl DS (1.21-1.97) were insoluble in water but soluble in chloroform, acetone, and dimethyl sulfoxide (DMSO). Acetylation with lower DS (DS 0.09-0.86) rendered HPAcS 20-95% soluble in water at 2.5% solid content. Glass transition temperature (T_g) of dry HPS was significantly lowered (63.8 and 1.8 °C for mid and high MS HPS). Acetylation and hydroxypropylation synergistically, lowered the T_g of starch derivatives to subzero temperatures. Intrinsic viscosity of starch derivatives was 32-100 mL/mg comparing to native high amylose starch at 80 mL/mg, suggesting a smaller hydrodynamic volume after derivatization. The water insoluble HPAcS was blended with hydrophobic plasticizers to form a viscoelastic gum base. Rheological analysis indicated the gum bases made with higher T_g HPAcS were stiffer. Gum bases all had at excellent stretch without breaking, resembling chewing gum bases.

Keywords

Hydroxypropylated starch, acetylated starch, starch ester, starch ether, highly substituted, hydrophobic starch, thermoplastic starch, starch gum base

Abbreviations

HPAcS, hydroxypropylated acetylated starch; HPS, hydroxypropylated starch; HP, hydroxypropyl: Ac, acetyl; MS, molar substitution; DS, degree of substitution; RE, reaction efficiency; T_g, glass transition temperature; TFA, trifluoroacetic acid; DMSO, dimethyl sulfoxide; TMS, tetramethylsilane; IPA, isopropyl alcohol; PO, propylene oxide; AA, acetic anhydride; GPC, gel permeation chromatography

Introduction

Starch is an abundant renewable polymer that has found uses in many food and non-food applications. However, shortcomings, e.g. insoluble in cold water and organic solvents, and susceptibility to degradation by enzyme, acid, heat, and shear, hurdled native starch from being a satisfactory industrial polymer (Shogren, 1996). Native starch is not thermoplastic owning to its high glass transition temperature (T_g). The high T_g allows narrow window for thermal processing without high amount of plasticizer (Kaseem et al., 2012). A practical approach to actualize starch's industrial potential is through chemically derivatizations. Chemically modified starch, particularly starch ethers and esters, have altered thermal behavior and solvent compatibility depending on type and extent of modification (Liebert et al., 2011).

Starch reaction with propylene oxide (PO) has been well known for many decades. The resulting product, hydroxypropylated starch (HPS), was chiefly produced at molecular substitution level (MS) 0.05-0.1 (one hydroxypropyl group per 10-20 glucose unit) (Tuschhoff, 1987) as food additives. Such low MS HPS was prepared in aqueous starch slurry with addition

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of 7-8% (starch wt) PO in presence of a gelatinization-inhibiting salt, e.g. 10-30% sodium sulfate (Han & BeMiller, 2005) and recovered as granular starch. Gelatinization temperature in low MS HPS was 5-18 °C lower than its native base starch. Upon cooling, HPS sets to a clear paste because hydroxypropyl groups provide disrupt interchain H-bond and their re-association and also improved hydrophilicity (Hjermstad, 2012). Hydroxypropylation would proceed to achieve MS up to 0.43 in aqueous medium with further addition of propylene oxide for longer reaction hours; however, hydroxypropylated starch material, primarily amylose, progressively leach out from the granule and renders dewatering extremely difficult (Shi & BeMiller, 2002). According to Aminian, Nafchi, Bolandi, & Alias (2013), the highest MS of granular HPS as was 0.66 and above which the starch was not recoverable from water. Hjermstad, (2012) pointed out 0.6 as the MS cutoff for HPS because above 0.6, starch granular structure and crystallinity are lost completely. HPS with MS > 0.6 can be synthesized in light alcohol water mixture to prevent swelling and settling, purified by alcohol wash, and recovered as an amorphous powder. A representative preparation of such HPS was disclosed by Hunt, Kovats, & Bovier (1981), in which the HPS was synthesized in aqueous isopropanol and the MS of HPS could potentially ramp higher with more reagent and longer hours (Han & BeMiller, 2005). Hydroxypropylation provide intermolecular plasticization that greatly lowers T_g of starch to allow thermal processing, e.g. hot press, extrusion, molding, casting, etc. possible without using external plasticizers (Hjermstad, 2012). HPS can be subsequently derivatized e.g. esterification, to gain more functions. Such preparation is known as dual-modification and it allows starch to gain both functional characters. Typical dual-modifications include cross-linked HPS (Gunaratne & Corke, 2007; Zhao et al., 2015), acetylated HPS (Granza et al., 2015), and acid thinned HPS (Li et al.,

2018); however, these studies were conducted using low MS HPS. There were very limited studies that dual-modified a high MS HPS.

In this study, we synthesized HPS to three MS levels and further acetylated the HPS to six degree of substitution (DS) and examined their properties. In theory, high MS hydroxypropyl starch is highly water soluble and of a significantly lowered T_g. Subsequent acetylation is expected to counter the hydrophilicity and further lower the Tg. Therefore, we hypothesized that thermoplastic starches with different hydrophobicity can be prepared by varying MS of hydroxypropyl group and DS of acetyl group. Such starch derivatives could find applications as coatings, adhesives, foams, or controlled release carriers (Zhang et al., 2013). We picked acetyl group as the secondary substituent because it is a common, low-cost, and rather mature method to produce hydrophobic starch. According to Xu, Dzenis, & Hanna (2005), acetylated starch of DS above 1.6 would revert hydrophilicity of native starch and render the starch water insoluble. Furthermore, acetyl is a feasible ester to prepare because it is a less bulky substituent and easy to achieve high DS. It was expected that the hydroxypropylated acetylated starches (HPAcS) we synthesized are capable to cover a wide range of T_g and water solubility. We also explored a possible application of the HPAcS as chewing gum bases.

Materials and methods

Materials

High-amylose (70% amylose) maize starch (Hylon VII) was obtained from Ingredion Inc. (Bridgewater, NJ). Acetic anhydride, propylene oxide, sodium hydroxide, isopropyl alcohol (IPA), chloroform, acetone, and calcium carbonate were purchased from Fisher Scientific (Waltham, MA). Triacetin, deuterium oxide, and DMSO-d_6 and tetramethylsilane (TMS) was

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purchased from Sigma-Aldrich (St. Louis, MO). Acetylated monoglyceride (Acetem 70) was kindly provided by DuPont USA (Wilmington, DE).

Preparation of starch derivatives

Preparation of HPS

HPS was prepared by using the method based on Hunt, Kovats, & Bovier (1981) (Fig 5.1). Briefly, 300 g starch was slurried in 400 mL 85% isopropyl alcohol (IPA) and reacted with 84 mL propylene oxide (PO) to produce low MS HP starch targeting MS approximately 0.6. HPS was washed, dried, re-slurried in fresh aqueous IPA, and further reacted with more PO to produce mid and high MS HPS (Fig 5.1). This set of HPS samples were denoted as made from "stepwise" method, because HPS at each MS level was isolated. In a separate set of experiment, mid and high MS HPS were prepared by adding the same amount of PO directly into the reaction without recovering low and mid MS HPS and re-slurry them into fresh reaction medium. This set of samples were denoted as made from "continuous" method. In another set of experiment, PO was added into initial reaction media in one-dose aiming at mid and high MS. This set of samples were denoted as made from "one-dose" method.

Preparation of HPAcS in anhydrous medium

HPS was subsequently acetylated according to Mark & Mehltretter, (1972) by reacting 50 g HPS in 150 mL acetic anhydride at 115 °C in presence of concentrated sodium hydroxide to achieve presumable maximum DS. The HPAcS paste was washed using methanol (500 mL) and precipitated with 500 mL water (Fig 5.1).

Preparation of HPAcS in aqueous medium

Another set of HPAcS was prepared using in aqueous synthesis according to Luo & Shi, (2012). After hydroxypropylation, an aliquot of low, mid, and high MS HPS pastes containing 10

g HPS were adjusted to pH 8.5 with HCl and diluted with water to solid content 25%. Acetic

anhydride was added dropwise to the mixture with intense stirring while the pH of the mixture

was maintained at 8-9 by a pH controller (Eutech Instruments, Vernon Hills, Illinois). As

reaction proceeded, HPAcS gradually precipitated and aggregated to a "doughball". Actual

consumption of acetic anhydride was recorded at the end-point of the reaction where there was

no more flocculent precipitation formed. The HPAcS "doughball" was spooned out, desalted by

dialysis, and freeze dried.

Preparation of hydroxypropylated acetylated starch ether ester

High-amylose starch (300 g)	Low MS HPS (200 g)	Mid MS HPS (100 g)	**HPAcS**
Slurry in 400 mL 85% IPA and 12 mL 50% NaOH + 70 g (27% dry starch wt) PO, stir for 48 h at 55 °C. Filter under vacuum	Slurry in 260 mL 85% IPA and 8 mL 50% NaOH + 46 g (25% dry starch wt) PO, stir for 24 h at 55 °C	Dissolve in 130 mL 85% IPA and 4 mL 50% NaOH + 56 g (60% dry starch wt) PO, stir for 48 h at 55 °C Dialyze & freeze dry	Methanol/water wash Dropwise add 6 mL 50% NaOH Heat to 115 °C
Starch Cake	Thick starch paste		Dispersed HPS
Slurry in 600 mL IPA and neutralize to pH 7 with acetic acid. Repeat once. Filter & air dry	+ 400 mL 85% IPA and 46 g (25% dry starch wt) PO, stir for 24 h at 55 °C Dialyze & freeze dry	**High MS HPS**	Mix with 150 mL acetic anhydride, heat to 60 °C
Low MS HPS	**Mid MS HPS**		HPS (50 g)

Figure 5.1. Flow diagram of two-stage synthesis of hydroxypropylated acetylated starch (HPAcS); hydroxypropyl starch (HPS) was first prepared to low, mid, and high molecular substitution (MS) in aqueous isopropyl alcohol (IPA) with propylene oxide (PO) and acetylated using acetic anhydride as a reactive solvent.

Characterization of HPS and HPAcS

DS determination by ^{1}H nuclear magnetic resonance (^{1}H NMR)

Sodium acetate anhydrous (250 mg) was weighed and dissolved in a 25 mL volumetric

flask containing deuterium oxide (20 mL) and deuterium chloride (DCl, 5 mL, 35%w/w). To

each 2 mL aliquot (contain 20 mg sodium acetate) of above solution, HPS (100 mg, d.b) was added. The HPS slurry was heated in a boiling water bath for 30 min with constant stirring to yield a transparent solution. The solution was transferred to a standard 5 mm NMR tube and analyzed by a Varian 400 MHz NMR spectrometer (Varian Inc., Palo Alto, CA) at 25 °C. Sixteen scans were acquired for each sample and the relaxation time was 1.0 s. Chemical shift was calibrated to a trace addition of tetramethylsilane (TMS) to 0 ppm. Hydroxypropyl content and MS was calculated using Equation 1 and 2.

$$\%HP = \frac{59.3 \times I1.13}{82.05 \times I2.08} \times 20/100 \tag{1}$$

Where $I_{1.13}$ and $I_{2.08}$ was signal areas of hydroxypropyl's and sodium acetate's methyl groups.

$$MS = \frac{162 \times \%HP}{59 - 58 \times \%HP} \tag{2}$$

Reaction efficiency of hydroxypropylation (%RE$_{HP}$) was calculated using equation 3.

$$\%RE_{HP} = \frac{\frac{Y}{(162 + 58 \times MS)} \times MS}{M_{PO}} \tag{3}$$

Where M_{PO} was mole of propylene oxide used; Y was yield of HPS, MS is degree of mole substitution.

Due to insolubility of HPAcS in aqueous solvent, DMSO-d_6 containing 5% TFA-d was used. HPAcS (30 mg, d.b) was exchanged in 1 mL deuterium oxide with constant stirring at room temperature overnight, freeze dried and reconstituted in 1 mL DMSO-d_6 TFA-d mixture and analyzed by NMR. DS and reaction efficiency of acetylation (%RE$_{Ac}$) was calculated using and 4 and 5.

$$DS = \frac{A}{B} \times MS \tag{4}$$

where B was signal area of acetyl methyl group at 2.10-1.93 ppm; A was HP methyl at 1.13 ppm; MS was the molecular substitution of HPS.

$$\%RE_{Ac} = \frac{\frac{Y}{(162+58\times MS)+(43\times DS)} \times DS}{M_{AA}} \qquad (5)$$

Where M_{AA} was mole of acetic anhydride used; Y was yield of HPAcS, MS is degree of mole substitution of HPS, DS was the acetyl degree of substitution of HPAcS.

Differential scanning colorimetry (DSC) analysis

HP starches and HPAcS that has been dried at 80 °C in vacuum oven for 2 hours were stored in a desiccator for 24 hours and weighed (Ca. 15 µg) into a DSC pan. The sample pan was heated from -30 to 100 °C at 10 °C/min and rescanned to 100 °C. In the cases of high MS starches, the scan began from -50 °C to accommodate expected low glass transition temperatures. DSC thermograms were analyzed using Universal Analysis software (TA Instruments, New Castle, DE).

Solubility of HPS and HPAcS in water

Solubility of starch derivatives was determined according to Aminian et al. (2013). Briefly, HPS and HPAcS (1 g d.b.) was weighed into centrifuge tubes and 40 mL distilled water was added. The tubes were heated at 30, 60, and 90 °C in a shaking water bath for one hour, cooled to room temperature, and centrifuged at $1700\times$ g for 20 minutes. Supernatant was poured to pre-weighed dishes and dried overnight at 105 °C. Solubility was determined as the ratio of dried supernatants to one-gram starch.

Capillary viscometry of HPS and HPAcS

Intrinsic viscosity of starch derivatives was determined based on method of Aminian et al., (2013) with minor modifications. Starch solutions (1%) were prepared by mixing HPS and

HPAcS in DMSO overnight at room temperature until all solutions became complete transparent. In the case of native starch, the slurry was heated in a boiling water bath for 20 min to ensure dissolution. An Ubbelohde type capillary viscometer was used to measure intrinsic viscosity. The temperature was controlled by at water bath at 25 °C. Starch solutions (12 mL) was transferred into the viscometer and equilibrated for five minutes. The starch solutions were further diluted to 0.3%, 0.5%, and 0.8% to measure specific viscosity and the intrinsic viscosity was obtained by extrapolating the specific viscosity to an infinite dilution.

Molecular weight determination by gel permeation chromatography (GPC)

Native high-amylose starch, HPS, and HPAcS (8 mg) were added into 2 mL DMSO and stirred overnight in 80 °C water bath, filtered through 0.45 micro filter and injected into a PL-GPC 220 instrument (Polymer Laboratories, Inc., Amherst, MA). Separation was performed on three Phenogel 10 μm columns arranged in order of 10^6 Å, 10^3 Å and 10^2 Å pore size (Phenomenex, Inc., Torrance, CA, USA), a guard column (Phenomenex, Inc., Torrance, CA), and recorded by a differential refractive index detector with dn/dc value 0.066. Eluent system was DMSO containing LiBr (0.55% w/v) at a flow rate of 0.8 mL/min. Column oven temperature was controlled at 80 °C. Pullulan standards and maltose were used for molecular weight calibration.

Rheological analysis of starch chewing gum base

Formulation of starch chewing gum base is modified from (Liu, 2010). Triacetin (3.5 g), hydrogenated soybean oil (0.5 g), and acetylated monoglyceride (0.5 g) were mixed in a 100-ml beaker and heated at 60 °C for approximately one hour to form a one-phase liquid plasticizer mixture. HPAcS (4.5 g) was mixed with calcium carbonate (1 g) and added to the plasticizer mixture. The mixture was conditioned at 60 °C overnight and kneaded to a uniform gum base.

The gum rested at room temperature for at least 24 hours before analysis. Stress sweep (300-10000 Pa) was carried out using smooth parallel plates (25 mm diameter; 2 mm gap) at 25 °C to find the liner-viscoelastic region (LVR), which was determined at 10% decrease of initial storage modulus (G'). Frequency sweep, temperature sweep, and creep recovery was conducted at using 500 Pa, which was in the LVR of all samples.

Extensibility of the gum base was determined by a TA.XT Texture-Analyzer (Texture Technologies Corp. and Stable Micro Systems, Ltd., Hamilton, MA) coupled to a double clamp geometry (TA-96). Starch gum base was rolled to 5 mm sheet using a rolling pin with 5 mm guide. Stripes (40 mm×20 mm) were cut from the gum sheet and clamped to the geometry with each end clamping 10 mm of the gum stripe. The gum was stretched from 20 cm to 170 mm (150 mm travel distance) at 3.3 mm/s.

Statistical analysis

Preliminary scouting synthesis was carried out for starch hydroxypropylation and acetylation to ensure levels of substitution fall into expected range. One formal full-scale synthesis according to Fig 5.1 was carried out. NMR, DSC, solubility, GPC and rheology analysis were carried out in duplicates. Texture profile was carried out in triplicates. Quantitative changes from above analysis were analyzed by one-way analysis of variance (ANOVA). A probability of $P \leq 0.05$ was considered significant. Statistical procedures were carried out by SAS 9.3 (SAS Institute; Cary, NC).

Results and discussion

MS and DS determination by proton NMR

Colorimetric method has long been exercised as a conventional determination for starch HP content. In such method, HPS was treated in hot concentrated sulfuric acid to liberate

propylene glycol, which subsequently dehydrated to propionaldehyde to produce a violet color with ninhydrin (Fu, Zhang, Ren, & BeMiller, 2019). The colorimetric method was fundamentally sound; however, required extreme care to avoid aberrant results. Xu & Seib (1997) developed a method using high-resolution proton NMR to determine HP content, in which HPS was exhaustively digested with thermal stable α-amylase, spiked with sodium acetate and analyzed with a high-resolution NMR. Methyl signal of HPS was standardized to sodium acetate signal and the degree of substitution can be calculated. The NMR method was more accurate (only half of the standard errors as compared to colorimetric method) and less time-consuming and therefore, was adopted to determine the HP content in our samples. Considering high substitution might hinder enzyme activity, DCl was used instead to depolymerize the HPS thus reduce its viscosity. Owning to retrogradation-prevention from high HP substituent, the DCl treated HPS yielded a complete transparent, free-flowing solution that produced NMR spectra (Fig 5.2a) highly consistent to literature (Xu & Seib, 1997).

After acetylation, HPAcS became largely insoluble in aqueous system; therefore, they were dissolved in DMSOd_{-6} to assess acetyl DS. Based on the reports (Chi et al., 2008a; Elomaa et al., 2004), starch substituted with carboxylic acids gave strong methyl resonance at lower ppm. As shown in in Fig 5.2b & c, acetyl methyl signal was found at 2.08 to 2.01 ppm and was clearly resolved from HP methyl signal. Starch ring proton signals on these spectra appeared broadened bands overlapping with HP methylene and methine signals and were not useful for acetyl DS calculation. Therefore, DS of acetyl was calculated as a direct ratio of acetyl to HP methyl signal intensities. The broadening of starch ring protons has been reported by Kono, Hashimoto, & Shimizu (2015) due to uneven electron negativity drawing by heterogenous substituent distribution on anhydrous glucose unit.

Substitution levels and yield of HPS were summarized in Table 5.1. RE of the "low MS HPS" was noticeably higher than conventionally reported values. According to Han & BeMiller (2005), typical hydroxypropylation for commercial food starch uses propylene oxide at up to 8.3% starch weight to produce MS up to 0.15, translating to 62% RE. In many cases, starch substitution was heterogenous and favored on amylose than amylopectin, because amylose is amorphous and prone to leaching from starch granule during reaction (Han & BeMiller, 2005; Luo & Shi, 2012). Therefore, higher MS and RE were anticipated for high-amylose starch. MS of HPS reached 1.20 by further hydroxypropylation with more PO. Viscosity increase was apparent as reaction proceeded, because HPS greatly swelled as MS increased. Therefore, more aqueous IPA was added to dilute the reaction. MS of hydroxypropylation achieved 1.68 after another dose of the PO addition; however, the RE of hydroxypropylation was significantly reduced. HPS prepared by the "continuous" method had similar MS values to "stepwise" method, suggesting that refreshing reaction medium is not necessary as long as the viscosity of reaction media was adjusted. Adding PO in one dose resulted a thick glutinous starch mass that caused difficulties in subsequent processes.

DS and RE of acetylation were reversely related to HP substitution (Table 5.2). HP substituent should not alter the amount of available hydroxyl reaction site because it carries an extra hydroxyl group which as well would esterify. Therefore, it is reasonable to attribute the decrease of acetylation efficiency to steric hindrance brought by bulkiness of HP substituent. Acetylation in aqueous medium resulted in much less DS than anhydrous reaction because starch material progressively precipitated and aggregated as DS increased thus prohibited contact of starch to acetic anhydride.

a

Anomeric H (1)   AGU H (2-6)   DMSO

b

CH$_3$COONa

HP CH$_3$ (9)   HP0.56

HP1.18

All other H   HP1.64

c

Acetate−CH$_3$ (10)

HP−CH$_3$ (9)

HP0.56Ac1.97

HP1.18Ac1.46

HP01.64Ac1.21

d

Acetate −CH$_3$ HP−CH$_3$ (9)

(10)   HP0.56Ac 0.86

All other H   HP1.18Ac 0.21

HP1.64Ac 0.09

6   5   4   3   2   1   0
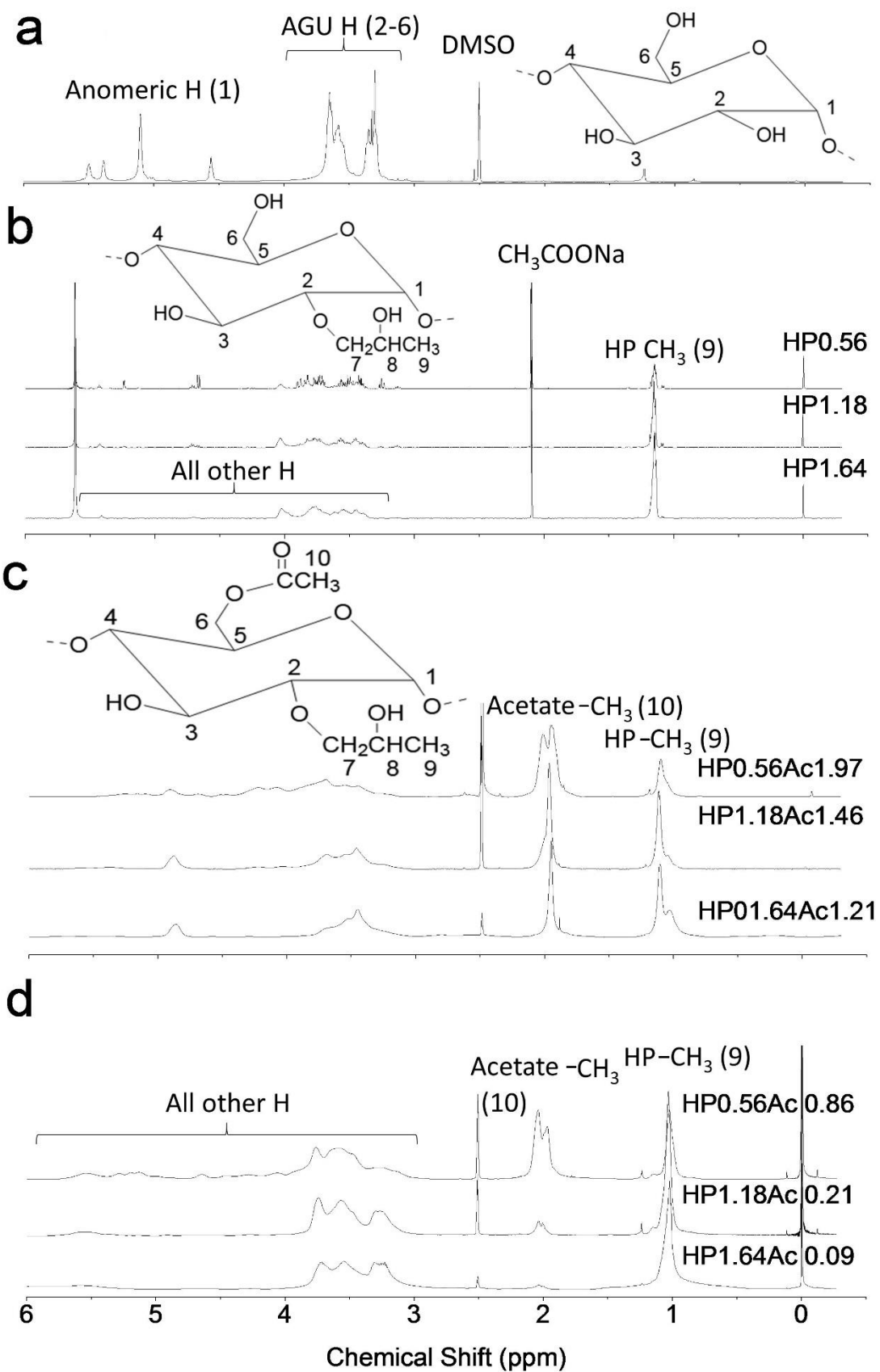
Chemical Shift (ppm)

Figure 5.2. Proton NMR spectra of native, hydroxypropylated (a), and hydroxypropylated acetylated (b & c) high-amylose starch; spectra were recorded in D$_2$O (b) and DMSO-d_6 (a, c, & d).

Table 5.1. Hydroxypropylated starch (HPS) synthesized by stepwise (isolating HPS at each molar substitution (MS) levels and reconstitute into reaction media) or continuous (adding more propylene oxide (PO) without refreshing the reaction media) procedure; %RE, reaction efficiency.

HPS samples	PO (Mole)	%HP	MS	Starting starch/HPS wt (g)	HPS yield (g)	%RE$_{PO}$
Low MS	1.21	17.2±0.4c	0.57±0.01c	300	322	78.1±1.6a
Mid MS (stepwise)	2.81	30.1±0.1b	1.20±0.02b	200	253	46.6±0.6b
High MS (stepwise)	3.77	38.1±0.9a	1.68±0.06a	100	136	23.4±0.5c
Mid MS (continuous)		29.6±0.4	1.16±0.02b			
High MS (continuous)		37.7±0.4	1.66±0.03a			
Mid MS (PO added in one-dose) *	Reaction viscosity too high (1600 Pa.s); did not proceed to characterization					
High MS (PO added in one-dose) *	Reaction viscosity too high (720 Pa.s); did not proceed to characterization					

*Viscosity determined by rheological analysis (10% strain; 1 Hz) using smooth parallel plates

(25 mm diameter; 2 mm gap) at 55 °C. Different letters indicate significant difference (n=2-4; $P<0.05$) between each starch type in each column.

Table 5.2. Hydroxypropylated acetylated starch (HPAcS) synthesized in anhydrous (acetic anhydride (AA) as reactive solvent) or aqueous (adding AA to HPS aqueous solution) acetylation media; %RE, reaction efficiency; MS, molar substitution; HPS, hydroxypropylated starch; DS, degree of substitution.

MS of HPS	AA (Mole)	%Acetyl	DS	Starting HPS wt (g)	HPAcS yield (g)	%RE$_{AA}$
Anhydrous acetylation						
0.56	1.36	29.9±0.5a	1.94±0.05a	50	70	35.8±0.6a
1.18	1.36	21.0±0.5b	1.43±0.04b	50	63	22.7±0.5b
1.64	1.36	17.0±0.3c	1.23±0.03c	50	58	16.9±0.3c
Aqueous acetylation						
0.56	0.09	16.2±0.4a	0.88±0.03a	10	10.4	45.2±1.2a

| 1.18 | 0.04 | 3.9±0.2b | 0.22±0.01b | 10 | 9.6 | 21.1±1.3b |
| 1.64 | 0.03 | 1.8±0.2c | 0.11±0.01b | 10 | 9.6 | 11.7±1.5c |

Different letters indicate significant difference (n=2-4; P<0.05) between each starch type in each column.

Solubility of HPS and HPAcS in water

As shown in Fig 5.3, starch solubility was greatly altered after modification. Unlike normal native starch that is typically insoluble in cold water and partially soluble once heated above gelatinization temperature (>70 °C), native high-amylose starch showed very low solubility (<5%) after heating even to 90 °C. This is consistent with many previous studies because gelatinization temperature of high-amylose starch was over 130 °C due to longer amylose chains. For HPS with a MS 0.56, cold water solubility was improved to over 30%, indicating a weakened granular structure of the starch imparted by HP modification; moreover, heating to 60 °C and 90 °C further increased the solubility to 48%. The solubility increase suggested HP substituent greatly lower temperature required for gelatinization and prevented amylose interchain association. Starch had 100% solubility at as MS increased to 1.18 and 1.64 with or without heating, indicating HP substituent completely prevented starch self-association. As reported by BeMiller (2009) & Fu et al. (2019), hydroxypropylation is the most effective modification to prevent starch retrogradation by interchain H-bond disruption. Hydroxypropylation at MS > 0.1 the starch can be up to 10% (10 g starch/100 mL water) soluble in cold water (Aminian et al., 2013). Solubility of HPAcS was negatively relative to acetylation DS. Acetylation of DS > 1.21 rendered starch virtually insoluble in water regardless of temperature. HP0.56Ac0.89, HP1.18Ac0.21, and HP1.64Ac0.09 samples showed 30-95% solubility although they were precipitated and recovered from aqueous synthesis medium. This disparity was possibly due to difference in solid contents (25% in synthesis vs 2% in solubility

test) and presence of sodium acetate (a salt byproduct that might reduce starch solubility). It was evident that maximum acetyl DS negatively related to HP content. One possible reason was because acetylation might have taken place on HP substituent and created more hydrophobic moieties.
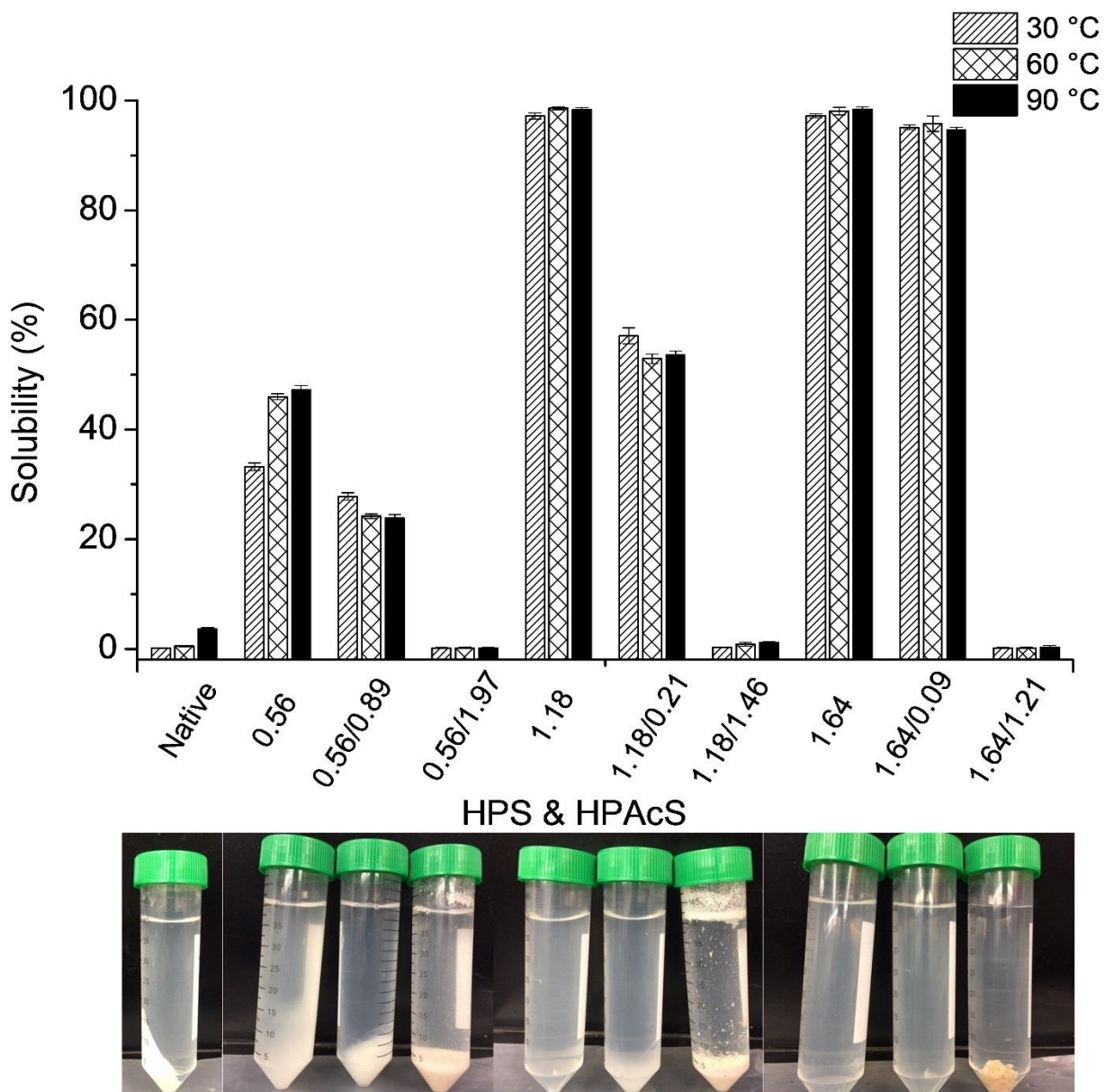

Figure 5.3. Solubility of 2% (starch wt. to volume) native, hydroxypropylated (HPS), and hydroxypropylated acetylated (HPAcS) high-amylose starches after heating in water at 30, 60, and 90 °C for one hour and cooled to 25 °C.

*Images were respective samples heated at 90 °C and centrifuged at $1700\times$ g for 20 min; first value in sample name was molar substitution of hydroxypropyl group; second value was degree of substitution of acetyl group.

Thermal properties of HPS and HPAcS

Molecular mobility of a dry unmodified starch is greatly constrained by hydrogen bond and as a result, (T_g) of native starch is about 224 °C. Because T_g of the native starch is close to its thermal decomposition temperature (approximately 300 °C), there was narrow margin for thermal processing (Xu et al., 2005). In practical starch bioplastic processing, i.e. extrusion, starch materials typically were heated to a temperature lower than 150 °C to avoid degradation; therefore, we scanned them to 140 °C to capture the T_g that would be practically meaningful (Shogren, 1996; Xu et al., 2005). As shown in Fig 5.4 & Table 5.3, glass transitions temperatures decreased with increasing hydroxypropylation MS. These changes can be explained as replacement of hydroxyl by HP prevented hydrogen bond formation and increased free volume thus allowed higher molecular mobility. Low DS (0.86, 0.21, and 0.09) acetyl substitution significantly reduced the T_g of their base HPS to various extents. It was noted high DS (1.97, 1.46, 1.21) acetyl substitution only brought about 10 °C further reduction on the T_g to their low acetyl DS counterparts. Probably because the intermolecular hydrogen bond formation was already inhibited in low DS HPAcS.

T_g is a critical property for starch bioplastics since they not only define processing temperatures but also determine useful temperature range for end applications. By varying substitution level of HP and acetyl, it is possible to obtain a series of thermoplastic starch of different hydrophobicity. For example, sample HP0.56Ac0.89 can be blended with a small amount of water and thermally processed, i.e. extrusion and hot press, to produce starch-based

plastics with moderate water resistance. Furthermore, sample HP0.56Ac0.89 was also

synthesized with high reaction efficiency which increased practicability for industrial-scale

production. In another example, sample HP1.18Ac1.46 had a dry T_g of 44.2 °C and was strictly

water insoluble. With addition of common plasticizers, i.e. triacetin, triglyceride, and propylene

glycol, etc., it is possible to create a gum base that soften at body temperature and useful as a
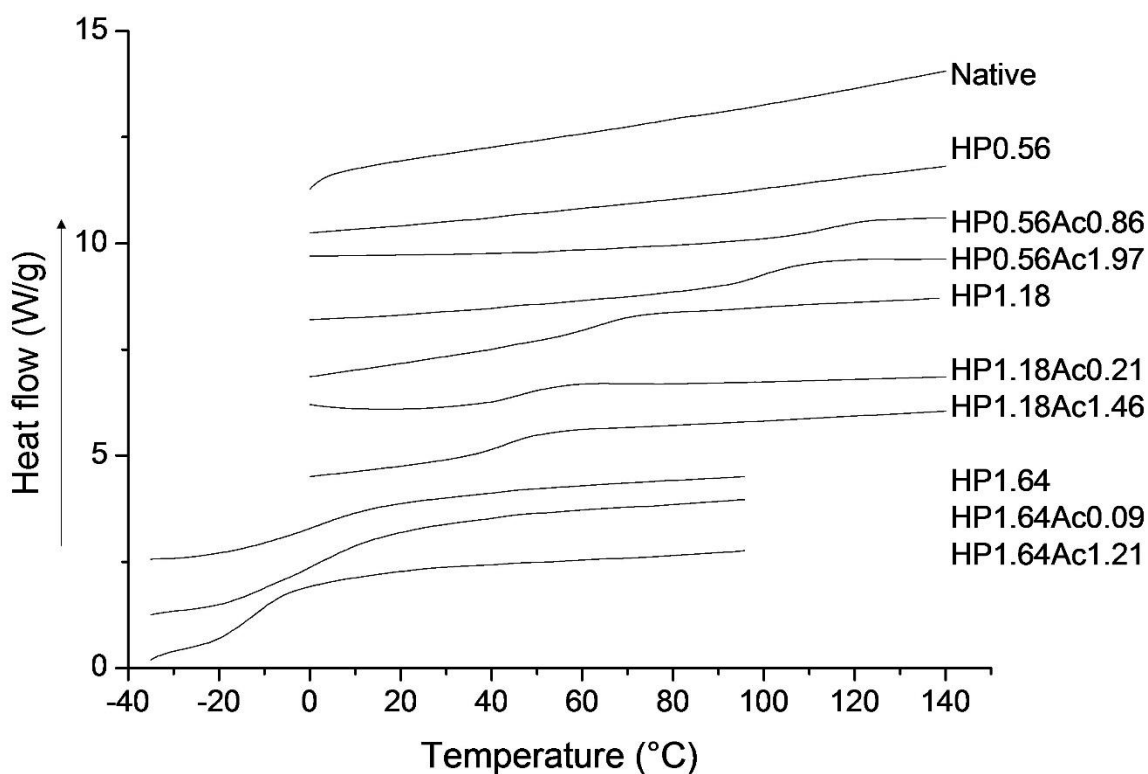
masticatory material.

Figure 5.4. Thermograms (second scan) of hydroxypropylated (HP) and hydroxypropylated acetylated (HPAc) high-amylose starch determined by differential scanning colorimetry; values in sample names indicate molar substitution of HP and degree of substitution of Ac.

Table 5.3. Glass transition (second scan) of native, hydroxypropylated (HP) and hydroxypropylated acetylated (HPAc) high-amylose starches determined by differential scanning colorimetry; values in sample names indicate molar substitution of HP and degree

of substitution of Ac; T_o, onset temperature; T_g, glass transition temperature T_e, end temperature; ΔCp, change in heat capacity; N/D, not detected.

	T_o (°C)	T_g(°C)	T_e(°C)	ΔCp (J/g·°C)
Native	N/D	N/D	N/D	N/D
HP0.56	N/D	N/D	N/D	N/D
HP1.18	56.9±0.6c	63.8±0.3c	70.7±0.5c	0.308±0.008c
HP1.64	-12.1±0.3e	1.8±0.1e	11.3±0.3e	0.512±0.014a
HP0.56Ac1.97	94.7±0.5b	98.9±0.4b	108.5±1.0b	0.282±0.006cd
HP1.18Ac1.46	38.0±0.1e	44.2±0.9e	49.3±0.6e	0.256±0.008d
HP1.64Ac1.21	-19.0±0.4g	-11.0±0.2g	-7.1±0.3g	0.304±0.006c
HP0.56Ac0.86	101.9±1.7a	110.4±1.2a	117.0±1.1a	0.310±0.006c
HP1.18Ac0.21	42.3±1.3d	47.0±0.4d	55.1±0.3d	0.282±0.006cd
HP1.64Ac0.09	-14.0±0.2f	-0.6±0.4f	13.0±0.2f	0.468±0.007b

Different letters indicate significant difference (n=2; $P<0.05$) between each starch type in each column.

Capillary viscometry and molecular size of HPS and HPAcS

Intrinsic viscosity measures volume occupied by individual polymer molecule and it is a widely accepted criterion for molecular size. Conventionally, hot water (>60 °C) or potassium hydroxide (1N) were used to make diluted starch solution for intrinsic viscosity determination (Heitmann & Mersmann, 1995; Leach, 1963). However, considering great likelihood of retrogradation and instability of ester bond in alkali, straight DMSO was used as solvent as it was reported to effectively dissolve all kinds of starches (Schmitz et al., 2009). As shown in Table 5.4, the intrinsic viscosity of native high-amylose starch was about 80.4 mL/g, whereas the value for modified starches ranged from 22.8 to 100.6 mL/g. Evidently, the intrinsic viscosity of high-amylose starch increased significantly when hydroxypropylation reached MS 0.56 and decreased as hydroxypropylation progressed, suggesting some degradation of starch polymers have occurred. The depolymerization of starch was likely due to beta-elimination (also known as

Peeling reaction) after prolonged residence in high alkaline conditions (BeMiller, 2019).

Acetylation in aqueous media further decreased starch intrinsic viscosity, which was possibly

due to localized high acidity when acetic anhydride was added. Starch acetylated in anhydrous

acetic anhydride at high temperature resulted in the lowest intrinsic viscosity, which was

reasonable because glyosidic bonds are vulnerable when heated in acidic environment. Gel

permeation chromatography was used to reveal change of hydrodynamic volume of starch

materials. The distribution of hydrodynamic volume (Fig 5.5) was converted to molecular weight

distribution that was summarized in Table 5.4, assuming molecular conformation of HPS and

HPAcS in DMSO was similar to dextrin standards. GPC analysis suggested starch occurrence

was present, and primarily in amylopectin fraction. The amylopectin fraction was reduced after

in-aqueous acetylation and disappeared after anhydrous acetylation (Fig 5.5). Polydispersity was

reduced after modification in general, suggesting a narrower distribution of molecular weight. It

was noted the very low molecular weight fraction was also reduced in the modified starch

samples, this fraction loss might due to dialysis process.

Table 5.4. Intrinsic viscosity, number (Mn) and weight (Mw) molecular weight, and polydispersity of hydroxypropylated (HP) and hydroxypropylated acetylated (HPAc) high-amylose starch determined by capillary viscometry and gel permeation chromatography; values in sample names indicate molar substitution of HP and degree of substitution of Ac.

Starch	Intrinsic viscosity (mL/g)	Mn ($\times 10^3$)	Mw ($\times 10^3$)	PD
Native	80.4±1.2b	47±0b	1223±28a	26.4±0.6a
HP0.56	100.6±2.9a	55±1b	1354±27d	24.6±0.8d
HP1.18	70.7±0.8c	54±2b	889±32b	16.9±0.6b
HP1.64	41.5±0.5e	45±1.8b	773±32c	16.8±0.7b
HP0.56Ac1.97	31.1±0.7f	57±12b	262±5e	4.7±1.1f
HP1.18Ac1.46	34.9±0.4f	59±5ab	251±0e	4.3±0.4f
HP1.64Ac1.21	22.8±0.5g	78±6a	235±6e	3.0±0.3f

HP0.56Ac0.89	78.0±1.6b	53±1.8b	687±17d	9.5±0.3e
HP1.18Ac0.21	59.4±0.8d	63±2.6ab	887±37b	13.7±0.6cd
HP1.64Ac0.09	32.2±0.5f	49±1.1b	754±17c	15.5±0.3bc

Different letters indicate significant difference (n=2; P<0.05) between each starch type in each
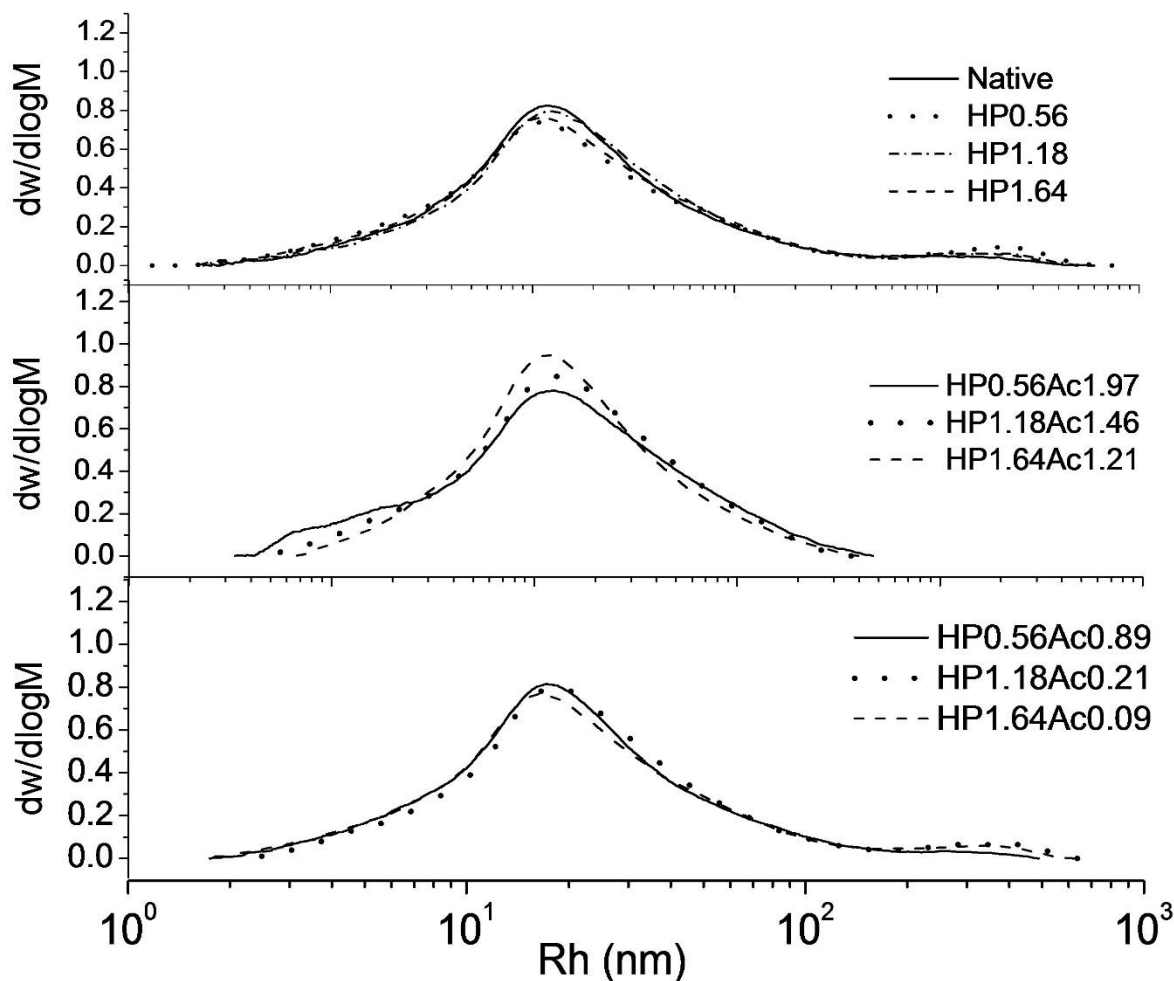
column.

Figure 5.5. Distribution of hydrodynamic volume of hydroxypropylated (HP) and hydroxypropylated acetylated (HPAc) high-amylose starch determined by gel permeation chromatography; values in sample names indicate molar substitution of HP and degree of substitution of Ac.

 Textural and rheological analysis of HPAcS chewing gum base

To explore potential uses of HPAcS, three highly hydrophobic HPAcS were selected to

formulate to chewing gum bases. Choice of plasticizers was based on a patented starch-based

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chewing gum formulation (Liu, 2010). As Fig 5.6 shows, the HPAcS formed off-white, opaque, and homogenous soft gums resembled chewing gum. Starch gums were stretchy and able to elongate determined by a texture analyzer. All gum samples did not break at maximum elongation distance (150 mm; 750% elongation); however, peak force during stretching was different and in the order of HP0.56Ac1.97 > HP1.18Ac1.46 > HP1.64Ac1.21. In starch-based gums, HPAcS and plasticizer amount were uniform for all three gums and therefore, peak force was affected by other intrinsic joint factors such as glass transition temperature of HPAcS and severity of depolymerization of starch backbone. As severity of depolymerization was not significantly different for all three HPAcS (Table 5.4), glass transition temperature probably was the most influential factor for the peak force of the gums.
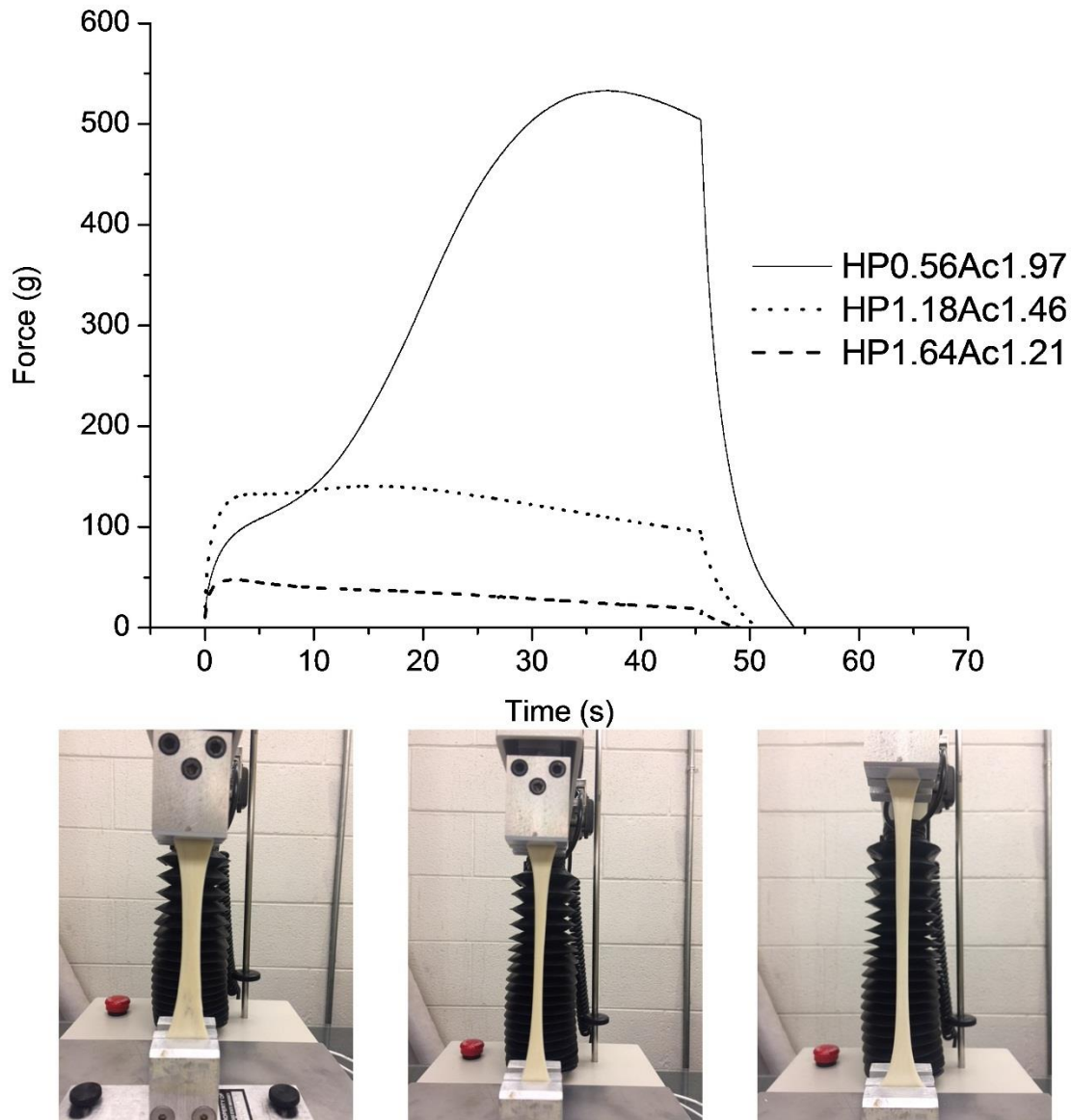
Figure 5.6. Elongation test of hydroxypropyl acetylated starch (HPAcS) based chewing gum base by texture analyzer; the gum curd was stretched from 20 mm to 150 mm at 3.3 mm/s; three images were HP0.56Ac1.97, HP1.18Ac1.46, and HP1.64Ac1.21, from left to right, respectively; values in sample names indicate molar substitution of HP and degree of substitution of acetyl

Oscillation stress sweep was carried out to characterize three gum bases to identify their

linear viscoelastic region (LVR) (Fig 5.7a). A typical LVR, where storage modulus (G')

remained independent of stress up to certain value and decrease as stress further elevated, was

observed for HP0.56Ac1.97 gum at 3 kPa. This critical value indicated the point where gel

structure began to fail, leading to reduction of stiffness. HP1.18Ac1.46 and HP1.64Ac1.21 based gums exhibited no observed critical stress within the stress range tested, indicating their structure was less prone to breaking under stress deformation. Initial G' was reversely related to MS of HP, suggesting higher MS resulted in softer gum. It was also noted G' was less than loss modulus (G'') over the range of stress applied, meaning the starch gums had more liquid-like component. They may behave like a thick paste and flow slowly due to gravitational force. Frequency sweep showed starch gums were sensitive to frequency change as the G' and G'' increased steadily, together with constant G''> G' (Fig. 5.7b), it is concluded that the starch gums were "true paste" rather than "gel". G' of starch gum base was highly dependent on temperature as shown in Fig 5.7c. Upon heating to 20 to 70 °C, G' undergone about 10-fold decline in every gum base. Dramatic stiffness decrease is very important in chewing gum processing, because compounding and blending the gum base with other ingredient require a thin mixture and the only practical way to "thin" the gum base is by heating (Fritz, 2006).
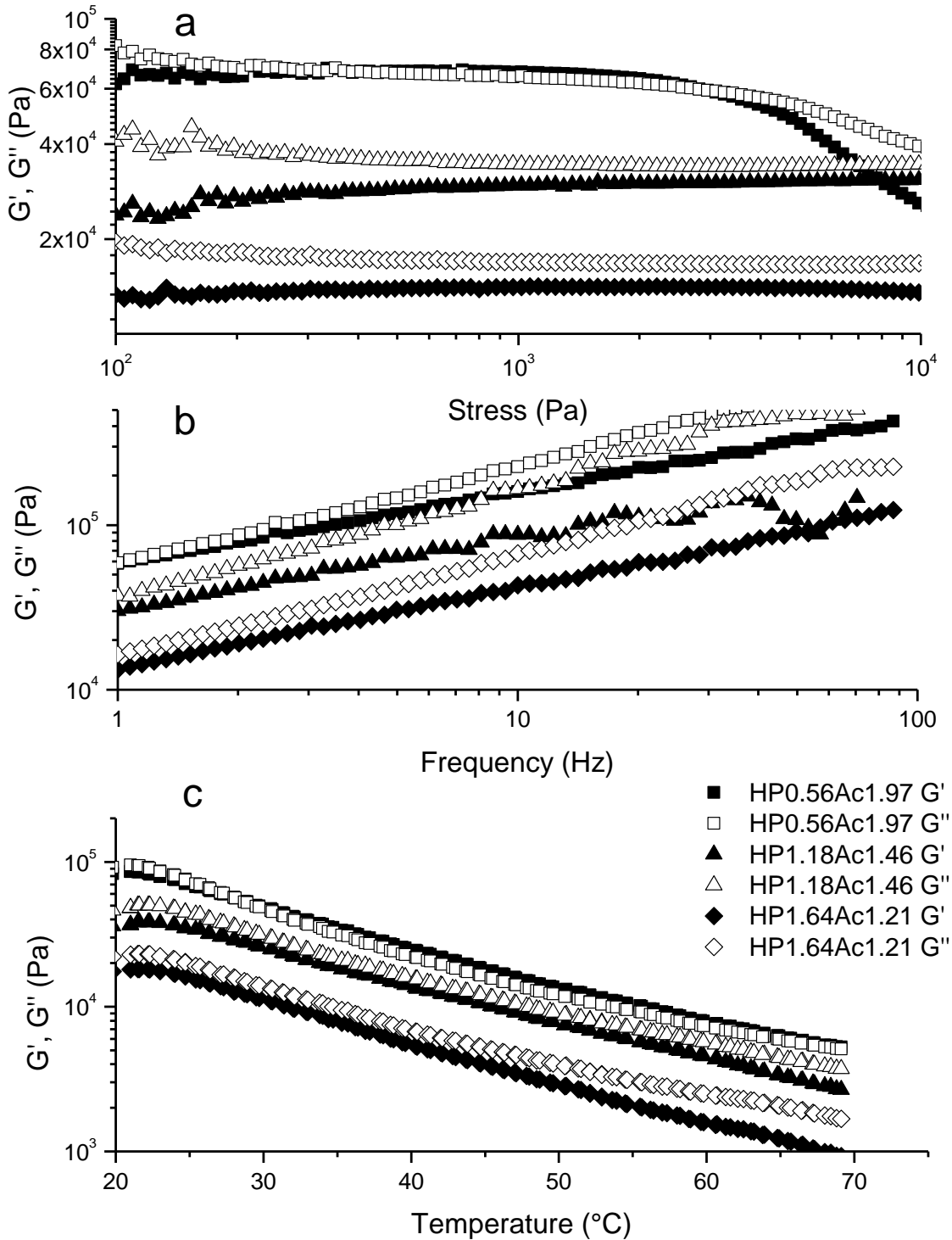
Figure 5.7. Rheological analysis of hydrophobic hydroxypropylated acetylated (HPAc) high-amylose starch formulated chewing gum base; values in sample names indicate molar substitution of HP and degree of substitution of Ac; formula contain: HPAc starch (4.5 g), triacetin (3.5 g), hydrogenated soybean oil (0.5 g), and acetylated monoglyceride (0.5 g)

Conclusions

High-amylose maize starch was hydroxypropylated to MS 0.56, 1.18, and 1.64 in aqueous isopropanol medium and further acetylated to high and low DS. Hydroxypropylation and acetylation synergistically lower glass transition temperature of the HPS. By varying MS and DS, it was possible to prepare thermoplastic starch of different hydrophobicity. Plasticized HPAcS samples exhibited unique rheological properties resembled chewing gum bases. Those gums behaved like thick pastes, softened greatly upon heating and exhibited excellent elongation. Stiffness of the starch gums was dependent on glass transition temperature, which was ultimately determined by levels of hydroxypropylation. Future works may focus on more economy approaches in synthesizing and recovering HPS and HPAcS.

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Chapter 6 - Overall conclusions

Maize starches of different amylopectin contents were successfully acetylated in aqueous slurry to achieve intermediate DS. High-amylose maize starch appeared to achieve the highest DS (1.7), comparing to waxy and normal maize starches when same amount of acetic anhydride was used. Acetylation took place primarily on hydroxyl group linked to carbon number 2 regardless of amylose content of base starch. The acetylated starches were further melted and reacted in OSA in absence of catalyst and organic solvent. In representative OSA modified starch acetate samples, reaction efficiency of OSA was around 18%, which translated to DS about 1.2 in the mixed starch ester. The OS substituent imparted thermoplasticity to starch materials by lowering its T_g to around 56 °C. Approaches to improve reaction efficiency of OSA such as pregelatinization of starch acetate, incorporating an inert diluent, and acid thinning of base starch, were tested. Incorporating triacetin as a diluent in the reactive melt effectively improved (doubled) reaction efficiency of OSA without harming DS of the products. The OSA modified starch acetate were further plasticized to mimic chewing gum base. Rheology analysis shows similarity of high-amylose starch-based chewing gum to commercial chewing gum in terms of stiffness and softening upon heating.

Highly water-soluble and thermoplastic hydroxypropylated starch (MS 0.56-1.64) were prepared by reacting propylene oxide and high-amylose maize starch. Glass transition temperature of the starch ethers were greatly lowered by high degree of etherification and was further synergistically lowered by acetylation. Depending on the extent of acetylation, solubility of starch ethers changed drastically. It was very applicable to produce thermoplastic starch of different water solubility through manipulating extent of etherification and acetylation. Water insoluble starch ether esters were formulated to chewing gum bases. The starch-based chewing

gum bases demonstrated excellent stretch on texture profile analyzer and their stiffness was in

range as chewing gum base.

Chapter 7 - Appendix list

Appendix A. DS determination using ^{1}H-NMR

The starch acetates and pre-gelatinized starch acetates (15 mg) were dried at 80 °C in vacuum oven for 2 h, boiled in 1 mL deuterium oxide for 10 min, freeze-dried, and dissolved in 1 mL dimethyl sulfoxide-d_6 (DMSO-d_6) to obtain a clear solution. The solution was analyzed by a Varian 400 NMR system (Varian Inc., Palo Alto, CA) at 25 °C. Sixteen scans were acquired for each sample and the relaxation time was 1.0 s. The DMSO signal was calibrated to 2.5 ppm (Gottlieb, Kotlyar, & Nudelman, 1997). DS of each starch acetate was calculated by the following equation (Chi et al., 2008).

$$DS = 4A/(3B + A)$$

where A is the methyl protons at 2.01-2.08 ppm; B is the sum of OH and H-1 protons for anhydroglucose unit moiety observed at higher than 4.5 ppm.
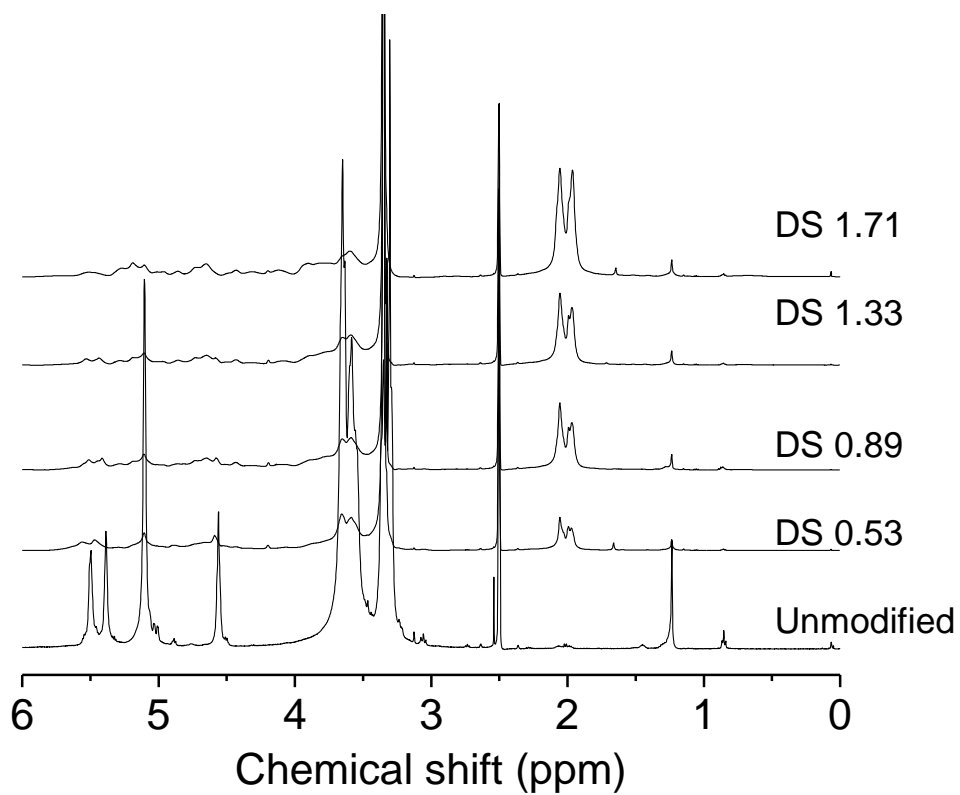
Figure A. 1. ^{1}H NMR spectra of acetylated high-amylose maize starches; DS, degree of substitution.

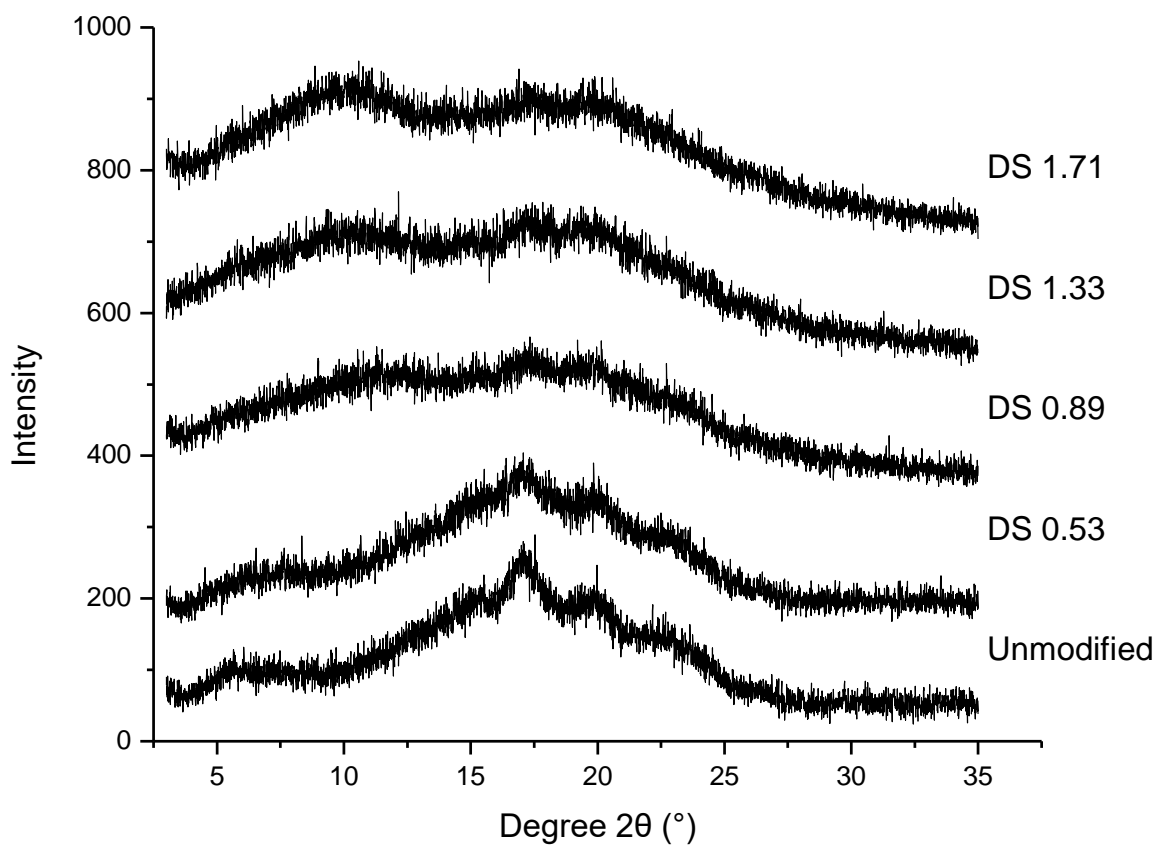**Appendix B. X-ray diffraction**

Figure B. 1. X-ray diffraction patterns unmodified and acetylated high-amylose maize starches; DS, degree of substitution.
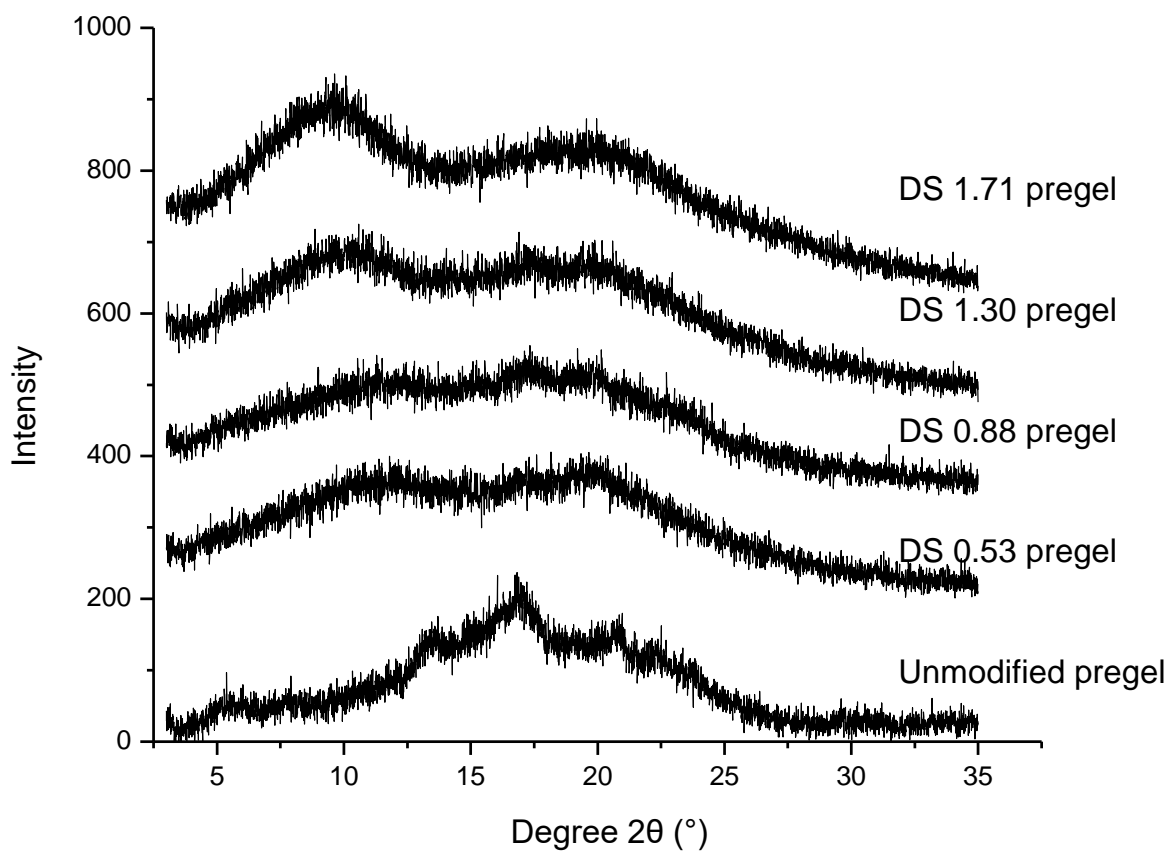
Figure B. 2. X-ray diffraction patterns of unmodified and acetylated pre-gelatinized (pregel) high-amylose maize starches; DS, degree of substitution;

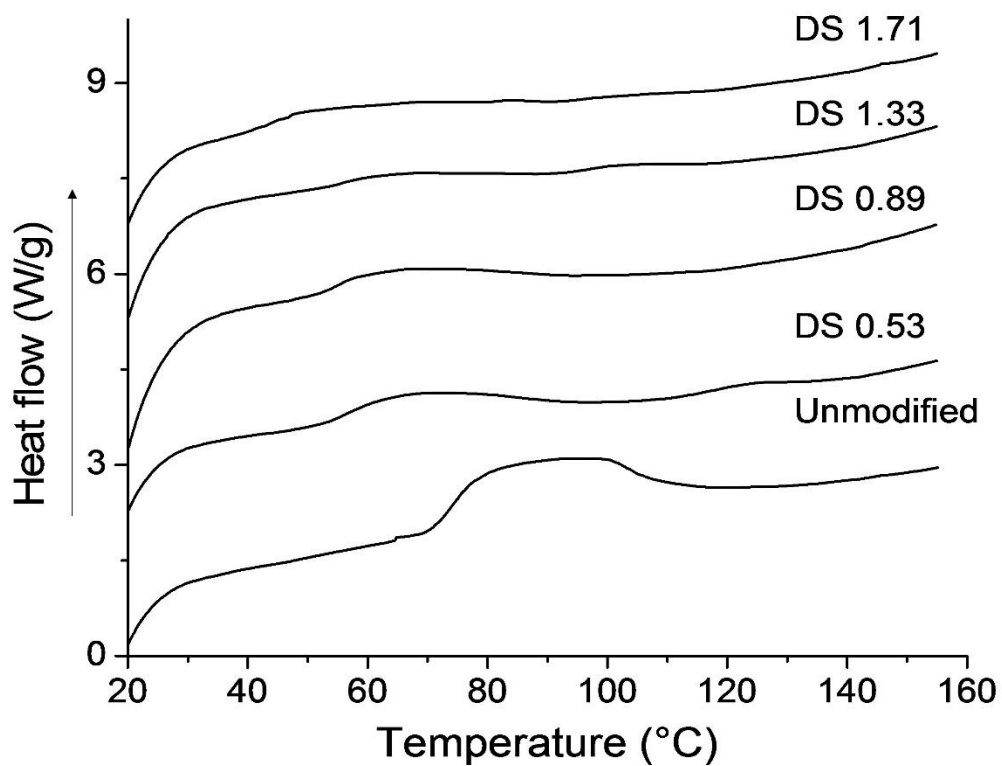# Appendix C. Differential scanning calorimetry analysis

Figure C. 1. Differential scanning calorimetry thermogram of unmodified and acetylated high-amylose starch in water (25% solid); DS, degree of substitution.
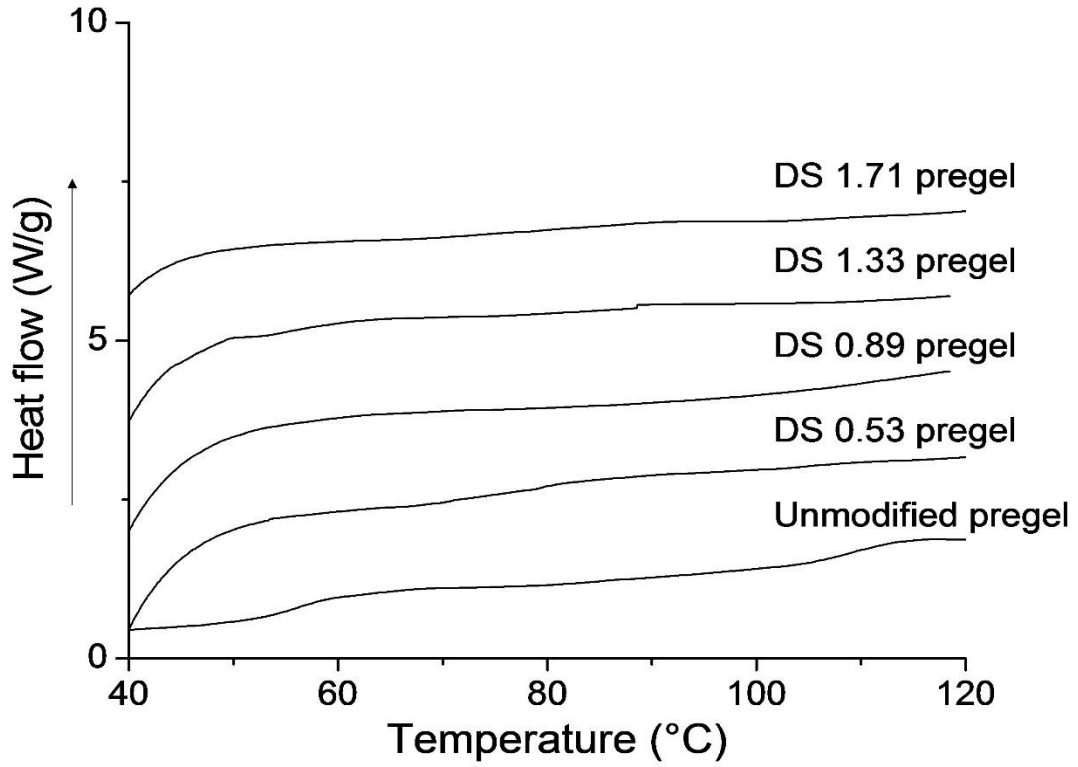
Figure C. 2. Differential scanning calorimetry thermogram of unmodified and acetylated pre-gelatinized (pregel) high-amylose maize starches in water (25% solid); DS, degree of substitution.
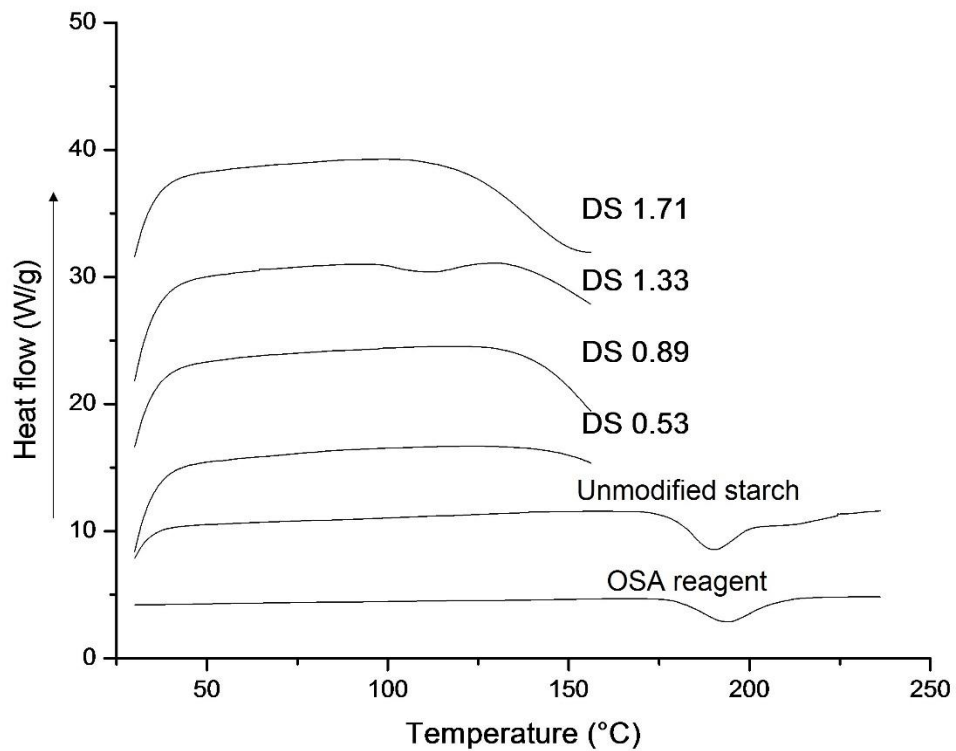
Figure C. 3. Thermograms of unmodified and acetylated high-amylose starch heated in 400% octenylsuccinic anhydride (OSA) by a differential scanning calorimeter; DS, degree of substitution

Appendix D. High performance liquid chromatography (HPLC) analysis of octenylsuccinic starch acetate

Contents of total OS and unbound OS in OS-Ac-starches were determined by a HPLC procedure adapted from Qiu, Bai, & Shi (2012) The method was modified accordingly to suit the high level of OS. An Agilent 1100 HPLC system (Agilent, Waldbronn, Germany) consisted of a G1311A pump, an auto-sampler with 10 µL injection loop, a G1315A diode array detector (DAD) and a Phenomenex Kinetex C18 column (2.6 µm, 100×4.6 mm; Torrance, CA) were used to perform the analysis. Mobile phase was a mixture of acetonitrile and water (50:50, v/v), acidified by 0.1% trifluoroacetic acid (TFA). Flow rate was 1 mL/min. Column temperature was kept at 25 °C.

Standard curve

OSA reagent (1.0000 g) was weighed into a 100-mL volumetric flask acetonitrile was added to the mark to obtain OSA solution. To each 25 mL volumetric flask, 1, 2, or 3 mL of OSA solution was pipetted. Acetonitrile (11.5, 10.5, or 9.5 mL) was added into the volumetric flask respectively and the flask was filled to mark with water. The mixtures were stirred overnight to convert OSA to OS acid and were referred to OS acid stocks. OS acid stocks (1, 2, 3, 4, or 5 mL) was added into 25 mL volumetric flasks which were then filled to mark with acetonitrile and water (50:50, v/v) to produce working standards. The final OS acid amount in the working standards (per 10 µL injection) were 0.16 to 2.4 µg. In addition, 0.16 µg standard was further diluted five times to obtain a 0.032 µg standard to cover the very low end of the curve.

Unbound OS content

OS-Ac-starch (Ca. 50 mg, dry weight) was weighted into 10 mL DMSO and stirred overnight to complete dissolution. An aliquot of the solution (0.2 mL) was diluted to 1.5 mL (final dilution

factor is 75×) with acetonitrile water (50:50, v/v) and filtered through a 0.45-micron syringe filter before injected into the HPLC. The %OS$_{unbound}$ was calculated from the equation below.

$$\%OS_{unbound} = \frac{75 \times W_{unbound}}{W} \times 100\%$$

where W$_{unbound}$ was the weight of unbound OS in µg; W was the dry weight of OS-Ac-starch.

Total OS content and DS of starch gum

OS-Ac-starch (Ca. 50 mg) was weighted into a 50-mL conical tube containing 4 N sodium hydroxide (5 mL) and stirred overnight. HCl (21 mL, 1 N) was added to neutralize the excessive alkali. One aliquot (1 mL) of the mixture was diluted 10 times using acetonitrile and water (50:50, v/v) before injection (final dilution factor was 260×). The %OS$_{total}$ was calculated using the formula below.

$$\%OS_{total} = \frac{260 \times W_{total}}{W} \times 100\%$$

where W$_{total}$ was the weight of total OS in µg; W was actual weight of starch gum in µg.

The DS of octenyl succinate in the starch gum was determined from the equation below.

$$DS = \frac{\left(162 + (43 \times AcDS)\right) \times \%OS_{bound}}{210 - 209 \times \%OS_{bound}}$$

where the AcDS the DS of acetylation of base starch; %OS$_{bound}$ the difference between %OS$_{total}$ and %OS$_{unbound}$.
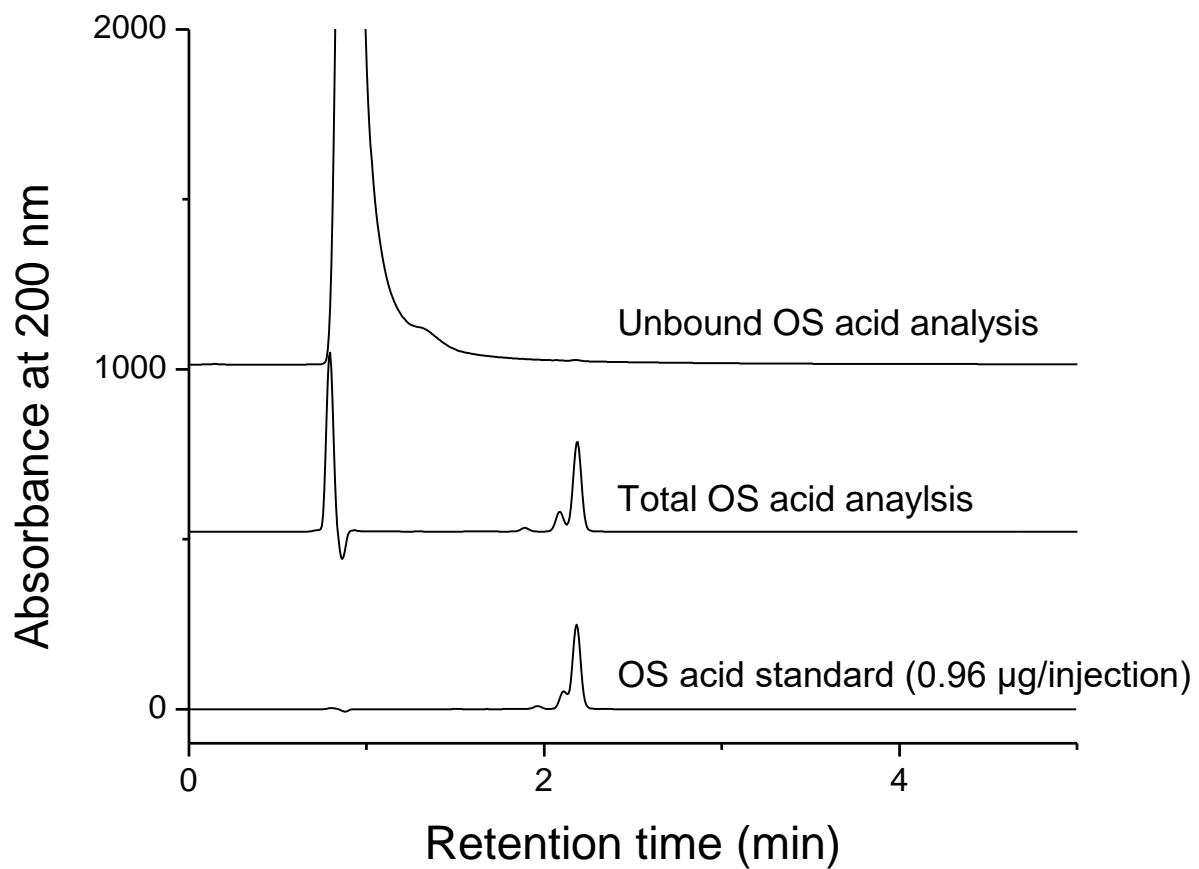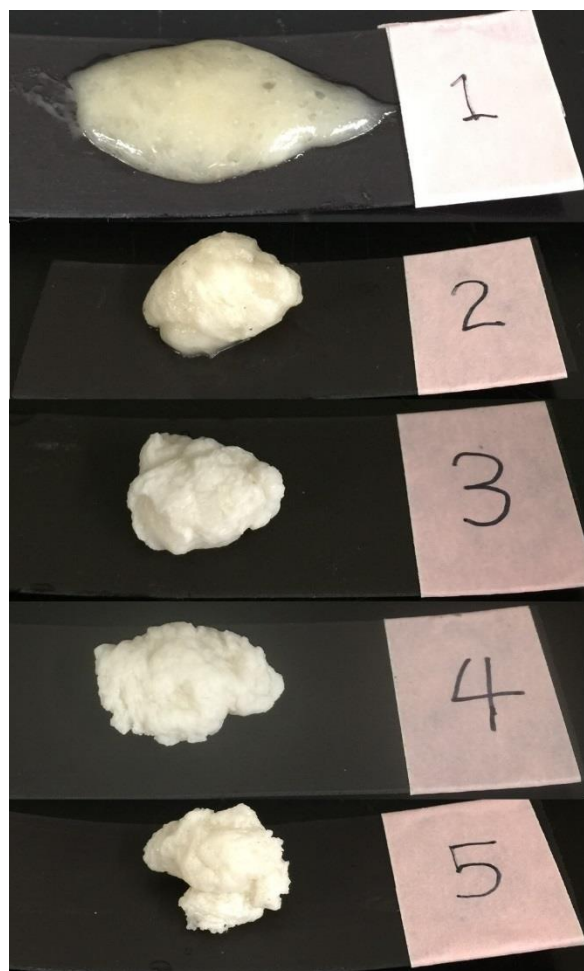
Figure D. 1. HPLC chromatograms of hydrolyzed octenylsuccinylated acetylated starch (1.33 acetyl 1.19 octenylsuccinyl).

Appendix E. Images of octenylsuccinylated acetylated starch (1.33 acetyl, 1.19 octenylsuccinyl)

* unpurified (1), after ethanol wash cycles (2-4) and soaked in water overnight (5)

Appendix F. DSC thermograms of starch acetate and octenylsuccinyl acetate at different moisture contents
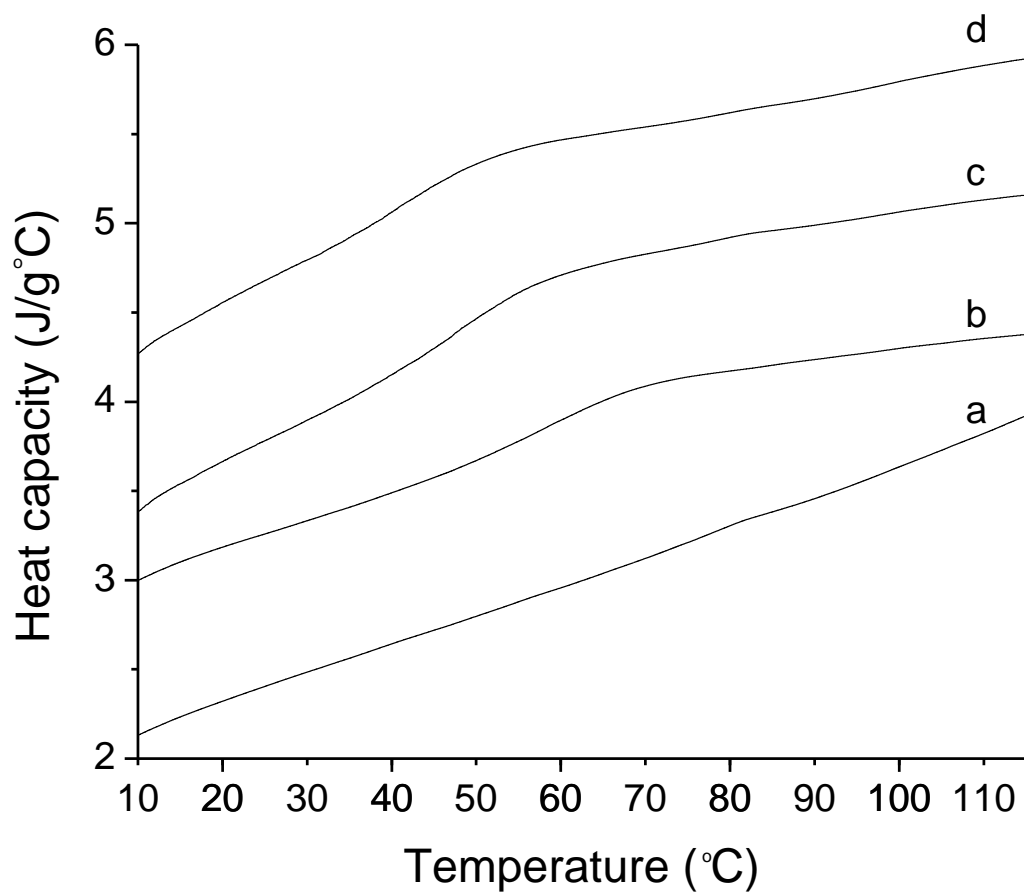
*Thermograms of starch acetate (DS 1.33), dry (a) and octenylsuccinylated acetylated starch (DS 1.33 for acetyl and DS 1.2 for octenylsuccinate groups), dry (b), 5.8% moisture (c), and 8.7% moisture (d); DS, degree of substitution.

Appendix G. Solubility and swelling power (SP) of AS and OS-Ac-starch in various diluents

Starches (pre-dried in a vacuum oven at 80 °C for 2 h, 100 mg) and different diluents (1 g) were into pre-weighed centrifuge vials. The mixtures were vortexed for 10 min, heated at 120 °C for 1 h and cooled to 25 °C, and centrifuged at 1000 $\times$ g for 15 min. Supernatant was removed to pre-weighed glass vials by pipetting. Swelling power was obtained by dividing weight of mixture with weight of AS. Solubility was determined by vacuum drying supernatant at 120 °C. In another set of experiment, different diluents were mixed with OSA at diluent to OSA 1:4, and 1:1 (w:w), with ethanol at 1:1 (w:w) to observe miscibility. All diluents showed no phase separation with OSA and ethanol; however, exhibited different SP and solubility to starch esters.

Diluents	Boiling point (°C)	Acetylated starch		OS-Ac-starch	
		SP	Solubility %	SP	Solubility %
Acetylacetone	140.0	3.34	1.23	7.32	5.73
Ethyl acetoacetate	180.8	3.61	1.21	7.16	6.10
Ethylene glycol diacetate	186	3.82	2.19	7.70	5.94
Propylene glycol diacetate	190	4.00	1.48	8.12	7.42
Diethyl malonate	199	4.23	1.47	8.00	7.58
Triacetin	259	4.60	7.27	7.75	9.52

Figure G. 1. Swelling power (SP) and solubility of acetylated high-amylose maize starch (DS 1.3) and octenylsuccinylated (DS 1.3) acetylated (DS 1.3) high-amylose maize starch (OS-Ac-starch) in various diluents.

Appendix reference

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