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Influence of reaction conditions on the location of reactions in waxy maize starch granules reacted with a propylene oxide analog at low substitution levels

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Abstract

A constant amount of an anionic propylene oxide analog was reacted with waxy maize starch under conditions in which temperature, pH, and swelling-inhibiting salt type and concentration were varied. Reaction sites were located utilizing a reflectance confocal laser scanning microscopy (R-CLSM) method. Results confirm that uniformity of reaction within granules and molar substitution (MS) levels are a function of reaction conditions. In most cases, increasing pH from 10.7 to 11.7 increased the extent of reaction. Changes in temperature between 44 and 54 °C had little effect. Reactions carried out in the presence of NaCl produced more derivatization than did reactions carried out in the presence of Na_2SO_4 under identical conditions. More reaction was observed in the presence of a lower concentration (0.395 m) of Na_2SO_4 as compared to a higher concentration (0.527 m). Salt concentration effects were not discernible when reactions were done in the presence of NaCl (0.471 and 0.629 m).

Keywords: Hydroxypropylstarch; Starch; Hydroxypropylation; Waxy maize

1. Introduction

Development of improved modified starch products is likely limited to the use of currently allowed reagents and conditions. Patterns of reaction in granules are important since one avenue available for the development of novel products in granular form is control of the location of reactions within granules (BeMiller, 1997). Assuming that reaction efficiency is a function of granule swelling, it is theoretically possible to control the location of reactions by changing granule swelling conditions. Verification requires reliable methods to locate reactions in granules. Two such methods are now available (Gray & BeMiller, 2004; Huber & BeMiller, 2001).

The objective of this research was to determine the effect, if any, of modification conditions related to granule swelling on location of derivatization in waxy maize starch granules reacted with an anionic propylene oxide analog at a low substitution level using one of these methods (Gray & BeMiller, 2004). Waxy maize starch was chosen for these experiments for two reasons: (1) to avoid loss of starch polymer molecules as much as possible (the primary material that leaches from hydroxypropylated starch granules during reaction and/or work-up appears to be amylose (Shi & BeMiller, 2002)) and (2) to minimize the background (waxy maize starch contains less protein, which forms silver salts, the basis of the method for locating reactions sites to be used, than does normal corn starch (Han & Hamaker, 2002)). All reaction-related variables in derivatization reactions, viz. pH, temperature, type of swelling-inhibiting salt, and concentration of swelling-inhibiting salt, potentially impact granule swelling, so all were selected for study. Representative industrial reaction conditions for hydroxypropylation were chosen as the midpoints; higher and lower temperatures, pH values, and salt concentrations were utilized to provide insight as to the effect of each on the efficiency of reaction.

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1.1. Impact of swelling on starch granule reactivity

Hauber, BeMiller, and Fannon (1992) concluded that, in order for reactions with granular starch to take place, granule swelling must first occur. That granule swelling has an effect on granule reactivity is supported by the finding that about 1.8 times more propylene oxide was needed to achieve the same MS when Na_2SO_4 was replaced by potassium citrate (a more effective swelling inhibitor) (Shi & BeMiller, 2000). Also, solutions of fluorescent amides of C_6, C_8, and C_{10} fatty acyl molecules were less able to penetrate common corn, waxy maize, and potato starch granules in the presence of sodium citrate than in the presence of Na_2SO_4 (Gray & BeMiller, 2001).

The importance of granule swelling to reactions with starch can be explained using base-catalyzed derivatization as an example. Formation of nucleophilic alkoxide ions is crucial for reactions to occur and is associated with dissipation of hydrogen bonds that hold the granule structure together. Thus, starch polymers, while becoming more reactive, become more flexible and amorphous regions become more hydrated and more swollen (Jenkins & Donald, 1997). As regions become more derivatized, they swell even more, eventually pulling apart crystallites.

1.2. Factors affecting starch granule swelling under derivatization conditions

Factors affecting starch swelling include temperature, starch:H_2O ratio, pH, presence or absence of a swelling-inhibiting or swelling-promoting salt, and the concentration of the salt. In addition, derivatization disrupts hydrogen bonding permanently, mostly by a steric effect, and makes further granule swelling more facile.

Because reaction of ethylene oxide with alkaline solutions is slower inside starch granules than in the extragranular phase, van Warners, Stamhuis, and Beenackers (1994) recommended that hydroxyethylation of starch be conducted at as high a starch/water ratio as possible. The reaction rate of starch with ethylene oxide increases with increasing moisture content of the starch because the rate of absorption of ethylene oxide increases exponentially with the moisture content of starch granules (van Warners, Lammers, Stamhuis, & Beenackers, 1990).

In theory, an increase in media pH (by the addition of NaOH, for example) causes swelling of starch granules via ionization of starch hydroxyl groups, which disrupts hydrogen bonding of chains and thereby effectively makes the starch molecules more flexible. Another explanation for the effect of base addition on granule swelling is that alkoxide ions repel one another, opening the spaces between starch chains (BeMiller & Pratt, 1981). Starch buffers changes in pH (Oosten, 1990) and can support pH levels up to at least pH 11 with little apparent structural change (BeMiller & Pratt, 1981; Hauber et al., 1992), even though the buffering effect means that starch hydroxyl groups are being converted into alkoxyl groups.

The impact of salts on gelatinization of granules is not completely clear. Swelling-inhibiting salts, particularly Na_2SO_4 and NaCl, are important to starch modification reactions because they prevent starch molecule leaching and inhibit gelatinization (Oosten, 1990; Pratt, 1975). Salts affect starch gelatinization according to their position in the Hofmeister (lyotropic) series. For example, Na_2SO_4, high on the lyotropic series, increases the gelatinization temperature (T_{gel}) of starch granules, while salts on the low end (NaI, for example) decrease the T_{gel} (some cause gelatinization at room temperature) (Sandstedt, Kempf, & Abbott, 1960). Salts in the middle of the series, such as NaCl, increase the onset temperature of gelatinization at low concentration (<1.5 M), but at concentrations >1.5 M lower the onset temperature (Evans & Haisman, 1982; Jane, 1993; Lii & Lee, 1993; Sandstedt et al., 1960; Wootton & Bamunuarachchi, 1980).

It appears that the impact of salts on starch gelatinization is controlled by two effects: (a) altering the structure of water (For example, ions with high charge density have been postulated to stabilize both the molecular structure of water and starch granules, while ions of low charge density both break water structure and destabilize granule structure (Jane, 1993)) and (b) affecting electrostatic interactions between starch and salt ions. (The starch granule is a weak-acid ion exchanger (Oosten, 1990), and thus attracts cations into the granule, and repels anions. Cations stabilize the granule to gelatinization, whereas anions disrupt hydrogen bonds (Oosten, 1982, 1983).) It has been postulated that salts not only change the T_{gel} of granules, but also the mechanism of gelatinization (Sandstedt et al., 1960).

The complexity of competing effects of salts (anion vs. cation) has been attributed to ion charge densities and overall salt concentrations (Jane, 1993), as well as to pH and temperature (Oosten, 1982, 1983). When sodium hydroxide is added to starch solutions containing NaCl or Na_2SO_4, gelatinization temperature increases. Perhaps this is due to the influx of sodium ions into the granule, which results in a higher Donnan potential that excludes anions from the granule (Oosten, 1979, 1982, 1983). However, above a critical pH value, anions are able to enter the granule and effect gelatinization. Heat, which effects a lowering of the Donnan potential, allows faster anion entry and thus accelerates the gelatinization process (Oosten, 1982, 1983).

1.3. Hydroxypropylation of starch

Reactions of starch with propylene oxide are generally conducted at 38–52 °C (100–125 °F) in the presence of sodium hydroxide. Swelling-inhibiting salts are added to prevent pasting. Reaction takes about 24 h to complete, and reaction efficiency is generally about 60% (Tuschoff, 1986).

2. Experimental

2.1. Starch and reagent sources

Waxy maize starch was a gift from Tate and Lyle North America (Decatur, IL). Sodium 3-chloro-2-hydroxy-1-propanesulfonate (propylene oxide analog, POA), sodium sulfate decahydrate, and silver nitrate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Propylene oxide was purchased from EM Science (Gibbstown, NJ). Sodium chloride was obtained from VWR International (West Chester, PA).

2.2. Derivatization of starch with propylene oxide analog

Waxy maize starch was modified with a constant amount of POA reagent (10% based on dry weight of starch) with varying temperature (44, 49, or 54 °C), pH (10.7, 11.2, or 11.7), salt type (Na_2SO_4 or NaCl), and salt concentration ('low' = 0.395 m Na_2SO_4 or 0.471 m NaCl or 'high' = 0.527 m Na_2SO_4 or 0.629 m NaCl). Samples are identified by the sequence *XXYZ*, where *XX* = temperature of reaction: 44 (44 °C), 49 (49 °C), or 54 (54 °C); *Y* = type of swelling-inhibiting salt used: S (Na_2SO_4) or N (NaCl); and *Z* = pH of reaction and concentration of swelling-inhibiting salt used: 1 (pH = 10.7; salt concentration = 'low'); 2 (pH = 10.7; salt concentration = 'high'); 3 (pH = 11.2; salt concentration = 'low'); 4 (pH = 11.2; salt concentration = 'high'); 5 (pH = 11.7; salt concentration = 'low'); 6 (pH = 11.7; salt concentration = 'high') (Table 1). Before addition of starch, POA was mixed with a constant amount of water (60 mL dd H_2O) in a 125-mL glass-stoppered Erlenmeyer flask at the appropriate reaction temperature and adjusted to the appropriate pH by concomitant addition of base (2 M NaOH) with a Radiometer Copenhagen (Copenhagen, Denmark) ABU80 autotitrator coupled with a PHM82 standard pH meter with an alkaline-resistant electrode (Red Rod pH 0-14, Radiometer Analytical S.A., Villeurbanne Cedex, France) until the pH stabilized (<30 min). The reaction vessel was removed from the water bath, and the appropriate amount of swelling-inhibiting salt was added. Starch (36%, w/v, db; based on initial water, 36 g/60 mL) was then added quickly with constant stirring. The reaction mixture was adjusted again to proper pH, and any salt necessary to maintain proper concentration in the reaction mixture was added. The reaction vessel was glass stoppered, and placed on a submersible magnetic plate stirrer in a heated water bath set at the appropriate temperature. After 24 h, the starch slurry was neutralized with dilute HCl, and the starch was isolated using a Büchner funnel. The starch cake was washed with ~50 mL of 70% (v/v) ethanol and air-dried. The cake was returned to the reaction vessel, which was filled with ~75 mL of 85% (v/v) ethanol, and the slurry was stirred magnetically for 24 h at room temperature. The starch was again recovered using a Büchner funnel and rinsed with ~50 mL 85% ethanol, followed by 100% ethanol, and allowed to dry on the filter disk. Control starches were produced in the same way, with the exception that no reagent was added.

Table 1
Reaction conditions for waxy maize starch granules modified with POA

Sample	Temperature (°C)	pH	Na_2SO_4 (m)	NaCl (m)
44S1	44	10.7	0.395	–
44S2	44	10.7	0.527	–
44S3	44	11.2	0.395	–
44S4	44	11.2	0.527	–
44S5	44	11.7	0.395	–
44S6	44	11.7	0.527	–
49S1	49	10.7	0.395	–
49S2	49	10.7	0.527	–
49S3	49	11.2	0.395	–
49S4	49	11.2	0.527	–
49S5	49	11.7	0.395	–
49S6	49	11.7	0.527	–
54S1	54	10.7	0.395	–
54S2	54	10.7	0.527	–
54S3	54	11.2	0.395	–
54S4	54	11.2	0.527	–
54S5	54	11.7	0.395	–
54S6	54	11.7	0.527	–
44N1	44	10.7	–	0.471
44N2	44	10.7	–	0.629
44N3	44	11.2	–	0.471
44N4	44	11.2	–	0.629
44N5	44	11.7	–	0.471
44N6	44	11.7	–	0.629
49N1	49	10.7	–	0.471
49N2	49	10.7	–	0.629
49N3	49	11.2	–	0.471
49N4	49	11.2	–	0.629
49N5	49	11.7	–	0.471
49N6	49	11.7	–	0.629
54N1	54	10.7	–	0.471
54N2	54	10.7	–	0.629
54N3	54	11.2	–	0.471
54N4	54	11.2	–	0.629
54N5	54	11.7	–	0.471
54N6	54	11.7	–	0.629

2.3. Reaction of waxy maize starch granules with propylene oxide

Waxy maize starch granules were reacted with propylene oxide in the following manner. First, the appropriate amount of salt was weighed out and dissolved in 60 mL of dd water with stirring. Starch (36 g, db) was added, and the slurry was stirred for ~5 min. The pH was adjusted to the desired value with 2 M NaOH with continued stirring. The reaction vessel was sealed tightly with a septum, and 1.28 mL of propylene oxide was added via a syringe. The flask was placed in water bath set at the appropriate temperature, and samples were stirred on a submergible magnetic stir plate for 24 h. The reaction mixtures were neutralized and washed in the same manner as described in Section 2.2.

2.4. Conversion of starch derivatives to silver salts

Exchange of anionic functional groups with $AgNO_3$ was done as previously described (Gray & BeMiller, 2004). Hydroxypropyl analog starch derivatives (0.6 g) were gently shaken in 35 mL of 0.25 M $AgNO_3$ for 24 h. The suspensions were centrifuged 10 min at 3000 rpm, and the supernatant was decanted. The exchange procedure was repeated twice more. Silver-exchanged starch pellets were washed with ~65 °C, 85% (v/v) aqueous ethanol for 30 min to remove excess $AgNO_3$. The pellet was resuspended in 85% (v/v) ethanol, washed twice more, and then recovered by vacuum filtration. Samples were protected from light throughout the entire procedure and storage with aluminum foil.

2.5. Reflectance confocal laser scanning microscopy (R-CLSM)

Starch granules were prepared for R-CLSM analysis (Gray & BeMiller, 2004) by dusting a small amount of Ag^+-exchanged starch granules on a paraffin wax-coated microscope slide. After lightly tapping the slide to remove excess starch granules (in order not to have fields with large clumps of granules), slides were passed through a flame to melt the wax and affix granules. Slides were then exposed to UV radiation for >15 h to fully reduce silver ions to silver atoms; 2–3 drops of 20% (w/v) aqueous sucrose solution were applied to the slides immediately before viewing.

Digital sections of starch granules were obtained using a Bio-Rad (Hercules, CA) MRC-1024 confocal laser scanning microscope coupled to a Nikon (Melville, NY) Diaphot 300 inverted microscope with a 40× objective lens (N.A. = 1.4). Light reflected from samples was collected by use of a blue reflectance filter. Any light reflected off silver atoms or silver atom clusters or refracted due to changes in refractive index was detected. A krypton–argon laser source (488 nm) operating at 10% capacity was used. Background reflected light was minimized by utilizing a $\lambda/4$-wave plate (45° polarizer). Once optimized, microscope settings were kept constant when viewing samples, except for the digital zoom level, unless otherwise mentioned. Images presented were collected using the Kalman imaging technique provided by the software, which averaged a variable number of 'slow' scans. Digital images were processed using LaserSharp Processing (BioRad), Confocal Assistant (v. 4.02, Todd Clark Brelje), ThumbsPlus (v. 4.10-S, Cerious Software, Inc., Charlotte, NC), and Adobe Photoshop v6.0 (Adobe Systems, Inc., San Jose, CA) software packages. Scale bars on images are 50 μm in length.

2.6. Determination of molar substitution (MS) values of propylene oxide analog-derivatized waxy maize starch granules

Starch granules that were reacted with POA were subjected to sulfur analysis (duplicate, Galbraith Laboratories, Inc., Knoxville, TN). MS was calculated as moles of sulfur per mole of glucosyl unit on a dry weight basis. Although analysis of a blank (carried through the process using Na_2SO_4 as the swelling-inhibiting salt but without POA) showed no residual sulfur, only those samples prepared in the presence of NaCl were analyzed.

2.7. MS determination of hydroxypropylated waxy maize starch granules

Starch granules that were reacted with PO (~35 mg) were placed in a 15-mL polypropylene centrifuge tube and dispersed in 5 mL of D_2O. The slurry was heated in a boiling water bath for >5 min, then cooled to room temperature. α-Amylase (EC 3.2.1.1, Sigma Chemical Co., St Louis, MO) (1.0 unit) was added. The samples were incubated for at least 48 h at room temperature. The digest was subsequently freeze dried and dissolved in 1 mL D_2O. Proton NMR of the samples was acquired with an INOVA 300-MHz NMR spectrometer. Integrated peaks were used for calculations using the equation $MS = (a/3)/((b-a)/7))$, where (a) = hydroxypropyl methyl group at 1.2 ppm and (b) = ring protons between 4.3 and 3.0 ppm.

3. Results and discussion

The strategy of this project was to modify starch granules with POA at various reaction conditions and determine how patterns of reaction were affected. Optimal reaction conditions for hydroxypropyl starch are 49 °C (120 °F), pH 11.2, 0.527 m Na_2SO_4 (17% $Na_2SO_4 \cdot 10\ H_2O$, 7.5% Na_2SO_4), 24 h (Villwock, 1996). In order to alter swelling conditions without adversely affecting starch granule integrity, the following conditions were used: pH = 10.7, 11.2, or 11.7; temperature = 44, 49, or 54 °C; salt type = Na_2SO_4 or NaCl; salt concentration = 'low' or 'high' (see Section 2). (Equimolal salt levels with respect to cation were used.) Starch granules were reacted using a constant H_2O:starch ratio and with a constant amount of sodium 3-chloro-2-hydroxy-1-propanesulfonate. The reagent concentration used was lower than that used in preparation of most products for food use so that subtle differences could be detected by R-CLSM and so that granule integrity would be maintained at highest swelling conditions. Molal concentrations were used because it was found that making up reaction mixtures by weight is most accurate (Villwock, 1996). Reaction mixtures were adjusted to constant pH because starch neutralizes alkaline solutions (Oosten, 1990), and addition of the same amount of base does not always give the same pH value.

3.1. Sample preparation

The anionic analog of propylene oxide (POA) used is this work is thought to react with starch in a manner similar to that of propylene oxide itself (Gray & BeMiller, 2004; Huber & BeMiller, 2001). POA could react with starch under alkaline conditions in two possible ways: (a) a direct nucleophilic displacement of a chloride ion from the reagent by an alkoxide ion of a starch molecule, or (b) the epoxide (i.e. the true PO mimic) could form via an intramolecular displacement of a chloride ion with the release of one mole of HCl per mole of epoxide derivative formed. Thus, without maintaining the alkalinity, reaction efficiency would decrease due to a decrease in the number of alkoxide ions present. Reaction pH dropped in alkaline conditions without starch present at the same rate as with starch present, indicating that the cause of the pH drop was not an abnormally fast reaction with starch. Once pH stabilized, little change was noticed over the remaining 24-h reaction. Therefore, the likely cause of pH change was base-catalyzed epoxide formation. Since adjusting each sample with NaOH until pH was stabilized was unfeasible (>2 h was required per sample), and because the stirring power of the submersible magnetic stir plate was not fast enough to prevent samples from gelatinizing upon base addition, the following reaction procedure was employed. Reagent was added to water, and pH was adjusted (while heated, which reduced significantly the time for pH stabilization) before any starch was added. Thus, each flask could be submerged while adjusting the pH. In this manner, stabilization of pH was achieved in 15–30 min. Since stirring conditions could not accommodate starch samples during pH adjustment, flasks were removed, and appropriate amounts of salt were added (keeping in mind addition of water associated with starch and sodium sulfate decahydrate), followed by starch addition (added quickly to minimize damage to granules (Tuschhoff, 1986)). Addition of starch resulted in a decrease in pH, so the pH was readjusted. Salt concentration was readjusted taking into account added base (this affected pH insignificantly). Samples were then stirred 24 h at the appropriate temperature in the water bath.

After isolating neutralized modified starch samples, they were suspended in 85% ethanol solution for 24 h. The purpose of this treatment was to remove any protein or phospholipid that might bind Ag ions and be detected from both the control and reacted samples, a treatment found to be effective in removal of anionic compounds in preliminary work (Gray & BeMiller, 2004). Furthermore, any reacted proteins were expected to be more soluble after reaction than before; thus, any contribution of proteins to observed reaction patterns can be considered conservative at best. Also, sulfur analysis of samples prepared in the presence of sodium sulfate proved that all sulfate ions, which would have bound silver ions, had been removed by washing.

3.2. Effect of altering swelling conditions on location of reaction

In the beginning, it was not known if changes in reaction patterns due to changes in reaction conditions would be too subtle to be detected by the R-CLSM method. To determine if changes could be detected, three dissimilar samples were viewed per salt type: 44S2 and 44N2 ($T=44$ °C, pH = 10.7, Na_2SO_4 conc. = 0.527 m/NaCl conc. = 0.629 m, respectively), 49S4 and 49N4 ($T=49$ °C, pH = 11.2, Na_2SO_4 conc. = 0.527 m/NaCl conc. = 0.629 m), 54S5 and 54N5 ($T=54$ °C, pH = 11.7, Na_2SO_4 conc. = 0.395 m/NaCl conc. = 0.471 m). Predicted swelling conditions during reaction of samples were as follows: 44S2/44N2 < 49S4/49N4 < 54S5/54N5.

Each sample differed in terms of intensity and location of Ag-reflection signals (Fig. 1). Thus, different reaction conditions produced observable different reaction patterns. In terms of amount of reflected light signal, samples appeared to follow the predicted extents of reaction as related to degrees of swelling, i.e. 44S2 < 49S4 < 54S5. Upon closer inspection, it appeared that 49S4 granules (Fig. 1B) (prepared under common industrial conditions) were mostly reacted around granule centers. Some granules had Ag present throughout the entire granules, but such granules were in the minority. In some cases, regions of unreactivity were noticed near the periphery of granules, a phenomonon described previously and possibly a result of highly associated amylopectin molecules (Gray & BeMiller,

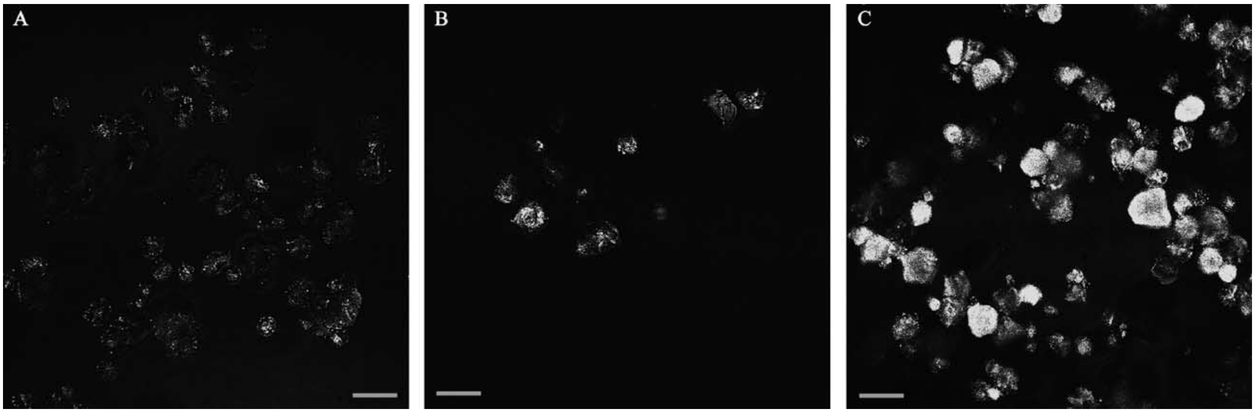

Fig. 1. R-CLSM images of waxy starch granules reacted with POA at varying swelling conditions in the presence of Na_2SO_4: (A) 44S2, (B) 49S4, (C) 54S5.

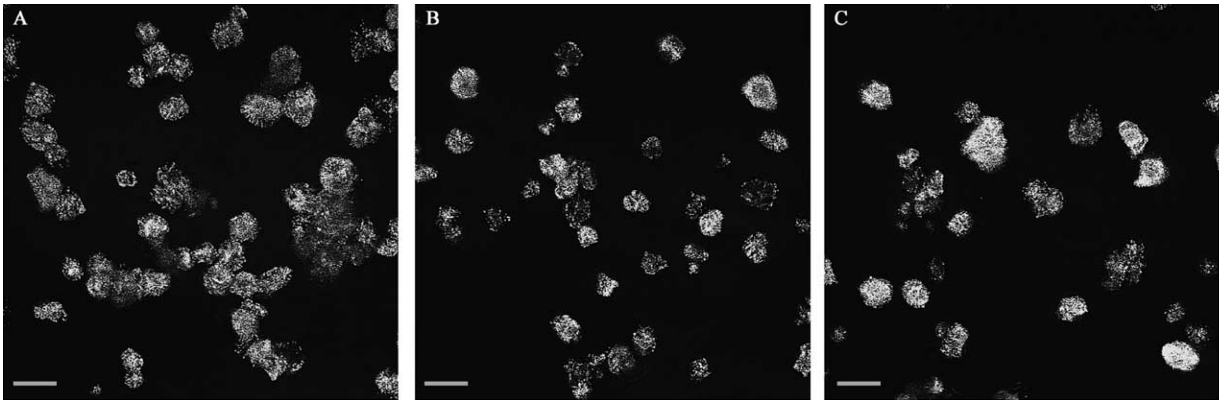

Fig. 2. R-CLSM images of waxy maize starch granules reacted with POA at varying swelling conditions in the presence of NaCl: (A) 44N2, (B) 49N4, (C) 54N5.

2004; Huber & BeMiller, 2001). 44S2 (Fig. 1A) granules appeared even less reacted. On the other hand, 54S5 (Fig. 1C) granules were highly reacted, usually in all parts of the granule. Silver appeared to be uniformly distributed throughout some granules.

Granule reaction patterns for samples reacted in the presence of NaCl can be seen in Fig. 2. They appeared to also differ depending on swelling conditions. As swelling conditions improved, granules appeared to possess more uniform reaction patterns as evidenced by a more even distribution of Ag through granules (i.e. less granule structural features were noticed) and reflections appeared to be more intense. Ag appeared to be the most uniformly distributed in 54N5 (Fig. 2C). Differences in effects of Na_2SO_4 and NaCl as swelling inhibiting salts are described below, but 44N2 (Fig. 2A) appeared to contain more Ag than 44S2 (Fig. 1A).

We questioned the observed apparent reaction patterns of 49S4 and 49N4, since they appear to be low in terms of extent of reaction, even though they were reacted under conditions that normally are applied industrially, i.e. conditions that presumably have been optimized for reaction efficiency. As mentioned above, reagent concentration used was lower than that used to make a typical product for food use. For example,

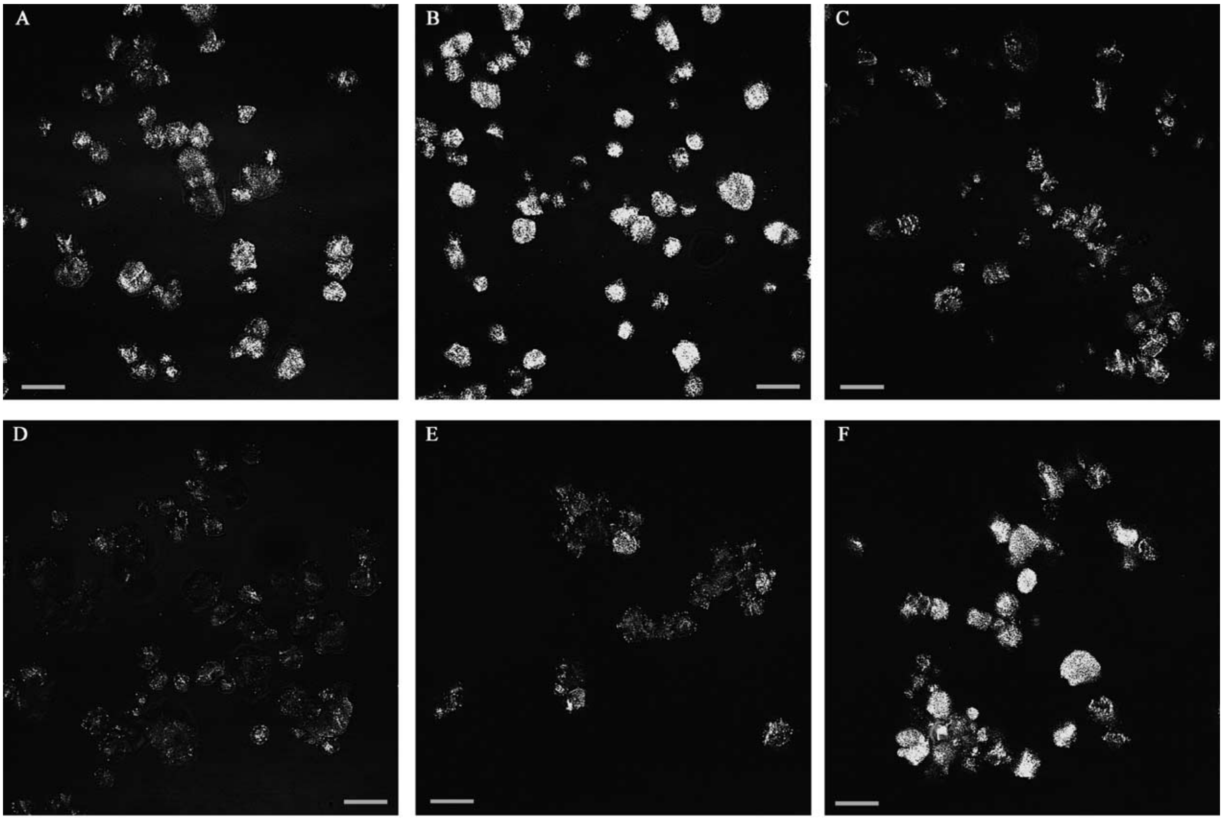

Fig. 3. R-CLSM images of waxy maize starch granules reacted with POA at 44 °C and varying pH levels in the presence of Na_2SO_4: (A) 44S1, (B) 44S3, (C) 44S5, (D) 44S2, (E) 44S4, (F) 44S6.

Tuschhoff (1986) reports most hydroxypropylated starch products are reacted with 5–10% propylene oxide (0.03–0.06 mol/mol starch; 0.08–0.16 MS, assuming 60% reaction efficiency) based on starch (db). In this study, starches were reacted with 10% of POA based on starch (db). The molecular weight of propylene oxide is only about 70% that of POA. Thus, assuming a 60% reaction efficiency (Tuschhoff, 1986), the expected molar substitution levels of starches reacted with POA would be about 70% of that produced by the same weight of PO (0.05 MS with respect to POA, 0.04 MS when considering just the propylene oxide contribution).

Gray and BeMiller (2004) and Huber and BeMiller (2001) found significantly different reaction patterns of granules reacted with different POA concentrations. Once reagent concentration was raised to a point where extensive reactions occurred in amorphous levels, granules appeared to 'open up' and react in previously unreactable regions. While the relatively low concentration of POA made detection of products difficult in some cases, using this reagent level ensured that reaction patterns in granules reacted under high swelling conditions were able to be distinguished from one another while maintaining granule integrity at those levels. Further, microscope conditions were optimized such that saturation of the highest reacted products was avoided as much as possible. Nonetheless, even at low reagent levels, relatively uniformly reacted products can be seen in some cases, such as 54S5 and 54N5.

Results from these experiments indicated that location and extent of reaction is a function of granule swelling conditions. Increasing pH and temperature likely enhanced swelling of starch granules and therefore allowed for heightened reactions. The relationship between patterns of reactions and granule swelling conditions was further investigated by differences in reaction patterns of all hydroxypropyl-analog samples prepared.

3.3. Effect of pH on location of reaction

Reaction pH (pH 10.7, 11.2, and 11.7) was varied to alter swelling conditions in order to determine if reaction patterns were related to pH. Thus, modified starch samples were prepared at constant reaction temperatures, salt concentration, and salt type and compared. Differences between the series due to other conditions are described in the appropriate sections.

In samples reacted at 44 °C in the presence of Na_2SO_4, increasing pH appeared generally to increase uniformity of reaction. Fig. 3A–C shows differences in granules reacted at increasing pH levels in the presence of 0.395 m Na_2SO_4; Fig. 3D–F shows differences in granules reacted in the presence of 0.527 m Na_2SO_4. As pH increased from 10.7 to

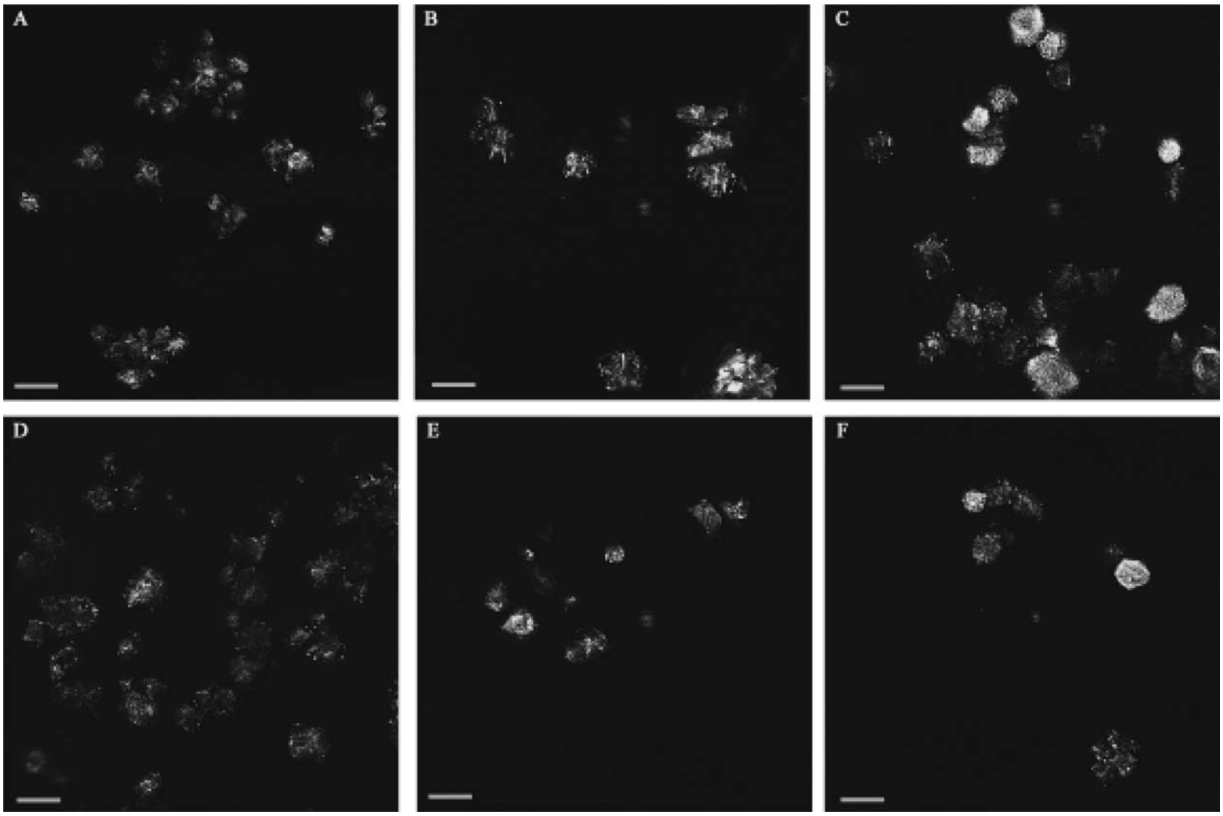

Fig. 4. R-CLSM images of waxy maize starch granules reacted with POA at 49 °C and varying pH levels in the presence of Na_2SO_4: (A) 49S1, (B) 49S3, (C) 49S5, (D) 49S2, (E) 49S4, (F) 49S6.

11.2, granules appeared to be more thoroughly reacted and homogenous (Fig. 3A and B). At the highest pH (11.7, Fig. 4C), reaction appeared to drop off, which was unexpected (see discussion below). In Fig. 3D–F, it can be seen that distribution of Ag in granules increased steadily with increasing pH. Granules reacted at pH 10.7 and 11.2 (Fig. 3D and E, respectively) appeared only slightly reacted, while at pH 11.7 (Fig. 3F), Ag appeared to be distributed throughout granules.

At 49 °C, in the presence of the low and high Na_2SO_4 concentrations, uniformity of derivatization again appeared to be a function of pH (Fig. 4A–F). In both cases (low and high salt), uniformity of reaction was heightened at high pH, with pH 11.7 (Fig. 4C and F) producing granules reacted to a high degree. However, apparent heterogeneity of reaction was noticeable, especially at high pH levels, where highly reacted granules could be found next to granules with less intense reflection, but this observation could be an artifact due to granules being in and out of the focal plane.

At the highest reaction temperature (54 °C), granules that were reacted in the presence of the low concentration of Na_2SO_4 again appeared to be influenced by increasing pH (Fig. 5A–C). At low pH, it appeared that only slight reaction occurred. However, increasing pH from 10.7 to 11.2 led to bright reflection patterns (Fig. 5B). Further, at pH 11.7 (Fig. 5C), Ag appeared to be thoroughly distributed in granules, producing a bright reflective signal. As with granules reacted at 49 °C, granule to granule differences were noticed in reactions done at pH 11.2 and 11.7. At high Na_2SO_4 concentration (Fig. 5D–F), granules reacted at pH 10.7 were bright, perhaps as much or more than were the granules of 54S3. As pH increased, the extent of reaction appeared to decrease. Observations from the high salt concentration were unexpected and are discussed below.

As pH was increased from 10.7 to 11.2 under the low concentration of NaCl at 44 °C (Fig. 6A and B), the extent of reaction appeared to increase. However, upon further increase of pH to 11.7 (Fig. 6C), extent of reaction appeared to fall. Although patterns were different, this observed trend was similar to that found for granules reacted at 44 °C in the presence of the lowest concentration of Na_2SO_4. Granules reacted in the presence of a high concentration of NaCl appeared to become more extensively reacted as pH increased (Fig. 6D–F), as evidenced by less noticeable structural features in samples reacted at higher pH compared with granules reacted at pH 10.7 in this series. Although an apparent increase in intensity of reflections was noticed as pH increased from pH 10.7 to 11.7, differences in intensities were subtle.

No clear influence of reaction pH was found when granules were reacted in the presence of the low concentration of NaCl at 49 °C (Fig. 7A–C). Granules reacted at pH 10.7 appeared to be the most extensively reacted, with fairly bright reflection signals observed.

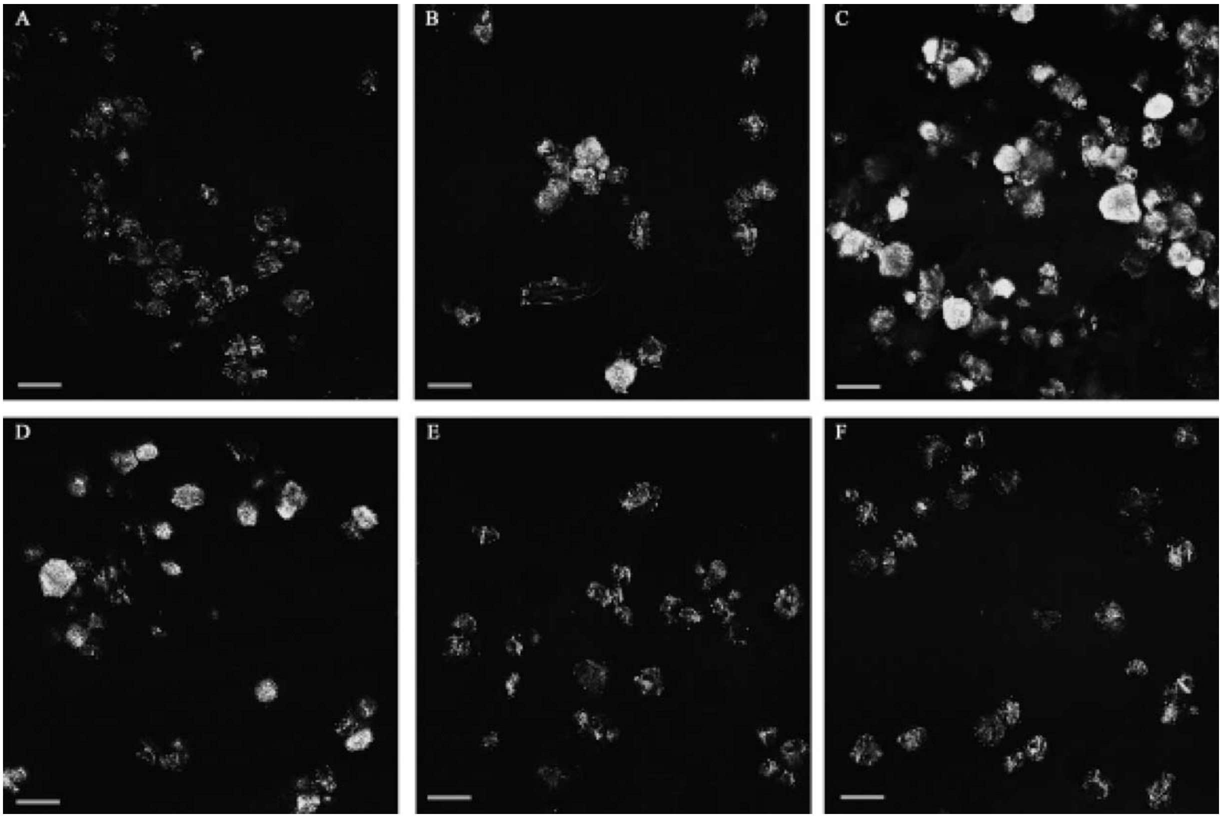

Fig. 5. R-CLSM images of waxy maize starch granules reacted with POA at 54 °C and varying pH levels in the presence of Na_2SO_4: (A) 54S1, (B) 54S3, (C) 54S5, (D) 54S2, (E) 54S4, (F) 54S6.

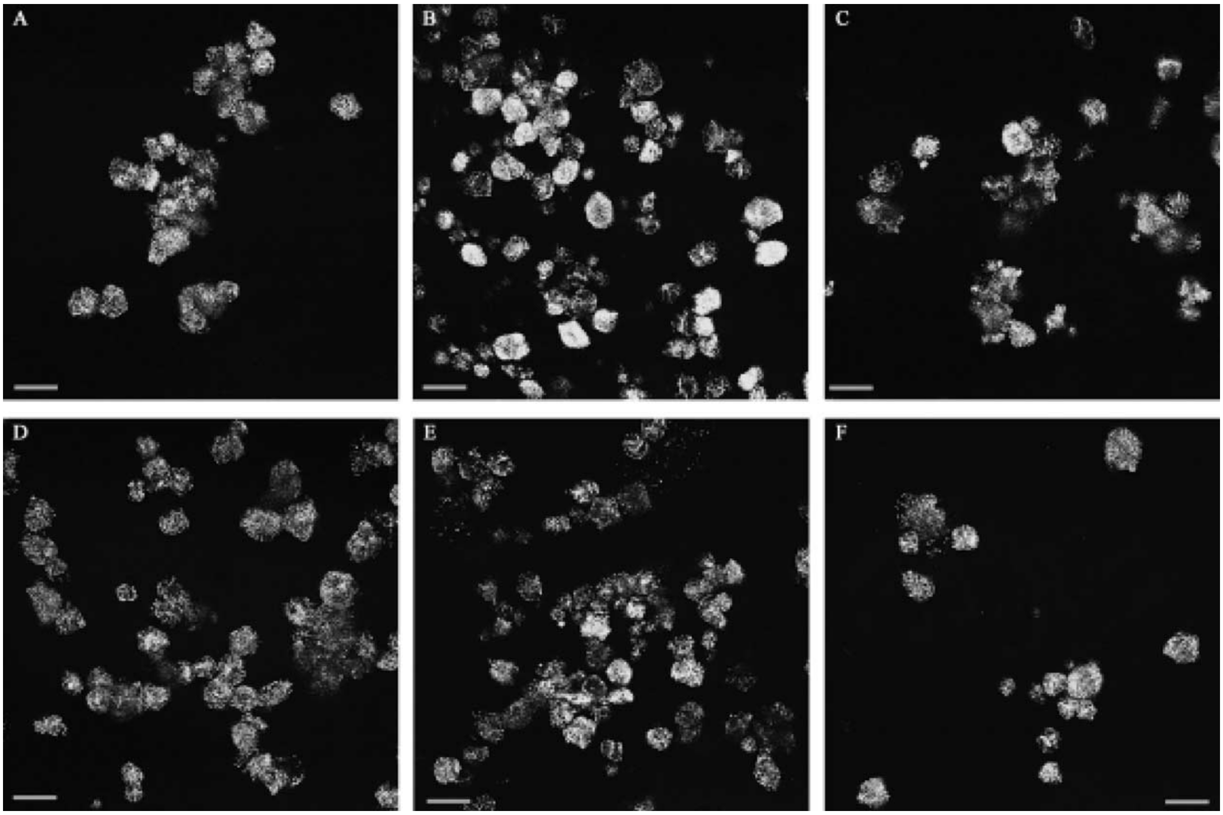

Fig. 6. R-CLSM images of waxy maize starch granules reacted with POA at 44 °C and varying pH levels in the presence of NaCl: (A) 44N1, (B) 44N3, (C) 44N5, (D) 44N2, (E) 44N4, (F) 44N6.

Granules reacted at pH 10.7 and pH 11.2 gave similar reflection patterns, with the sample reacted at pH 11.7 perhaps producing a more intense reflection signal than that reacted at pH 11.2. At the high concentration of NaCl (Fig. 7D–F), extent of reaction appeared to be nonresponsive to increasing pH. At best, reaction was more extensive at pH 10.7, where reflective light signals from granules appeared to be slightly more intense.

Extent of reactions in granules appeared to increase with increasing pH when granules were reacted in the presence of the low concentration of NaCl at 54 °C (Fig. 8A–C). At pH 11.7, granules yielded bright reflection patterns and appeared to be reacted throughout. In the presence of the high concentration of NaCl, granules reacted at pH 10.7 and 11.2 (Fig. 8D and E) appeared to be similar in extent of reaction, with pH 11.2 appearing to result in slightly more reaction than pH 10.7, as evidenced by unreacted regions near the periphery of granules in pH 10.7. At pH 11.7 (Fig. 8F), granules appeared to be modified to a greater extent, indicating a relationship between increasing pH and extent of reaction for these samples.

3.4. Discussion of effect of pH

An apparent clear relationship between pH increase and extent of reaction as detected by R-CLSM was found when granules were reacted under the following conditions: 44 °C, high Na_2SO_4 concentration; 44 °C, high NaCl concentration; 54 °C, low Na_2SO_4 concentration; 54 °C, low NaCl concentration; and 49 °C, high and low Na_2SO_4 concentrations. Under these conditions, an increase in pH appeared to result in a higher degree of reaction. Granules reacted at 44 °C in high concentrations of Na_2SO_4 and NaCl and at 54 °C in low concentrations of Na_2SO_4 and NaCl were the expected swelling extremes (lowest and highest swelling conditions for the respective salt series). Increasing pH should increase reaction efficiency due to an increase in starch alkoxide concentration (Van Warners et al., 1994); in addition, the association of polymers would be reduced and granules would be more opened up. It was also expect that as reaction occurred, this concerted process would accelerate due to introduction of more hydroxypropylsulfonate groups (as regions opened up), which in turn would effect greater hydration, swelling, and opening up, and so on.

Increasing pH appeared to be associated with an increase in extent of reaction, which might seem contradictory to previous studies that implied no significant swelling was obtained as pH was increased below pH 11. However, these experiments cannot necessarily be compared since the effect pH on granule swelling was measured at room temperature in the previous work (BeMiller & Pratt, 1981). At temperatures above room temperature, as investigated in this study, the effect of pH on granule swelling might increase as temperature increases (Oosten, 1982, 1983).

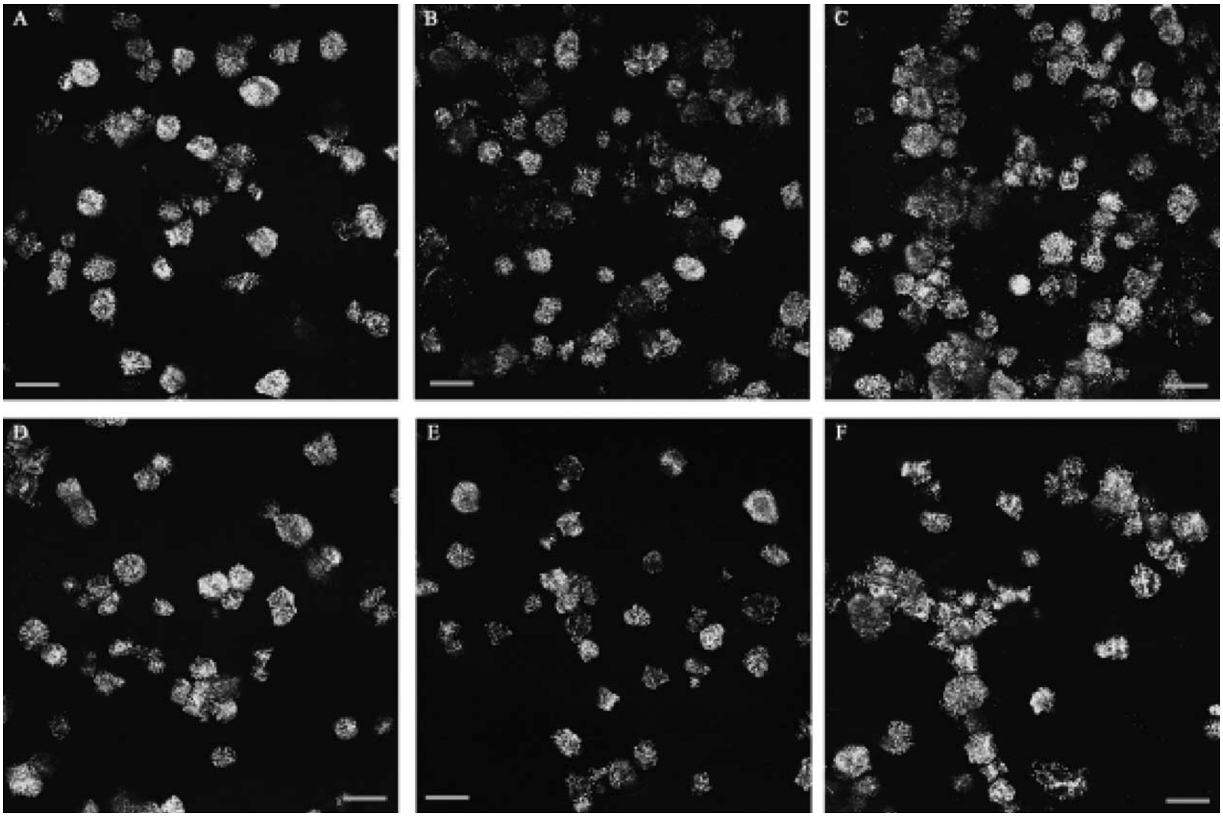

Fig. 7. R-CLSM images of waxy maize starch granules reacted with POA at 49 °C and varying pH levels in the presence of NaCl: (A) 49N1, (B) 49N3, (C) 49N5, (D) 49N2, (E) 49N4, (F) 49N6.

Second, the highest pH value studied by BeMiller and Pratt (1981) and Hauber et al. (1992) was pH 11, whereas higher alkalinity concentrations were used in the current study (pH 11.2 and 11.7, the intermediate and extreme pH values used in this study). It is likely that the effect of pH on granule swelling would increase as concentration of alkali increased and as the pK_a of hydroxyl groups is approached. Third, waxy maize starch was used in the current study, and its granules have been found to be structurally different than those of common corn starch in several ways, including a lower gelatinization temperature. Further, BeMiller and Pratt (1981) found an increase in water sorption by waxy maize starch with increasing pH. In the authors' opinion, it would not take much swelling to have an impact on reactivity (at least in the case of propylene oxide or an analog thereof), since reaction rate will increase as more substituent groups are added to starch molecules, opening up the granule for more extensive reactions as the granule swells more.

Series in which there was not a clear positive correlation of reaction extent with increasing pH included 54 °C, high Na_2SO_4 concentration and 49 °C, low and high NaCl concentration. In the former case, a reverse trend was noticed. POA side reactions, including those with salts or base (Van Warners et al., 1994), may have been more prevalent under these conditions and might have altered reaction patterns enough so that no correlation with pH was noticed. Neither do we have an explanation for apparent results for 49 °C, low and high concentrations of NaCl.

It is not completely clear whether, in the case of granules reacted at 44 °C and in the presence of the low concentrations of Na_2SO_4 and NaCl, the extent of reaction is positively correlated with increasing pH. While an influence of pH appears to be associated with a higher degree of reaction when comparing pH 10.7 and 11.2 in the presence of both types of salts, the trend was apparently reversed when granules reacted at pH 11.7 were investigated. Perhaps at pH 11.7, granules were reacted to such an extent that granule integrity of the more derivatized granules was compromised (low concentration of swelling inhibiting Na_2SO_4 was used in that case) either during reaction, ethanol washing, exchange with $AgNO_3$, or under microscope viewing, leaving only less reacted granules intact. As discussed in the temperature section below, 44 °C might be a condition where extensive reactions could proceed due to a slower reaction at low temperatures. However, extensive damage was not noticed during filtering procedures, and while damaged granules could be seen in microscope fields of view (granules were placed in 20% sucrose solution to minimize reflection of light caused by refractive index differences between the inside and outside of starch granules), it is unlikely that extensive pasting occurred during viewing without being noticed, especially considering that, as shown below, increasing temperature in these

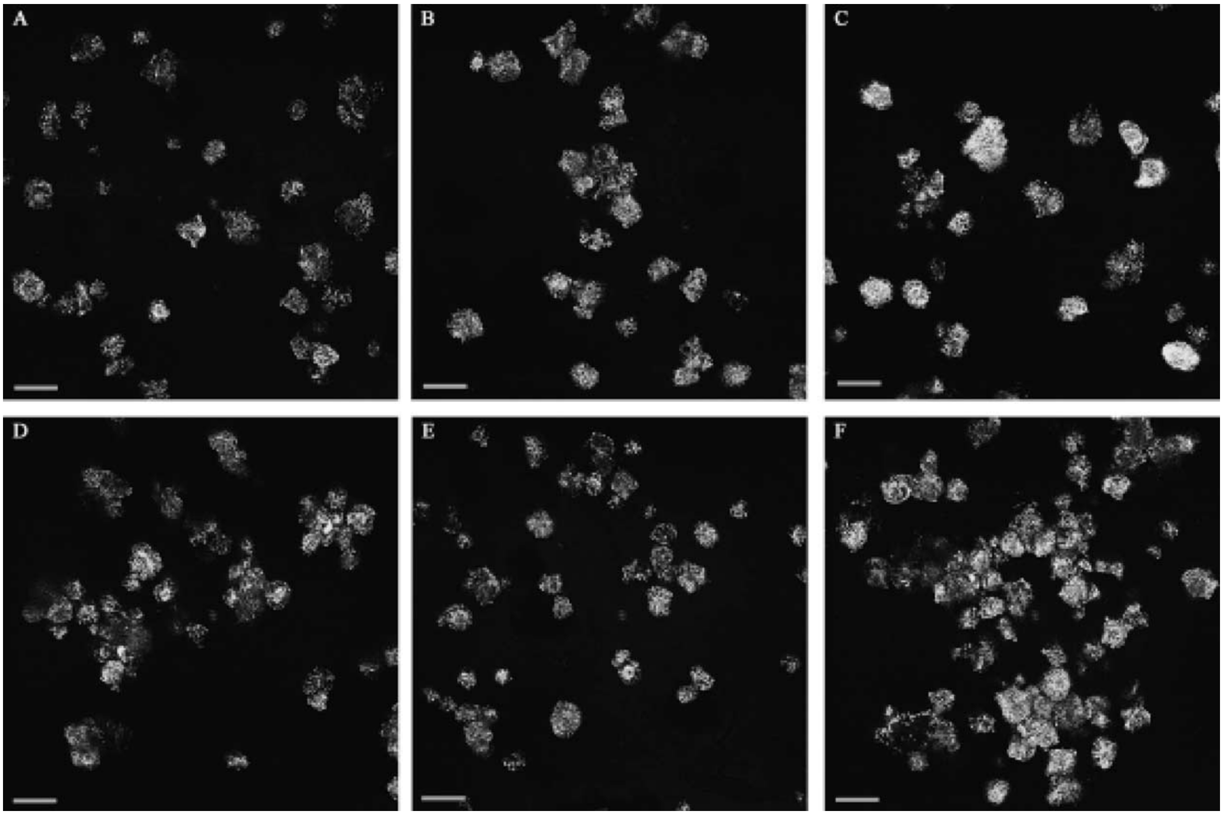

Fig. 8. R-CLSM images of waxy maize starch granules reacted with POA at 54 °C and varying pH levels in the presence of NaCl: (A) 54N1, (B) 54N3, (C) 54N5, (D) 54N2, (E) 54N4, (F) 54N6.

types of samples (pH 11.7, low Na_2SO_4/NaCl concentrations) influenced reactivity.

Another possible explanation is that differences in intensities of reflected light from granules that also have differences in uniformity of reaction may not reflect differences in extent of reaction, i.e. MS values, because observed reflections from Ag in starch granules probably is due to clusters of Ag atoms (Gray & BeMiller, 2004). As uniformity of reaction increases, less clustering would occur, and thus, perhaps less observed intensity of reflection signal would result. Further, as starch molecules are derivatized with POA, this effect might be increased due to anionic sulfonate groups which would likely repel one another, cause a higher degree of swelling, and decrease the likelihood for clustering of Ag atoms once the granules are treated with $AgNO_3$. Thus, the apparent decreased reaction might be artifactual. Whatever the case may be, it is clear that pH had a significant effect on patterns of granule reaction at 44 °C.

While differences in salt concentration are discussed in another section, in general, effect of pH differed due to salt concentration at the pH in which a uniform reaction occurred. An example is the 44 °C series in the presence of Na_2SO_4, where extent of reaction increased with increasing pH level to the point of uniformity at pH 11.2 in the presence of the low salt concentration. At the high salt concentration, pH 11.7 was required to approach an apparently uniform reaction. Thus, it appears that the reaction/swelling promoting effect of pH overcame the swelling-inhibiting effect of Na_2SO_4 (Oosten, 1982, 1983). Overall, pH increased the extent of reaction, either by increasing the degree of swelling, increasing the efficiency of the POA reaction, or most likely, a combination of the two. In some series, extent of reaction did not increase with increasing alkali concentration, and thus reaction extent was likely more influenced by factors other than pH.

3.5. Effect of temperature

By holding all other reaction conditions constant, the effect of temperature on reaction location was studied. In this case, granules were reacted at common industrial etherification conditions, and the temperature of the water bath was set at 44, 49, or 54 °C.

Increasing temperature of reaction slurries at pH 10.7 in the presence of the lowest concentration of Na_2SO_4 significantly affected observed reaction patterns of starch granules, although variations were apparently negatively correlated. While granules reacted at 44 °C in the presence of the low concentration of Na_2SO_4 seemed to have reaction sites located throughout granules, extent of reaction appeared to decrease as the pH was increased to 11.7. On the other hand, in the presence of the high concentration of Na_2SO_4 at pH 10.7, extent of reaction appeared to increase with increasing temperature. Differences in reaction patterns of granules reacted at 44 and 49 °C were subtle. 54S6 appeared to be extensively reacted.

At pH 11.2, granules reacted in the presence of the low Na_2SO_4 concentration at 44 °C appeared to be more extensively reacted than those reacted at 49 and 54 °C and to have Ag distributed throughout entire granules. Granules reacted at 54 °C appeared to possess more Ag than granules reacted at 49 °C. In the case of the high Na_2SO_4 concentration, increasing temperature from 49 to 54 °C appeared to have little if any affect on distribution of reaction sites.

For granules reacted at pH 11.7 in the presence of the low Na_2SO_4 concentration, an apparent reaction pattern dependence on reaction temperature was clearly observed. While reaction seemed localized to surface regions in 44S5, increasing temperature caused reaction sites to be more uniformly distributed throughout granules. On the other hand, in the presence of the high concentration of Na_2SO_4, the reverse trend was found; 44S2 appeared to be highly reacted, and an observable apparent diminishing extent of reaction was noticed with increasing temperature.

At pH 10.7 in the presence of the low concentration of NaCl, extent of reaction appeared to increase from 44 to 49 °C. However, samples reacted at 54 °C appeared to be significantly less reacted than were the previous two. Under the high salt concentration, a similar pattern was found, although it appeared to be more subtle. When comparing samples reacted at 44 and 49 °C, reaction appeared to be slightly more extensive at the higher temperature. When the temperature was raised to 54 °C, however, apparent extent of reactivity decreased.

At pH 11.2 in the presence of the low concentration of NaCl, an apparent inverse relationship between extent of reaction and increasing temperature was observed. A similar effect, but to a lesser degree, was noticed in granules reacted in the high concentration of NaCl.

All granules reacted in the presence of the low concentration of NaCl at pH 11.7 appeared to be extensively reacted. However, 54N5 appeared to be the brightest. A slight effect of heat on the extent of reaction was noticed. A similar effect was not apparent for granules reacted in the presence of the high concentration of NaCl. Instead, a slight decrease in brightness of reflections was noticed as the reaction temperature increased, even though it was difficult to distinguish reaction patterns in terms of brightness of reflection. However, more unreacted granule regions were noticed as the temperature increased in the presence of the high concentration of NaCl.

3.6. Discussion of the effect of temperature

Granules reacted at different temperatures had significantly different reaction patterns, i.e. different distributions of silver atoms as detected by R-CLSM, but relationships were not clear. An increase in apparent extent of reaction was positively correlated with an increase in temperature in only a few cases: pH 10.7, high Na_2SO_4 concentration; pH 11.7, low Na_2SO_4 concentration; and pH 11.7, low NaCl concentration. Correlations of extent of reaction with an increase in temperature with Na_2SO_4 present were found for the highest and lowest swelling conditions. A same type of correlation was found for the influence of pH on extent of reaction. In other series, extent of reactions in granules seemed to be unaffected (pH 11.2, high Na_2SO_4 concentration), slightly responsive (pH 11.2, low Na_2SO_4 concentration and pH 10.7, high NaCl concentration), or perhaps reduced (pH 10.7, low Na_2SO_4 concentration; pH 11.7, high Na_2SO_4 concentration; pH 11.2, low/high NaCl concentrations; and pH 11.7, high NaCl concentration) by increases in reaction temperature.

The influence of temperature on extent of reaction is seemingly a straightforward concept. Heat increases granule swelling, which involves ‘stripping’ of starch chains away from one other and making hydroxyl groups free for base-catalyzed reactions (Donovan, 1979); thus, previously unreactable regions should be more prone to reaction with increasing temperature. Additionally, increasing temperature should effect an increase in rate of reaction with POA, which should cause more extensive reaction in a given time (for example, more substituted starch polymers cause more swelling, which allows for a higher molar substitution level, which further increases swelling, etc.). From a Donnan potential perspective, increasing temperature would decrease Donnan effects due to a lower adsorption of sodium cations at higher temperatures (Oosten, 1982, 1983). Thus, repulsive forces would be diminished, and hydroxide anions might more easily enter the granule and cause swelling.

However, the effect described was only observed in a few cases when reaction temperature was increased for a given pH. Complicating matters are side reactions in which POA is involved. Besides reaction of POA with starch molecules, reactions can also occur between POA and alkali (forming the propylene glycol analog), the propylene glycol analog, and salts (Na_2SO_4 or NaCl) (Van Warners et al., 1994). (Reactions between hydroxyl groups of POA substituted starch and POA are also possible, but this would be detected by the R-CLSM method and be measured as MS.) Heat will likely increase the rate of each reaction just mentioned, including the reaction with starch. The effect of increasing temperature on selectivity of reactions is not known for POA, but in the case of ethylene oxide, no significant temperature effect on selectivity of reaction was found (Van Warners et al., 1994). Perhaps increasing temperature speeds up the reaction of starch with POA to an extent that reaction sites are concentrated in more accessible granule areas, while at an optimal temperature, or at a temperature lower than the optimal temperature, reaction could be more uniform and extensive due to a slower reaction. A more plausible explanation is that, under conditions that produced the most extensive apparent reaction patterns in

POA-modified starch granules, reaction sites were less clustered than in granules reacted to a lesser degree, thus lowering the amount of the apparent Ag-binding. For now, unexpected results remain unexplained. Overall, increasing temperature increased the extent of reaction in starch granules in some cases, while additional factors were seemingly involved in others.

3.7. Effect of salt type

General trends in differences between the type of swelling-inhibiting salt used in reactions were noticed. For example, granules reacted in the presence of NaCl appeared to be more extensively reacted than those reacted in the presence of Na_2SO_4. When comparing series of reaction products, the following trends were noticed: As pH increased from pH 10.7 to 11.7, products of reaction in the presence of Na_2SO_4 and NaCl followed similar trends at 44 °C, high/low salt concentration and 54 °C, high/low salt concentrations, some of these results being unexpected. For example, granules reacted at 44 °C appeared to be more extensively reacted as pH increased from 10.7 to 11.2, but appeared to be less reacted at pH 11.7 than at the lower pH values. This pattern was observed for granules reacted in the presence of both Na_2SO_4 and NaCl. In the case of 49 °C, low and high concentrations of salt, positive correlations with pH were found for Na_2SO_4, but not for NaCl.

As the reaction temperature was raised from 49 to 54 °C, apparent extent of reaction increased for granules reacted at pH 11.7 with the high concentrations of Na_2SO_4 and NaCl. A similar trend of apparent decreasing extent of reaction with increasing temperature was found for both salts in the case of pH 11.7 at the high salt concentration. Other similarities between the two salts were observed for pH 10.7, low salt concentration and pH 11.2, low and high salt concentrations, where increasing temperature of reaction did not effect an observed increase in apparent extent of reaction, although patterns differed between the two sets. For example, increasing reaction temperature at pH 11.2 in the low Na_2SO_4 concentration apparently caused the extent of reaction to drop as the temperature was increased from 44 to 49 °C, but slightly recover when the temperature was raised to 54 °C; in the case of pH 11.2, low NaCl concentration, apparent extent of reaction appeared to decrease with increases in temperature.

3.8. Discussion of effect of salt type

The observation that granules derivatized in the presence of NaCl apparently contained more Ag than did granules derivatized in the presence of Na_2SO_4 is likely related to the well-known fact that NaCl is less effective as a swelling-inhibiting agent than is Na_2SO_4. Sulfate ions are believed to penetrate granules less effectively than do chloride ions due to increased repulsive forces associated with the sulfate anion (more electronegative) (Oosten, 1982, 1983). In fact, at high concentrations, NaCl lowers the onset temperature of gelatinization (Evans & Haisman, 1982; Jane, 1993; Lii & Lee, 1993; Sandstedt et al., 1960; Wootton & Bamunuarachchi, 1980). Since granules are more able to swell in the presence of NaCl, more extensive reaction should take place inside the granule matrix. While this may seem beneficial, in practice, this property causes problems in that gelatinization can occur at higher MS levels. For example, common corn starch reacted at 49 °C with 0.27 mol of propylene oxide/mole of starch in the presence of NaCl at a molal concentration equivalent to the normally used molal concentration of Na_2SO_4 gelatinized after 2 h of reaction (Villwock, 1996). The same results would have been expected in this study. Despite the complexity of results, they do indicate that factors other than salt type are responsible for apparent differences in reactions in cases where an expected difference between effects of Na_2SO_4 and NaCl was not observed.

The effects of both salts were expected to be responsive to pH changes, since they are ionic species. Perhaps for cases in which unexpected results were observed, results were complicated by the effect of alkali in increasing the salt effect (increasing gelatinization temperature) due to the Donnan potential (Oosten, 1982, 1983). This effect likely depends on both temperature and alkali concentration. For example, in samples reacted at 49 °C in the high and low concentrations of Na_2SO_4, extent of reaction apparently increased with increasing pH, which is explainable due to increased swelling at higher alkalinity. However, in the case of granules reacted in presence of NaCl (high and low concentrations at 49 °C), apparent extent of reaction was not a function of pH. Perhaps this observation is related to the lesser electronegativity of chloride ions. Thus, chloride ions can enter the granule at low pH, swelling can proceed there, and reaction extent is relatively stable across the pH range examined. However, when more electronegative sulfate ions are present, a critical alkali concentration may be required to achieve a similar degree of reaction.

As for temperature, increasing temperature is expected to affect swelling patterns, reagent adsorption, and reaction rate (Evans & Haisman, 1982). Thus, a higher MS is expected as temperature increases. More extensive reaction was found in some cases (as discussed above). In addition, reactions of POA with salt are expected to increase with increasing temperature (Van Warners et al., 1994).

3.9. Effect of salt concentration

A significant decrease in apparent extent of reaction upon increasing salt concentration was noticed for the following conditions (Na_2SO_4): 44 °C, pH 10.7 and 11.2; 49 °C, pH 10.7, 11.2, and 11.7; and 54 °C, pH 11.2 and 11.7. An apparent inverse effect of salt concentration was observed in two cases: 44 °C, pH 11.7 and 54 °C, pH 10.7. Thus, increasing pH to the extreme level at low temperature apparently diminished the effect of salt concentration, as did decreasing pH to the extreme low level at high temperature.

As for reactions conducted in the presence of NaCl, clear differences between concentrations were difficult to distinguish, except in a few cases. Those cases included the following conditions: 44 °C, pH 11.7 and 44 °C, pH 11.2, in which a lower salt concentration apparently resulted in more extensive reaction. In all other cases, the effects of salt concentration on reaction patterns, if any, were too subtle to differentiate visually.

3.10. Discussion of effect of salt concentration

With regards to reactions carried out in the presence of Na_2SO_4, in almost every case, more extensive reaction appeared to have occurred for the low concentration of Na_2SO_4 as compared to the high concentration. This is logical, given the reported effect of increasing effectiveness of the salt to inhibit starch granule gelatinization (and presumably swelling) with increased concentrations (Jane, 1993). In two cases, an apparent inverse relationship was seen between the extent of reaction and Na_2SO_4 concentration, which was unexpected. This finding might be related to the influence of alkali on the adsorption of anions (Oosten, 1982, 1983) or other factors coming more into play at these conditions.

Differences between the high and low concentrations of NaCl were not easily distinguishable, a result confirmed by MS determination (Table 2). NaCl is known to be less effective as a starch granule swelling inhibitor salt than is Na_2SO_4, so more extensive reactions might be allowed in the presence of NaCl. Also, at a critical concentration of salt (~1–1.5 M), NaCl begins to promote swelling instead of inhibit it (Evans & Haisman, 1982; Jane, 1993; Lii & Lee, 1993; Sandstedt et al., 1960; Wootton & Bamunuarachchi, 1980). While the salt concentration used in this study was lower than that critical concentration, it is assumed that factors other than lowering of T_{gel} might be involved, since at concentrations $>6\%$, extent of gelatinization began to increase with increasing salt concentration, while the onset gelatinization temperature had not yet decreased (Wootton & Bamunuarachchi, 1980). It is possible that this critical amount was approached when the NaCl concentration was raised to a level at least enough to compensate for the increased swelling due to lack of salt in the low salt concentration case.

Table 2
MS values of POA-modified waxy maize starch reacted under different conditions

Sample[a]	MS	Sample[a]	MS
44N1	1.6	54N1	1.3
44N2	1.6	54N2	1.2
44N3	1.6	54N3	1.9
44N4	1.8	54N4	2.0
44N5	2.0	54N5	2.2
44N6	2.1	54N6	2.1
49N1	1.6		
49N2	1.8		
49N3	1.7		
49N4	1.9		
49N5	2.0		
49N6	2.3		

[a] See Table 1 for reaction conditions.

3.11. MS determination of waxy maize starch reacted with POA

MS values (Table 2) were determined by measuring the amount of incorporated sulfur. (Only those samples prepared in the presence of NaCl were analyzed, even though analysis of a blank carried through the procedure using Na_2SO_4 showed that all sulfate ions had been removed by washing.) The results confirm that salt concentration had little or no effect on the reaction when NaCl was used; when the salt concentration was raised from 0.471 to 0.629 m, the MS decreased in two cases, increased in six cases, and was unchanged in one case. pH had a considerable effect; average MS values for the two NaCl concentrations increased as the pH increased from 10.7 to 11.2 to 11.7. At 44 °C, the three values were 1.6, 1.7, and 2.05; at 49 °C, 0.855, 1.8, and 2.15; at 54 °C, 1.25, 1.95, and 2.15. The pH data also show that temperature had only a small effect.

3.12. MS determination of hydroxypropylated waxy maize starch

To ascertain if altering reaction conditions could result in different MS values of starch reacted with propylene oxide, three conditions were chosen: 44 °C, pH 10.7, high Na_2SO_4 concentration; 49 °C, pH 11.2, high Na_2SO_4 concentration; 54 °C, pH 11.7, low Na_2SO_4 concentration which represented theoretically different swelling conditions (signified by $44S2_{po}$, $49S4_{po}$, and $54S5_{po}$, respectively, with the subscript po indicating that this is a normal reaction with propylene oxide). Results are given in Table 2. MS values increased as swelling conditions increased between $44S2_{po}$, $49S4_{po}$, and $54S5_{po}$.

The fact that samples were increasingly modified with PO as swelling conditions increased in the presence of Na_2SO_4 confirms visual results from R-CLSM with starches reacted with POA. Differences in reaction patterns observed between 49S4 and 54S5 POA samples using R-CLSM appeared greater than MS values might indicate for corresponding PO samples ($49S4_{po}$ and $54S5_{po}$). Our interpretation is that, while small differences were found between the two samples in terms of MS values, in the case of $49S4_{po}$ (PO) and 49S4 (POA), reactions may have been limited to amorphous regions. As swelling conditions were increased, the granule was apparently opened up to more extensive reaction, although this is only slightly reflected in MS values. Further, the potential reaction efficiency was likely approached, which could explain the relatively small

Table 3
MS values of PO-modified waxy maize starch reacted under different conditions

Sample	pH	Temperature (°C)	Na_2SO_4 (m)	NaCl (m)	MS
$44S2_{po}$	10.7	44	0.527	–	0.029
$49S4_{po}$	11.2	49	0.527	–	0.061
$54S5_{po}$	11.7	54	0.395	–	0.068
$49N4_{po}$	11.2	49	–	0.629	0.059

difference in MS values between $49S4_{po}$ and $54S5_{po}$ as compared with a large increase between $44S2_{po}$ and $49S4_{po}$ (Table 3).

4. Summary

It was found that patterns of reactions in starch granules are affected by altering swelling/reaction conditions. Since, in the case of hydroxypropylation, the incorporation of substituents causes a further increase in swelling, comprehension is difficult, especially considering that a qualitative method was used. Nonetheless, trends in reaction patterns were found when granules were reacted under different conditions. Further, general findings were supported by NMR data of starch granules reacted with PO under the same conditions.

In most cases, increasing pH increased extent of reaction on a granule basis. The effect could be due to either increasing the degree of swelling during reaction, or increasing the efficiency of the POA reaction, or a combination of the two. Temperature apparently had less of an effect on extent of reaction than did pH. However, in some cases, increasing temperature apparently increased extent of reaction. The type of swelling inhibiting salt used during the reaction seemed to impact reaction patterns in that granules reacted with POA in the presence of NaCl appeared to be more extensively reacted as compared to granules reacted in the presence of Na_2SO_4, when all other factors were held constant. This most likely occurred becuase Na_2SO_4 is a more effective swelling inhibitor than is NaCl. However, in many cases, similar trends were seen among salt types as pH and temperature were increased for the POA reaction. While differences in reaction patterns between the high and low concentrations of NaCl were not easily distinguishable, in almost every case, a more extensive reaction was seen for the low Na_2SO_4 concentration as compared to the high concentration. Increasing swelling conditions and MS values was confirmed for granules reacted with PO.

Increasing extent of reaction can be achieved by increasing the concentration of reagent molecules in the reaction system, and this has been observed visually (Gray & BeMiller, 2004; Huber & BeMiller, 2001). However, there are legal constraints on reagent levels for modified starch products intended for human consumption. Results confirm that altering reaction conditions can impact uniformity of reaction on a granule basis, as well as influencing MS levels.

It is possible that effects investigated in this study were difficult to observe visually due to the low MS values of the products. Wootton and Manatsathit (1983) found little effect of water binding capacity of hydroxypropylated maize starches at low MS values; in fact, at MS values below 0.07, water binding capacity decreased with increasing MS. Using a higher MS value might more clearly reveal effects of each parameter on reaction patterns in granules, but differences in reaction patterns between samples reacted under different conditions might be less discernable as MS increases.

The results presented here are for waxy maize starch. It is almost certain that other starch granules, such as those containing amylose and especially those containing phosphate ester groups (e.g. potato starch), would react differently.

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