Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 64 (2006) 158–162
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Influence of reaction conditions on MS values and physical properties of waxy maize starch derivatized by reaction with propylene oxide

Jung-Ah Han, James N. BeMiller *

Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN, USA

Received 7 April 2005; received in revised form 18 October 2005; accepted 1 November 2005
Available online 13 December 2005

Abstract

Waxy maize starch was hydroxypropylated under different swelling conditions (pH, temperature, salt type, salt concentration) to determine the effect of reaction conditions on the extent of modification. MS, pasting temperatures, and paste characteristics of the products were determined. pH had the greatest effect on the extent of reaction. MS values increased as the pH was raised from 10.7 to 11.7. In most cases, samples hydroxypropylated in the presence of NaCl had lower or equal MS values as compared to those reacted in the presence of Na_2SO_4, and reactions carried out in the presence of NaCl were more sensitive to changes in pH than were those conducted in the presence of Na_2SO_4. The lower MS values for samples reacted in the presence of NaCl was most notable at the lowest pH value. Temperature had less effect on reaction efficiency. No substantial differences were found in pasting temperature or peak viscosity. Under conditions that promoted greatest swelling, viz. highest pH (11.7) and highest temperature used (54 °C), some granules reacted in the presence of NaCl gelatinized, i.e. NaCl failed to prevent pasting. Therefore, it was confirmed that Na_2SO_4 better protects against gelatinization than does NaCl. The concentration of Na_2SO_4 (0.395 vs. 0.527 m) only slightly affected MS values.

1. Introduction

Gray and BeMiller (2005) applied a new method of locating reaction sites in starch granules (Gray and BeMiller, 2004) to a study of the influence of reaction conditions (pH, temperature, and swelling-inhibiting salt type and concentration) on the location of substituent groups in waxy maize starch granules derivatized with an anionic analog of propylene oxide, for which there is evidence that it reacts similarly to propylene oxide (Gray and BeMiller, 2004). Their results indicated that altering reaction conditions impacted both MS levels and uniformity of reaction within granules. In general, it was found that conditions that allowed greater swelling of granules resulted in greater reaction efficiency, i.e. higher molar substitution (MS) values. Higher pH values, the presence of NaCl as compared to Na_2SO_4, and lower concentrations of Na_2SO_4 all resulted in more derivatization (as observed by confocal laser scanning microscopy (CLSM)). Temperature and concentration of NaCl had little effect. However, actual MS values could not be determined by the usually used ^{1}H-NMR method because of the nature of the derivatizing group. While their method is quite useful for locating reaction sites within granules, it provides only qualitative rather than quantitative information about extents of reaction (because its results are visual and because granules are far from uniform in their degrees of reaction). Also, Gray and BeMiller (2004) pointed out that it is likely that the method only visualizes clusters of silver atoms (and, hence, clusters of substituent groups). It may, therefore, underestimate evenly distributed groups. In addition, the method requires use of an anionic analog of propylene oxide rather than propylene oxide itself.

It was the objective of this project to use propylene oxide itself and to determine actual MS values of waxy maize starch modified under the same 36 reaction conditions used by Gray and BeMiller (2005) to obtain information about the relative effects of extrinsic variables on reaction efficiency.

2. Materials and methods

Waxy maize starch was provided by Tate & Lyle North America (Decatur, IL). Sodium sulfate was 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).

Abbreviations Starch, Hydroxypropyl; Starch, Hydroxypropylation; Starch, Waxy maize.

* Corresponding author. Tel.: +1 765 494 5684; fax: +1 765 494 7953.
E-mail address: bemiller@purdue.edu (J.N. BeMiller).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.11.008

3. Reaction of waxy maize starch granules with propylene oxide

Waxy maize starch (33 g, db) was reacted in duplicate reactions with propylene oxide (2.6 mL) under conditions identical to those used by Gray and BeMiller (2005). Temperature (44, 49, or 54 °C), pH (10.7, 11.2, or 11.7), salt type (Na_2SO_4 or NaCl), and salt concentration were varied.

The appropriate amount of salt was weighed and dissolved in 90 g of water with stirring. Starch was added, and the slurry was stirred for ~5 min. With continued stirring, the pH was adjusted to the desired value with 2 M NaOH. The reaction vessel was sealed tightly with a septum, and propylene oxide (2.6 mL) was added via a syringe. The flask was placed in a water bath set at the appropriate temperature, and the samples were stirred on a submergible magnetic stir plate for 24 h. After that, the starch slurry was neutralized with dilute HCl and washed with 50% acetone to minimize loss of molecules via leaching (Stapley and BeMiller, 2003). The cake was returned to the reaction vessel, which was filled with 50% acetone (~75 mL), and the slurry was stirred magnetically for 2 h at room temperature. The starch was recovered in a Büchner funnel, rinsed with 50% acetone (~50 mL), and allowed to dry on the filter disk (Gray & BeMiller, 2005).

4. RVA analysis

The pasting characteristics of modified waxy corn starch were determined with a Rapid Visco Analyser (Model 4, Newport Scientific, Warriewood, Australia) (RVA) using standard profile 1. A 13-min analysis was used: equilibration to 50 °C for 1 min, heating to 95 °C in 222 s, holding at 95 °C for 150 s, cooling to 50 °C in 228 s, holding at 50 °C for 120 s. Waxy maize starch (2.1 g, db) and 27.9 g of distilled water (sample + water = 30 g) were combined and stirred in the aluminum sample container for 20–30 s before inserting the container into the RVA. Analyses were done in triplicate.

5. MS determination

Modified starch (~35 mg) was dispersed in 5 mL of D_2O, and the slurry was heated in a boiling-water bath for 15 min. α-Amylase (1.0 unit) (EC 3.2.1.1, Sigma Chemical Co., St Louis, MO) was added, and the mixture was incubated for at least 48 h at room temperature. The digest was then freeze dried and dissolved in 1 mL D_2O. Proton NMR of the samples was acquired with a 300-MHz NMR spectrometer (Inova, Varian Corp., Palo Alto, CA). 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 (Villwock and BeMiller, 2005).

6. Results and discussion

To minimize loss of starch polymers via leaching during the modification process, waxy maize starch and washing with 50% acetone were used. The modification conditions used were identical to those used by Gray and BeMiller (2005) in reactions with an analog of propylene oxide. The conditions were chosen such that the mid temperature (49 °C), mid-pH (11.2), and the higher Na_2SO_4 concentration (0.527 m = 7% Na_2SO_4, 17.5% $Na_2SO_4 \cdot 10H_2O$) were similar to industrial hydroxypropylation conditions.

7. Effect of pH on MS

Table 1 gives MS values and pasting characteristics as a function of reaction pH (10.7, 11.2, 11.7). At all combinations of temperature, salt type, and salt concentration, MS values increased as the pH increased from 10.7 to 11.7. The increases in MS values in reactions conducted in the presence of NaCl were greater than those conducted in the presence of Na_2SO_4, i.e. reactions conducted in the presence of NaCl were more sensitive to pH changes than were those conducted in the presence of Na_2SO_4. The differences in MS values when the pH was raised from 10.7 to 11.2 were greater (usually much greater) than when the pH was raised from 11.2 to 11.7 in all cases except at the lowest temperature.

Apparent (see later) optimal conditions for reaction efficiency all involved a reaction pH of 11.7 and temperatures of 49 or 54 °C. They were 0.395 m Na_2SO_4 and 49 or 54 °C, 0.471 m Na_2SO_4 and 49 °C, 0.471 m NaCl and 49 °C, and 0.629 m NaCl and 49 °C.[1] In general, these conditions are those of probable greatest swelling, i.e. highest pH, highest temperature, lowest anion concentration. Under these apparent optimal conditions, reaction efficiencies were 64–66%, confirming the previous report (Gray and BeMiller, 2005).

From the above results, it is apparent that pH affects hydroxypropylation of waxy maize starch significantly, with higher pH resulting in a more efficient reaction (higher MS values). This effect is probably due to an increase of starch alkoxide concentration (Van Warners et al., 1994). More alkaline conditions might also reduce starch polymer associations and open up granules.

8. Effect of temperature on MS

The effect of reaction temperature on MS of hydroxypropylated waxy maize starch is given in Table 2. Three temperatures (44, 49, and 54 °C) were used to determine if temperature can affect the reaction pattern. (Commercial modifications are done at ~49 °C.) As found by Gray and BeMiller (2005), temperature had less effect on the extent of

[1] Samples prepared at pH 11.7 and 54 °C at both 0.471 and 0.629 m NaCl showed greatly reduced values for pasting temperature, peak viscosity, and breakdown as compared with values for samples prepared at pH 11.2 and all other preparations. These results may have been due to a greater swelling of granules and a more homogeneous distribution of substituent groups within granules, but are likely due, at least in part, to granule gelatinization. (That some starch granules were gelatinized during the modification process was confirmed by polarized light microscopy.) Therefore, these preparations are not included subsequent discussions.

Table 1
Effect of reaction pH on MS, pasting temperatures, and paste characteristics of hydroxypropylated waxy maize starch

			Pasting characteristics		
	pH	MS	Pasting temp.	Peak viscosity	Breakdown
Unmodified waxy corn	–	–	72.25	2265	1146
*44°, 0.*395 m Na_2SO_4					
	10.7	0.055	69.95	2398	1153
	11.2	0.065	68.75	2471	1256
	11.7	0.092	68.65	2576	1310
*49°, 0.*395 m Na_2SO_4					
	10.7	0.064	68.50	2407	1222
	11.2	0.104	67.35	2502	1224
	11.7	0.118	66.30	2627	1332
*54°, 0.*395 m Na_2SO_4					
	10.7	0.067	68.50	2290	1073
	11.2	0.102	68.70	2339	1125
	11.7	0.118	66.35	2409	1128
*44°, 0.*527 m Na_2SO_4					
	10.7	0.056	69.95	2372	1150
	11.2	0.067	68.65	2448	1184
	11.7	0.084	68.65	2495	1255
*49°, 0.*527 m Na_2SO_4					
	10.7	0.070	68.65	2384	1175
	11.2	0.101	67.50	2448	1226
	11.7	0.111	67.15	2516	1268
*54°, 0.*527 m Na_2SO_4					
	10.7	0.072	68.70	2258	1037
	11.2	0.097	68.70	2376	1144
	11.7	0.112	67.45	2427	1127
*44°, 0.*471 m *NaCl*					
	10.7	0.032	69.95	2282	1139
	11.2	0.064	68.65	2480	1428
	11.7	0.095	67.50	2733	1401
49°, 0.471m NaCl					
	10.7	0.053	69.90	2435	1235
	11.2	0.099	67.50	2517	1267
	11.7	0.118	66.35	2646	1505
*54°, 0.*471 m *NaCl*					
	10.7	0.064	68.75	2301	1091
	11.2	0.102	67.10	2351	1108
	11.7	0.116	62.70	1862	766
*44°, 0.*629 m *NaCl*					
	10.7	0.037	69.90	2360	1177
	11.2	0.065	68.70	2426	1226
	11.7	0.097	67.50	2710	1276
*49°, 0.*629 m *NaCl*					
	10.7	0.062	68.70	2363	1189
	11.2	0.100	67.50	2436	1243
	11.7	0.116	66.50	2559	1337
*54°, 0.*629 m *NaCl*					
	10.7	0.063	68.70	2296	1074
	11.2	0.104	67.30	2377	1117
	11.7	0.112	60.75	1571	533

reaction than did pH. The effect of temperature was greatest when the temperature was raised from 44 to 49 °C.

Gray and BeMiller (2005) speculated that increasing temperature accelerated the reaction of starch with chemical reagent to an extent that reaction sites are concentrated in more accessible granule areas. According to Donavan (1979), raising the temperature induces more granule swelling, which makes more hydroxyl groups accessible to base-catalyzed reactions. Oosten (1982) explained that increasing temperature would decrease Donnan effects due to a lower adsorption of sodium ions, thus repulsive forces would be diminished and hydroxide anions might more easily enter granules and cause swelling. In the present study, when the temperature was increased from 44 to 49 °C, MS values of all samples increased significantly. When the temperature was increased from 49 to 54 °C, MS values changed less, even slightly decreasing in some cases. Two explanations for the latter observation are proposed: (1) increased reaction of the reagent with the aqueous alkaline medium rather than with starch polymers; (2) increased leaching of more highly derivatized starch molecules during

Table 2
Effect of reaction temperature on MS of hydroxypropylated waxy maize starch

Temp. (°C)	pH 10.7	pH 11.2	pH 11.7
*0.*395 m Na_2SO_4			
44	0.055	0.065	0.092
49	0.064	0.104	0.118
54	0.067	0.102	0.118
*0.*527 m Na_2SO_4			
44	0.056	0.067	0.084
49	0.070	0.101	0.111
54	0.072	0.097	0.112
*0.*471 m *NaCl*			
44	0.032	0.064	0.095
49	0.053	0.099	0.118
54	0.064	0.102	0.116
*0.*629 m *NaCl*			
44	0.037	0.065	0.097
49	0.062	0.100	0.116
54	0.063	0.104	0.112

Pasting temperatures and paste characteristics are given in Table 1.

the reaction, resulting in a lower MS for the remaining granular starch and an apparent lower reaction efficiency.

9. Effect of swelling-inhibiting salt type on MS

We expected that the starch reacted in the presence of Na_2SO_4 would be less substituted because Na_2SO_4 is known to be a more effective swelling inhibitor than is NaCl (Villwock and BeMiller, 2005). However, at 44 °C and pH 10.7, apparent reaction efficiencies for reactions conducted in the presence of sulfate ions were greater than those for samples reacted in the presence of chloride ions. For reactions done at pH 11.2 and 11.7, MS values were about equal or slightly less for reactions conducted in the presence of Na_2SO_4 as compared to those conducted in the presence of NaCl under otherwise identical conditions. Gray and BeMiller (2005), who used both salts with a lesser amount of propylene oxide (1.28 vs 2.60 mL) for modification of waxy corn starch, also reported that there was no significant difference in MS values between the samples reacted with propylene oxide in the presence of the two salts at pH 11.2 and 49 °C. However, using the propylene oxide analog, they obtained evidence that reaction in the sample reacted in the presence of Na_2SO_4 was less homogeneous with regards to intragranular distribution of substituent groups.

10. Effect of swelling-inhibiting salt concentration on MS

According to Villwock and BeMiller (2005), at higher Na_2SO_4 concentrations, granule swelling is restricted by lyotropic effects, which reduces the number of sites available for reaction with propylene oxide. MS values of modified waxy maize starch prepared using the higher concentration of Na_2SO_4 (Table 2) were lower than those of products prepared using the lower concentration of Na_2SO_4 only for reactions conducted at pH 11.7. The differences as a function of salt concentrations for reactions conducted in the presence of Na_2SO_4 were greater than those conducted in the presence of NaCl. Gray and BeMiller (2005) reported that reactions conducted in the presence of Na_2SO_4 at the higher concentration seemed to result in less uniformly substituted products. In this work, reactions that employed the higher concentration of Na_2SO_4 gave products with slightly greater MS values at pH 10.7. That the effect of Na_2SO_4 is an all-or-none effect, i.e. is independent of concentration above a certain minimum concentration (Villwock and BeMiller, 2005) is confirmed by the results in Table 2 except for reactions conducted at pH 11.7.

11. The relationship between MS and pasting characteristics

Significant differences in pasting temperatures as a function of MS values were not found. However, all hydroxypropylated products had reduced pasting temperatures as compared to unmodified waxy maize starch (Seow and Thevamalar, 1993). (For comparison, the pasting characteristics of unmodified waxy maize starch are given in Table 1. Values for final viscosity and setback are not given because there was no consistent trend in them.) As the reaction pH increased and, thus, MS values increased, the pasting temperature decreased. After hydroxypropylation, the pasting curve changed only slightly. With one exception,[1] peak viscosity increased, but to a small extent. That the pasting characteristics of waxy maize starch are little affected by modification supports the findings of Han et al. (2005) and Liu et al. (1999).

At 44 and 49 °C, the higher concentration of Na_2SO_4 resulted in lower peak viscosities and reduced breakdown. A clear trend was not evident when NaCl was used. Usually, the breakdown in hydroxypropylated starch products is larger than that in unmodified starch because the susceptibility of swollen starch granules to disruption by stirring is increased by the modification (Liu, Ramsden, and Corke, 1999; Shi and BeMiller, 2000). However, we found that the breakdown value in hydroxypropylated waxy maize starch was, in general, not much greater than that in unmodified waxy maize starch and in some cases less. Overall, the best combination of greatest peak viscosity, least breakdown, and least setback was given by products prepared at pH 11.7 and 54 °C in the presence of Na_2SO_4 (either concentration).

12. Conclusions

Increasing pH promoted derivatization of waxy maize starch with propylene oxide. Temperature had much less effect on the reaction; however, in a few cases, increasing temperature increased extent of reaction. At pH 10.7 and 11.2 (with the exception of the sample reacted in the presence of the lower concentration of Na_2SO_4 at 54 °C), samples prepared in the presence of NaCl had lower MS values than did samples reacted in the presence of Na_2SO_4, contrary to expectation, assuming that reaction efficiency is correlated with the degree of swelling. The explanation may be increased loss of more highly derivatized starch molecules into the

reaction medium. The optimum conditions (of those used in this study) with regards to reaction efficiency and paste characteristics are pH 11.7, 54 °C, and Na_2SO_4 as the swelling-inhibiting salt.

References

Donavan, J. W. (1979). Phase transitions of the starch–water system. *Biopolymers*, *18*, 263–275.

Gray, J. A., & BeMiller, J. N. (2004). Development and utilization of reflectance confocal laser scanning microscopy to locate reaction sites in modified starch granules. *Cereal Chemistry*, *81*, 278–286.

Gray, J. A., & BeMiller, J. N. (2005). Influence of reaction conditions on the location of reactions in waxy maize starch granules reacted with a propylene oxide analog at low substitution levels. *Carbohydrate Polymers*, *60*, 147–162.

Han, J.-A., Lee, B.-H., & Lim, S.-T. (2005). Utilization of hydroxypropylated waxy starches in Korean waxy rice gel cake to retard retrogradation. *Cereal Chemistry*, *82*, 88–92.

Liu, H., Ramsden, L., & Corke, H. (1999). Physical properties and enzymatic digestibility of hydroxypropylated *ae*, *wx*, and normal maize starch. *Carbohydrate Polymers*, *40*, 175–182.

Oosten, B. J. (1982). Tentative hypothesis to explain how electrolytes affect the gelatinization temperature of starches in water. *Starch/Stärke*, *34*, 233–239.

Seow, C. C., & Thevamalar, K. (1993). Internal plasticization of granular rice starch by hydroxypropylation: Effects on phase transitions associated with gelatinization. *Starch/Starke*, *45*, 85–88.

Shi, X., & BeMiller, J. N. (2000). Effect of sulfate and citrate salts on derivatization of amylose and amylopectin during hydroxypropylation of corn starch. *Carbohydrate Polymers*, *43*, 333–336.

Stapley, J. A., & BeMiller, J. N. (2003). Hydroxypropylated starch: Granule subpopulation reactivity. *Cereal Chemistry*, *80*, 550–552.

Van Warners, A., Stamhuis, E. J., & Beenackers, A. A. C. M. (1994). Kinetics of the hydroxyethylation of starch in alkaline salt-containing aqueous slurries. *Industrial and Engineering Chemistry Research*, *33*, 981–982.

Villwock, V. K., & BeMiller, J. N. (2005). Effects of salts on the reaction of normal corn starch with propylene oxide. *Starch/Stärke*, *57*, 281–290.