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Effect of sulfate and citrate salts on derivatization of amylose and amylopectin during hydroxypropylation of corn starch

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Abstract

Common corn starch was modified in 0.56 M sodium sulfate solution and in 0.31 M potassium citrate solution. It was found that about 1.8 times the amount of reagent (propylene oxide) was needed to get a same molar substitution (MS) when potassium citrate was used. Hydroxypropylated starches were fractionated on a size-exclusion column to separate amylose from amylopectin, and MS values of the whole starch, the amylose, and the amylopectin were determined. In all preparations, amylose was derivatized to a greater extent than was amylopectin. The data indicate that, with common corn starch: (1) the greater the overall derivatization, the greater was the preference for derivatization of amylose; and (2) the preference for amylose derivatization was greater for corn starch modified in potassium citrate solution than in sodium sulfate solution when the MS values for the two preparations were essentially the same. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Starches are often modified to provide better functionalities. Native starches generally produce weak-bodied, cohesive gels or rubbery pastes with a tendency to retrograde. Modification (chemical and/or genetic) is used to reduce negative characteristics and enhance positive ones. It was previously found that, in a commercial hydroxypropylated potato starch, amylose was modified to a greater extent than was amylopectin, presumably because amylose is located in accessible amorphous regions (Kavitha & BeMiller, 1998).

Salt is needed in some chemical modifications of granular starch to protect granules from gelatinization under conditions of high pH and elevated temperature. Sodium sulfate is most often used for this purpose because it provides a good balance between protection and reaction efficiency; but sulfate, which can be converted to hydrogen sulfide by anaerobic reduction during wastewater treatment, can have an undesirable effect on the environment. Villwock (1996) studied the mechanisms of inhibition by salts of starch gelatinization under alkaline conditions, and the effect of the salts on reaction efficiency of hydroxypropylation of common corn starch. It was found that citrate salts were extremely effective in protecting starch granules from swelling, but at the expense of reaction efficiency. Although the lower reaction efficiency is a negative factor with respect to citrate salts, their biodegradability is advantageous when environment is a concern. In addition, use of citrate salts may provide a way to modify the pattern of substitution with regards to preference for derivatization of the two components of starch: amylose and amylopectin.

This project was undertaken: (1) to determine relative amounts of derivatization of amylose and amylopectin upon hydroxypropylation of common corn starch (hereafter referred to as corn starch); and (2) to determine whether, when starch granules are modified in different salt solutions resulting in different degrees of swelling, the availability of amylose and amylopectin for modification is affected. The latter aspect is important because one way to produce products with different characteristics while maintaining M.S. values within allowable limits is to control reaction sites within starch granules (BeMiller, 1997).

2. Materials and methods

2.1. Materials

Common corn starch was obtained from A.E. Staley Mfg. Co. (Decatur, IL, USA). Potassium citrate was obtained from Sigma Chemical Co. (St. Louis, MO,

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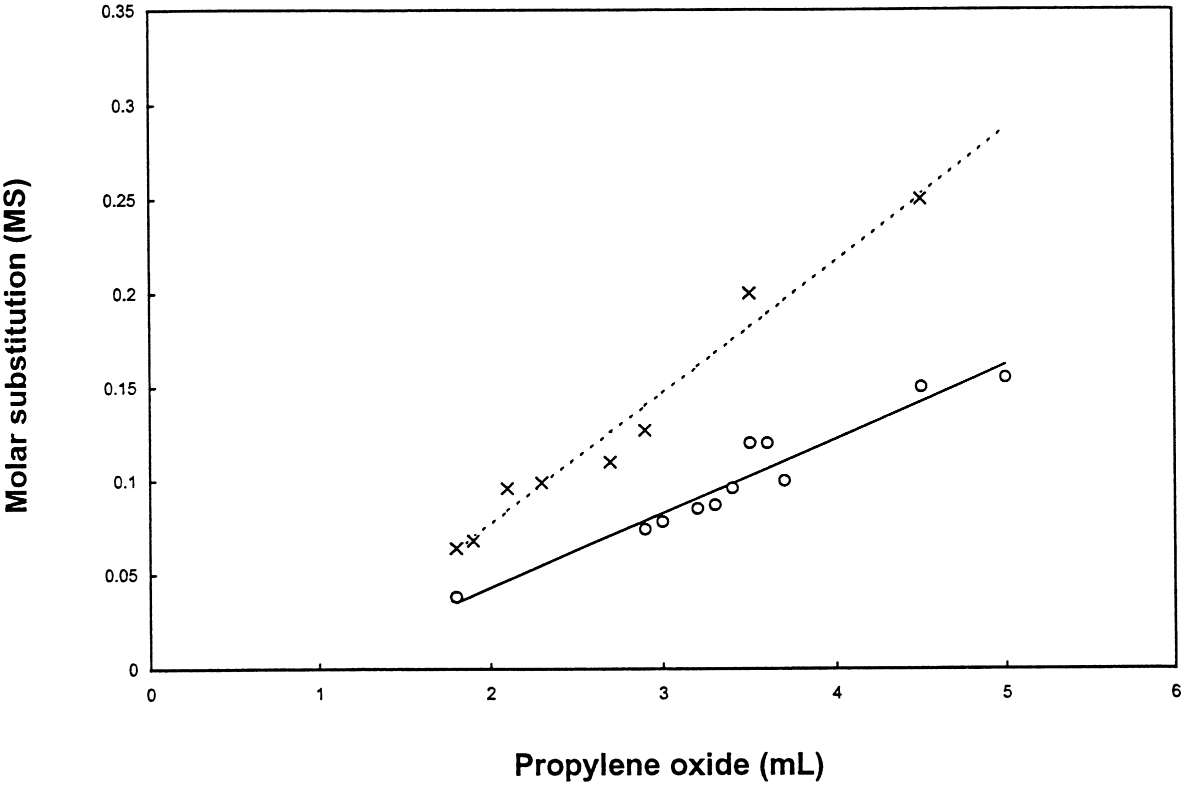

Fig. 1. Reaction efficiencies in the presence of 0.56 M sodium sulfate (crosses) and 0.31 M potassium citrate (circles).

USA). Sodium hydroxide and hydrochloric acid were obtained from Mallinckrodt, Inc. (Paris, KY, USA). Sodium sulfate was obtained from J.T. Baker Chemical Co. (Philipsburg, NJ, USA). Deuterium oxide was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Trifluoroacetic acid and dimethyl sulfoxide (DMSO) were obtained from Fisher Scientific Co. (Fair Lawn, NJ, USA). Sepharose CL-2B was obtained from Pharmacia Biotech (Piscataway, NJ, USA).

2.2. Modification (Villwock, 1996)

In a 125-ml Erlenmeyer flask, 33.3 g of starch was dispersed in 50 ml of distilled water, and the mixture was stirred with a Teflon stir bar. Potassium citrate (5.18 g) or sodium sulfate (4.30 g) was added. After the salt was dissolved, 4.1 ml of 7% (1.75 M) sodium hydroxide solution was added dropwise with fast agitation. The Erlenmeyer flask was sealed with a rubber septum secured with Parafilm (American National Can Co., Neenah, WI, USA). Propylene oxide (different amounts from 1.8 to 5.0 ml) was injected into the flask, which then was placed in a water bath at 49°C for 24 h while stirring, after which the slurry was neutralized with dilute hydrochloric acid and filtered. The modified starch was washed 3 times with water and air dried.

2.3. Molar substitution (MS)

Modified starch (about 0.1 g) was placed in a 500-ml round-bottom flask fitted with a condenser, and 50 ml of 1.5 M trifluoroacetic acid (TFA) solution was added. The flask was heated at 80°C with stirring. After 6 h, TFA was removed by evaporation under reduced pressure at 70°C, and any residual TFA was removed by placing the flask in a desiccator over sodium hydroxide pellets at room temperature for 1 day. The flask was rinsed with about 10–15 ml of distilled water, and the hydrolyzate was freeze-dried. The hydrolyzate (20 mg) was dissolved in 0.5 ml of deuterium oxide, and the solution was again freeze-dried. The deuterium oxide exchange was repeated once. The deuterated sample was dissolved in 1 ml of deuterium oxide and analyzed by proton NMR (Inova 300 MHz NMR). Integration at 1.2 ppm (a) and integration of peaks between 3.1 to 4.1 ppm (b) were used in the calculation of MS: $\text{MS} = (a/3)/[(b - 3a/3)/6] = 2a/(b - a)$ (Villwock, 1996).

2.4. Fractionation of starches

The three hydroxypropylated starches (I and III, modified in the presence of sodium sulfate; II, modified in the presence of potassium citrate) were fractionated using size-exclusion chromatography (SEC) as described by Kavitha and BeMiller (1998). Starch (70 mg) was dissolved in 7 ml of 90% (v/v) DMSO by stirring in a 96°C water bath for 1 h and then at room temperature for 24 h. Ethanol (100%, 42 ml) was added with stirring. After centrifugation and washing twice with 70% ethanol, the precipitated starch

Table 1
MS values of modified starches and their fractions

Preparation	Salt	Whole starch, MS	Amylose (Am), MS	Amylopectin (Ap), MS	(Am − Ap)/Ap × 100 (%)
I	Na_2SO_4	0.096	0.109	0.090	21
II	K_3citrate	0.099	0.121	0.096	26
III	Na_2SO_4	0.186	0.225	0.171	32

was redissolved in 5 ml of distilled water by heating in a boiling water bath for 20 min. After filtration through a 0.45-μm filter, the solution was applied to a Sepharose CL-2B column (3.0×90 cm^2) and eluted with degassed 0.02% sodium azide solution at a flow rate of about 20 ml/h. Each fraction (7 ml) was tested by adding 0.1 ml of iodine solution (2 g I_2 and 20 g KI per liter) to 0.5 ml aliquants. Amylose (blue in iodine test) and amylopectin (red-brown in iodine test) fractions were pooled, dialyzed, and freeze-dried. Column chromatography was repeated to get sufficient amounts of amylose and amylopectin for NMR analysis. For starches I and II, the MS values of amylose, amylopectin and starch were measured directly in triplicate from their NMR integrals.

For starch III, the MS values for amylopectin and total starch were also obtained directly from their NMR spectra, but the MS of amylose was calculated using the equation

$$0.72\ (\text{MS of amylopectin}) + 0.28\ (\text{MS of amylose})$$

$$= \text{MS of starch (in decimal form)}$$

This indirect calculation was used because, as a result of the amylose of starch III having a higher MS (0.225), some derivatizing groups were oligomers of propylene glycol, and the chemical shifts of the methyl groups vicinal to the ether linkage of the chained hydroxypropyl units were shifted sufficiently downfield towards those of the sugar ring protons as to interfere with their integration.

2.5. RVA analysis

The pasting and set-back of modified and unmodified corn starches were evaluated with a Rapid Visco-Analyzer (RVA) (Model RVA-4, Newport Scientific, Warriewood, Australia) using the standard profile I. A 13-min analysis was used: equilibration to 50°C for 1 min, heating to 95°C in 3 min-42 s, holding at 95°C for 2 min-30 s, and cooling to 50°C in 3 min-48 s, holding at 50°C for 2 min. Modified starches were passed through a 250-μm sieve and conditioned at 43% relative humidity for one week before testing. Unmodified common corn starch used as the control was treated in the same way. Starch (2.5 g) was suspended in distilled water (sample + water = 28 g) in an aluminum sample container and stirred manually for 20–30 s before inserting into the RVA. Analyses were done in duplicate.

3. Results and discussion

MS values as a function of the amount of propylene oxide added for modification of corn starch in the presence of sodium sulfate and potassium citrate are shown in Fig. 1. From regression lines, it was calculated that about 1.8 times the amount of propylene oxide was needed to get the same MS when potassium citrate was used in place of sodium sulfate. In this work, both the cation and anion concentrations of sodium sulfate (1.12 and 0.56 M, respectively, approximate industrial level) were higher than those of potassium citrate (0.93 and 0.31 M, respectively); still the strong lyotropic effect of citrate ions retarded starch granules from swelling more effectively than the higher concentration of sulfate ions (on the assumption that reaction efficiency is a function of granule swelling [Hauber, BeMiller & Fannon (1992)]); therefore, more propylene oxide was needed in potassium citrate solution to get the same level of modification, a finding consistent with that reported by Villwock (1996).

From the results reported in Table 1, it appears that, when the total modification levels were almost same (0.099 vs. 0.096), a greater percentage of amylose was modified when potassium citrate was used than when sodium sulfate was used, i.e. in the presence of potassium citrate, derivatization of amylose was ca. 26% greater than was derivatization of amylopectin, while in the presence of sodium sulfate, derivatization of amylose was ca. 21% greater than was derivatization of amylopectin. This result indicates that, when starch granules were modified in an alkaline citrate solution (less swollen), the difference in the availability for derivatization of amylose and amylopectin became more pronounced, i.e. there was a greater preference for derivatization of amylose. This observation is significant because hydroxyalkylation of amylose is more important than hydroxyalkylation of amylopectin as the goal is a reduced tendency to retrograde.

Comparison of the MS values for preparation III (modified in sodium sulfate solution) with those of preparation I (also modified in sodium sulfate solution) (Table 1) indicates that, when modifications were done in the same salt solution but to a higher level (by adding more reagent), there is an even greater preference for derivatization of amylose, for the preference for amylose derivatization increased from 21% more to 32% more. This phenomenon may be the result of derivatization opening up the areas containing amylose even more than they were

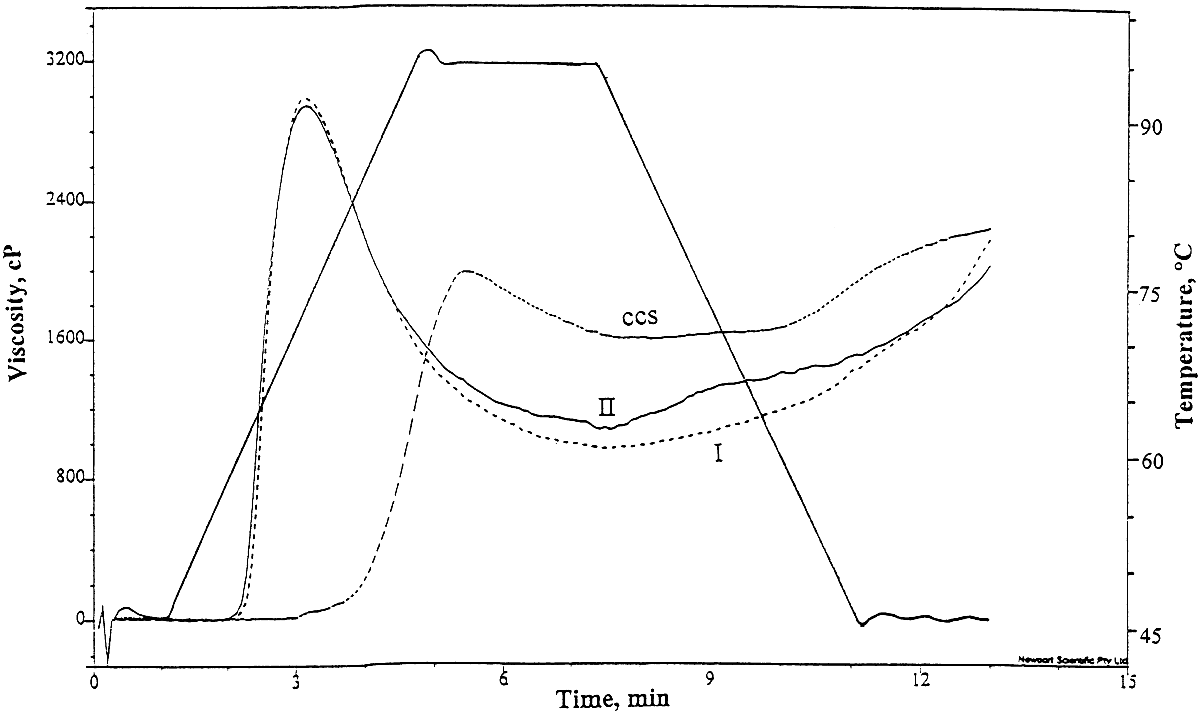

Fig. 2. RVA profiles for native and hydroxypropylated common corn starch: unmodified (ccs) and modified in the presence of sodium sulfate (I) and potassium citrate (II).

before substitution, making amylose even more available for reaction.

The pasting and setback properties (RVA profiles) of preparations I and II are given in Fig. 2. The pasting behavior of the two modified starches were almost the same. However, their was a very slight difference in the holding at 95°C and cooling phases, with the starch modified in the presence of citrate ions showing less setback, a result congruent with its higher amylose MS.

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