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Effects of the order of addition of reagents and catalyst on modification of maize starches

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ABSTRACT

The objective of this research was to determine if adding reactive reagents to starch granules before addition of alkali (TRF method) would produce products that are different than those obtained by adding alkali before addition of reagent. Normal (NMS) and waxy (WMS) maize starches were each reacted with acetic-adipic mixed anhydride (AAMA), phosphoryl chloride ($POCl_3$), sodium trimetaphosphate (STMP), acetic anhydride (AA), succinic anhydride (SA), and octenylsuccinic anhydride (OSA). Almost no or no starch polymer molecule modification occurred when the TRF method and AAMA, AA, or $POCl_3$ were used; less than half as much reaction when SA was the reagent used, and about the same amount of reaction when STMP or OSA were the reagents used (for different reasons). It was concluded that most AAMA, AA, SA, and $POCl_3$ reacted with surface protein molecules when the TRF method was used and that OSA molecules were driven into the structured internal water of granules.

1. Introduction

The universal procedure for modifying a starch using crosslinking or stabilization reactions is to add a base to a starch-in-water slurry to raise its pH to the desired level, followed by addition of the derivatizing reagent. Phosphoryl chloride, a very reactive and rapidly reacting reagent, is known to react with the first starch molecules it encounters, i.e., the molecules at granule surfaces (Huber & BeMiller, 2001; Gray & BeMiller, 2004). It can be assumed that other rather reactive reagents would react in a similar manner. We therefore wondered whether allowing reagents, especially quite reactive reagents that are likely to react with the first ionized starch hydroxyl groups they encounter, to penetrate more evenly throughout granules before adjusting the pH to an alkaline value might result in more efficient reactions or modified starches with the substituent groups more evenly distributed throughout granules and, therefore, with different properties.

2. Materials and methods

2.1. Materials

Commercial normal and waxy maize starch and normal and waxy maize kernels were donated by Tate & Lyle, NA (Decatur, IL USA). Acetic anhydride, succinic anhydride, 2-octen-1-ylsuccinic anhydride, phosphoryl chloride, adipic acid, sodium trimetaphosphate, and thermolysin (from *Baccillus thermoproteolyticus*, p1512) were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Laboratory-isolated starch

Starch was isolated from whole maize kernels using the method described by Wongsagonsup, Varavinit, and BeMiller (2008) and Sui, Shah, and BeMiller (2011), which is a standard laboratory procedure for isolation of maize starch involving steeping whole maize kernels in a solution of sodium bisulfite/sulfurous acid, grinding the softened kernels, sieving the resulting mixture, mixing with 70% ethanol, and centrifugation to remove remaining protein.

2.2.2. Standard/conventional reaction (adding alkali before addition of reagent)

Starch was dispersed in reverse-osmosis and distilled (ROD) water with stirring at room temperature. The starch slurry was adjusted to the specified pH value with 1 M NaOH. Reagent was added with vigorous stirring. During reaction, the pH was maintained using an auto-titrator (TIM 854, Radiometer, Brønshøi, Denmark). After reaction, the reaction mixture was neutralized with dilute HCl. The modified starch was collected by centrifugation, washed three times with ROD water and once with 100% ethanol, and air dried.

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2.2.3. Alternative reaction (adding alkali after impregnating granules with reagent)

Starch was dispersed in ROD water with stirring at room temperature. Reagent was added to the starch slurry with vigorous stirring, which was maintained for 3 h. Then, the starch slurry was adjusted to the specific pH value with 1 M NaOH. The pH was maintained at the same value as for the standard reaction via use of an auto-titrator. The time of reaction (after adjustment of the system pH) was also the same. After reaction, the reaction mixture was neutralized with dilute HCl. The modified starch was collected by centrifugation, washed three times with ROD water and once with 100% ethanol, and air dried.

2.2.4. Incubation of starch with thermolysin

The two commercial starches were treated with thermolysin (Sigma–Aldrich Corp., St. Louis, MO, USA) for 30 min at 23 °C as described by Mu-Forster and Wasserman (1998) to remove surface protein.

2.2.5. Crosslinking with phosphoryl chloride ($POCl_3$)

Starch (20.0 g, db) was dispersed in ROD water (40 mL) with stirring at rt. The pH of the slurry was adjusted to 11.3 with 1 M NaOH either before or after adding phosphoryl chloride (0.01% of the weight of starch added slowly and dropwise as a 3% solution in 1,4-dioxane). The reaction was maintained at pH 11.3 for 1 h using an auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with 40 mL of ROD water and once with 100% ethanol, and air-dried.

2.2.6. Crosslinking with sodium trimetaphosphate (STMP)

STMP (0.040 g) and sodium sulfate (1.00 g) were dissolved in 5 mL of ROD water. Starch (20.0 g, db) was dispersed in ROD water (35 mL) with stirring at rt. The pH of the slurry was adjusted to 10.0 with 1 M NaOH either before or after adding the sodium trimetaphosphate-sodium sulfate solution dropwise. The reaction mixture was maintained at pH 10.0 for 1 h at room temperature using an auto-titrator. The dispersion was put in a Petri dish and dried at 40 °C until the moisture content was ~10%. The modified starch was then heated at 130 °C for 2 h. After cooling to room temperature, the starch was dispersed in 40 mL of ROD water. The reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with 40 mL of ROD water, and dried at 40 °C overnight.

2.2.7. Modification with acetic-adipic mixed anhydride (AAMA)

After adipic acid (0.20 g) was clearly dissolved in acetic anhydride (5.6 mL), the acetic-adipic mixed anhydride (AAMA) reagent was diluted with 28 mL of 1,4-dioxane. Starch (25.0 g, db) was dispersed in ROD water (46 mL) with stirring at rt. The pH of the slurry was adjusted to 9.0 with 1 M NaOH either before or after adding the diluted acetic–adipic mixed anhydride solution (6.1 mL) dropwise. The reaction was maintained at pH 9.0 for 2 h using an auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.0 with dilute HCl. The modified starch was collected by centrifugation, washed three times with 40 mL of ROD water and once with 100% ethanol, and air dried. The dried preparations were stored in a refrigerator.

2.2.8. Esterification with acetic anhydride (AA)

Starch (20.0 g, db) was dispersed in ROD water (50 mL) with stirring at room temperarure. The pH of the slurry was adjusted to 8.2 with 1 M NaOH either before or after adding acetic anhydride (AA) (1.7 mL) dropwise. The reaction was maintained at pH 8.2 for 6 h using an auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with 40 mL of ROD water and once with 100% ethanol, and air-dried.

2.2.9. Esterification with succinic anhydride (SA)

Starch (20.0 g, db) was dispersed in distilled water (45 mL) with stirring at rt. The pH of the slurry was adjusted to 8.5 with 1 M NaOH either before or after adding succinic anhydride (SA) (0.80 g) dropwise. The reaction was maintained at pH 8.5 for 6 h using an auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with 40 mL of ROD water and once with 100% ethanol, and air-dried.

2.2.10. Esterification with octenylsuccinic anhydride (OSA)

Starch (20.0 g, db) was dispersed in ROD water (45 mL) with stirring at rt. The pH of the slurry was adjusted to 8.5 with 1 M NaOH either before or after adding 2-octen-1-ylsuccinic anhydride (OSA) (0.6 mL) dropwise. The reaction was maintained at pH 8.5 for 6 h using an auto-titrator. After reaction, the reaction mixture was neutralized to pH 6.5 with dilute HCl. The modified starch was collected by centrifugation, washed three times with 40 mL of ROD water and once with 100% ethanol, and air-dried.

2.2.11. Pasting characteristics

Pasting characteristics of the modified starches were determined with a Rapid Visco-Analyzer (Model 4, Newport Scientific, Warriewood, Australia) using standard profile 1. Modified starch (1.96 g, db) and distilled water (26.04 g) (sample + water = 28.00 g) (7.0% starch; OSA products were measured at 5.0% starch) were mixed and stirred within the analyzer's aluminum sample canister. A 13-min analysis was used with a programmed heating and cooling cycle. The sample was heated to and held at 50 °C for 1 min; the temperature was raised to 95 °C within 3.7 min and held there for 2.5 min, then cooled to 50 °C within 3.8 min, and finally held there a further 2 min. Triplicate analyses were performed on each sample, and the results were averaged.

2.2.12. Differential scanning calorimetry (DSC)

Thermal properties of starch samples were investigated with a differential scanning calorimeter (DSC; TA 2910, TA Instruments, Wilmington, DE, USA). The starch sample (5.0 mg, db) was placed in a pan; ROD water was added to a total of 20.0 mg (starch to water ratio = 1:3, w/w). The pan was sealed and equilibrated at room temperature for at least 2 h before the scan. Samples were heated from 20 °C to 120 °C at a heating rate of 10 °C/min. A sealed empty pan was used as a reference, and indium was used as a calibration standard. Triplicate analyses were performed on each sample, and the results were averaged.

After being stored 7 days at 4 °C, gelatinized samples were rescanned from 20 °C to 140 °C at a heating rate of 10 °C/min. Thermal transitions for gelatinization and retrogradation were characterized by T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature) and ΔH (enthalpy). Triplicate analyses were performed on each sample, and the results were averaged.

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

Starch samples (0.6 g) were shaken in 0.25 M $AgNO_3$ solution in the dark for 24 h at room temperature, then recovered by centrifugation. The exchange procedure was repeated twice more. Silver ion-exchanged starch pellets were washed with 85% (v/v) aqueous ethanol for 30 min to remove excess $AgNO_3$. The washing procedure was repeated twice more. The starch pellet was recovered by vacuum filtration. Starch granules were prepared for R-CLSM analysis by dusting a small amount of Ag^+-exchanged starch granules on a microscope slide. Slides were exposed to a UV light source for >24 h to fully reduce silver ions to silver atoms. Otherwise, samples were