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Effect of reaction solvents on the multi-scale structure of potato starch during acid treatment

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ABSTRACT

Potato starch was treated with 0.36% HCl in ethanol and water for various time periods, and its structural changes were evaluated and compared in this study. Acid-ethanol treated starch (AET-s) had relatively low average molecular weight (M_w) and z-average radius of gyration (R_g), and its solubility was higher than that of the counterpart acid-water treated starch (AWT-s). The granular appearance and differential scanning calorimetry (DSC) profile demonstrated that acid in ethanol and in water exhibited different attack pathways on the granules. No significant difference in crystallinity was observed for AET-s; however, the ratio of absorbance 1022/995 cm^{-1} and the peak intensity detected by small-angle X-ray scattering (SAXS) were increased with increasing treatment time. These results suggested that ethanol-acid treatment simultaneously attacked on the amorphous and crystalline regions, and the degradation extent on crystalline regions caused by ethanol-acid treatment was higher than that observed by acid-water treatment.

1. Introduction

Acid treatment has been used to modify starch granule structure and produce "soluble starch" or glucose syrup for many years. The acid-modified starches are normally prepared by treating starch granules with dilute aqueous solution of hydrochloric or sulphuric acid at 25–55 °C for various time periods [1,2]. The molecular weight and viscosity of acid-modified starches vary with the different hydrolysis conditions used; however, their yields decrease consistently with increasing treatment time and acid concentration. Alcohol-acid treatment that involves suspending starch granule in alcoholic solution of mineral acid has recently been attracted considerable attention because it can yield high recovery (ranging from 100% to 80%) of soluble starch by using little amount of acid [3,4]. Studies have shown that the molecular weight distribution of these acid-alcohol modified starches is narrower and more homogenous than that of native starch. Moreover, their average degree of polymerization (DP) exhibits a limiting value which progressively decreases in the following order: methanol > ethanol > 2-propanol > 1-butanol [5,6]. It also shows that the limiting DP values significantly decrease as the ratio of higher alcohol increases when treated in the mixture of the above alcohols [7]. Therefore, it is proposed that the susceptibility of starch granules to acid is affected by the alcoholic solution.

Starch is a plant polysaccharide that occurs naturally in the form of insoluble, semi-crystalline granules. Each granule has a layered organization with alternating amorphous and semicrystalline growth rings of similar 120–400 nm thickness [8]. The semicrystalline growth rings are characterized by alternating crystalline and amorphous lamellae with a repeat period of 9–11 nm [9,10]. On the molecular scale, starch granules are composed of two glucose polymers: highly branched amylopectin and linear amylose. The branches of amylopectin within the granules are often arranged as double helices (A-type or B-type) and located in the crystalline lamellae [11], whereas the amorphous lamellae mainly contain amylose, branch points and chains not organized as double helices. When treated with acid in water, starch granules exhibit two distinct hydrolysis stages: the first stage corresponds to the relatively fast hydrolysis of amorphous regions; the second stage is attributed to the slow hydrolysis of the crystalline lamellae. Starch granules obtained from various botanical origins show different granule morphologies (sizes and shapes), amylose contents and amylopectin architectures (branch chain length distribution and

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placement of branches), as well as the crystalline structure, which can also influence their susceptibility to acid treatment [12–14].

Potato starch, having B-type crystalline structure, is generally considered to be more acid-resistant than maize starch in water [13,15,16]. In a previous study, Lin et al. found that potato starch under acid-methanol treatment showed higher degradation rate than that of maize starch, both in amylose and amylopectin, which was explained to be associated with the reaction solvent during acid treatment [3]. However, there is a dearth of information on the effect of reaction solvent (alcohols) on the multi-scale structure of starch, particularly the crystalline structure. Moreover, it is not well understood why starch treated by acid in alcohol has a distinct two-stage molecular degradation pattern, which are the initial slow degradation rate followed by a faster rate [17,18]. Thus, the objective of this work is to compare the structure changes (including short- and long- range order) of starch granules caused by acid hydrolysis in alcoholic and aqueous solution and to understand the roles of reaction solvents on the degradation pattern of starch granules. To this end, starch samples, subjected to acid treatment in ethanol, have been characterized using high-performance size-exclusion chromatography (HPSEC), scanning electron microscopy (SEM), fourier transform infrared spectrometry (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), and small-angle X-ray scattering (SAXS). Control samples treated by acid in water were used for comparison purpose. We used ethanol as the representative of alcohols since it had wide application in food industry. The results also could be usefully applied to the production of microcrystalline starch or starch nanocrystals for food industry.

2. Materials and methods

2.1. Materials

Potato starch, anhydrous ethanol (<0.03% water), and sodium bicarbonate ($NaHCO_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and Dimethyl Sulfoxide (DMSO) was purchased from Aladdin Reagents Co., Ltd. (Shanghai, China). All other chemicals and reagents used in the study were of analytical grade.

2.2. Preparation of acid-treated starches

Starch (10 g) was suspended in 40 mL anhydrous ethanol or water, followed by addition of 0.4 mL 36% (w/w) HCl, and kept at 50 °C for different periods (4, 8, 12, 24, 48, 72, and 96 h) with constant stirring. Starch suspension was then naturalized with 5.6 mL $NaHCO_3$ (1 mol/L), cooled in an ice bath for 15 min, and centrifuged at $3500 \times g$ for 10 min. The precipitate was washed with 50% ethanol until NaCl was not detected by $AgNO_3$ solution (0.1 mol/L). The precipitate was dried in an air oven at 40 °C for 24 h.

2.3. Molecular weight analysis of acid-treated starches

The weight-average molecular weight of native and acid-treated starches was determined by HPSEC-MALLS-RI according to the procedure described previously with minor modifications [19]. Starch samples (25 mg, db) were dissolved in 5 mL 100% DMSO containing 50 mM $NaNO_3$ by stirring in a boiling water bath for 1 h, and then continuously stirring at 25 °C for 24 h. The solutions were filtered through a 0.45 μm filter (Millipore, Billerica, MA, USA), and 100 μL of the filtrate was injected into a high-performance liquid chromatography system (HPLC) consisting of a Shimadzu LC-20A pump (Shimadzu Scientific Instruments, Inc., Kyoto, Japan), a multi-angle laser light-scattering detector (MALLS; Dawn DSP, Wyatt Technologies, Santa Barbara, CA, USA), a Waters 2414 refractive-index detector (RI), and two columns (Styragel HMW7 and HMW6; Styragel, Waters, Milford, MA, USA) used in tandem at 40 °C. The 50 mM $NaNO_3$-DMSO (100%) solution was used as mobile phase at a low rate of 0.6 mL/min. The laser source used was He-Ne, $\lambda = 658$ nm with a K-5 flow cell. A dextran standard, T40 ($M_w = 4.19 \times 10^4$ g/mol) and T2000 ($M_w = 2.05 \times 10^6$ g/mol) was used to verify the accuracy of the instrument. Data obtained from the MALLS and RI detectors were analyzed by Astra software version 5.3.4 (Wyatt Technologies). The M_w and z-average radius of gyration (R_g) were calculated by the second-order Berry method. An RI of 1.479 and a dn/dc value of 0.066 were used in this calculation.

2.4. Solubility determination

Water solubility of the processed starches was determined according to the method of De Kerf et al. [20] with a minor modification. A dispersion of 500 mg starch sample in 10 mL water was placed in a test tube. After continuous stirring at 65 °C for 30 min, the mixture was centrifuged at $8000 \times g$ for 5 min, and then an aliquot of supernatant was evaporated overnight at 130 °C and weighed. The solubility was expressed as the percentage of the dried supernatant related to the initial weight of the dry starch. One point, which should be emphasized, was that 65 °C was selected for the determination of starch solubility according to the previous reference [3], since native starch granules were usually not soluble in cold water and the solubility of starch granules was determined at certain high temperature (60–100 °C).

2.5. Attenuated total reflectance (ATR)-FTIR

The infrared spectra were obtained using a Nicolet iS10 spectrometer (Thermo Scientific,Waltham, MA, USA) with a deuterated triglycine sulfate (DTGS) detector equipped with an attenuated total reflectance (ATR) single-reflectance cell. Each spectrum was recorded in the region of 900–1200 cm^{-1} with 4 cm^{-1} resolution by 64 scans. The spectrum of water recorded under the same conditions was obtained as the background. The absorbance ratios of 1047 cm^{-1}/1022 cm^{-1} and 1022 cm^{-1}/995 cm^{-1} were obtained from the deconvolution spectra with a half-width of 15 cm^{-1} and an enhancement factor of 2.1 with triangular apodization.

2.6. Differential scanning calorimetry (DSC)

Thermal analysis of native and acid-treated potato starches was performed by a DSC 7000 instrument (Seiko Instruments Inc., Chiba, Japan). The prepared sample of 3.0 mg and distilled water (6 μL) were combined in an aluminum pan, and the mixture was sealed and equilibrated at 4 °C for 24 h. The thermal behavior of the starch samples was studied by heating samples at a rate of 8 °C/min from 20 to 100 °C under ultrahigh-purity nitrogen atmosphere. An empty pan was used as a reference.

2.7. X-ray diffraction (XRD)

X-ray diffraction measurements of samples were performed with a Bruker D8-Advance XRD instrument (Bruker AXS Inc., Karlsruhe, Germany) equipped with Cu-Kα_1 radiation ($\lambda = 0.15405$ nm) produced in a sealed tube at 40 kV and 40 mA. The diffractograms were collected over a 2θ range of 3°–40° with a step of 4°/min and step size of 0.02°. The relative crystallinity of the starches were analyzed with the Jade 5.0 software (Materials Data Inc., Livermore, CA, USA) and quantitatively estimated according to the method of Nara et al. [21].