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Heat pretreatment improves the enzymatic hydrolysis of granular corn starch at high concentration

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ABSTRACT

Heat pretreatment was investigated as a way to improve the α-amylase-catalyzed hydrolysis of granular corn starch at high concentration (45%, w/w). Native and preheated starches were hydrolyzed in the granular state for 1 h at 40 °C using a commercially available α-amylase. The initial dextrose equivalent (DE) value of the native granular starch (8.0%) increased significantly (to 12.6%) after pretreatment at 60 °C for 15 min. Microscopic analysis of the starch granules indicated that heat pretreatment increased the number and size of the pores and pinholes on the granule surface, which facilitated the adsorption and subsequent penetration of enzyme molecules. Furthermore, the amylose present in amorphous regions leached rapidly when corn starch was incubated at 60 °C, suggesting weaker interactions within the granules. The DE value of the hydrolysate showed a linear dependence on the amount of amylose that leached during the course of heat pretreatment (R^2 = 0.9771). Mobilization of amylose chains within the granule, caused by the heat pretreatment, may have allowed the enzyme to make greater use of amylose, which is its primary substrate during starch granule hydrolysis. In addition, a 35% reduction in the K_m value showed that heat pretreatment increased the affinity of α-amylase for the starch granules. This can be attributed to the mobilization of amylose chains within the granule and expansion of the surface pinholes. These effects demonstrate that proper heat pretreatment of granular starch before amylolysis has great potential in industry.

1. Introduction

Corn starch is a relatively abundant raw material that is available at low cost. It can be processed into starch sugars, alcohol, organic acids, and other valuable commodities. Conventional processes for the production of glucose syrups and bioethanol typically include the acid or enzymatic hydrolysis of gelatinized starch, which involve starch liquefaction and saccharification [1]. Liquefaction is a key step in the starch hydrolysis process used to produce syrups, including those used in the industrial production of microbial fermentation products. Liquefaction is commonly achieved through the dispersion of insoluble starch granules in an aqueous solution, followed by partial hydrolysis at a relatively high temperature using thermostable α-amylases, which are endoglucanases that catalyze the hydrolysis of internal α-1,4-glycosidiclinkages [2].

Native starch slurries derived from conventional corn wet milling contain approximately 40–45% dry solids. They are generally diluted to 25–35% dry solids before they are heated above the liquefaction temperature [3,4]. Diluting the slurry increases the amount of water that must be evaporated from the final product, which increases processing costs and complicates the process layout [5]. The benefits of using more concentrated slurries are that maintaining a higher substrate concentration during the enzymatic hydrolysis can increase both productivity and enzyme stability [6]. Unfortunately, conventional jet cookers cannot be used at higher substrate concentrations due to the exponentially increased viscosity of the slurry. Therefore, different processes are needed to handle more concentrated starch slurries. Several authors have used extruders [6], high-speed shearing devices [5] and ultrasonic pretreatment [7] to process more concentrated starch slurries. Alternatively, low-temperature processes, in which granular starch is enzymatically hydrolyzed below the gelatinization temperature, have also been described [8–10]. These low-temperature systems have been reported to be able to process higher concentrations of dry solids (up to 45%) [11]. After sufficient partial hydrolysis and

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granular starch dissolution has occurred, the temperature is either raised and held above the gelatinization temperature to complete the liquefaction process, or kept at the low-temperature to convert uncooked starch to glucose and other sugars.

This low-temperature process could reduce the energy requirement [12] and handle high-viscosity starch slurries during cooking and gelatinization. However, starch, in its native form, has limited industrial utility. Starch macromolecules can be hydrolyzed in the granular state, but attempts to hydrolyze native granules invariably result in a slowly and often poorly hydrolyzed product [13]. Granular starch amylolysis systems require relatively long incubation times, which are associated with high energy costs. That is because the enzymatic hydrolysis of granular starch is generally performed as a two-phase (solid-solution) reaction in which the enzymes must diffuse toward the solid substrate, bind to it, and then cleave its glycosidic linkages [14]. Physical and chemical modifications are commonly used to produce starches with special properties. The physical modification of starch using moisture, heat, shear, or radiation has gained wider acceptance because these processes do not produce chemical waste. Heat treatment is a common method of physical modification that changes the physicochemical properties of starch. Oates [13] suggested that the hydrolysis of native starch can be improved by increasing the incubation temperature to approximately 60 °C. Because of the large scale on which granular starch is processed, even seemingly small improvements in efficiency can produce great economic advantages. Further increases in the degree of native starch conversion would be very useful in the industrial production of bioethanol and starch sugars from high-concentration starch slurries.

Granular starch amylolysis systems require one or more enzymes with granular starch hydrolyzing activity, known as granular starch hydrolyzing enzymes. These enzymes may include an α-amylase and/or a glucoamylase, between which the action of α-amylase does is not effectively decrease the resistance of native starch granules [15]. In other words, the endolytic activity of α-amylase is a main restriction on granular starch amylolysis. Furthermore, if conversion to maltodextrins is desired, it is preferably that no enzyme other than an α-amylase be used. Thus, the objective of this study was to determine the effects of heat pretreatment on the susceptibility of granular corn starch, the most commonly used starch material in industry, to enzymatic hydrolysis by α-amylase at high concentration (45%, w/w).

2. Materials and methods

2.1. Materials

Normal corn starch containing 12.3% moisture was obtained from Hebei Yufeng Industry Group Co., Ltd (Hebei, China). The α-amylase (Cleatflow AA) was obtained from Genencor International (28,000 U/mL; Palo Alto, CA, USA). Isoamylase was purchased from Sigma Chemical Co. (500,000 U/mL; St. Louis, MO USA). Soluble starch, dinitrosalicylic acid (DNS), hydrochloric acid, sodium hydroxide, citric acid, disodium hydrogen phosphate, glycerin, iodine, and potassium iodide were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

2.2. α-Amylase activity assay

α-Amylase activity was determined following by the procedure of Z. Li et al. [16]. One unit of a-amylase activity was defined as the amount of enzyme that caused a 10% reduction in the starch-iodine color under the assay conditions.

2.3. Heat pretreatment and starch amylolysis

Corn starch slurries (45%, dry basis, w/w) were preheated for 15 min, with constant shaking, in a water bath at 25 °C, 50 °C, 60 °C, 62.5 °C, or 65 °C. The starch slurry was then dried at 40 °C for 6 h. Native corn starch without heat pretreatment was used as a control.

α-Amylase (500 U/g of dry starch) was added to the starch slurry with or without heat pretreatment. The samples were incubated in an incubator shaker (160 rpm) at 40 °C. After 1 h, the hydrolysis was stopped by adjusting the pH of each slurry to 1.5–1.6 with 1 M HCl. After centrifugation at 2400*g* for 15 min, the concentration of reducing sugars in the supernatant was determined. Then, the pH of each starch suspension was adjusted back to pH 6–7 by washing the starch with distilled water. The starch residues were dried at 40 °C for 6 h and used for further characterization by light microscopy, scanning electron microscopy and X-ray diffraction.

In separate experiments, corn starch slurries preheated at 60 °C for 1, 3, 5, 7.5, 10, 15, 20, 30 or 60 min were hydrolyzed with α-amylase using the same procedure. Samples of native corn starch hydrolyzed without heat pretreatment are reported as having been preheated for 0 min.

2.4. Dextrose equivalence of the starch hydrolysate

The reducing sugar content of the starch hydrolysate was determined using the DNS method [17]. The degree of amylolysis is expressed as a dextrose equivalent (DE), which is defined as the total reducing sugars expressed as dextrose and calculated as a percentage of the dry substance.

2.5. Light microscopic analysis

Native, preheated, and hydrolyzed starches were diluted with 50% (v/v) glycerin solution and promptly examined using an optical microscope (Model BX51,Olympus Co., Japan) fitted with a polarized light filter and a digital camera. Observations were conducted under normal visible light and cross-polarized light using a 40 × objective.

2.6. Scanning electron microscopic analysis

Native, preheated and hydrolyzed starch samples were coated with gold-palladium by using a sputter coater (Denton Vacuum, LLC, Moorestown, NJ), and then viewed at 2400× and 5000× magnification with a scanning electron microscope (SEM) (S-3800N, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV.

2.7. Differential scanning calorimetric analysis

The thermal properties of samples preheated for 15 min at 25 °C, 50 °C, or 60 °C were determined from differential scanning calorimeter (DSC) curves obtained using a Pyris 1-DSC (PerkinElmer Corp., Norwalk, CT, USA). Native corn starch that did not undergo heat pretreatment was used as a control. Each starch sample (2 mg, dry basis) was placed in an aluminium DSC pan, and then deionized water was added to each sample to achieve a solid-water ratio of 1:2 (w/w). The sample pans were hermetically sealed, then equilibrated at 4 °C for 24 h. This equilibration resulted in an even distribution of water without microbial fermentation, making the starch slurries more uniform. Scans were performed from 40 °C to 90 °C at a constant rate of 5 °C/min. Enthalpy changes (ΔH) evaluated based on the area of the main endothermic peak were expressed in terms of J/g of dry starch. A sealed, empty crucible was used as a reference.

2.8. X-ray diffraction analysis

X-ray diffractograms of native, preheated and hydrolyzed starch powders were obtained with a copper anode X-ray tube using a Rigaku D-Max-2200 X-ray diffractometer (Rigaku Denki Co. Tokyo, Japan). X-ray diffraction patterns were acquired at room temperature over the 2θ