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An improved two-step saccharification of high-concentration corn starch slurries by granular starch hydrolyzing enzyme

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ABSTRACT

Starch saccharification is a key step in the industrial production of syrups. A two-step saccharification of raw corn starch at high concentration (45%, w/w) using granular starch hydrolyzing enzyme (GSHE) was investigated to improve the efficiency of this process. The results showed that the two-step saccarification (reaction at 65 °C for 30 min, followed by reaction at 70 °C for 90 min) was more effective than a single-step saccarification involving reaction at 65 °C for 120 min. After saccharification, both the glucose content and dextrose equivalent (DE) value were 10% greater using the two-step saccharification than using a single-step saccharification. Assessing the mechanism indicated that the reduction in peak viscosity of the starch slurry was more obvious using the two-step saccharification, the water activity was higher, and the mobility of water was stronger. Thus, the two-step saccharification offers great advantages in the production of syrups using granular starch hydrolyzing enzyme.

1. Introduction

The enzymatic hydrolysis of starch into sugars is an important industrial process that consists of three steps: gelatinization, liquefaction and saccharification (Myat and Ryu, 2014). During the industrial production of syrups, thermostable α-amylase is typically added to starch slurries (about 30–35% w/v) at temperatures between 90 and 165 °C. These slurries are held at 90 °C for 1–3 h, and then cooled to 60 °C with the addition of glucoamylase (Robertson et al., 2006). The temperature requirements of the conventional method are a considerable economic drawback; the conventional process uses a large amount of energy during the liquefaction steps (Gibreel et al., 2009). Furthermore, heating starch slurries with relatively low solid contents would increase amount of energy required to evaporate the excess water when concentrating the mash after subsequent saccharification (Li et al., 2015). Thus, one of the most effective ways conduct the enzymatic hydrolysis of starch into sugars is to increase the initial concentration of starch slurry, thereby reducing the moisture content of the slurry.

The gelatinization of the starch, generally the first step in the production of syrups, is accompanied by swelling and disruption of the starch granules and melting of their crystalline structure (Mandala and Bayas, 2004). Substantial effort has been devoted to the optimization of starch hydrolysis, such as the simultaneous use of two enzymes (Karakatsanis et al., 1997; Liakopoulou-Kyriakides et al., 2001); the use of starch pretreatments, such as mechanical activation (Chen et al., 2010; Huang et al., 2007); the use of ultrasound (Barton et al., 1996; Nitayavardhana et al., 2008); and the use of microwaves (Lewandowicz et al., 1997). Because of the processes related to starch gelatinization, it is very hard to hydrolyze starch at high concentration. However, increasing the concentration of the initial starch slurry would increase productivity (Baks et al., 2008; De Cordt et al., 1994). There is a need to improve this step so that it makes the processes of syrup production more efficient.

An alternative process, in which granular starch hydrolyzing enzyme (GSHE) is applied to convert granular or uncooked starch to glucose and other sugars, has been suggested (Bialas et al., 2010). With the view of reducing the energy requirement, there is currently considerable research on the use of GSHE (Sun et al., 2007). The GSHE is used commercially in a simultaneous saccharification and fermentation process for ethanol production (Wang et al., 2005, 2007). However, the GSHE is rarely applied in the starch-sugar industries.

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Since the GSHE is beneficial for handling high viscosity starch slurries, processes involving the GSHE are used to produce starch sugars from high-concentration starch slurries. Furthermore, slight granule swelling increases the specific area of the granule and opens up the granule surface, which is beneficial for enzymatic hydrolysis, and starch granules swell with increasing temperature. However, if starch granules swell excessively, enzymatic hydrolysis is suppressed. In the present study, the saccharification of high-concentration starch slurries by the GSHE was examined, and a two-step saccharification process was devised to improve saccharification efficiency. The relative saccharification mechanisms were also analyzed. The results could provide a deeper understanding of the impact of this two-step procedure on the enzyme hydrolysis process, and provide a strategy and theoretical basis for the production of starch sugar at high concentration.

2. Materials and methods

2.1. Materials

Normal corn starch (containing 12.3% moisture) was obtained from Shandong Dazong Co., Ltd (Shangdong, China). Enzymes used in the present study were commercial preparations. The GSHE (Stargen 001) was purchased from Genencor International (Palo Alto, CA). The Glucose oxidase-peroxidase kit was purchased from Beijing Leadman Biochemical Co., Ltd.

2.2. Hydrolysis of starch granules

A starch slurry (45%, w/w, dry basis) was prepared, brought to a temperature of 65 °C, then adjusted to pH 4.0 using hydrochloric acid. After 15 min, GSHE (50 U/g starch) was added to the sample over a period of 30 min with constant shaking at 300 rpm. After addition of the enzyme was complete, the temperature was adjusted to 70 °C and the mixture was allowed to react for 90 min. At regular time intervals, samples were removed from the reaction and tested for glucose content and dextrose equivalent (DE). The control process involved reaction of the sample at 65 °C for 120 min.

2.3. Determination of glucose content and DE value

The glucose content was measured using a glucose oxidase-peroxidase kit (McCleary et al., 1994; McCleary and Codd, 1991). DE values were determined by measuring the amount of reducing sugar using the dinitrosalicylic acid method (Miller, 1959; Zhao et al., 2008).

2.4. Changes in viscosity during saccharification

The changes in viscosity as a function of time during starch saccharification were determined by using a Brabender Viscoamylograph (C.W. Brabender Instruments, Inc., South Hackensack, NJ). A starch sample (45% w/w, dry basis) was stirred in the Brabender sample canister during a programmed heating and cooling cycle that consisted of heating from 50 °C to 65 °C at 1.5 °C/min and then held at 65 °C for 30 min; heating from 65 °C to 70 °C at 1.5 °C/min and then maintained at 70 °C for 90 min; then heating up to 90 °C at 1.5 °C/min; held at 90 °C for 5 min, cooled from 90 °C to 60 °C at −2 °C/min and held at 60 °C/min for 5 min. The control process consisted of an identical sample was reacted only at 65 °C or 70 °C for 120 min.

2.5. Morphology of the starch granule

The microstructure of the starch granules was studied by scanning electron microscopy (SEM). After reaction for 120 min, samples were taken out at different time intervals and freeze dried for 72 h. The dried samples were coated with gold-palladium using a sputter coater (Denton Vacuum, LLC, Moorestown, NJ) and viewed at 2400× magnification with a SEM (S-3500N, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV.

2.6. Determination of water activity

Water activity was measured with the UNIFAC-LARSEN model (Achard et al., 1992) water activity meter (FA-st/1 from GBX France Scientific Instrument). After the pH reached 4.0, the enzyme was added to the starch slurry to initiate saccharification. Slurry samples were taken over time and their water activities were determined.

2.7. Determination of water molecule mobility

GSHE was added to a starch slurry (45%, w/w, dry basis) to initiate saccharification at 65 °C. Then, samples of fixed size were removed at specific time intervals and poured into a 10 mm diameter NMR tubes. The tubes were then sealed and immediately transferred to the NMR probe. The T_2 values of the water protons were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Hughes, 1977; Loria et al., 1999). Experimental parameter settings were as follows: the pulse gap between the 90° and 180° pulses was set to 200 μs. Eight scans, each containing 1024 echoes, were averaged to obtain the final signal. All measurements were performed in triplicate. Relaxation curves were obtained by fitting the data to the exponential model described in Eq. (1), where T_2 is spin-spin relaxation time and A_0 is a relative measure of the amount of the water fraction corresponding to T_2.

$$A = A_0 e^{\left(\frac{t}{T_2}\right)} \quad (1)$$

2.8. Analysis of products

The products obtained after saccharification, primarily glucose and maltose, were analyzed using an HPLC system (Merck Hitachi, Germany) equipped with a Chromater-545 refractive index detector and an Amino APS-2 Hypersil column (BioRad, USA) thermostated at 30 °C. Each supernatant was collected, filtered through a 0.22 μm membrane, and a 20 μL aliquot applied the amino column. Acetonitrile solution was used as the mobile phase at a flow rate of 1 mL/ min.

2.9. Statistical analysis

All analyses were carried out in triplicate. The glucose contents, DE values, viscosities, water activities, and T_2 values are represented as mean ± standard deviation. Simple Pearson's correlations and regression analyses were evaluated using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA). Duncan's least significant test was used to compare means. Differences resulting in P values < 0.05 were considered significant.

3. Results and discussion

3.1. Effects of two-step reactions on the degree of saccharification

The degree of saccharification of raw corn starch was observed by measuring the glucose content and DE value over time. Glucose