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Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes

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ABSTRACT

Native granular starches (corn, cassava, mung bean, and sago) were hydrolyzed using a mixture of alpha-amylase and glucoamylase at 35 °C for 24 h. Hydrolyzed starches were analyzed for the degree of hydrolysis and for physicochemical and functional properties. Corn starch showed the highest degree of hydrolysis, as evidenced by the presence of distinct pores penetrating deep into the granules. Enzymatic erosion occurred mainly at the surface for cassava, whereas isolated porous structures were observed in hydrolyzed mung bean and sago starch. The amylose content was significantly lower in all starches except for sago starch. The powder X-ray diffraction of all starches showed no significant changes after hydrolysis, but hydrolyzed starches showed a more crystalline nature. The action of enzymes caused significant changes in some pasting properties and in the swelling/solubility of starches. Evidently, enzymes were able to hydrolyze granular starches to a variable degree at sub-gelatinization temperature, and produced a relatively high degree of conversion.

Keywords: Sub-gelatinization temperature; Enzymatic hydrolysis; Amylase; Glucoamylase; Corn; Mung bean; Cassava; Sago

1. Introduction

Starch is the most abundant form of storage polysaccharides in higher plants. In starch granules, amylose and amylopectin are densely packed in a semicrystalline state with inter- and intra-molecular bonds, they are insoluble in cold water, and are often resistant to chemicals and enzymes. Starch from any source can be used as an inexpensive source for the production of fermentable sugars containing glucose, fructose or maltose, all of which are widely used in food industries. In addition, these sugars can be fermented to produce bio-ethanol.

In the course of conventional enzymatic liquefaction, slurry containing 15–35% starch is gelatinized, where it is heated to 105 °C to physically disrupt the granule and open the crystalline structure for the enzyme action (Singh and Soni, 2001). This increases the viscosity of the slurry by 20-fold (Robertson et al., 2006), and therefore makes mixing and pumping difficult. The gelatinized starch is liquefied with thermostable alpha-amylase, and is then saccharificated with glucoamylase at a much lower temperature of 50–60 °C. The whole process requires a high-energy input, which increases the production cost of inverted sugar products.

In view of energy costs, effective utilization of natural resources and viscosity (handling) problems, direct hydrolysis of starch below gelatinization temperature is desirable. In recent years, the importance of the enzymatic liquefaction of raw starch without heating has been well recognized, mainly due to energy savings and the effective utilization of biomass, which reduces the overall cost of starch processing (Robertson et al., 2006). This has generated a worldwide interest in the discovery of amylases that are capable of digesting raw starches and that do not require gelatinization (i.e. amylases that directly hydrolyze raw starch in a single step or that liquefy starch at moderate temperature, much below the gelatinization temperature).

It is more difficult for amylases to act on raw starch granules than on gelatinized starch. Previous studies (Iefuji et al., 1996) indicate that the saccharification of raw starch by

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amylolytic enzymes might be related to the extent of adsorption of enzyme to the starch granules. According to Leloup et al. (1990) there are several steps involved in the enzymatic reaction which are: (1) the diffusion to the solid surface, (2) the adsorption of the enzyme and finally (3) the occurrence of the catalysis. The adsorption step is essential prior to the subsequent catalytic activity. Therefore the enzyme needs to pass across the boundary between the aqueous phases and solid phases before attaching to the granule. The penetration of hydrolyzing enzymes and other large molecules, however, is restricted and only possible through pores or channels (Oates, 1997). Amylases also must functionally bind glucan chains through several glucose units to their subsites (Oates, 1997). These subsites are found at the active centre of the enzyme and are capable of interacting with one glucose residue in the substrate. There are 4–9 subsites at the active centre. The number and the position of the subsites are unique for each type of amylase (Meagher et al., 1989). The hydrolysis occurs layer by layer with an attacked layer of granule being completely hydrolyzed (Wang et al., 1995). Besides that, a structural support which is known as the specialized starch granule-binding domains has been identified to exist in some amylases and glucoamylases. This binding site is separate from the active centre site and it is considered as essential for granule hydrolysis (Hayashida et al., 1990).

Most raw starch digesting enzymes reported to date hardly digest native (raw) starch or cannot produce high yields of fermentable sugars. Therefore, enzymes that can digest raw starch are economically attractive because they would increase the range of starch sources for direct hydrolysis. With technological advancements in biotechnology and enzyme engineering, a new generation of amylolytic enzymes has been discovered. These granular starch-hydrolyzing enzymes have been used in low-energy processes and can effectively hydrolyze starch that has not been cooked. The commercial enzymes are available in a blend of alpha-amylase and glucoamylase, which can hydrolyze insoluble granular (uncooked) starch into fermentable sugars by enabling the depolymerization of starch to glucose in a simultaneous saccharification and fermentation (SSF) process for alcohol production.

The objectives of this research were (1) to compare the action of a blend of amylolytic enzymes for their ability to hydrolyze different starch in its native (raw) granular state (at sub-gelatinization temperature) to fermentable sugars and (2) to examine the effect of hydrolysis on some physicochemical and functional properties.

2. Experimentals

2.1. Materials

Corn (*Zea mays*), cassava (*Mannihot esculenta* Crantz) and sago (*Metroxylon sagu*) starches were obtained from SIM Company Sdn. Bhd. (Penang, Malaysia). Mung bean (*Vigna radiata*) starch was obtained from Pearl Island Packaging Sdn. Bhd. (Penang, Malaysia).

2.2. Enzyme

The commercial enzyme used in the present study was procured from Genencor International (Palo Alto, CA). The enzyme contains alpha-amylase from *Aspergillus kawachi* and a glucoamylase from *Aspergillus niger*. The specific gravity of the enzyme is 1.10–1.15 g/mL and the optimum pH ranged from 4.0 to 4.5. The recommended temperature is 20–40 °C and the minimum activity is ≥456 GSHU/g (GSHU is defined as granular starch-hydrolyzing units). The enzyme's activity was determined by reaction at 37 °C with soluble starch (1%) that was buffered with sodium acetate (pH 4.4). Aliquots were taken after 10 min to determine the amount of D-glucose released. The glucose concentration was determined using the dinitrosalicylic acid method (Miller, 1959). The activity of enzyme was 3736 unit/g starch. The enzyme activity units are given as provided by the enzyme manufacturers. The assay protocol for determining enzyme activity can be obtained from the enzyme manufacturers.

2.3. Determination of moisture content

The moisture content of starch samples were determined by using IR-30 Moisture Analyzer (Denver Instrument, Colorado, USA). Starch (5 g) were spread uniformly on the pan and heated at 105 °C.

2.4. Starch hydrolysis

The starch slurry (25%, w/v) was prepared in 400 mL of sodium acetate buffer. The enzyme (3736 unit/g starch) was added (1%, w/v) into the samples. Samples were then incubated in an incubator shaker (JEIO Tech, SI-600R, Seoul, Korea) at 35 °C at a speed of 150 rpm. After 24 h, hydrolysis was stopped by adjusting the pH to 1.5–1.6 with 2 M HCl. This step was done quickly to minimize further hydrolysis of the starch. Preliminary experiments have established that the enzyme deactivation method does not appear to cause significant starch hydrolysis. The pH of starch suspensions was adjusted back to a pH of 5–6 by washing and filtering the starch with distilled water. Starch residues were collected and dried at 40 °C for 2 days.

2.5. Dextrose equivalent (DE)

The reducing sugar value was measured using the dinitrosalicylic acid method Miller (1959) to determine its dextrose equivalent (DE). A small aliquot was withdrawn from each batch of starch slurry at various time intervals, up to 24 h hydrolysis time. Absorbance was measured at 504 nm by using a UV/visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). Glucose was used as the standard. Each analysis was performed in duplicate. DE was calculated as follows:

$$\mathrm{DE} = \frac{\text{g reducing sugar expressed as glucose}}{\text{g dry solid weight}} \times 100\%$$

2.6. Scanning electron microscopy

The microstructure of starch granules was viewed with a field emission scanning electron microscope (FESEM Leo Supra 50VP, Carl-Ziess SMT, Oberkochem, Germany). Starch granules were mounted on aluminum specimen stubs with double-sided adhesive tape and sputter, with a 20–30 nm layer of gold, using Sputter Coater [Polaron (Fisons) SC515, VG Microtech, Sussex, UK]. The accelerating voltage of the SEM is 5 kV.