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Hydrolysis of granular corn starch with controlled pore size

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ABSTRACT

Native corn (*Zea mays* L.) starch granules were hydrolyzed using glucoamylase at 50 °C for 1–8 h. The degree of hydrolysis over time was analyzed by the concentration of glucose released into solution. The pore sizes of hydrolyzed starch granules increased gradually with the degree of hydrolysis, as evidenced by scanning electron micrographs. It was deduced that every pore on the surface of granules was formed by hydrolysis of one enzyme molecule, so the size of pores distributed on the surface of starch granules was almost homogeneous for the same hydrolysis time. The specific surface area (S_{BET}), porosity, adsorptive capacity and mean pore radius of porous starch granules were determined to analyze the effect of digestion time on granule properties.

1. Introduction

Starch is produced by green plants for energy storage and is synthesized in a granular form. It is typically a mixture of amylose and amylopectin. Amylose is primarily linear with α-1-4 linked glucosyl units. Amylopectin is a highly branched molecule, with (1→4)-linked α-D-glucosyl units in chains joined by (1→6) linkages (Olsen, 2008). Starch granules are not soluble in water at room temperature as they are densely packed with semi-crystalline structures. In the process of conventional enzymatic liquefaction and saccharification, slurries containing 15–35% starch are gelatinized at 105 °C to physically disrupt the granule and open the crystalline structure for the enzyme action. In recent years, direct hydrolysis of raw starch at sub-gelatinization temperature has been conducted as a means to reduce energy costs, effectively utilize natural resources and reduce viscosity problems. However, it is more difficult for enzymes to act on raw starch granules than on gelatinized starch. When raw starch is treated with the enzyme, the native starch granules degrade, leaving behind porous or broken starch granules.

The morphology of starch granules partially hydrolyzed strongly depends upon the botanical source from which they are obtained (Pérez and Bertoft, 2010). Yamada et al. (1995) Zhao and Whistler (1994) and Zhao et al. (1996) firstly, prepared porous starch granules from normal and waxy maize (*Zea mays* L.) starch with amylase (from *Aspergillus* sp.) treatment. They suggested that the crystal region may be more sensitive to amylase than the amorphous one, based on analysis by X-ray diffraction (XRD), differential scanning calorimetry (DSC) and gel permeation chromatography (GPC). Aggarwal and Dollimore (1998) further demonstrated that the porous granules obtained from corn, wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and potato (*Solanum tuberosum* L.) starch by partial hydrolysis with glucoamylase showed a higher gelatinization temperature than their native counterparts. Afterwards, Lacerda et al. (2008a, b) observed lower degradation of corn starch partially hydrolyzed by fungal-amylase at 40 °C and suggested that the partial degradation of the starch granules was concentrated in the amorphous regions. Shariffa et al. (2009) investigated the effect of mild heat treatment (below gelatinization temperature) towards the susceptibility of granular starch to enzymatic hydrolysis. The results showed that heating treatment below gelatinization temperature was effective in enhancing the degree of hydrolysis of granular starch. More recently, raw granular starches hydrolyzed by a mixture of alpha-amylase and glucoamylase at 35 °C for 24 h showed that enzymatic erosion occurred mainly on the surface of cassava (*Manihot esculenta* Crantz) starch granule, whereas isolated porous structures were observed in hydrolyzed corn, mung bean (*Vigna radiata* (L.) R. Wilczek) and sago starch (*Metroxylon sagu* Rottb.) (Uthumporn et al., 2010).

Development of porous carbohydrate ingredients for use in flavour and drug encapsulation was an important stepin the development of controlled-release agents (Zeller et al., 1999).

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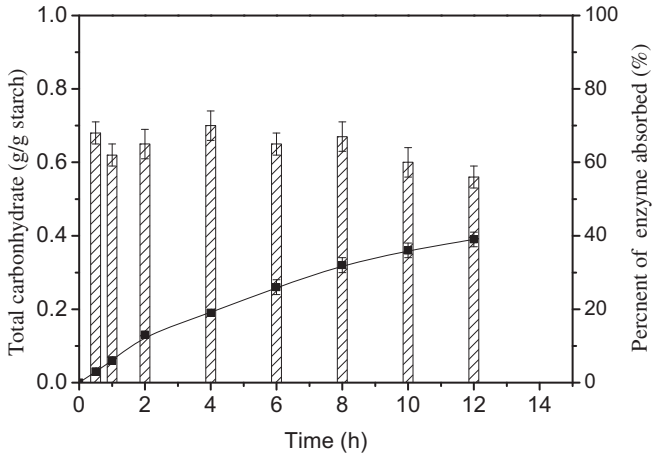

Fig. 1. Amount of glucose released (solid line) and percent of glucoamylase absorbed (sparse column) during hydrolysis of corn starch granules with enzyme (11 GSHU/g starch) at 50 °C and pH 4.6.

Porous starch possessed slow-release characteristics compared with native starch due to the adsorbent characteristics of porous starch (Kazunori et al., 2001; Yao and Yao, 2002). Karim et al. (2008) demonstrated a dual treatment of starch granules involving enzymatic pretreatment followed by hydroxypropylation for specific applications. Their results indicated that partial enzyme hydrolysis of starch in the granular state appeared to enhance the subsequent hydroxypropylation due to more substituent reagent access to the interior or subsurface of the starch granule. Poonam and David (2000) demonstrated that the size of the pores formed increased as the concentration of the enzyme was increased, until the pores started fusing into each other and breaking the walls of the starch granule. Anthony et al. (2010) gave an overview of the various mathematical models describing the kinetics of ungelatinized starch digestion. The porous material containing abundant pores is necessary for adsorption or encapsulation of different matter. The pore size of starch granules is also important for its application. However, few attempts have been focused on preparation of granular starch with size-controlled pores.

The purpose of this paper is to elaborate a method to control the pore size of the starch granule by glucoamylase treatment in a mild condition. The properties of porous starch were analyzed in details. Granular starch with size-controlled pores can be used as a carrier for specific purposes in food, pharmaceutics, etc.

2. Materials and methods

2.1. Materials

Corn starch was obtained from Guanzhen Starch Company (Shanxi, China).

Commercial glucoamylase (Zhaodong Sun Shine Co., Ltd., Zhaodong City, Heilongjiang Province, activity 50,000 IU/ml) was produced by *Aspergillus niger*. Here, IU/g refers to the amount of the enzyme required to produce 1 mg of glucose from gelatinized starch per hour under the specified conditions, and at 40 °C, pH 4.6. GSHU (granular starch-hydrolyzing units) is defined as the amount of enzyme liberating 1.0 mg of glucose from granular starch in 1 min at pH 4.6 and 50 °C.

All other reagents were analytical grade. The water used in experiment was deionized.

2.2. Methods

2.2.1. Preparation of porous starch

The starch slurry (25% w/v) was prepared in 100 mL of sodium acetate buffer (pH = 4.6). The enzyme (11 GSHU/g starch) was added into the samples. Samples were then stirred in a magnetic stirrer at 50 °C. After 0.5, 1, 2, 4, 6, 8 h, hydrolysis was stopped by adding 5 ml NaOH solution (4%). The starch suspension was filtered and washed with distilled water three times. This step was done quickly to minimize further hydrolysis of the starch. Starch residues were collected and dried at 45 °C for 24 h with a vacuum drier. The extent of enzymatic hydrolysis was determined by measuring reducing sugar of supernatant using the dinitrosalicylic acid method (Miller, 1959). All reported data are averages of experiments performed at least in duplicate.

2.2.2. Determination of the amount of glucoamylase adsorbed onto the granules

An amount of enzyme was added to the starch slurry (25% w/v) and incubated at 50 °C. Three aliquots (0.3 ml) were removed at

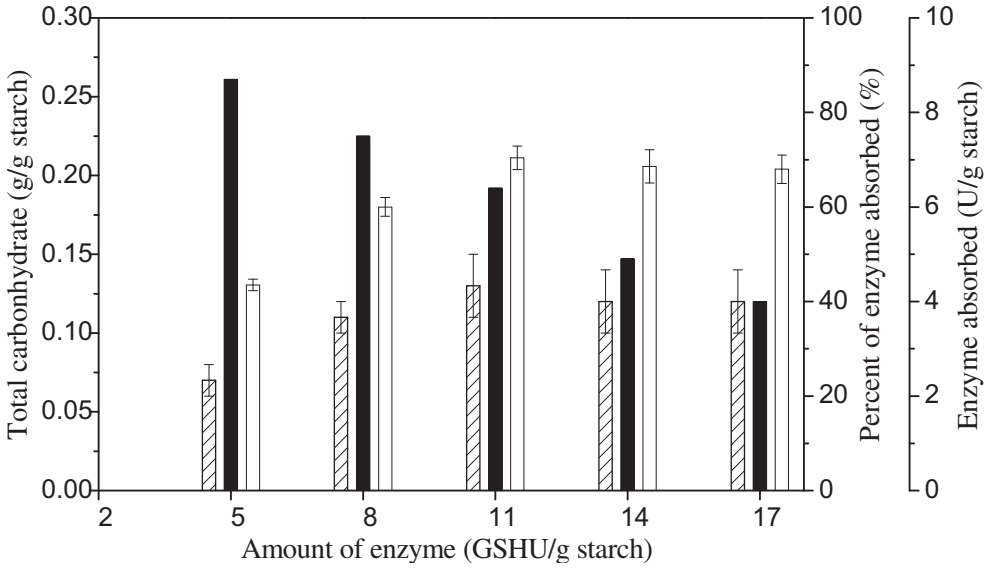

Fig. 2. Amount of glucose released (sparse), percent of enzyme absorbed (black) and enzyme absorbed by per gram starch granule (white) during hydrolysis of corn starch granules with different amount of glucoamylase for 2 h at 50 °C and pH 4.6.