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Short communication

Structural changes of cassava starch granules hydrolyzed by a mixture of α-amylase and glucoamylase

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ABSTRACT

The structural changes of cassava starch granules were studied by scanning electron microscope (SEM), X-ray diffraction (XRD), and differential scanning calorimeter after starch granules were hydrolyzed by a mixture of α-amylase and glucoamylase. The surface of starch granules was porous after hydrolysis treatment. Enzymatic erosion occurred mainly at the surface for cassava starch. The BET-specific surface area of hydrolyzed cassava starch improved 10.7 times compared with that of native starch. The powder XRD intensity of hydrolyzed starch was higher than that of native starch. The crystallinity in the hydrolyzed cassava starch increased due to hydrolysis. Compound enzymes could hydrolyze cassava starch granules at sub-gelatinization temperature, and could produce porous starch.

1. Introduction

Starch is a mixture of two polysaccharides, amylose and amylopectin, and contains small amounts of non-carbohydrate constituents such as lipids, phosphates and proteins (Buleon, Colonna, Planchot, & Ball, 1998; Jenkins, Cameron, & Donald, 1993). In starch granules, amylose and amylopectin are densely packed at a semi-crystalline state with inter- and intra-molecular bonds (Miles, Morris, Orford, & Ring, 1985). Susceptibility of starch to enzyme attack is influenced by factors such as amylose and amylopectin content (Holm & Bjorck, 1988; Ring, Gee, Whittam, Orford, & Johnson, 1988), particle size, crystalline structure, and enzyme inhibitors. Among these, granular structure is believed the most important one (Zhang & Oates, 1999). Typically, enzymatic hydrolysis of starch yields low degree of conversion to fermentable sugars containing glucose, fructose, or maltose, all of which are widely used in food industries.

Cassava starch is widely used because of its unique thickening properties, high purity, low cost, and ability to form clear viscous pastes. Unfortunately, it displays single functionality and low additional value in the industry, thus chemical and physical modifications are often made to overcome these problems and expand its use. In view of energy costs and effective utilization of natural resources, direct enzymatic hydrolysis of starch below gelatinization temperature is desirable. In recent years, starch can be hydrolyzed by α-amylase together with glucoamylase to obtain porous starch as the final product (Kimura & Robyt, 1995; O'Brien & Wang, 2008; Sarikaya, Higasa, Adachi, & Mikami, 2000; Uthumporn, Zaidul, & Karim, 2010; Wang, Powell, & Oates, 1995; Yamada, Hisamatsu, Teranishi, Hasegawa, & Hayashi, 1995; Yan & Zhengbiao, 2010; Yao & Yao, 2002). Further modification can increase the efficiency of native starch hydrolysis (Luo et al., 2008; Shariffa, Karim, Fazilah, & Zaidul, 2010). The structural changes of porous starch were studied by different methods. The pores of starch could be formed during the growing of granules because of enzymatic hydrolysis. Randomly distributed cavities on the surface of potato starch granules were detected microscopically (Apinan et al., 2007; Baldwin, Adler, Davies, & Melia, 1994; Baldwin, Davies, & Melia, 1997; Baldwin, Adler, Davies, & Melia, 1998; Castro & Aguilera, 2007; Zhao, Madson, & Whistler, 1996). The volume, shape, and size of open pores are the major parameters influencing the penetration rate of different compounds into the granule interior as well as the release of the granule interior content to the granule surface.

In this paper, cassava starches were hydrolyzed by a mixture of α-amylase and glucoamylase. The structural changes of hydrolyzed cassava starch were studied by scanning electron microscope (SEM), X-ray diffraction (XRD), and differential scanning calorimeter (DSC). Hydrolyzed cassava starch resulted in voids

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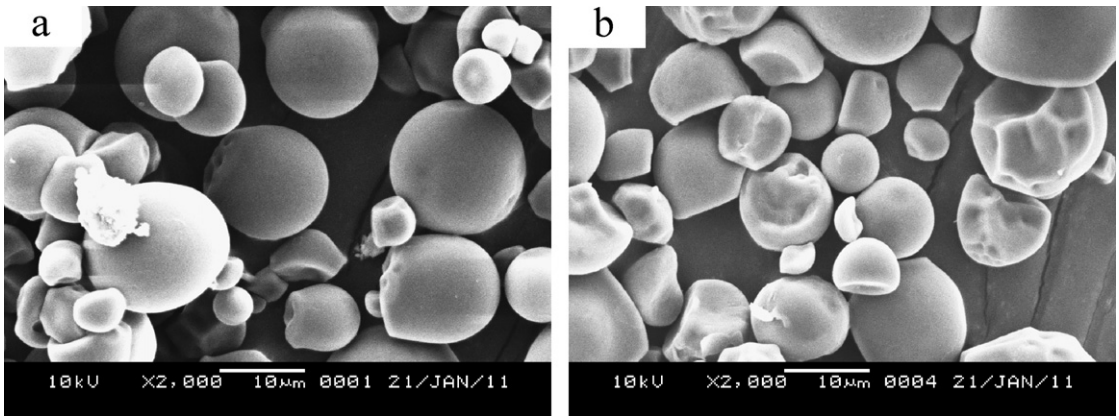

Fig. 1. Scanning electron microscopy of native (a) and hydrolyzed starch (b).

in the granules. The specific surface area increased more than 10 times compared with that of native starch.

2. Experimental

2.1. Materials

Cassava starches were obtained from Liuzhou OBO Technology Co., Ltd. Commercial glucoamylase and α-amylase was procured from Shanghai Kaiyang Biotechnology Co., Ltd. The optimum pH ranged from 4.0 to 4.5 and the recommended temperature is 40–60 °C. The minimum activity of glucoamylase is ≥100,000 u/g and the α-amylase activity is 4000 u/g. The enzymatic activity was determined by the reaction at 37 °C with soluble starch (1.0%) buffered with sodium acetate (pH 4.4).

2.2. Preparation of porous starch

Cassava starch (50.0 g), glucoamylase (0.42 g), and amylase (0.08 g) were suspended in 100 mL pH 4.5 sodium acetate buffer solutions and stirred at 200 rpm for 16 h at 45 °C. After 16 h, hydrolysis was stopped by adjusting the pH to 1.5–1.6 with 2.0 mol/L HCl. This step was done quickly to minimize further hydrolysis of the starch. Preliminary experiments have established that the enzyme deactivation does not appear to further cause significant starch hydrolysis. The pH of starch suspensions was adjusted back to pH of 5–6 by washing and filtering the starch with distilled water. Washing was repeated three times to remove residual enzymes, then the product was collected and dried at 40 °C for two days.

2.3. Structural changes of starch granules

The structural changes of starch granule morphology during the enzyme treatment were investigated. After treatment, the granules were coated with Pt–Pd using a Model MSP-1S magnetron sputter coater (Vacuum Device Inc., Tokyo, Japan). The coated samples were then analyzed using a Hitachi S-3000N SEM (Hitachi Co., Tokyo, Japan) at an operating voltage of 15 kV. Twenty frames of pictures of each sample were taken to represent the structure of the granules.

The BET-specific surface area of samples was determined by nitrogen gas adsorption–desorption at 77 K with saturation pressure of 106.65 kPa using an ASAP 2020 Automated Gas Sorption System. The BET surface area was assessed within the range of relative pressures from 0.05 to 0.3. The total pore volume was calculated by measuring the amount of N_2 adsorbed at a relative pressure of 0.99. The specific surface area of samples was obtained through the adsorption–desorption process.

Crystallinity patterns of starch granules were examined by XRD, as described by Lauro, Forssell, Suortti, Hulleman, and Poutanen (1999). The dried starches were conditioned overnight at room temperature in 100% relative humidity. The starches were scanned by XRD (D-MAX/2000, Rigaku, Japan). Diffractograms were recorded from the reflection made in the angular range of 10–40° (2θ) at 0.05° with a count time of 2 s. The Cu K_α-radiation (λ = 1.5406 A), generated at 40 kV and 30 mA, was made monochromatically by using a 15 μm Ni foil. Scattered radiation was detected using a proportional detector.

Starches were loaded into an aluminum pan and hermetically sealed. An empty aluminum pan was used as reference and the calorimeter was calibrated with indium. A Perkin-Elmer DSC-7 analyzer (Norwalk, CT), equipped with thermal analysis software (Perkin–Elmer Corporation, Norwalk, CT), was used to analyze starch thermal properties. All experiments were conducted at a scanning rate of 10 °C/min from −50 to 200 °C. The transition temperatures reported are the onset (T_0), peak (T_p) and conclusion (T_c) of the endotherm. Indium was used for calibration. The enthalpy (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak, and was expressed in terms of joules per unit weight of dry starch (J/g).

3. Results and discussion

3.1. Morphology of starch granule

Fig. 1 shows the SEM photographs of native and hydrolyzed starch granules. From SEM micrographs, the surfaces of native starch granules are smooth without scratches, while the surfaces of hydrolyzed starch granules were extensively eroded with numerous cracks. O'Brien and Wang (2008) found that the porous structure formed during an enzyme attack would be larger and deeper into granules because of more extensive hydrolysis and the presence of pores and pinholes on the surface of starch. SEM micrographs for cassava showed that enzymatic erosion occurred mainly at the surface. This is consistent with a previous report by Franco, Cabral, and Tavares (2002). Most cassava granules were in truncated form. Truncatures are weak points in the granule structure that lead to increased susceptibility (Valetudie, Colonna, Bouchet, & Gallant, 1993). Hydrolysis seems to proceed in a layered manner.

The surface pores of hydrolyzed starch are openings to the granule interior, mostly extending to the central cavity. Compound enzymes could directly access a loosely organized region in the center of starch by channels and cavities, which might lead to an alteration in the granule morphology. The cassava starch contained more hole-shaped granules than the native starch. These hole-shapes were found more frequently in larger granules. The

Table 1
The BET specific surface area of native and hydrolyzed samples.

Starch species	BET specific surface area (m^2/g)
Native starch	2.39
Hydrolyzed starch	25.58

Table 2
DSC characteristics of native and hydrolyzed starch.

Starch species	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Native starch	68.8	78.1	87.4	11.8
Hydrolyzed starch	62.5	67.6	73.4	16.4

hole-shaped granules could result from granular swelling followed by collapse. The swelling could have occurred early in the reaction when the starch granules underwent swelling in a concentrated solution of compound enzyme.

3.2. BET-specific surface area variation

Table 1 shows the BET-specific surface area changes in native starch and hydrolyzed starch. The measured specific surface area values varied from 2.39 to 25.58 m^2/g. The specific surface area of hydrolyzed starch increased 10.7 times compared with that of native starch. This is attributed to the micro-porous formation in the starch surface. The specific surface area increased after cassava starches were hydrolyzed by compound enzymes.

3.3. Crystallization structure variation

XRD patterns of native and hydrolyzed starches are shown in Fig. 2. Native and hydrolyzed starch showed an A-pattern with major reflections at $2h = 15.3$ and 23.4, and an unresolved doublet at 17 and 18. The crystalline types of native and hydrolyzed starch were not markedly changed. Higher diffraction intensities were obtained after enzyme treatment. The crystalline peak of hydrolyzed starch became bigger compared with that of native starch. The data suggest that the amorphous region of the granule was hydrolyzed more extensively than the crystalline region. This observation agrees with Gallant, Mercier, and Guilbot (1972), who suggested that amylolysis primarily occurs in the amorphous region of starch granules. In this paper, increased cassava starch crystallinity is attributed to the preferential hydrolysis of the amorphous domains by compound enzyme. When compound enzyme penetrates the starch granule through channels and cavities, it could disrupt the amorphous phase. The crystalline peak may increase in the cassava starch (Table 2).

3.4. Enthalpy variation

The influences of enzymatic treatment on transition temperatures and enthalpy are in Table 1. The onset temperatures of hydrolyzed starch shifted slightly to low temperatures compared with that of native starch. The enthalpy of hydrolyzed starch was higher than that of native starch. Cooke and Gidley showed that the ΔH value primarily reflected the loss of the double helical order rather than loss of crystalline register. The increase in ΔH of hydrolyzed starch suggests that some double helices present in non-crystalline regions of the granule may have been disrupted under enzyme hydrolysis. An increase in enthalpy confirms that the increased crystallinity of hydrolyzed starch results from hydrolysis, and this result supports the XRD data.

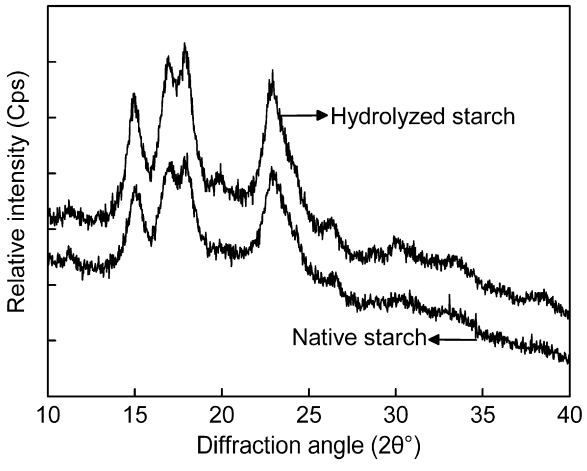

Fig. 2. X-ray diffraction pattern of native and hydrolyzed starch.

4. Conclusions

Cassava starch was hydrolyzed by a mixture of α-amylase and glucoamylase to obtain porous starch, which can be used as an adsorbent carrier. The structural changes of starch granules were observed by SEM, XRD, and DSC. The surfaces of hydrolyzed starch granules were extensively eroded with numerous cracks. Enzymatic erosion of hydrolyzed starch occurred mainly at the surface. The BET-specific surface area of hydrolyzed starch increased 10.7 times compared with that of native starch. The hydrolysis mainly occurred in the amorphous region from the XRD pattern. The crystallinity in the hydrolyzed cassava starch increased after the cassava starch was hydrolyzed by compound enzymes.

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