IMPROVEMENT OF GLUCOSE PRODUCTION IN ENZYME REACTION SYSTEM UTILIZING ACID-TREATED SAGO STARCH AS SUBSTRATE

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ABSTRAK

Pati sagu yang tidak diperlakukan berada sebagai struktur kristalin yang sangat kompak, reaksi enzimatik dan produk yang dihasilkan sangat rendah. Untuk itu, kombinasi perlakuan pati sagu menggunakan asam dan pemanasan di bawah temperatur gelatinisasi dapat membuat granula pati sagu mengembang dan membentuk lubang pada permukaan granula, yang kemudian dapat meningkatkan *accessibility* pati sagu untuk diserang oleh enzim. Pada kondisi optimum dan setelah 24 jam terjadi hidrolisis secara enzimatik campuran 24 % konsentrasi pati sagu tanpa perlakuan dan perlakuan menggunakan asam sebagai substrat dan 100 units/mL enzim dapat dihidrolisis menjadi glukosa sebesar 53,3 % dan 71,9 % berturut-turut.

INTRODUCTION

Products from hydrolysis of starch such as maltodextrin, corn syrup, glucose syrup and high fructose syrups have wide application in the food, textile, brewing and pharmaceutical industries. Since these products have mainly been derived from corn, barley and potato starch, it should be possible to obtain similar products from the processing of sago starch.

The use of enzymes for the breakdown of starch has some distinct advantages over the less specific method of acid hydrolysis. Enzymatic hydrolysis can be completed at relatively low temperatures and pressures whilst maintaining a high pH, thus minimizing the formation of undesirable components due to thermal effects and low pH. Recently, glucose has been produced from direct conversion of raw starches using the novel raw starch degrading enzyme to replace conventional method in the glucose syrup production [1]. Prospect of glucose syrup production from raw starches especially raw sago starch is interesting for the future. This prospect is supported by the abundant raw sago starch production in East Asia such as Indonesia and Malaysia. It is estimated that about 60 million ton of sago starch extracted from sago palms are produced per annum in South-east Asia[2]. However, raw starches exist as the compact crystalline structure.

The enzyme reaction rate and yield of products from raw sago starch was reported to be too low for industrial application[3]. The bioconversion of sago starch was limited by high paste viscosity and resistance of the raw granule to enzymatic hydrolysis[4]. In order to increase the susceptibility of raw sago starch and improve glucose production, it has to be treated with acid below gelatinization temperature. The present work reports improvement of glucose production in the enzyme reaction system utilizing acid-treated raw sago starch as substrate.

METHODOLOGY

Preparation of RSDE Solution

Raw starch degrading enzyme (RSDE) from *Acremonium* sp. endophytic fungus was prepared. The enzyme was concentrated by ultrafiltation to 100 units/mL.

Preparation of Acid-Treated Sago Starch

Ten percent raw sago starch slurried in 0.1 M HCl buffer solution at various pH between 2.0 – 3.5 and kept at temperatures of 50, 55, 60 and 65 °C. The solution was held for 1 – 3 hrs at the incubation temperatures. After the incubation, the starch was washed thoroughly with tap water to remove the acid and filtered by muslin cotton, dried at room temperature and powdered manually.

Measurement of Physical Properties of Sago Starch

Brabender Viscoamylograph (model VA-V) was used to determine the physical properties of the treated starch. 27.6 g of starch (dry weight basis) were weighed and slurried in distilled water to give a total volume of 460 mL. The slurry was transferred to a Brabender Viscoamylograph and heated from 30

°C to 95 °C in 40 min, held at 95 °C for 30 min and cooled to 50 °C in 30 min. The gelatinization temperature range, peak viscosity, breakdown, setback and consistency were determined from the Brabender amylogram.

Enzymatic Hydrolysis of Starch Granules

Enzymatic hydrolysis of starch was carried out in the reaction mixtures consisting 1 mL of 2 % (w/v) substrate in 0.1 M acetate buffer pH 5.5 and 1 mL of enzyme solution. Incubation was carried out at 55 °C for 30 min and the reaction was stopped by heating in boiling water for 5 min. The reducing sugar produced was determined by the Miller's method [5].

Determination of Glucose Conversion

The amount of glucose produced was analyzed by HPLC using the NH_2-18C column (25 cm x 6.5 mm, Merck-Germany). The column was maintained at 38°C with 80% (v/v) acetonitrile (HPLC grade) in deionized water as the mobile phase at 1.2 mL/min.

RESULTS AND DISCUSSION

Effect of Acid-Temperature Pretreatment on the Rheological Properties of Sago Starch

Raw sago starch exists as compact crystalline structure and produce high viscosity when gelatinized. The susceptibility of the raw starch to be attacked by enzyme is low. Thus, the sago starch must be treated before using enzyme for industrial application. Table 1 show the rheological properties of acid-treated and untreated sago starch as determined by Brabender amylograph.

The acid-treated and untreated sago starch shows different initial temperatures of gelatinization. For acid-treated sago starch granules the gelatinization temperature raised to 69.5 °C, whereas that of untreated, it was 68.5 °C as well as for typical gelatinization profile of starch[6,7]. In addition the gelatinization profiles of raw sago, arrowroot and cassava showed gelatinization temperatures in the range 68-90 °C, 75-90 °C and 68-90 °C, respectively[8].

Other researchers reported lower values for wheat and corn starches in the range 56-66 °C[9,10]. The acid-treated and untreated sago starches differed from each other with respect to viscosity (untreated > acid-treated sago starch). The acid-treated sago starch exhibited lower breakdown viscosity compared to the untreated sago starch as shown by vm-vr values (100 BU compared to 320 BU). Low breakdown indicates the stability of the swollen granules against disintegration during cooking[11]. The acid-treated sago starch has more tendency to be in fluid form as denoted by the negative consistency value (- 140 BU) than that of the untreated sago starch. The untreated sago starch produces viscous gel with value 360 BU.

Enzymatic Hydrolysis of Acid-Treated Sago Starch

The percentage of enzymatic hydrolysis on acid treated sago starch was expressed as reducing sugars produced. Figure 1A shows the effect of acid-treatment on sago starch at temperatures 50 to 65 °C for 2 h in acetate buffer solution pH 2.0. The result indicate that acid-treatment of sago starch below gelatinization temperature had a great impact on the biological behavior, similar to that reported by for potato starch after incubating for 2 h at temperature 3% below the gelatinization peak temperature (°K). Incubating the sago starch granules below gelatinization temperature swell the starch granules to some extent and form pores on the granules surface. Some of these pores are of sufficient size that enzymes can gain entry into the granule interior, thereby increasing the rate of reaction.

The result showed that temperature 65 °C and pH 2.0 was most suitable to improve the enzymatic reaction compared to other temperatures and pH treatment (Figure 1B). It was observed that the hydrolysis did not differ after 2 h of treatment period (Figure 1C). The treatment of sago starch in acetate buffer pH 3.5 at temperature 65 °C at various times (1, 2, 4, 6, 8, 10 and 12 h) did not show any effect on the degree of hydrolysis.

Table 1. Rheological properties of acid-treated and untreated sago starches

Starch sources	Gelatinization temperature (°C)	Max. viscosity on heating to 95°C (vm) (BU)	Viscosity after 30 min at 95°C (vr) (BU)	Viscosity on cooling to 35°C (ve) (BU)	Breakdown viscosity (vm-vr)	Setback viscosity (ve-vm) (BU)	Consistency (ve-vr) (BU)
Acid-treated sago	69.5	320	220	80	100	-240	-140
Untreated sago	68.5	640	320	580	320	-60	360

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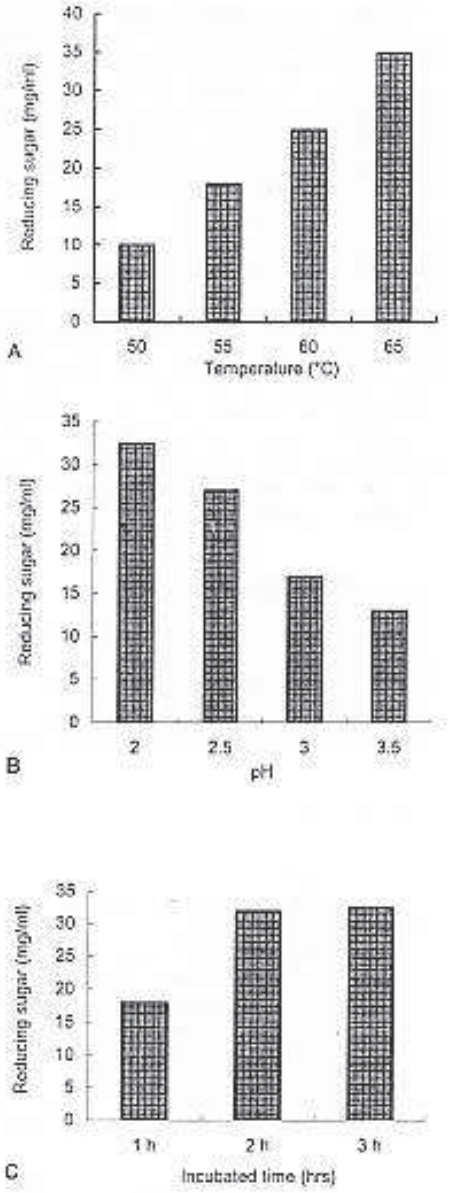

Figure 1. Enzymatic hydrolysis of acid treated sago starch at different pH and temperatures. The reaction mixtures containing 1 mL of 2% (w/v) acid-treated sago starch in 0.1 M acetate buffer at pH 5.5 and 1 mL of RSDE was incubated at 55 °C for 30 min (A: sago starch treated with acid at and incubated for 2 hrs 50-65 °C; B: sago starch incubated at 65 °C for 2 hrs in acid at pH range 2 – 3.5; C: sago starch treated with acid at pH 2.0 and incubated at 65 °C for 1-3 hrs).

Glucose Production

To maximize glucose production during the enzymatic degradation of acid-treated sago starch, the main experimental variables (enzyme and substrate concentrations) were optimized. Figure 2 shows that as the enzyme concentration was increased, the quantity of glucose released increased proportionally. The enzyme concentration needed to obtain the best degradation was 100 units/mL. Less than 100 units/mL enzyme concentrations the degree of hydrolysis is low as well as α-amylase and glucoamylase[2,3,12,13].

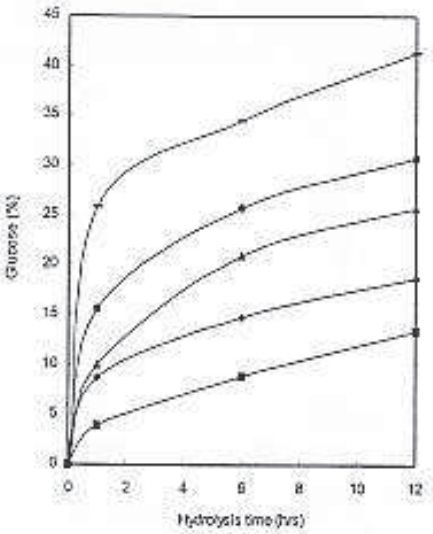

Figure 2. Enzymatic hydrolysis of acid treated sago starch (treated at pH 2.0, 65 °C for 2 hrs) with RSDE at different concentrations. The reaction mixtures containing 1 mL of 2% (w/v) acid-treated sago starch dissolved in 0.1 M acetate buffer of pH 5.5 with different concentrations of RSDE were incubated at 55 °C for 30 min. Enzyme concentrations; ■: 20; ◆ 40; ▲: 60; ●: 80; ∞: 100 units/mL)

The effect of acid-treated sago starch concentrations (2~30% (w/v)) on the production of glucose was determined. The results indicated that at higher concentration of starch (> 24% (w/v)) and 100 units/mL of enzyme, the degree of conversion to glucose become lower (Figure 3). Similar phenomenon was shown for raw starch degrading glucoamylase produced by *Rhizopus* sp. A-11 on degradation of cassava, wheat and corn starches at concentrations more than 15, 13 and 15% (w/v), respectively. The most suitable acid-treated concentration was 24% (w/v) and 100 units/mL of enzyme which yielded highest glucose production[14].

Figure 4 showed glucose production in a reaction mixture containing 5 mL of 24 % (w/v) acid-treated and untreated sago starch in 0.1M acetate buffer pH 5.5 and 500 units of enzyme incubated at 55 °C for 24 h. Under this condition, the acid-treated and untreated sago starch was degraded to glucose at 71.9% and 53.3%, respectively.

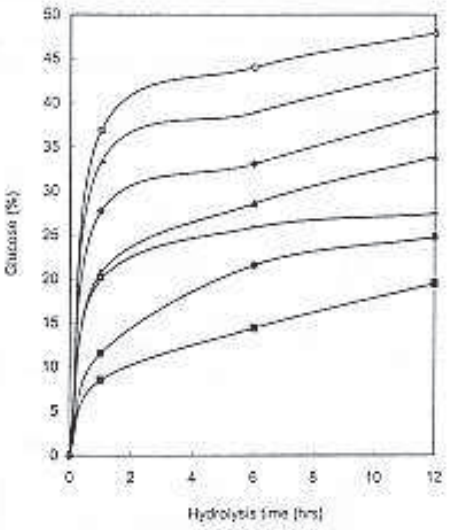

Figure 3. Enzymatic hydrolysis of different concentrations of acid-treated sago starch with RSDE (100 units/mL). The reaction mixtures containing 100 units of enzyme and different concentrations of acid treated sago starches in 0.1 M acetate buffer of pH 5.5 were incubated at 55 °C for 30 min. Acid-treated sago starches concentrations used were; ■: 2; ●: 4; ▲: 8; ▲: 16; ◆:2;– :24; ☒ : 30 % (w/v).

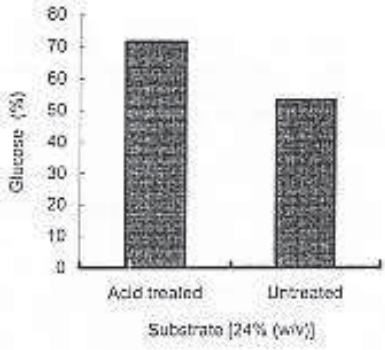

Figure 4. Production of glucose by RSDE using acid-treated and untreated sago starches. The reaction mixture containing 5 mL of 24% (w/v)) acid-treated or untreated sago raw starches as substrate and 500 units of enzyme in a total reaction volume of 10 mL was carried out under optimum conditions (pH 5.5, 55°C) for 24 h.

The results suggested that the enzymatic hydrolysis of raw starch by raw starch degrading enzyme produced by *Acremonium* sp. was found to be dependent on the nature of the substrate (acid-treated and untreated sago starch). The use of acid in combination with heating below gelatinization temperature greatly increased the extent and rate of hydrolysis of sago starch granules by the enzyme. Glucose production was increased approximately by 134% as compared to untreated sago starch.

CONCLUSIONS

It is clearly shown that high yield of glucose production by hydrolysis of acid-treated sago starch can be achieved with raw starch degrading enzyme from *Acremonium* sp. The difference between the acid-treated and untreated sago starch hydrolysis obtained from these treatments could be explained by differences of the surface of granule sago starch and this may influence the accessibility and diffusion of enzyme to attack the starch.

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