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Improvement of Glucose Production by Raw Starch Degrading Enzyme Utilizing Acid-Treated Sago Starch as Substrate

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Abstract: The native sago starch exists as a compact crystalline structure and is not efficiently hydrolyzed by Raw Starch Degrading Enzyme (RSDE). In order to enhance its hydrolysability, the starch was treated with acid and heated below its gelatinization temperature, thus increasing the accessibility of the sago starch granule to enzymatic attack. Results showed that treatment of sago starch with acid at pH 2.0 and temperature 65°C for 2 hours greatly enhanced its conversion rate to glucose from 53.3% to 71.9%. It is clearly shown that high yield of glucose is produced during hydrolysis of acid-treated sago starch using the Raw Starch Degrading Enzyme from *Acremonium* sp. The difference between the acid-treated and untreated sago starch in this study could be due to the differences on the surface of the sago starch granule which may influence the accessibility and diffusion of enzyme into the starch during hydrolysis.

Keywords: Glucose production, Raw Starch Degrading Enzyme, sago starch

INTRODUCTION

Products from hydrolysis of starch such as maltodextrin, corn syrup, glucose syrup and high glucose syrup have a wide application in the food, textile, brewing, and pharmaceutical industries (Griffin and Brooke, 1989). These products are mainly derived from corn, barley and potato starch. In Malaysia, sago starch is considered as one of the most important sources of starch. Wang *et al.* (1996) reported that about 60 million tonnes of sago starch extracted from sago palms are produced per annum in South-east Asia. Attempts have been made to produce glucose from direct conversion of raw starches using the novel raw starch-degrading enzyme to replace conventional methods in glucose syrup production (Yetti *et al.*, 2000a). However, the raw sago starch exists as large granules with compact crystalline structure. As a result, the enzyme reaction rate and yield of products from raw sago starch was reported to be too low for industrial application (Wang *et al.*, 1995).

Sakano *et al.* (1986) and Takao *et al.* (1986) reported that bioconversion of sago starch was limited by the resistance of the raw granule to enzymatic hydrolysis. A new RSDE, which is able to degrade large starch granules, was isolated and prepared (Yetti *et al.*, 2000b). The RSDE was able to hydrolyze raw sago starch to glucose at a conversion rate of 53.3%. It has been reported elsewhere that treatment of raw

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starches with acid at below its gelatinization temperature would enhance its digestibility by enzymes. Therefore, in order to increase the susceptibility of raw sago starch to enzymatic hydrolysis and improve glucose production, sago starch was treated with acid below its gelatinization temperature. In this paper the effect of acid treatment on the production of glucose using raw sago starch as the substrate was studied.

MATERIALS AND METHODS

Materials

Sago starch was obtained from Gamex Factories, Malaysia. Other chemicals and reagents were obtained from BDH laboratories Poole, England.

Preparation of RSDE

Raw starch degrading enzyme (RSDE) from *Acremonium* sp. endophytic fungus was prepared according to the methods of Yetti *et al.* (2000c). The enzyme was concentrated using ultra filtration to 100 units/mL.

Preparation of Acid-Treated Sago Starch

Ten percent of sago starch slurries in 0.1M HCl buffer solution were adjusted to pH 2.0, 2.5, 3.0 and 3.5 and kept at 50, 55, 60 and 65°C for 1 - 3 hrs. After the incubation, the starch was washed thoroughly with tap water to remove the acid and filtered using a muslin cloth. It was then dried at room temperature and powdered manually.

Measurement of Rheological Properties of Sago Starch

Brabender Viscoamylograph (Model VA-V) was used to determine the rheological properties of the untreated and treated starches. 27.6 g of starch (dry weight basis) was weighed and slurried in distilled water to a total volume of 460 mL. The slurry was transferred to the 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 amylogram.

Determination of Starch Structure

Starch samples used were previously dried in an oven at 110°C for 4 hours. The samples were individually mounted on circular aluminium stubs with double-sided sticky tape and sputter coated with gold palladium using Scanning Electron Microscope (SEM) Coating Unit (Polaron E5100, Belgium). The samples were then dried using a Critical Point Dryer (CPD 030, Bal-tec, Switzerland). The starch granules were examined and photographed using a Scanning Electron Microscope (Jeol 64100, Japan) at an accelerating potential of 15kV.

Enzymatic Hydrolysis of Starch Granules

Enzymatic hydrolysis of acid-treated starches was carried out in reaction mixtures consisting of 1 mL of 2% (w/v) substrate in 0.1M acetate buffer at pH 5.5 and 1 mL of concentrated RSDE. Incubation was carried out at 55°C for 30 min and the reaction was stopped by heating in boiling water for 5 min. The amount of reducing sugar produced was determined by the method of Miller (1959).

Determination of Glucose Conversion

The amount of glucose (%) produced was analyzed by High Performance Liquid Chromatography (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 a flow rate of 1.2 mL/min.

RESULTS AND DISCUSSION

Rheological Properties

Raw sago starch exists as a compact crystalline structure and produced high viscosity when gelatinized. The susceptibility of the raw starch

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

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

to be hydrolyzed by the RSDE is relatively low, although the newly isolated enzyme shows high specificity towards raw starch with large granule size (Yetti *et al.*, 2000b). In this study, raw sago starch was treated with acid at pH 3.5 for 6 hrs at room temperature and the rheological properties compared with untreated sago starch determined by Brabender viscoamylograph. Treatment of sago starch granule with acid slightly shifted the gelatinization temperature from 68.5°C to 69.5°C. Similar result has also been reported by Lund (1983) and Merca and Juliano (1981) for typical gelatinization profile of starch. In addition, Perez *et al.* (1998) in their study on the gelatinization profiles of raw sago, arrowroot and cassava starches concluded that their gelatinization temperatures were in the range 68-90°C, 70-90°C and 68-90°C, respectively. Other researchers reported lower values for wheat and corn starches, which are in the range 56-66°C (Hoover and Vasanthan, 1994) and 64-72°C (Hoover *et al.*, 1991), respectively.

Table 1 shows changes in the rheological properties of acid treated and untreated sago starch. The acid-treated sago starch exhibited a lower breakdown viscosity compared to the untreated sago starch as shown by vm-vr values (100 BU compared to 320 BU). Breakdown viscosity indicates the stability of the swollen granules against disintegration during cooking. The acid-treated sago starch has more tendencies 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 a viscous gel at 360 BU.

Effect of low acid treatment (i.e. pH 3.5) on the morphological structure of sago starch was further examined. The granules of the untreated and treated sago starch (pH <3.5) were viewed using SEM. The micrographs of the starch granules are shown in Figures 1 and 2. The untreated sago starch granule appeared to be oval-shaped with certain areas being concave and truncated whereas the acid-treated sago starch granules had fissures on their surfaces, irregular indentations and tiny protrusions. The cracks on the surfaces of the acid-treated sago starch will allow the RSDE to penetrate more.

Hydrolysability of Acid-Treated Sago Starch at Different pHs and Temperatures

The enzymatic hydrolysis of acid-treated sago starch at different pHs and temperatures are shown in Figure 3 (A, B and C). The progress of the reaction was expressed as the amount of reducing sugar produced during incubation. This study indicated that acid-treatment of sago starch below gelatinization temperature prior to hydrolysis had a great impact on the hydrolysability of the starch (Figure 3A), similar to that reported by Eerlinger *et al.* (1997) for potato starch after 2 hrs of incubation at a temperature of about 3 degrees below the gelatinization peak temperature (°K). This could be explained based on the fact that incubation of the sago starches below gelatinization temperature

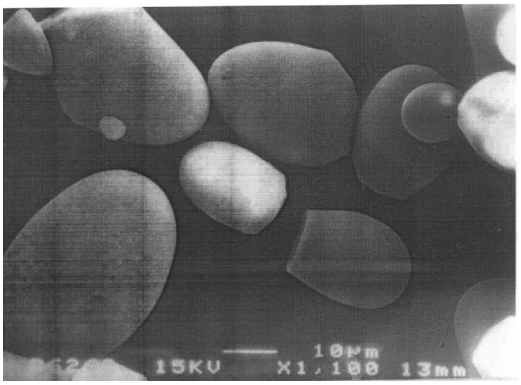

Figure 1: Morphological structure of untreated sago starch granules (control)

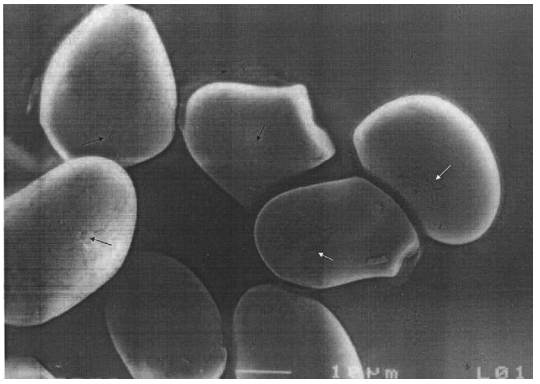

Figure 2: Morphological structure of acid-treated sago starch granules Sago starch was treated at pH 3.5 for 6 hrs. Fissures on sago starch granules

causes the starch granules to swell to a certain extent and form pores on the granule's surfaces. Some of these pores are of sufficient sizes that allow the entrance of RSDE into the interior, thereby increasing the rate of reaction. Treatment of sago starch at 65°C was found to be the most suitable condition as it improves the enzymatic reaction.

pH was also found to affect the hydrolysability of the sago starch. The lower the pH, the higher is the capability of the enzymes to enter the granules. Even though, the morphological structure of starch granules treated between pH 2.0 – 3.5 could not be distinguished clearly (data not shown), starch treated at pH 2.0 gave a better hydrolisability rate. It was also observed that enzymatic hydrolysis of treated sago starch did not increase after 2 hrs of hydrolysis (Figure 3C).

Varying the amount of RSDE and substrate concentrations can maximize production of glucose during the hydrolysis of acid-treated sago starch. Figure 4 shows that as the amount of RSDE was increased, the quantity of glucose produced was also increased. The RSDE concentration needed to obtain the highest degradation was 100 units/mL. Degree of hydrolysis was low when the enzyme concentrations were less than 100 units/mL. Similar results were reported by Wang *et al.* (1995, 1996, 1997) and Govindasamy *et al.* (1995) using commercial α-amylase and glucoamylase.

The effect of different concentrations (2 – 30 %) of acid-treated sago starch on the production of glucose by using 100 units/mL of enzyme was determined. The results indicated that the higher the concentrations of acid-treated sago starch (> 24% w/v) the lower the degree of glucose conversion (Figure 5). The concentration that yielded the highest glucose production was 24 % (w/v) using 100 units/mL of enzyme.

Figure 6 shows the glucose production in a reaction mixture containing 5 mL of 24 % (w/v) acid-treated or untreated sago starches in 0.1M acetate buffer at pH 5.5 and 500 units of RSDE incubated at 55°C for 24 hrs. Under these conditions, the acid-treated and untreated sago starches were degraded to glucose at 71.9% and 53.3%, respectively. These results suggested that the enzymatic hydrolysis of raw starch by RSDE produced from–*Acremonium* sp. was found to be dependent on the nature of the substrate (i.e. whether the sago starch is acid-treated or untreated). The use of acid in combination with heating below gelatinization temperature greatly increased the extent and the rate of hydrolysis of sago starch granules by the enzyme. Glucose production was increased

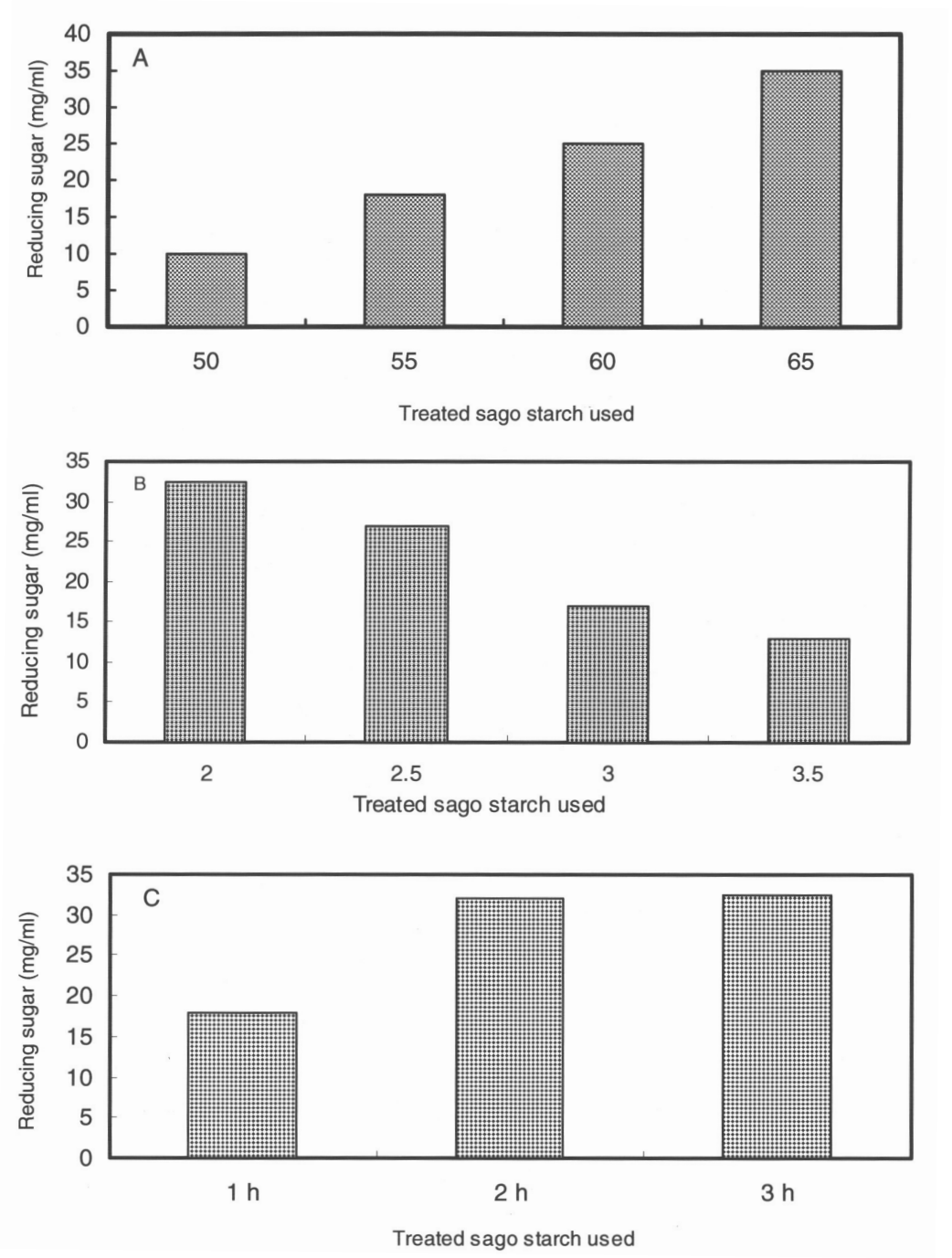

Figure 3. Enzymatic hydrolysis of acid treated sago starch. A: sago starch treated at pH 2.0 and temperature between 50-65°C for 2 hrs. B: sago starch treated at temperature 65°C and pH between 2.0-3.5 for 2 hrs. C: sago starch treated at pH 2.0, temperature 65°C for 1-3 hrs. The reaction mixtures containing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

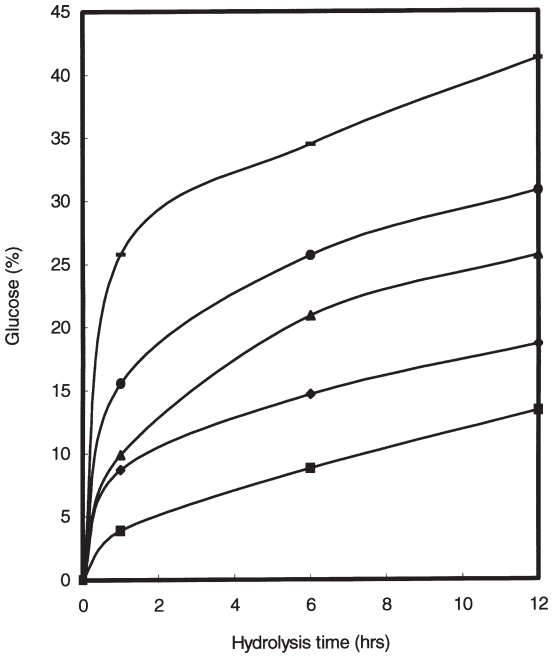

Figure 4: Enzymatic hydrolysis of acid treated sago starch (treated at pH 2.0, 65°C for 2 hrs) with RSDE at different concentrations. The reaction mixtures containing 1 mL of 2% (w/v) acid-treated sago starch dissolved in 0.1M acetate buffer at pH 5.5 with different concentrations of RSDE were incubated at 55°C for 30 min. Enzyme concentrations; ■: 20; ◆:40; ▲: 60; ●: 80;▭: 100 units/mL)

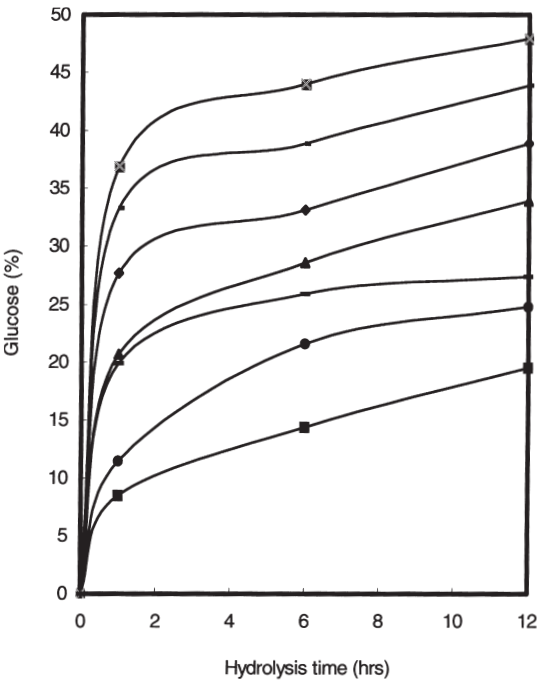

Figure 5. Enzymatic hydrolysis of different concentrations of acid-treated sago starch with RSDE (100 units/mL). The reaction mixtures containing 100 units of enzyme and different concentrations of acid treated sago starches in 0.1M acetate buffer of pH 5.5 were incubated at 55°C for 30 min. Acid-treated sago starches concentrations used were; ■: 2; ●: 4; ▭ : 8; ▲; 16; ◆: 20;–:24; ☒ and 30% (w/v)

approximately by 135% as compared to using untreated sago starch as the substrate.

CONCLUSION

The use of combined treatment of sago starch with acid and heating below gelatinization temperature enhances its hydrolysability by swelling the sago starch granule to some extent and causes the formation of pores on the granule's surface, thus increasing the accessibility of the starch to enzyme attack. Therefore this type of treatment can be employed to increase the hydrolysability of starches and enhance its conversion into glucose.

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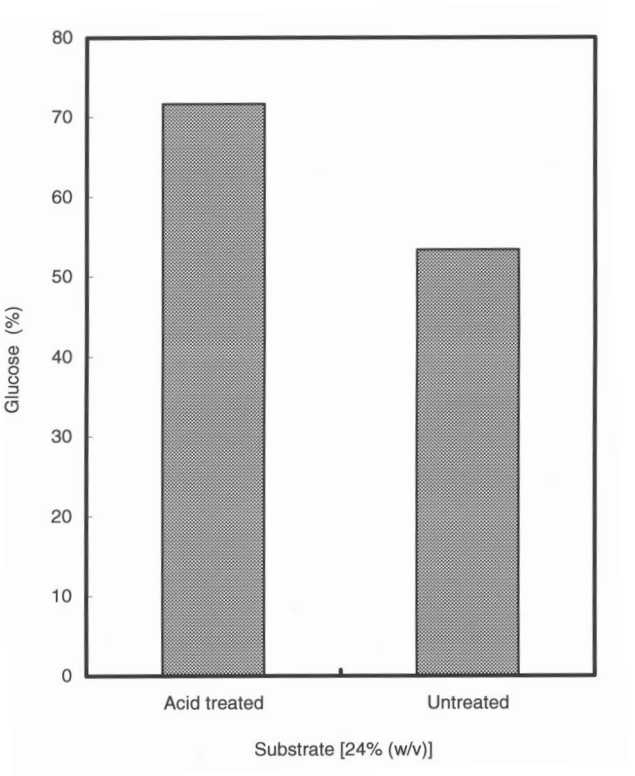

Figure 6. Production of glucose by RSDE using acid-treated and untreated sago starches. The reaction mixture containing 5 mL of 24% (w/v) acid-treated or untreated sago raw starches as substrate and 500 units of enzyme in a total reaction volume of 10 mL was carried out under optimum conditions (pH 5.5, 55^{o}C) for 6 hrs.

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