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A Study of Optimal Conditions for Reducing Sugars Production from Cassava Peels by Diluted Acid and Enzymes

Kanlaya Yoonan and Jirasak Kongkiattikajorn

ABSTRACT

Cassava peels were dried and milled into small pieces. 1.5%(w/v)Cassava peel was hydrolysed by 0.1 M sulfuric acid at 135°C under pressure 15 lb/inch^2 for 90 min producing 66.28%yield of reducing sugars while 0.025 M hydrochloric acid produced 63.29% yield of reducing sugars under the same conditions. Hydrolysis by 0.25 M acetic acid could produce 30.36% yield of reducing sugars under the same conditions. Enzymatic hydrolysis of 1.5% cassava peels by a-amylase at pH 6.0, 90°C for 2 hr produced 40.79% yield of reducing sugars. After that, the reaction mixture was continuously hydrolysed by amyloglucosidase at pH 4.0, 50°C for 24 hr, which produced 70.11% yield of reducing sugars. Hydrolysis of 1.5% cassava peel by cellulase under optimal condition at pH 5.0, 50°C for 24 hr produced 43.39% yield of reducing sugars. Hydrolysis of 1.5% cassava peels by xylanase under optimal conditions at pH 4.0, 50°C for 9 hr produced 2.64% yield of reducing sugars while hydrolysis by pectinase under optimal condition at pH 5.0, 40°C for 3 hr produced 9.01% yield of reducing sugars. From this study, it was shown that cassava peels could produce reducing sugars by dilute acid hydrolysis and that several enzymes digestion.

Key words: cassava peels, acid, enzymes, hydrolysis, reducing sugars

INTRODUCTION

The bioconversion of crops and residues to fuels and chemicals is receiving increased interest due to the perceived need for the reduction of consumption and importation of petroleum fuels. These wastes may be hydrolyzed by acids or enzymes to lower molecular weight carbohydrates and finally to monomeric sugars. Most of the effort has been aimed at producing ethanol by hydrolysis of lignocellulosic materials and subsequent fermentation of sugars in the hydrolyzate. Cassava peels, the main by-product from processing tuberous roots of cassava for human consumption, could be used to be the source of fermentable sugars. The mature root possesses three distinct regions: a central vascular core, the cortex (flesh), and the phelloderm(peels). The peels is 1-4 mm thick and may account for 10-12% of the total dry matter of the root (Nartey, 1979). The analyses of the chemical composition of cassava peels indicate the following chemical composition: dry matter, 86.5-94.5%; organic matter, 91.1%; nitrogen 1.0 %; neutral detergent fibre 57.4%; acid detergent fibre 28.4%; hemicellulose 29.0%; cellulose 20.8%; acid insoluble ash 2.8%; acid detergent lignin 5.0%; calcium 0.7%; phosphorus 0.10%; magnesium 0.15%; sulphur 0.08%; copper 11

Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand.

ppm; zinc 20 ppm; and manganese 86 ppm (Baah *et al.*, 1999). Cassava peels have been evaluated as a feedstuff for animals (Adegbola and Asaolu, 1986; Obioha and Anikwe, 1982; Osei *et al*., 1990). The aim of the study was to obtain soluble reducing sugars by diluted acid and enzymatic hydrolysis of cassava peels and to determine the optimal conditions of each treatment. The composition of sugars released allowed a comparison of efficiency of different diluted acid and hydrolytic enzymes.

MATERIALS AND METHODS

Substrate

Cassava peels from the factory of cassava starch production were milled to flour in the size of 63-425 micrometre by hammer mill and dried overnight at 55°C in an hot-air oven. The moisture content was found to be 11.2%.

Acid hydrolysis of cassava peels

The following condition were used in the hydrolysis of cassava peels by dilute acid. The concentration of cassava peels was 1.5%(w/v) in 0.01-0.25M of sulfuric acid, hydrochloric acid and acetic acid. The temperature was varied from 105-135°C and reaction time was varied from 15-90 min.

Enzymatic hydrolysis of cassava peels

1.5% cassava peels in 0.02 M citrate-phosphate buffer was added with 10mU of a-amylase. The enzymatic reaction was studied to determine the optimal condition by varying temperature between 70-100°C, pH between 3-7 and incubation time between 1-4 hr. After amylase hydrolysis, the reaction mixture was added with 10 mU of amyloglucosidase to determine the optimal condition of the enzyme by varying temperature between 40-70°C, pH between 3-7 and incubation time between 16-48 hr. 1.5% Cassava peels in 0.02 M citrate-phosphate buffer was added with 10 mU of cellulase. The enzymatic reaction was studied to determine the optimal conditions by varying temperature between 40-70°C, pH between 3-7 and incubation time between 3-48 hr. 1.5% Cassava peels in 0.02M citrate-phosphate buffer was added with 10 mU of xylanase. The enzymatic reaction was studied to determine the optimal condition by varying temperature between 40-70°C, pH between 3-7 and incubation time between 3-15 hr. 1.5% Cassava peels in 0.02 M citrate-phosphate buffer was added with10 mU of pectinase. The enzymatic reaction was studied to determine the optimal condition by varying temperature between 30-60°C, pH between 3-7 and incubation time between 1-5 hr. The enzymes in this study were purchased from Sigma-Aldrich, Inc, USA. The hydrolysates were centrifuged to remove any suspended or unhydrolysed materials. Each experiment was done triplicate. The reducing sugars present in the hydrolysates were measured by Nelson-Somogyi method (Nelson, 1944).

Enzyme unit (U) definition

For a-amylase, one unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20°C. For xylanase, one unit will liberate 1 micromole of reducing sugar measured as xylose equivalents from xylan per min at pH 4.5 at 30°C. For cellulase, one unit will liberlate 1 micromole of glucose from cellulose in one hr at pH 5.0 at 37°C (2 hr incubation time). For pectinase, one unit will liberlate 1 micromole of galacturonic acid from polygalacturonic acid per min at pH 4.0 at 25°C.

% reducing sugars yield was represented the amount of g of reducing sugars per 100 g of cassava peels.

All determinations were replicated three times to estimate mean values and standard deviations.

RESULTS

1.5% cassava peels in diluted acids were hydrolysed under varying conditions and then determined %reducing sugars yield was determined. Figure 1 shows that optimal % reducing sugar yields could be obtained at 66.3, 63.3 and 34% from hydrolysis at 135°C for 60 min with 0.1 M sulfuric acid, 0.025 M hydrochloric acid and 0.25 M acetic acid%, respectively. Thus, dilute sulfuric produced highest yield of reducing sugars. On the other hand, acetic acid could produce as much reducing sugars as did dilute sulfuric acid and hydrochloric acid but was needed at the concentration that was much lower than the other two acids. So, the diluted hydrochloric seemed to be suitable for cassava peels hydrolysis.

The optimum conditions for amylase reaction was found to be 2 hr incubation at pH 6.0, 90°C , which produced 40.89% yield of reducing sugars as shown in Figure 2. After that the reaction for amyloglucosidase was determined and the optimum conditions was found to be 24 hr incubation at pH 4.0, 50°C, which produced 75.41% yield of reducing sugars as shown in Figure 3.

The optimum conditions for cellulase reaction was found to be 24 hr incubation at pH 5.0, 30-70°C, which produced 43.39% yield of reducing sugars as shown Figure 4.

The optimum condition for xylanase reaction was found to be 9 hr incubation at pH 4.0, 50°C, which produced 2.64% yield of reducing sugars as shown Figure 5.

The optimum conditions for pectinase reaction was found to be 3 hr incubation at pH 4.0, 40°C which produced 9.01% yield of reducing sugars as shown in Figure 6.

DISCUSSION

When the cassava is used for bioconversion process to produce cassava starch, the residue is cassava peels. Hydrolysis of the hemicellulose fraction results in hydrolysate rich in hexoses and pentoses and with a large amount of xylose (Almeidae Silva *et al*.,1995). From this study, the best enzymatic reaction was found to be a-amylase followed by amyloglucosidase, producing the highest production of reducing sugars. This might

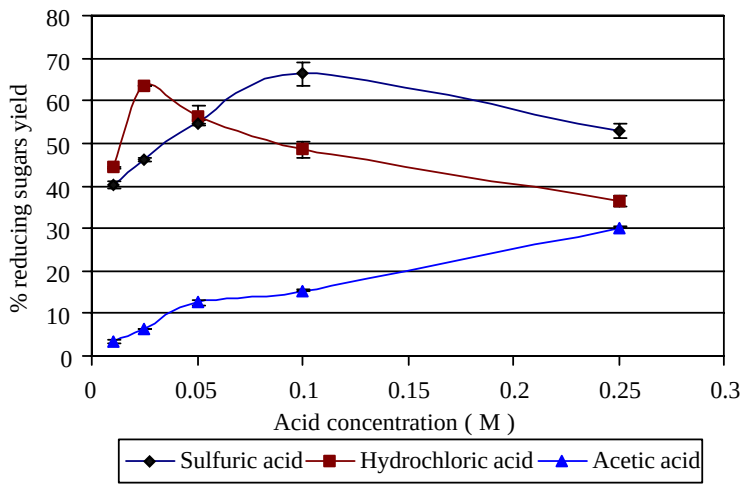

Figure 1 % reducing sugar yield from 1.5%cassava peels were hydrolysed by various concentration of diluted acid at 135°C for 90 min.

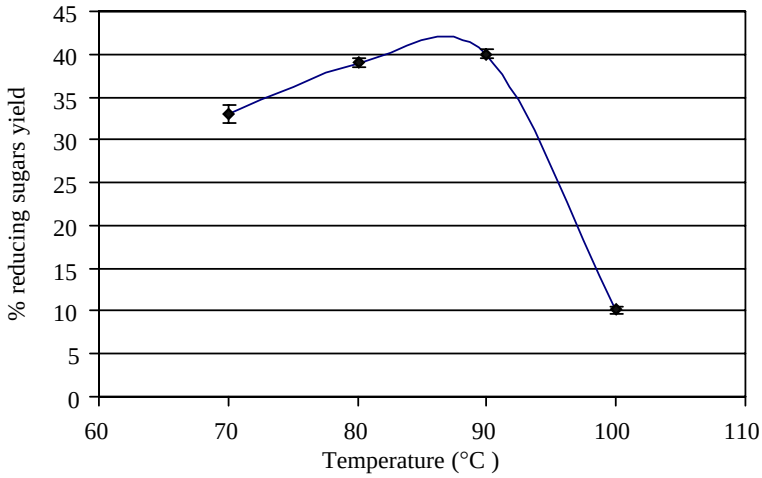

Figure 2 % reducing sugars yield from 1.5%cassava peels were incubated with amylase at pH 6.0, 70-100°C for 2 hr.

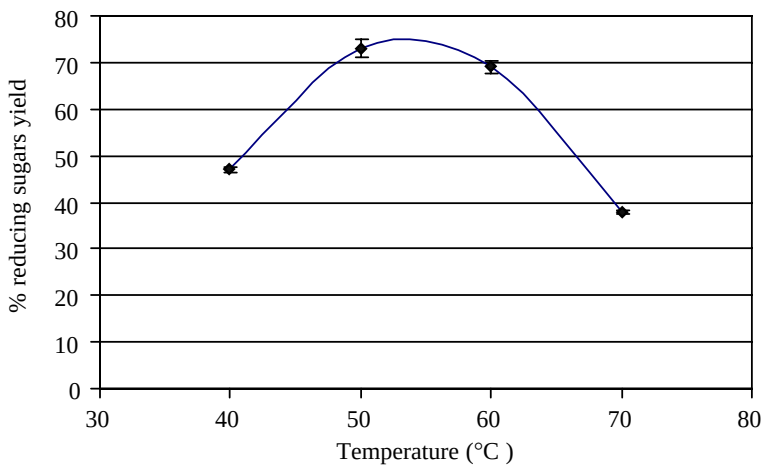

Figure 3 % reducing sugars yield from hydrolysate from amylase reaction above incubated with amyloglucosidase at pH 4.0, 40-70°C for 24 hr.

be due to large amount of starch in the cassava peels. Cellulose is known to be highly resistant to enzymatic hydrolysis but these difficulties can be overcome by employing a suitable chemical or mechanic al pretreatment prior to hydrolysis (Abasaeed *et al*., 1991). In comparison between acid and enzyme hydrolysis, it was shown that the cassava peels could be used to produce sugar by enzyme amylase and amyloglucosidase better than diluted acids.

This might be due to sugar product decomposed under experimental conditions although the methods for diluted acid hydrolysis appeared simpler than the enzymatic methods. Degradation of the sugars produced in the acid hydrolysis might be minimized by selectively

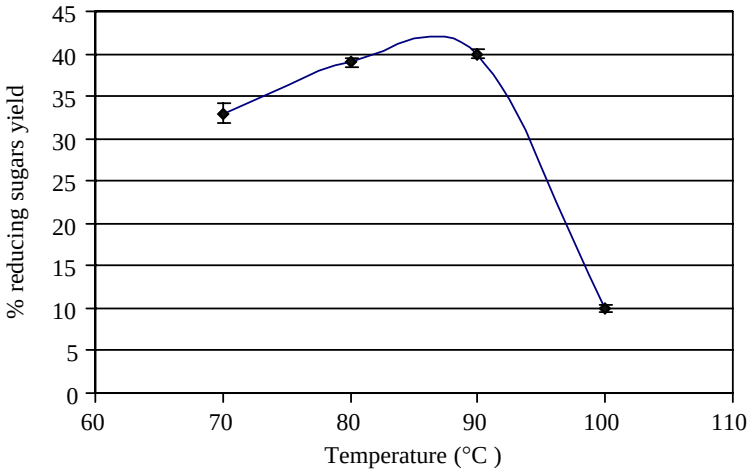

Figure 4 % reducing sugars yield from 1.5%cassava peels incubated with cellulase reaction at pH 5.0, 30-70°C for 24 hr.

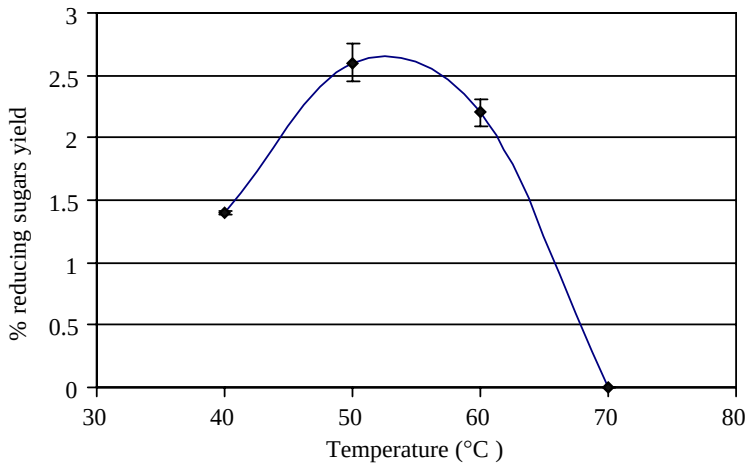

Figure 5 % reducing sugars yield from 1.5%cassava peels incubated with xylanase reaction was at pH 4.0, 40-70°C for 9 hr.

removing these sugars from the reactor during period of time of hydrolysis before further hydrolysis in the reactor. However, one of the major drawbacks of this process is that the acid hydrolysis equipment must be designed to withstand corrosive conditions at the high temperatures and pressures employed.

From the experiment, it was found that under the optimal conditions, a-amylase and amyloglucosidase treatment were capable of producing the amount of reducing sugars from cassava peels more than the other hydrolytic enzymes and diluted acid hydrolysis. However, in this experiment, the cassava peels pretreated by milling to flour in the size of 63-425 micrometre were taken part in hydrolysis by these hydrolytic

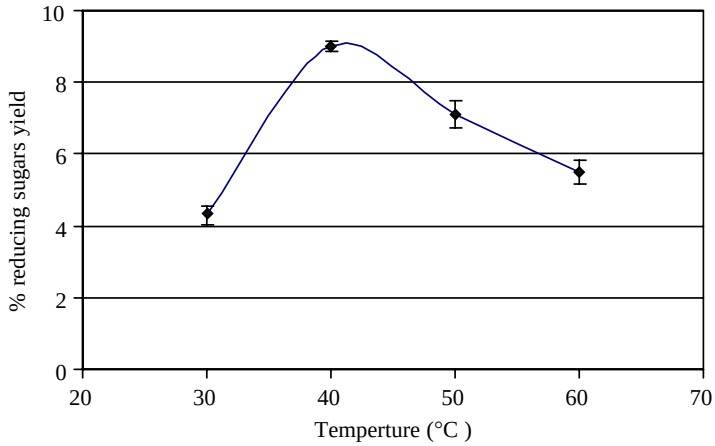

Figure 6 % reducing sugars yield from 1.5%cassava peels were incubated with pectinase at pH 4.0, 30 - 60°C for 3 hr**.**

enzymes to produce reducing sugars. Therefore the hydrolysis by combining of these hydrolytic enzymes should be produce higher yield of sugars and from these results, hydrolysis by different enzymes could produce sugars in difference amount suggesting different contents of cassava peels compositions and the reducing sugar products might be used for further applications.

CONCLUSIONS

The results showed that three diluted acids; sulfuric acid, hydrochloric acid and acetic acid; were evaluated for the acid hydrolysis for the production of reducing sugars from cassava peels. 0.1 M sulfuric acid was found to yield significantly more reducing sugars than hydrochloric acid and acetic acid. Under optimal conditions. a-amylase and amyloglucosidase were capable of producing the amount of reducing sugars cassava peels more than the other hydrolytic enzymes; cellulase, xylanase and pecticnase. In this study, comparison of the reducing sugars produced in the reaction mixture of acid hydrolysis and enzymatic hydrolysis it was found that the enzymatic treatment of a-amylase and amyloglucosidase reaction produced reducing sugars more than the acidic hydrolysis, however the enzyme reaction need longer incubation time than the acidic reaction while the acid treatment has to be used under the severe condition.

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