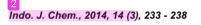
Production of Reducing Sugar from Cassava Solid Waste by Simultaneous Ultrasonication and Acid Hidrolysis

By Wasinton Simanjuntak



PRODUCTION OF REDUCING SUGAR FROM CASSAVA SOLID WASTE BY SIMULTANEOUS ULTRASONICATION AND ACID HYDROLYSIS

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ABSTRACT

In this study, acid hydrolysis of cassava solid waste under ultrasound irradiation for reducing sugar production was studied. A series of experiments was carried out under a 40 kHz ultrasound irradiation, with the main purpose to examine the effect of pHs, hydrolysis times, and temperatures on the concentration of reducing sugar produced. As a control an untreated sample was subjected to thermal hydrolysis under the optim 4n condition obtained from the hydrolysis experiments under ultrasound treatment. The results obtained indicated that the highest reducing sugar concentration was obtained from the experiment undertaken at pH = 2.0; hydrolysis time of 90 min, and temperature of 80 °C. Experiment conducted at this optimum condition was found to produce reducing sugar with the concentration of 801 mg/L. This concentration is around 11 times over that produced from control sample, demonstrating that simultaneous ultrasonication and hydrolysis is far more effective than conventional thermal hydrolysis. This hydrolysis due to the cavitations of the ultrasound stimulated degradation of starch molecules, leading to accelerated hydrolysis due to the cavitations of the molecules, as evidently shown by the result of SEM characterization of the original and ultrasonicated sample.

Keywords: cassava solid waste; ultrasound; reducing sugar

ABSTRAK

Penelitian ini dilakukan untuk mempelajari hidrolisis limbah padat industri tapioka (onggok) menghasilkan gula reduksi di bawah pengaruh ultrasonikasi. Serangkaian percobaan dilakukan menggunakan ultrasonikasi dengan frekuensi 40 kHz, dengan tujuan mempelajari pengaruh pH, waktu, dan suhu hidrolisis terhadap konsentrasi gula reduksi yang dihasilkan. Sebagai kontrol, satu sampel dihidrolisis dengan pemanasan pada kondisi yang sama dengan kondisi optimum yang diperoleh dari percobaan dengan ultrasonikasi. Hasil yang diperoleh menunjukkan kondisi optimum adalah pH = 2,0; waktu hidrolisis 90 menit, dan suhu 80 °C, dengan kadar gula reduksi yang dihasilkan sebesar 801 mg/L. Konsentrasi ini sekitar 11 kali konsentrasi yang dihasilkan dari sampel kontrol, yang menunjukkan bahwa hidrolisis dengan bantuan ultrasonikasi jauh lebih efektif dibanding hidrolisis termal. Keunggulan ini diyakini sebagai akibat terjadinya perubahan struktur sampel akibat perlakuan ultrasonikasi, yang ditunjukkan dengan jelas oleh hasil karakterisasi sampel dengan SEM.

Kata Kunci: limbah padat tapioka; ultrasonikasi; gula reduksi

INTRODUCTION

In response to uncertain fuel supply due to depletion of fossil fuel together with continuous increased energy demand and the need to reduce carbon dioxide emission, development of renewable and environmentally friendly energy sources has become one of the most challenging efforts around the globe. Currently, many types of renewable energy sources are intensively explored; one of them is bioethanol [1]. This compound is considered as one of the most promising biofuels today because raw material for its production is provided by many types of source crops which can be cultivated renewably in most climates around the world. In addition, combustion of bioethanol produces less CO_2 gas, which is an obvious advantage over fossil fuels. Another environmental benefit that should be acknowledged is that the use of bioethanol in principle is CO_2 neutral because in the growing phase of the source crops, CO_2 is absorbed by the plant and O_2 is released in the same volume with that of CO_2 produced from the combustion of this fuel. Furthermore, bioethanol can be blended with gasoline in various ratios to produce gasohol [2] and used as the main reactant of transesterification of vegetable oils for production of biodiesel [3], which currently is another important renewable fuel.

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In addition to the main product of the source crops, various agro-industrial residues are potential raw material for bioethanol production, such as sugarcane bagasse, corn stoves, and cassava solid waste. Cassava solid waste is an attractive raw material for bioethanol production for several reasons. Cassava is known as fast growing plant and reaches mature stage within 9-12 months of age, ensuring its role as sustainable source crop. This crop is known as one of the prime sources of starch which has been used as food and raw material for various purposes, such as production of copolymer [4] and bioethanol [5].

In the production of starch flour from cassava tubers, cassava solid waste is produced which tables, cassava solid waste is produced which tables, cassava solid waste is produced which tables, cassava solid waste can serve as an important substrate for production of bioethanol. Furthermore, utilization of cassava solid waste is beneficial in terms of the cost of raw material, competition between food industry and bioethanol industry, environmental safety, and creation of value added for cassava farm.

In utilization of starchy materials for bioethanol production, it should be recognized that starch is nonfermentable carbohydrate, and for this reason, the starch must be 3 onverted into reducing sugar through hydrolysis in the presence of an acid or enzyme as catalyst. While enzymatic hydrolysis continues to gain popularity [8-10], currently acid hydrolysis remains as the most widely method applied, primarily due to its simplicity and cost effectiveness since no supporting substrates is required as in the case with enzymatic method. Acid hydrolysis is commonly carried out at pH of 1.0 to 4.0 using strong mineral acids, primarily sulfuric acid and hydrochloric acid, under temperature range of around 100 to 135 °C, [11-14]. The need to carry out hydrolysis at relatively high temperature is considered as the prime disadvantage of acid hydrolysis.

In an attempt to increase glucose yield produced by hydrolysis of starch, another approach that has been applied is pretreatment of the starch prior to hydrolysis, with the main purpose to alter the structural characteristics of the starch to make it more amenable to hydrolysis. Currently, several pretreatment methods have been introduced, such as hot water pretreatment [15], pressured steam blast [16], microwave irradiation [5,17], and ultrasound irradiation or ultrasonication [18].

Ultrasound is known to increase chemical reactivity of macromolecules through an effect known as cavitation, which causes the molecules mor 13 usceptible to further processing. In particular, the efficiency of ultrasonication in the processing of vegetal materials has been reported in many studies. For examples, this technique has been applied to enhance isolation of lignin from wheat straw [19] and isolation of cellulose from kenaf and eucalyptus plants [20] Ultrasonication has also been used as pretreatment prior to hydrolysis for promoting reducing sugar production from cellulose [21-22], and lignocelluloses [23-2 20] More specifically, this technique has been applied as a pretreatment to enhance the production of reducing sugar from different starch samples, such as corn starch [25], cassava chip slurry [18], and grain sorghum [26].

Based on the findings on the utilization of ultrasound to enhance the degree of hydrolysis of starch materials, this study was carrie 23 put to investigate simultaneous ultrasonication and hydrolysis of cassava solid waste for production of reducing sugar. A series of experiments was conducted using 40 kHz ultrasound energy, with the main objective to examine the effect of three common hydrolysis variables, namely pH, time, and temperature on the degree of hydrolysis of cassava solid waste in term of the concentration of reducing sugar produced.

EXPERIMENTAL SECTION

Materials

Cassava solid waste was obtained from local cassava com19 y in Bandar Lampung. Before use, the sample was dried at 105 °C in an oven for 6 h, and then grounded into fine powder. The chemicals used were reagent grade sulfuric acid, dinitrosalicylic acid, sodium hydroxide, phenol, sodium sulfite, and sodium potassium tartaric, purchased from Aldrich.

Instrumentation

The instruments used were 40 kHz Bandelin Sonorex Technique ultrasonicator, EVO MA 10 Carl Zeiss Scanning Electron Microscopy, and Varian Cary 100 UV-Vis spectrophotometer for reducing sugar determination.

Procedure

As previously stated, this study aims to investigate the effects of pHs, hydrolysis time, and temperatures on the contrastration of reducing sugar. For these purposes, the experiments were conducted at different pHs of 1, 2, 3, and 4, hydrolysis time up to 300 min with 30 min interval, and temperatures of 50, 60, 70, 80, and 90 °C.

In the experiment, 20 g of cassava solid waste was dispersed in 250 mL of deionized water in a 500 mL beaker glass. The pH of the sample was adjusted to specified value using 5% H₂SO₄ solution, and then placed in the ultrasonicator filled with water.

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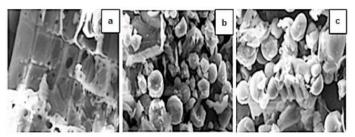


Fig 1. SEM images of the samples investigated (a) the original sample, (b) sample subjected to ultrasonication for 1 h, (c) sample subjected to ultrasonication for 3 h

The temperature of the water in the ultrasonicator was set to specified value, and then the hydrolysis was conducted for specified time. After the completen of the experiment, the sample was filtered and the concentration dipeducing sugar in the filtrate was determined by 3,5-dinitrosalicylic acid (DNT) method, using glucose solution as standard [27]. A standard curve was drawn by measuring the absorbance of known concentrations of glucose solutions at 550 nm, and the absorbance was plotted against the concentration of the standard to produce a line equation.

The DNS solution was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid 1720 mL distilled water and the solution was transferred into a 19 mL volumetric flask. Into the solution, an amount of 1 g of NaOH, 0.2 g of phenol, 0.05 g of Na₂SO₃, and 1 mL of 40% sodium potassium tartaric solution, and the volume of the solution was fixed to the mark with distilled water.

For reducing sugar determination, an aliquot of 0.25 mL of sample was placed in test tube, and then 0.25 mL distilled water an 22 mL DNS solution were added into the sample. The mixture was heated at 90 °C for 10 min in a water bath. The sample was cooled to a room temperature and the absorbance of the sample at 550 nm was recorded. The concentration of reducing sugar was calculated by substituting the absorbance of the sample into the line equation obtained from the standard curve.

RESULT AND DISCUSSION

Effect of Ultrasonication on Sample Morphology

To examine the effect of ultrasound treatment on the structure of the cassava solid waste, the untreated sample and those subjected to ultrasound treatment were characterized using SEM. The images obtained and presented in Fig. 1 show significant effect of the treatment on the morphology of the samples.

As can be seen, SEM images show that the nonpretreated sample has an intact structure, marked with contiguous and smooth surface (Fig. 1a). The pretreated sample, on the other hand, displays very evident disrupted surface structure, transforming the surface into porous structure characterized by the existence of spherical grains. This porous structure increases the surface area accessible to acid, leading to increased efficiency of hydrolysis process. It is then expected that ultrasonication will facilitate the simultaneous hydrolysis of the sample, leading to increased concentration of reducing sugar produced.

In the study by Shewale and Pandit [26], it was reported that application of ultrasound pretreatment of grain sorghuin increased the reducing sugar concentration by 10-25%, depending on the sonication time and ultrasound power applied. The result was attributed to the increased availability of more starch molecules for hydrolysis due disruption of the protein matrix and lipid complex surrounding the glucose granules. In another study, Huang et al. [28] subjected corn starch grantles to ultrasound pretreatment prior to hydrolysis and reported that the degree of hydrolysis ficreased significantly and suggested that the result was attributed to the effect of ultrasound on the structure of starch. It is also suggested that the amorphous regions of the starch molecules are more susceptible to attack by the ultrasound pretreatment.

Effect of pH on Reducing Sugar Production

The first set of hydrolysis experiments was conducted in order to investigate the influence of the pH on the glucose concentration produced. For this purpose, a series of experiments was carried out at different pHs of 1.0, 2.6 3.0, and 4.0. Each experiment was run at constant temperature of 50 °C and for constant time of 30 min. The concentrations of reducing sugar produced as a function of pH are presented in Fig. 2. As a control, the reducing sugar content of the untreated sample was measured in a similar way and the result obtained was 1.5 mg/L, indicating that the reducing sugar content of the untreated sample could be neglected, and the reducing sugar produced can be considered as the result of the

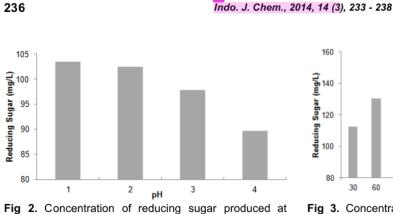


Fig 2. Concentration of reducing sugar produced at different pHs

treatmen 21 plied.

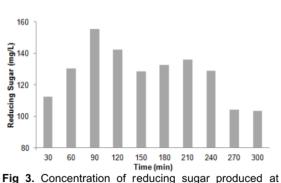
As shown in Fig. 2, the highest concentration of reducing sugar (103.4 mg/L) was achieved from the experiment at pH = 1, but no significant difference to that obtained from the experiment with pH = 2 (102.5 mg/L). On the other hand, sharp reduction of the concentration of reducing sugar was found for the experiments at higher pHs. This was most likely due to decreased amount of acid available for hydrolysis as pH of the sample was increased. Taking these results into account, together with consideration of the amount of acid required, the pH = 2 was considered as optimum pH and therefore applied for the rest of the experiment, carried out to investigate the effect of hydrolysis time and temperature.

As previously stated, the effect of pH in hydrolysis of starch materials has been reported in many investigations. Commonly acid hydrolysis is carried out at pH of 1.0 to 4.0 [8-11], and in this respect, the optimum pH obtained in this study is in accordance with the previous findings reported by others.

Effect of Hydrolysis Duration

To assess the influence of the duration of hydrolysis on the glucose (reducing sugar) concentration produced, a series of hydrolysis experiments was carried out in a similar way, keeping the temperature constant at 50 °C and pH of 2, with varied hydrolysis times up to 3 h with interval of 30 min. The reducing sugar concentration achieved 16 different times is presented in Fig. 3.

As shown in Fig. 3, the concentration of reducing 45 ar produced increased sharply up to 90 min, in which the highest concentration of reducing sugar was achieved, and then decreased at longer hydrolysis times. It is then clear that prolonged hydrolysis tend to reduce the degree of hydrolysis of the starch in the sample. The trend of the results observed in this study is in accordance with the result of previous study reported by others [29], in which it was concluded that elevated



different times

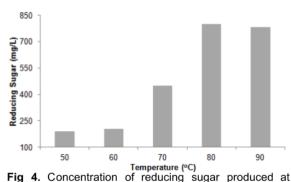
concentration of reducing sugar during the hydrolysis of starch led to significant decrease of t 8 hydrolysis rate. Another reason for decreased concentration of reducing sugar is the possibility of reducing sugar degradation as a consequence of prolonged time, as also observed by others [30]. According to the results obtained, it was concluded that the optimum time is 90 min, and this time was applied in the experiments to study the effect of temperature.

Effect of Hydrolysis Temperature

The effect of temperatures was evaluated by conducting the hydrolysis experiments at five levels of temperature, while pH and time were maintained at constant value of 2.0 and 90 min, 6 espectively. The experimental results are presented in Fig. 4, showing that the concentration of reducing sugar produced increased steadily following the temperatures. no significant different However. between the concentrations achieved at 80 and 90 °C. Considering the trend observed, the temperature of 80 °C is considered as the optimum temperature with the concentration of red 4 ng sugar achieved is 801 mg/L.

In the context of reducing sugar production from starch material, the temperature of hydrolysis is acknowledged as a variable with very significant role. In conventional acid hydrolysis, a wide range of temperature has been reported in many investigations. Sovorawet and Kongkiattikajorn [14], for example, studied hydrolysis of cassava stalk at different temperatures and reported that the temperature of 135 °C as the optimum temperature. This optimum temperature is practically similar to the optimum temperature for hydrolysis of maize starch, in which the optimum temperature of 132 °C was reported [12]. In another study [13] it was reported that the optimum reducing sugar from sago waste was achieved at 120 °C, in which the experiment was carried out in an autoclave. In earlier study [31] it was found that the

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different temperatures

optimum temperature for reducing sugar production from pure cassava starch was 98 $^\circ\text{C},$ carried out at pH of 0.8 for 4.5 h.

In term of the temperature, the current study demonstrated that hydrolysis of starch can be achieved at much lower temperatures than those required in conventional thermal hydrolysis. In addition, the hydrolysis process can be carried out at atmospheric pressure, which is another advantage worthy to consider. As previously stated, to compare the efficiency of ultrasonication, an untreated sample was subjected to thermal hydrolysis in which the optimum condition obtained from ultrasonication experiments was applied. The concentration of reducing sugar produced from the control sample was only 71.2 mg/L, while from the experiment involving ultrasonication the concentration of reducing sugar achieved was 801 mg/L, or more than 11 times than that produced from untreated sample. In this respect, the present study has demonstrated that simultaneous ultrasonication and acid hydrolysis offers the potential to optimize the production of reducing sugar from cassava solid waste.

CONCLUSION

The present study demonstrated that the simultaneous ultrasonication and acid hydrolysis of cassava solid waste is considerably more effective than thermal hydrolysis for product on of reducing sugar. The results obtained indicated that the highest reducing sugar concentration was obtained from the experiment undertaken at pH = 2, hydrolysis time of 90 min, and temperature of 80 °C. Experiment conducted at this optimum condition was found to produce reducing sugar with the concentration of 801 mg/L, which is 11 times over that produced from the same condition but without ultrasonication. This beneficial performance is attributed to the significant change of the structure of the sample

due to cavitation of the sample as evidently demonstrated by the results of SEM characterization.

ACKNOWLEDGEMENT

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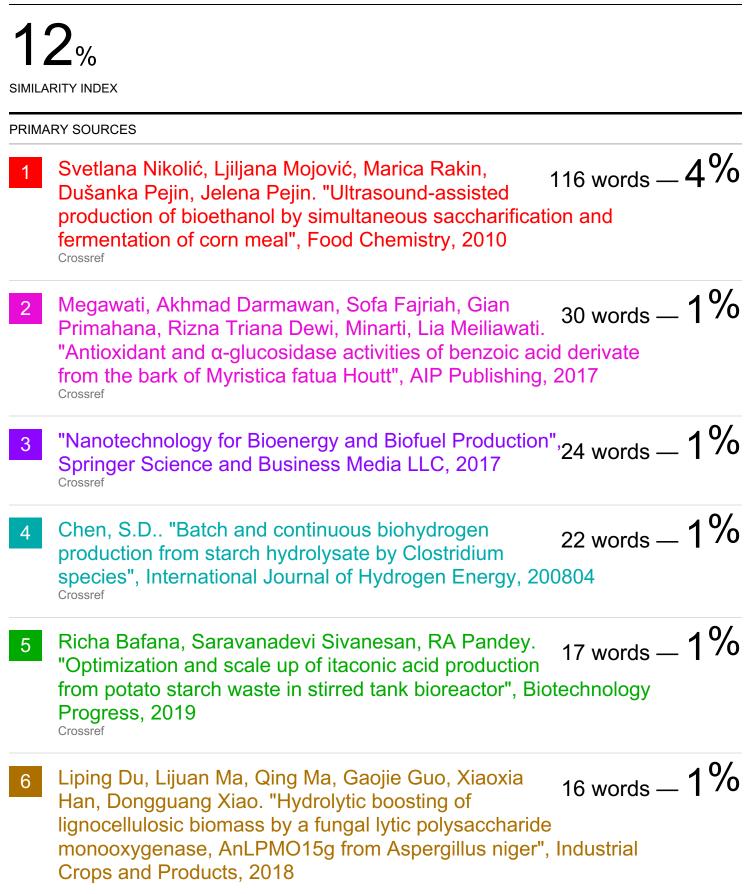
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