Reducing Sugar Production from Cassava Pulp Using Enzymes and Ultra-filtration I: Enzymatic Hydrolyzation

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Cassava pulp, which is comprised of mostly carbohydrate (66%) and fiber (15%), was used as raw material in glucose production. The hydrolysis of cassava pulp was accomplished by using acids or enzymes. The hydrolyzation of cassava by hydrochloric acid produced hydrolyzate with a reducing sugar content of 61.68 $\pm$ 0.62% in 2.5 hours. This paper aimed to increase the hydrolyzation of cassava pulp by using enzymes, mixed enzymes and sequences in combination with ultra-filtration to enhance the efficiency of the process. It was found that the combination of 285.6 MWU of alpha-amylase and 0.21 DU of glucoamylase per gram of cassava pulp with a pH of 5.0 at a temperature of 50^{o}C gave a hydrolyzate with higher reducing sugar content than using either enzyme alone. The hydrolyzation rate was 0.133 mg of reducing sugar per minute. Reducing sugar was produced at the rate of 24.1 g/100g of cassava pulp when hydrolyzed for 4 hrs. The application of the ultrafiltration system with an average pressure of 98 kPa, a flow rate passed the membrane surface of 130 ml/sec and a filter area of 28.27 cm^2 gave a yield of 29 g reducing sugar /100 g cassava pulp, which was 20% better than the non-ultrafiltration system.

Key words: Cassava pulp, reducing sugar and ultra-filtration.

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การผลิตน้ำตาลรีดิวซ์จากกากมันสำปะหลังโดยการใช้เอนไซม์และอัลทราฟิวเทรชัน I: การย่อยด้วยเอนไซม์

สุนีย์ โชตินีรนาท ชิดพงศ์ ประดิษฐสุวรรณ โปรดปราน สิริธีระศาสน์ และ
สุเมธ ตันตระเธียร (2547)

วารสารวิจัยวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **29(2)**

ได้ใช้กากมันสำปะหลัง ซึ่งองค์ประกอบส่วนใหญ่คือ คาร์โบไฮเดรต (66%) และไฟเบอร์ (15%) เป็นวัตถุดิบในการผลิตน้ำตาลกลูโคส การย่อยกากมันสำปะหลังด้วยกรดไฮโดรคลอริกเป็นเวลา 2.5 ชั่วโมง ให้ปริมาณน้ำตาลรีดิวซ์ 61.68±0.62% บทความนี้มีวัตถุประสงค์เพื่อเพิ่มประสิทธิภาพการย่อยกากมันสำปะหลังโดยเอนไซม์แอลฟาอะไมเลส และกลูโคอะไมเลส และเพิ่มประสิทธิภาพการย่อยโดยนำอัลทราฟิลเทรชั่นมาประยุกต์ใช้ จากการทดลองพบว่าภาวะที่เหมาะสมที่เอนไซม์ทั้งสองน่าจะทำงานร่วมกันได้คือ ค่าความเป็นกรดด่าง 5.0 ที่ 50 องศาเซลเซียส การใช้เอนไซม์ผสมสามารถผลิตน้ำตาลรีดิวซ์ได้มากกว่าการใช้เอนไซม์-แอลฟาอะไมเลสหรือกลูโคอะไมเลส เพียงชนิดเดียว อัตราส่วนของแอลฟาอะไมเลสต่อกลูโคอะไมเลสที่เหมาะสม คือ แอลฟาอะไมเลส 285.6 MWU ต่อกรัมกากมัน กลูโคอะไมเลส 0.21 DU ต่อกรัมกากมันมีความเร็วในการผลิตน้ำตาลรีดิวซ์เท่ากับ 0.133 มิลลิกรัมน้ำตาลรีดิวซ์ต่อมิลลิลิตรต่อนาที ทำการย่อยเป็นเวลา 4 ชั่วโมงได้น้ำตาลรีดิวซ์ 24.10 กรัม/100 กรัมของกากมันสำปะหลัง การใช้อัลทราฟิวเทรชันในกระบวนการย่อยกากมันสำปะหลังที่ความดันเฉลี่ย 98 kPa อัตราไหลผ่านแผ่นกรอง 130 มิลลิลิตรต่อวินาที พื้นที่การกรอง 28.27 ตารางเซนติเมตร พบว่าปริมาณน้ำตาลรีดิวซ์ที่ได้เพิ่มขึ้นเป็น 29 กรัม/100 กรัมกากมันสำปะหลัง ซึ่งให้ผลดีกว่าระบบที่ไม่ใช้อัลทราฟิวเทรชันร้อยละ 20

คำสำคัญ กากมันสำปะหลัง น้ำตาลรีดิวซ์ อัลทราฟิวเทรชัน

INTRODUCTION

Thailand has grown cassava for her own consumption and for export for many years. Cassava has become an important economic plant of Thailand. Cassava starch is used in many industries including the food industry. Cassava pulp is the by-product from the starch production industry. With its high starch content, cassava pulp has the potential to be a good substrate for many fermentation industries.

The hydrolyzation of cassava starch has been studied and the hydrolyzed products used for ingredients in many food products. Many studies have reported the enzymatic hydrolyzation of cassava starch.[1,2] The utilization of cassava pulp as a substrate for hydrolyzation is overlooked. Since the carbohydrates in cassava pulp composed of starch and cellulose, different enzymes are necessary to complete the hydrolyzation. The limitations of hydrolyzation are selecting the type of enzymes and controling the concentration of end product. The accumulation of the end product, glucose, slows down the hydrolyzation process. To shorten the time of the starch hydrolyzation, mixed enzymes of α-amylase and glucoamylase are used.[3]

Enzymatic hydrolyzation of cassava starch as well as cassava pulp is limited by the effectiveness of α-amylase and glucoamylase in producing high concentrations of end product. The accumulation of the end product could be minimized by the application of ultra-filtration. The ultra-filtration removes hydrolyzation end product from the system while still keeping the enzymes in the system. Previous studies described the advantages of the application of ultra-filtration to separate glucose from the hydrolyzation system.[4,5]

The objective of this experiment was to apply an ultra-filtration system for increasing the reducing sugar production from the enzymatic hydrolyzation of cassava pulp.

MATERIAL AND METHOD

Dried cassava pulp (kindly provided by ThaiWa Co., Bangkok) was milled and sieved through a 25 mesh sieve. The enzymes for hydrolyzation were α-amylase (Tenase® L-340, Solvay Enzyme, Inc., Indiana, USA.) and glucoamylase (Diazyme® L-300, Solvay Enzyme, Inc., Indiana, USA). The products from enzymatic hydrolyzation were measured as reducing sugar by the Somogyi-Nelson method.[6]

Hydrolyzation of cassava pulp with hydrochloric acid

5 grams of cassava pulp in 200 ml of water was added to 20 ml HCl and refluxed for 2.5 hr. The hydrolyzate was cooled and neutralized with 5N NaOH and the volume was adjusted to 500 ml. The reducing sugar content was determined by the Somogyi-Nelson method.[6]

Effects of pH on the enzyme activity

Cassava pulp was suspended in 0.05 M citrate buffer for a pH level of 3.0, 0.05 M acetate buffer for pH 4.0 and 5.0 and 0.05 M phosphate buffer for pH 6.0 and 7.0 at the concentration of 2.4 g/dl. The enzyme α-amylase (1,360 MWU* per gm of cassava) and glucoamylase (1 DU** per gm of cassava) were added to the suspension separately. The total volume was 3.5 ml. The suspension was shaken and incubated at 30°C for 1 hour. The enzyme activities were terminated by boiling for 15 minutes. The supernatant was analyzed for reducing sugar.[6] The experiment was repeated for 3 times.

The stability of the enzymes on the pH was examined by incubating the enzymes in various pHs for 1 hr. at 30°C prior to hydrolyzing. The α-amylase and glucoamylase were diluted to 1,360 MWU and 1 DU per gm of cassava pulp, respectively. The hydrolyzation was carried on in 0.05 M phosphate buffer pH 6.0 for glucoamylase and in 0.05 M acetate buffer pH 4.0 for α-amylase. The experiment

was conducted in the same manner as above.

Note:

* 1 MMU can be described as the amount of enzyme used for hydrolyzing 1 mg of starch for 30 min following the standard method of modified Wohlgemuth method at pH 6.0, 40°C.

** 1 DU can be described as the amount of enzyme used for hydrolyzing starch to produce glucose 1.0 gram in 1 hr at pH 4.2, 60°C.

Effect of temperature on the enzyme activity

Cassava pulp was suspended in buffer and treated in the optimum pH from the prior experiment. The suspensions were incubated with enzymes at temperatures of 30, 40, 50, 60 and 70°C to find the optimum temperature. The stability of the enzymes at various temperatures was checked by incubation of the enzymes at each temperature for 1 hr prior to hydrolyzing. The treated enzymes were used to hydrolyze cassava pulp at the optimum temperature.

Hydrolyzation of cassava pulp with mixed enzymes

The cassava pulp 2 g/dl in 0.05 M acetate buffer pH 5.0 was hydrolyzed with α-amylase 8160 MWU and glucoamylase 6 DU per ml. The ratios of each enzyme by volume were varied as 1:0, 0.8:0.2, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.2:0.8 and 0:1 for the total volume of 1.0 ml. Enzyme and cassava pulp suspension were mixed and made up to the volume of 50 ml. The suspensions were incubated at 50°C and shaken at 100 rpm for 1 hr. The reducing sugar was determined.

The best ratio of glucoamylase and α-amylase as determined above was used to hydrolyze the cassava pulp at various concentrations from 1-6 g/dl to select for the proper cassava pulp concentration for the hydrolyzation. The total volume was 50 ml. The hydrolyzation was carried on with the incubation at 50°C, shaken at 100 rpm for 15 min. The reducing sugar produced was determined and the rate of reducing sugar production was calculated.

Reducing sugar production in the Ultra-filtration system

Ultra-filtration was applied to detain the enzymes in the hydrolyzation system in order to improve the yield of hydrolyzation. The ultra-filtration system consisted of a 3-liter tank, pump, valves, pressure gauge and polyethersulfone filter with a molecular weight cut off at 10K, 6.0 cm in diameter. The system was set up as in Figure 1.

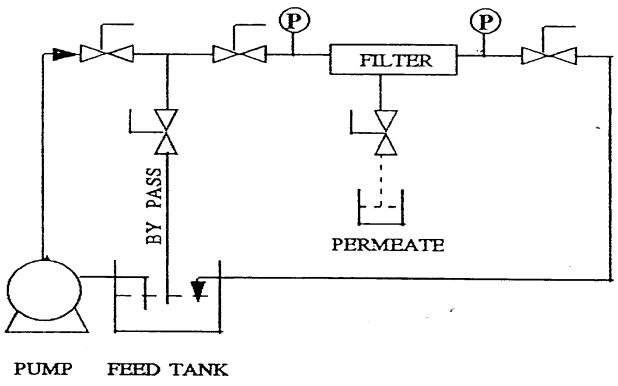

Figure 1. Chart and flow direction of Ultra-filtration system.

The appearance rejection coefficient was determined by

$$\sigma_{obs} = 1 - C_p/C_b$$

when σ_{obs} = appearance rejection coefficient
C_p = permeate concentration
C_b = initial concentration of the solution

The production of reducing sugar in the ultra-filtration system was compared with the hydrolyzation of the optimum ratio of enzymes and cassava pulp concentration for 10 to 240 min with no ultra-filtration applied.

RESULTS AND DISCUSSIONS

Cassava pulp was chemically analyzed. It contained 3.39% protein, 0.24% fat, 15.25% fiber and 66.22% as calculated carbohydrate. The pulp was hydrolyzed chemically by reflux with hydrochloric acid for 2.5 hours. The hydrolyzate was determined for reducing sugar as 61.68 $\pm$ 0.62%. The reducing sugar content of hydrolyzate was less than the result calculated from the theory, 68.82 %, which means that some of the reducing sugar was converted to furfural compounds.[7]

Conditions and the enzyme activity of α-amylase and glucoamylase

The cassava pulp was hydrolyzed by the α-amylase and glucoamylase for 1 hr. Then, the hydrolyzates were examined for reducing sugar content. Hydrolyzation with α-amylase produced a hydrolyzate containing the highest amount of reducing sugar, 1.52 mg/ml at pH 6.0. Hydrolyzation with glucoamylase produced 6.1 mg/ml at pH 4.0 as shown in Figure 2. The results agreed with Solvay.[7] After the incubation of the enzymes in various pH for an hour, the maximum activity of alpha-amylase was reduced at least 27% as compared with the maximum activity of the untreated enzymes. Glucoamylase showed about a 25 percent reduction of maximum activity after incubation at the maximum pH (Figure 3). At pH 5.0, both enzymes were stable at the incubation of 50°C. The pH 5.0 was selected for further experiment.

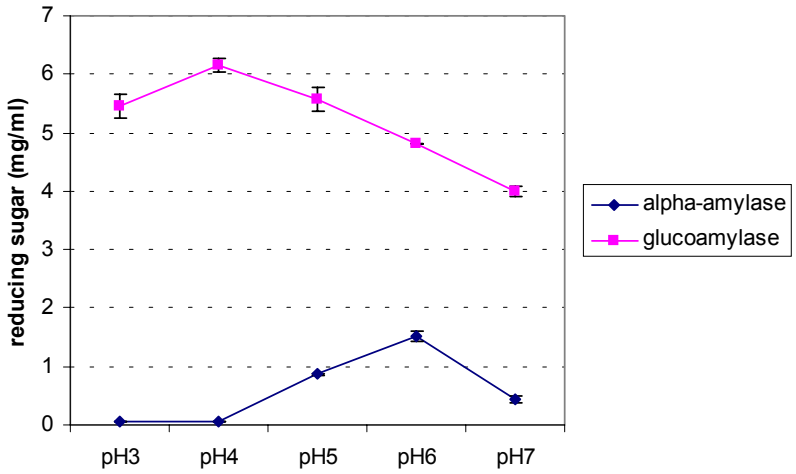

Figure 2. Effect of pH on the activity of enzymes using cassava pulp as substrate at 30°C for 1 hour.

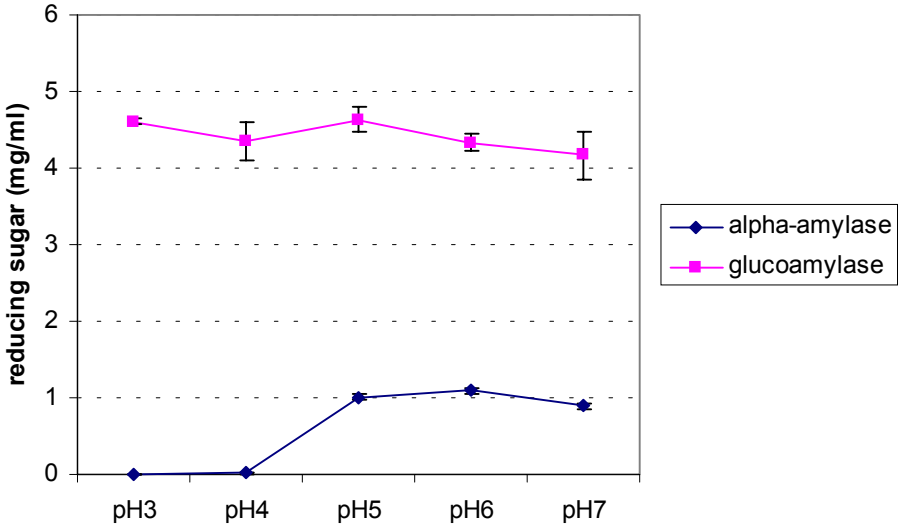

Figure 3. Activity of enzymes after one hour incubation in various pH solutions treated with α-amylase and glucoamylase to hydrolyze cassava pulp at pH 4.0 and 6.0, respectively, for 1 hour.

Effect of temperature on the enzyme activity

α-Amylase showed the highest enzyme activity at 50°C with a reducing sugar production of 2.0 mg/ml. Glucoamylase produced the highest amount of reducing sugar at 60°C at 9.0 mg/ml, while at 50°C it produced only 8.8 mg/ml (Figure 4). After incubation for 1 hr. at various temperatures, α-amylase was stable at the temperature of 40°C. At 50 and 60°C the reducing sugar declined to 28 percent. Glucoamylase started to loose its activity (15 percent loss) at 40°C. There was almost no enzyme activity from either enzymes at 70°C (Figure 5). The temperature of 50°C was chosen for further experiments because the higher temperature is more preferable for working in the ultra-filtration system.

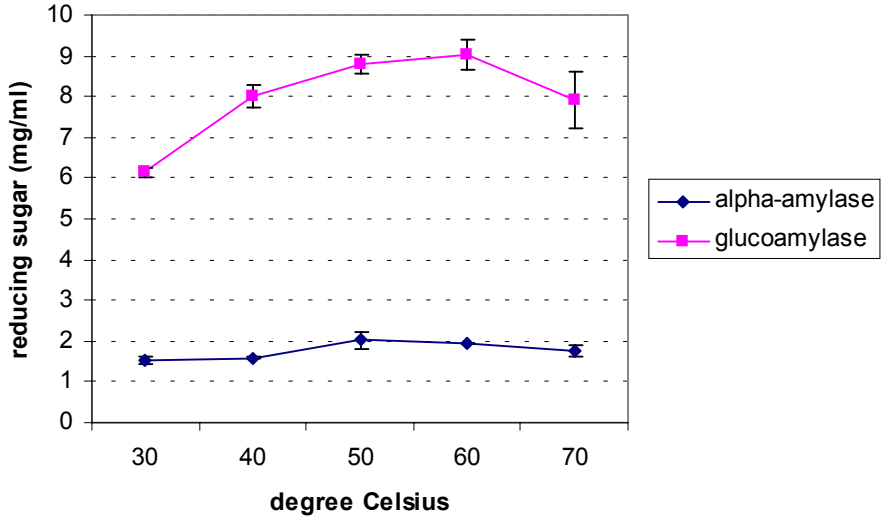

Figure 4. Effect of temperature on the activity of enzymes using cassava pulp as substrate in buffer solution of pH 4.0 and 6.0 for α-amylase and glucoamylase.

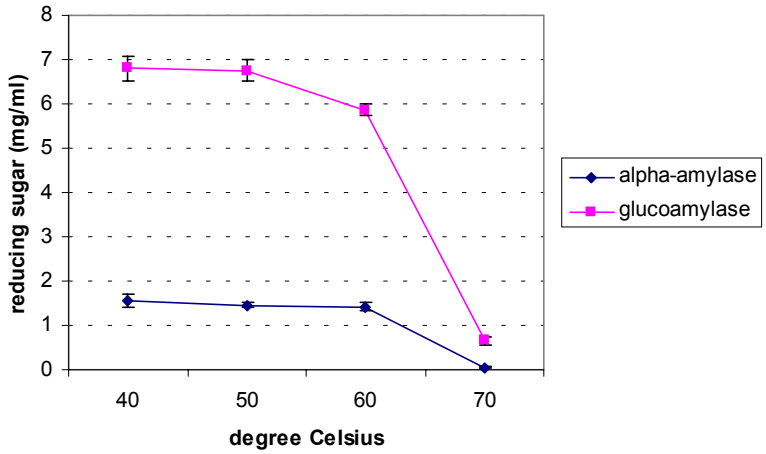

Figure 5. Activity of enzymes after incubated at various temperatures for 1 hour with hydrolyzed cassava pulp at 50°C at optimum pH of each enzyme.

Hydrolyzation of cassava pulp by α- amylase and glucoamylase combined

A mixed enzyme hydrolyzation showed better activity than single enzyme hydrolyzation (Figure 6). The 0.5:0.5 ratio of α-amylase and glucoamylase gave the highest reducing sugar from hydrolyzation at 9.88 mg/ml. The activity of α-amylase and glucoamylase on cassava pulp was about 4 times higher than the amount produced by α-amylase and 1.3 times higher than glucoamylase alone. The results agreed with Fuji *et. al.*[9] who explained that α-amylase supplied the non-reducing end groups to glucoamylase. The cooperation of the two enzymes is a synergism which increases the end product output.

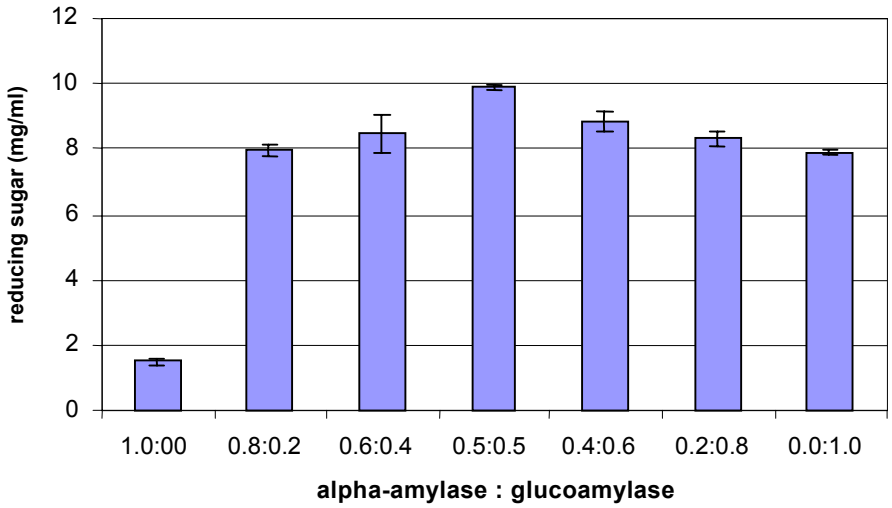

Figure 6. Reducing sugar production in the hydrolyzation of cassava pulp at various ratios of α-amylase and glucoamylase.

The use of the ultra-filtration system with the various conditions described above was explored. It was found that the production of reducing sugar reached its maximum level in a very short time. The enzymes were diluted to slow down the reaction. The 0.5 ml of α-amylase (1428 MWU/dl) and 0.5 ml of glucoamylase (1.05 DU/dl) were used to hydrolyze cassava pulp with a concentration of 1-6 g/dl. It was found that a concentration of cassava pulp at 5 g/dl in the suspension gave the highest reaction rate and produced reducing sugar at 0.133 mg/ml min as shown in Figure 7.

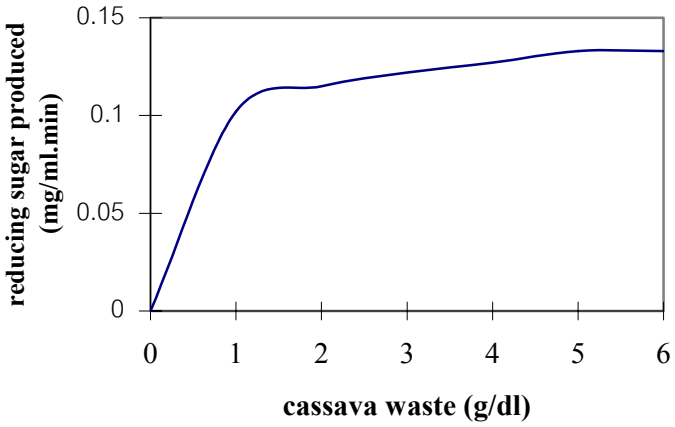

Figure 7. Rate of hydrolyzation of cassava pulp with α-amylase (1428 MWU/dl) and glucoamylase (1.05 DU/dl) at equal volume.

Reducing sugar production in the Ultra-filtration system

The hydrolyzation conditions were set using the optimal conditions obtained from the previous experiments. Cassava pulp, 5 g/dl, was hydrolyzed with α-amylase 285.6 MWU (1428 MWU/dl) and glucoamylase 0.21 DU (1.05 DU/dl) per gram of cassava pulp in 0.05M acetate buffer with pH 5.0 solution, incubated at 50°C. The reducing sugar was produced in the first 50 min at a rate of 0.135 mg/ml minute. After 50 minutes the production level declined. This might be because of the higher concentration of product. The starch that was left in the system might have been unavailable to the enzymes because it was surrounded by cellulose that is found in high concentrations amount in cassava pulp. This would also slow down the reducing sugar production.[10] The total reducing sugar production produced at 240 min was 24 g/100 g cassava pulp (Figure 8). The experiment was then rerun with the direct flow ultra-filtration system introduced after 50 min of hydrolyzation with the following conditions: the pressure in the system was 98 kPa and the flow rate of solution across the filter surface was 130 min/sec. The buffer solution at pH 5.0 was added to the system at the same rate as the effluent to control total volume of fluid in the system. With ultra-filtration, the reducing sugar production was increased to 90 min before the production slowly decreased until no production at 240 min. The total reducing sugar production at 240 min was 29 g/100 g cassava pulp, which was a 20% increase compared to the system without ultra-filtration.

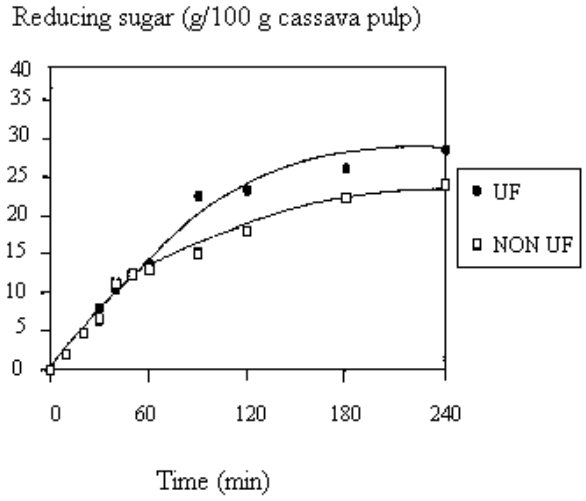

Figure 8. Reducing sugar produced by hydrolyzation using enzymes with and without ultra-filtration.

The filter sheet used in this system had a molecular weight cut off of 10K. This filter sheet was selected to keep the enzymes with MW of 93,000 and 63,000 for α-amylase and glucoamylase respectively,[11] in the system while letting the glucose molecules, MW 180, permeate through. The filter sheet had an appearance rejection coefficient for glucose of 0.090, while the appearance rejection coefficient of 1.0 means that the filter has the ability to completely detain that compound. This means that the filter sheet could let glucose when more than 90% of the solvent permeate through at the pressure 98 kPa.

The permeate flow rate of the system was reduced from 1.57 ml/min to 1.0 ml/min in 14 min of filtration. After that, the permeation rate decreased slowly to 0.5 ml/min at 100 min of filtration and then remained constant. The decrease of the permeation rate depended on the gel formation on the surface of filter sheet. The formation of gel on the surface of the filter sheet was quick at the early stage of filtration and then slowly increased until stable. Some other type of filtration, like cross flow, could be applied to avoid the gel formation.[12]

CONCLUSIONS

The proper conditions for hydrolyzation of cassava pulp were α-amylase 285.6 MWU/g cassava pulp and glucoamylase 0.21 DU/g cassava pulp buffered at pH 5 at a temperature of 50°C. The maximum activity was 0.133 mg reducing sugar/ml. min. Hydrolyzation of cassava pulp for 240 min produced reducing sugar at a rate of 24.10% by weight of the cassava pulp. Ultra-filtration with filter MW cut off at 10 K and with a pressure in the system of 98 kPa, and a flow rate across the filter of 130 ml/sec, increased the production of reducing sugar to 29% by weight of the cassava pulp. The initial permeate rate was 1.57 ml/min and reduced to constant at 0.5 ml/min. The hydrolyzation system with ultra-filtration produced the reducing sugar at a rate 20% higher than the system without ultra-filtration.

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