



## Production of Biofuel from Cassava

A. A. Bakky\*, M. R. Hoque and M. S. Islam

Dept. of Biochemistry and Food Analysis  
Patuakhali Science and Technology University, Patuakhali

\*Corresponding email: bakky.90@gmail.com

### Abstract

An experiment was conducted to produce ethanol/biofuel from cassava flour which reduce both consumption of crude oil and environmental pollution. Cassava flour hydrolysate with standard glucose and sucrose solutions were used as controls. The effects of yeast concentrations (5, 15 and 20%) and fermentation time (24, 48, 72, 96 and 120 h) on alcohol yield from CFH were also studied. The maximum carbon-dioxide evolved during fermentation was 8.57 g recorded by cassava flour hydrolysate while the conversion efficiency of sugars to alcohol was 247.6, 97.14 and 92.51% for cassava flour hydrolysate, standard glucose and sucrose solutions, respectively. Alcohol produced was mostly ethanol with traces of methanol. However, yeast concentrations did not showed any significant effect but fermentation time had significant impact on alcohol yield which suggests that the high yield of alcohol could be obtained from cassava flour hydrolysate.

**Key words:** Biofuel (ethanol), Cassava, Production

### Introduction

Cassava (*Manihot esculenta*) is native to the North region of Brazil, then spread throughout the Indian sub-continent by Portuguese and Spanish explorers. Cassava is rarely seen and cultivated in Bangladesh. Cassava, sometimes also called manioc, is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tonnes (Kuiper *et al.*, 2007). Cassava is highly efficient in producing starch due to the fresh roots contain about 30% starch which is one of the best fermentable substances for the production of ethanol. Bioethanol is the most important biofuel, accounting for more than 90% of the total biofuel use. Cassava tubers are also used to produce cassava chips and bioethanol. Azmi *et al.* (2010); Tan and Khatijah (2000) reported that the bioethanol industry contributing about 37.3% higher profit compare to cassava chips industry in Malaysia. Bioethanol can also be used in mixtures with fuels for motor vehicles. It can increase the octane index; reducing it between 10 and 15% the CO. Ethanol can be mixed with unleaded gasoline between 10 to 25% without difficulty (Zamora *et al.*, 2010). Ethanol could therefore replace MTBE (methyl-tert-butyl ether), an oxygenated product used in Mexico since 1989, although it has reduced CO<sub>2</sub> emissions it has proved to be a groundwater pollutant and has a carcinogenic effect. So, by investing into bioethanol industry we can increase annual profitability of Bangladeshi economics. For instance, developing new bioethanol productions area in Bangladesh, it can reduce the dependence of bioethanol importation from foreign countries. Therefore, fermentation is the oldest way for humans to produce bioethanol, and this is also the

traditional way of making alcoholic beverages (Kuiper *et al.*, 2007). Bioethanol can be produced from biomass by the hydrolysis process and followed by sugar fermentation processes. Biomass wastes contain a complex mixture of carbohydrate polymers from the plant cell walls known as cellulose, hemi cellulose and lignin. The cellulose and the hemi cellulose portions are broken down (hydrolyzed) by enzymes or dilute acids into sucrose sugar that is then fermented into bioethanol. In order to produce sugars from the cassava, the cassava is pre-treated with acids or enzymes to reduce the size of the feedstock and to open up the plant structure (Nitayavardhana *et al.*, 2010). There are three principle methods of extracting sugars from cassava which are acid hydrolysis, dilute acid hydrolysis and enzymatic hydrolysis (Akihiko *et al.*, 2008). Leng *et al.* (2008) also reported that the cassava-based ethanol is energy efficient as indicated by an energy output to input ratio of 1.28 and a major contribution to energy consumption and sulphur dioxide and CO<sub>2</sub> emissions primarily comes from ethanol conversion phase as a result of the combustion of coal to produce energy (Hu *et al.*, 2004). There is a current upsurge of interest in the search for renewable biomass (cassava) for the production of transportation fuels like bioethanol, arising especially from the environmental concerns due to the toxic gas emission from petroleum fuels, squeezing petroleum resources and fossil fuels (Shanavas *et al.*, 2010). For the above context, the present study was to evaluate the yeast concentration, fermentation time and substrate concentration on bioethanol production from cassava flour using yeast (*Saccharomyces Cerevisiae*).

### Materials and Methods

The research was conducted at the laboratory of the Department of Biochemistry and food Analysis, Patuakhali Science and Technology University, Dumki, Patuakhali during the period from December 2015 to March 2016. Only one cassava genotype was used in the research cv. "Sree Sahya". The methodology described by Ocloo and Ayernor (2008).

### Cassava flour preparation

Ten kilograms of fresh, mature cassava roots without any rot or infection were used for the preparation of cassava flour in this research. They were first washed to remove the soil and other waste particles. Then they were peeled and they were again washed. After that they were grated in a mechanical grater and then dried in the sun on a thin layer. Then it was pulverized in a blender machine. At last it was milled finely in a laboratory grinder. Thus the cassava flour is prepared.

### Experimental set up

Five hundred grams of cassava flour was mixed with 2500 ml of water to form slurry. The mixture was allowed to boil until gelatinized at 70°C and allowed to cool. About 125 g of rice was added to the gelatinized mash, stirred and the mixture allowed to cool gradually to 50°C for the amylase in the malt to convert the gelatinized starch to sugars. Thinned liquor was then heated to 70°C and the last batch of 125 g rice malt added to further convert the un-hydrolyzed starch to sugars. The mixture was boiled briefly and immediately filtered using cloth and a Laboratory test sieve of aperture 180 µm (Endecotts Ltd, London, England). The sweet wort produced was boiled again to arrest further enzyme action and then cooled. Here Cassava flour hydrolysate having a reducing sugar content of 8.58% was obtained.

### Experimental design

Yeast concentrations (5, 15 and 20%) and fermentation time (24, 48, 72, 96 and 120 h) were also used for the present research. Various bakers' yeast concentrations were added to the CFH at pH 4.5–5.0 and temperature of 28–30°C. The mixtures were allowed to ferment and samples taken from day 1 to 5 for alcohol content analysis. The time taken for the fermentation to be completed was plotted against the yeast concentrations.

### Alcohol production process

The cassava flour hydrolysate produced alongside the standard glucose and sucrose solutions (of 8.72%) were fermented in aspirator bottles (previously sterilized to exclude other microorganisms) containing about 1500 ml sugar syrup and 100 ml of 15% yeast inoculum (7.5 grams of dry baker's yeast rehydrated in 50 ml of distilled water at 37°C for 10 min). The bottles were topped with tubes to allow carbon dioxide (CO<sub>2</sub>) to escape. Fermentation was done for 5 days at 28–30 °C. At the end of the fermentation period, the

alcohol was separated from the extract using simple distillation procedure at 78.3–80°C.

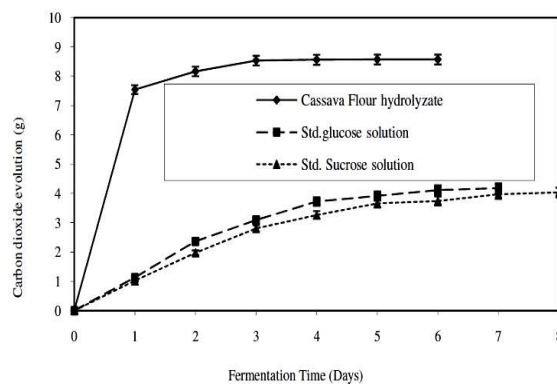
### Distillation process

About 100 ml of the fermented cassava flour hydrolysate was measured into a volumetric flask at 20°C and was washed into the distillation flask with 50 ml water. The sample was distilled slowly into the same 100 ml volumetric flask at temperatures between 78.3–80°C. About 95 ml distillate was collected and then made up to the 100 ml mark using water at 20°C.

## Results and Discussion

### Rate of fermentation

The amount of carbon dioxide evolved in the course of the fermentation by CFH was higher than that recorded by Ocloo and Ayernor (2008) for standard glucose and sucrose solutions. These results were statistically significant ( $p < 0.05$ ) (Figure 1).



**Fig. 1.** Time course of fermentation of CFH compared with standard glucose and sucrose solutions of the same concentration

After 3 days of fermentation at room temperature (28–30°C), there was no further change in the carbon dioxide evolution for CFH. This suggested that fermentation was completed within this period, indicating maximum alcohol production. In the study of Ueda *et al.* (1981) on production of ethanol from raw cassava starch by a nonconventional fermentation method it was reported that the rate of fermentation or carbon dioxide evolution ceased after 5 days of fermentation at 30°C. The duration of fermentation however depends on the method used for starch liquefaction, saccharification and fermentation, yeast type and concentration and also the conditions of fermentation (Briggs *et al.*, 1981). The observed period of fermentation of CFH could be attributed to the presence of readily fermentable sugars and the nutrients—that is the wort composition. Wort composition was reported to have some great influences on the speed of fermentation and the extent of fermentation. In the case of standard glucose and sucrose solutions, evolution of carbon dioxide ceased

after 6 and 7 days of fermentation at 28–30°C, respectively. The difference in their fermentation period was due to the fact that glucose as a readily fermentable sugar was easily metabolized by the yeast into alcohol and carbon dioxide with increasing rate whereas, sucrose, a non-reducing sugar had to be converted initially into invert sugar before the conversion of sugars into alcohol and carbon dioxide, hence the observed longer period. The maximum carbon dioxide evolved in the course of the fermentation of CFH, standard glucose and sucrose solutions were 8.57, 4.18 and 4.03 g respectively. The carbon dioxide evolved by CFH fermenting medium was almost twice the values for the fermenting medium of standard glucose and sucrose solutions. The high carbon dioxide evolved by CFH medium could be attributed to the conversion of limitdextrins, which were not considered as reducing/fermentable sugars.

**Conversion efficiency of CFH comparison to standard glucose and sucrose solutions**

The concentrations of reducing sugar that were converted in the course of fermentation were obtained by subtracting the reducing sugar concentration left after fermentation from the initial reducing sugar concentration before fermentation. The values obtained were 6.49 for CFH and 7.78 and 7.65 for standard glucose and sucrose solutions by Ocloo and Ayernor (2008) respectively. These reducing sugars gave rise to alcohol contents of 8.22, 3.98 and 3.65 v/v, respectively (Table 1). However, the theoretical/predicted alcohol production values were 3.29% v/v, 3.97% v/v and 3.85 v/v for CFH, standard glucose and sucrose solutions, respectively. The values observed for standard glucose and sucrose solutions were comparable to the theoretical/predicted alcohol content values calculated. However, contrary to this observation, the alcohol content obtained for CFH was

about two times higher than the theoretical/predicted alcohol content calculated (Table 1). The alcohol fermentation efficiency or yield (%) is expressed here as the ratio of the actual alcohol produced to theoretical/predicted alcohol based on fermented sugar ( $\times 100$ ). Alcohol fermentation efficiency or yield (%) = actual alcohol produced to alcohol based on fermented sugar ( $\times 100$ ).

The fermentation efficiency values obtained for standard glucose and sucrose solutions were comparable and also higher than that reported by Maiorella *et al.* (1981) which was 90–95%. However, the value obtained for CFH was twice more than those reported by Ocloo and Ayernor (2008). The high alcohol content obtained in the course of fermentation was therefore reflected in the fermentation efficiency value. The difference observed was due to the secondary conversion of the limit-dextrins (high molecular saccharide produced due to inability of  $\alpha$ -amylase to hydrolyze 1–6 linkage found in amylopectin) in the CFH. Limit-dextrins could have an average chain length of four to five glucose units per molecule. Though limit-dextrins were not hydrolyzed into glucose by the malt enzymes prior to fermentation, they were however converted by the yeast into fermentable sugars, which were later converted into alcohol and carbon dioxide. The fermentation efficiency could also be attributed to the conversion of other non-reducing sugars present in the CFH. According to Mark *et al.* (1963), dextrin conversions occur in the primary and secondary phases of fermentation. The fermentation efficiency value for CFH could vary depending upon the method, the enzymes and the type of yeast used in the conversion process. Ueda *et al.* (1981), reported alcohol yields of 82.3 and 99.6% from their study on production of ethanol from raw cassava starch by a non-conventional method.

**Table 1.** Conversion efficiency of cassava flour hydrolyzate, standard glucose and sucrose solutions

Substrate	Reducing sugars (%)		Alcohol formed (%v/v)	Theoretical /predicted alcohol production (%v/v)
	Before fermentation	After fermentation		
Cassava flour hydrolyzate	8.58	2.12	8.22	3.29
Glucose	8.58	0.79	3.98	3.97
Sucrose	8.58	0.97	3.65	3.85

Determinations are averages of 5 batches of production

**Effect of yeast concentration and fermentation time on the yield of alcohol**

Yeast concentration between 5–20% of bakers' yeast converted CFH to alcohol in nearly equal amounts after fermentation was completed (120 h) for each fermenting medium (Table 2). This means that the yeast concentration did not affect significantly ( $p > 0.05$ ) on alcohol yield while yeast concentration had significant ( $p < 0.05$ ) affected on fermentation time to be taken to complete. The alcohol yield was achieved highest when

the fermentation was completed over the period of 72, 96 and 96 for 20, 15 and 5% yeast concentrations, respectively. However, alcohol yield decreased at the fermentation time of 120 hrs except 15% yeast concentration. The results obtained supported the fact that the speed of fermentation depends on the yeast concentration, the higher the concentration, the shorter the fermentation period required to achieve maximum alcohol yield (Kordylas, 1990). Ueda *et al.* (1981)

reported of 5 days fermentation period for raw cassava root starch using 15% yeast suspension.

**Table 2.** Effect of yeast concentration and fermentation time on alcohol yields

Yeast conc. (%) (bakers yeast)	Fermentation time (h)				
	24	48	72	96	120
	Alcohol (% v/v)				
20	7.81	8.15	8.46	8.24	8.22
15	7.57	7.63	8.09	8.22	8.22
5	7.29	7.58	8.01	8.19	8.11

About 50 ml of yeast suspension was added to 1500 ml of cassava flour hydrolyzate while initial sugar concentration was 8.58%

**Conclusion**

From this research work, it was evident that alcohol of high yield was produced during the fermentation of cassava flour hydrolysate. The conversion efficiency of sugars to alcohol was 247.6% for cassava flour hydrolysate. This suggested that the limit-dextrins in the cassava flour hydrolysate were converted by the yeast during the fermentation process. The yeast concentrations used had no significant effect (p > 0.05) on the yield of alcohol.

**References**

Akihiko, K., Akhiko, K., Mitsuyoshi, U., Yoshinori, M., Pilanee, V., Warunce, T., Takamitsu, A. and Yutaba, M. 2009. Production of ethanol from Cassava pulp via fermentation with a surface-engineered yeast strain displaying glucoamylase. *Renewable Energy*, 34: 1354–1358.

Azmi, A. S., Ngoh, G. C., Mel, M. and Hasan, M. 2010. Single-step bioconversion of unhydrolyzed cassava starch in the production of bioethanol and its value-added products (Chapter 2. pp. 33–50

Briggs, D. E., Hough, J. S., Stevens, R. and Young, T. W. 1981. *Malting and Brewing Science*. 2<sup>nd</sup> Ed. Chapman and Hall Ltd. Volume-1.

Hu, Z. Y., Zhang, C. and Pu, G. Q. 2004. Life cycle assessment on energy environment and economy of cassava-based ethanol and gasoline mixed fuel. *Journal of Internal Combustion Engine*, 25(1): 13–16.

Kordylas, J. M. 1990. *Processing and preservation of tropical and subtropical foods*. Macmillan Education Limited, Houndmills pp. 105107.

Kuiper, L., Ekmekci, B., Hamelinck, C., Hettinga, W., Meyer, S. and Koop, K. 2007. Bio-ethanol from Cassava. A report presented at the Upstream Committee Annual Meeting. Ecofys Netherlands BV. Project Number

PBIONL062937. Online available at <http://www.ecofys.com>.

Leng, R. A. 2008. The potential of feeding nitrate to reduce enteric methane production in ruminants. A Report to the Department of Climate Change Commonwealth Government of Australia.

Maiorella, B. L., Wilke, C.R. and Blanch, H. W. 1981. Alcohol production and recovery. *Advanced in Biochemical Engineering*, 20: 43.

Zamora, L. L., Calderon, J. A. G., Vazquez, E. T. and Reynoso, E. B. 2010. Optimization of ethanol production process from cassava starch by surface response. *Journal of the Mexican Chemical Society*, 54(4): 198–20

Mark, H. F., McKetta, J. J. J. R., Othmer, D. F. and Standen, A. 1963. *Kirk-othmer encyclopaedia of chemical technology*. 2<sup>nd</sup> Edition. John Wiley and Sons, Inc. New York, 8: 422–427.

Ocloo, F. C. K. and Ayernor, G. S. 2008. Physical, chemical and microbiological changes in alcoholic fermentation of sugar syrup from cassava flour. *African Journal of Biotechnology*, 7(2): 164–168.

Nitayavardhana, S., Shrestha, P., Rasmussen, M.L., Lamsal, B. P., van Leeuwen, J., Khanal, S. K. 2010. Ultrasound improved ethanol fermentation from cassava chips in cassava-based ethanol plants. *Bioresourch and Technology*, 101(8): 2741–2747

Shanavas, S., Padmaja, G., Moorthy, S. N., Sajeev, M. S., Sheriff, J.T. 2011. Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes. *Biomass Bioenergy*, 35: 901–909.

Tan, S.L. and Khatijah, I. 2000. Present situation and future potential of cassava in Malaysia. Paper presented at the 6<sup>th</sup> Asian Cassava Workshop, Ho Chi Minh City, Vietnam, Feb 21– 25, 2000.

Ueda, S., Zenin, C. T., Monteiro, D. A. and Park, Y. K. 1981. Production of ethanol from raw cassava starch by a non-conventional fermentation method. In: John Wiley and Sons Inc. *Biotechnology and Bioengineering*, 23: 291–299.