Evaluation of the Potentials of Some Cassava Varieties in Nigeria for Bio-Ethanol Production

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Abstract

Suitability of four cassava varieties (98/2101, 98/0505, TME 419, and TMS 4(2) 1425) for ethanol production was investigated. The total starch and amylose contents of variety 98/2101 were higher than those of the other three varieties. Variety 98/2101 also gave the highest ethanol productivity and yield. This was followed by variety 98/0505 while TMS 4(2) 1425 gave very low ethanol concentration and yield. The final ethanol concentration and yield were influenced by the age of variety 98/2101. The optimum age for harvesting this variety for ethanol production is 12 months. Above 12 months, some of the tubers started rotting and even ethanol yield per cassava flour decreased. On the whole, the starch contents of the varieties investigated were lower than the values reported for other varieties in other parts of the world. Efforts should therefore be made to develop cassava varieties with higher starch contents in Nigeria.

Keywords: Cassava varieties, Fuel ethanol, *Koji*, Amylase enzymes, Bio-fuels

Introduction

Nigeria is the world largest producer of cassava (FAO, 2004). However, most of the produced cassava are either consumed as food in various forms or exported as chips which are bulky and cheap in international markets. In order to sustain cassava production in Nigeria, it is necessary to expand markets for the cassava by developing methods of processing the cassava into value-added products in the country.

The interest in production of bio-ethanol as an alternative fuel is increasing due to the non-renewable nature of fossil fuels and the environmental problems associated with their use (Barnola *et al,* 1987; Genthon et al, 1987). However, in order to produce bio-ethanol as bio-fuel, the production cost has to be as low as possible. The cost of raw material (carbon source) represents more than 70% of the total production cost (Krishnan, et al, 2000). Therefore production of bio-ethanol at competitive price requires the use of cheap raw material. Although Nigeria is the world largest producer of cassava, the cost of cassava tuber in Nigeria is still much higher than their cost in other countries such as China (Zhang et al, 2003). Use of cassava as the raw material for bio-ethanol production in Nigeria requires drastic reduction in prices as well as cultivation of varieties that can easily be converted to bio-ethanol with high ethanol yields per unit amount of cassava flour. Some research institutes in Nigeria such as International Institute for Tropical Agriculture and Institute for Tropical Tuber Research have been involved in development of improved cassava varieties. However, they have focused mainly on development of varieties for improved yield, and resistance to biotic and abiotic factors. Ethanol yield from cassava tubers depend on such factors as their water content, the starch contents as well as on their amylose and amylopectin ratios.

The aim of the present study is therefore to evaluate the potentials of some Nigerian cassava varieties for bio-ethanol production.

Materials and Methods

Cassava varieties used in this study: Stems from four different cassava varieties (98/2101, 98/0505, TME 419, and TMS 4(2) 1425) were obtained from Ebonyi State Agricultural Development Program (EBADEP) in the month of April 2006. They were planted at OGB Biotechnology Research and Development Centre, Emene Industrial Layout, Enugu on 4th April 2006. Tubers from the same varieties were also harvested from EBADEP in the month of August 2006. The tubers were peeled, washed and soaked in water overnight (12 h) to avoid coloration. The following morning the tubers were removed from water, drained and sliced into chips. The chips were sun dried to a constant dry weight and ground into flour using a manual grinding machine. The flour was sieved using a muslin cloth and used for the experiments.

Determination of physical properties of the cassava tubers: One kilogram of fresh cassava was harvested from each variety. The outer brown skin was carefully removed and weighed. The inner rind was carefully removed and their thickness measured. The fresh weight of pulp was noted and then sun-dried to constant weight. The moisture contents and their dry mater contents were calculated from the fresh and dry weights.

Determination of starch contents of the cassava flour: The total starch contents of the flour from the four cassava varieties (98/0505, 98/2101, TME 419 and TMS 4(2) 1425) were measured by the Potassium iodide/iodine method (Bailey and Olis, 1986). The assay was done by adding 0.5 ml of appropriately diluted gelatinized starch solution to 10 ml of colour reagent (I- KI indicator). The absorbance of the solution was read at 660nm wavelength and the absorbance readings were converted to starch concentration, using pre-determined calibration curve.

Determination of amylose/amylopectin ratio: The materials and apparatus include milled cassava 100mg, volumetric flasks 100ml, ethanol (95 %), NaOH (1 M), boiling water bath, timer, distilled water, acetic acid (1 M), iodine solution (0.2 %), I_2 in 2% KI, and spectrophotometer.

One hundred milligrams (100 mg) of milled cassava sample was added into a 100 ml volumetric flask (X 2). One (1) ml of 95% ethanol was added into each flask. Sample adhering to the wall of the flask was washed down with 9 ml of 1 M NaOH. The mixture was heated in a boiling water bath for 10 min. It was then cooled to room temperature. After cooling, it was diluted to 100ml with distilled water. Then, 5 ml of the sample suspension was added to 50 ml of distilled water in another 100 ml volumetric flask. One milliliter (1 ml) of 1 M acetic acid was added to acidify the sample. One and a half milliliter (1.5 ml) of iodine solution was added and then distilled water was added to make the volume up to 100 ml. The suspension was mixed and left for 20 min. A blank sample (0.09 M NaOH solution) was used to zero the spectrophotometer at 620nm. The absorbance of the sample was read at 620 nm. The amylose content was determined by referring to an amylose standard curve.

The amylopectin content was determined by subtracting the amount of amylose from the total starch content of each variety.

Ethanol production

Enzyme production: The enzyme used for cassava starch hydrolysis was produced by solid state cultivation of *Aspergillus awamori* using cassava flour from the four test varieties supplemented with 20% rice bran. Cassava flour 80 g each from the four test varieties was mixed with 20 g of rice bran. The mixture was wetted with 60 ml of water and steamed over a moderate flame for 30 minutes. It was allowed to cool to room temperature and then inoculated with 20 ml of *A. awamori* spores (1.232×10^7spores/100g of substrate). The resulting fermented substrate containing hydrolytic enzymes (*koji*) was used as the enzyme source and substrate for ethanol production.

Fermentation: Twenty grams (20g) of the cassava *koji* was weighed from each test variety into 200 ml of water in a 500ml Erlenmeyer flask. It was inoculated with a seed culture of *Saccharomyces cerevisiae*. The ethanol content was measured every 24 hours for a period of 96 hours.

Determination of ethanol concentration: The ethanol concentration was measured by the boiling iodometric method of Adetuyibi (1989).

The reagents used for measurement of ethanol concentration were 10% potassium iodide, 10% potassium dichromate, 1:1 dilution of concentrated sulphuric acid, 10% potassium sulphate, 1 M solution of sodium thiosulphate and 0.4% soluble starch. Ten milliliter (10 ml) of 10% potassium dichromate was pippeted into a big test tube and then 10 ml of 1:1 dilution of concentrated sulphuric acid was added. In a 50 ml flat bottom flask, 10 ml of 10% potassium sulphate was added, and then 10 ml of sample (fermented broth containing ethanol) was added to the flask. The potassium dichromate and sulphuric acid mixture in a test tube was connected to the potassium sulphate and sample mixture in the flask by means of a glass delivery pipe. The flask was heated with a low flame to a boiling point and the ethanol in the sample was distilled into the test tube and it was absorbed by the potassium dichromate and sulphuric acid. This made the colour change from yellow to greenish. After a known volume (6 ml) of the ethanol has distilled into the test tube, distillation was stopped. The ethanol sulphuric acid and potassium dichromate mixture was transferred into a 100 ml measuring cylinder. The total volume was made up to 60 ml. The 60 ml was then transferred into a 500 ml flat bottom flask and 5 ml of 10% potassium iodide was added. The flask was swirled to mix and then allowed to cool to room temperature. After cooling, 2 ml of 0.4% soluble starch was added. The mixture was titrated with 1 M solution of sodium thiosulphate until a blue colour just appeared. The ethanol concentration in the sample was calculated from a reference standard.

Effect of the age of cassava variety 98/2101 on ethanol production: Cassava tubers were harvested from the 98/2101 variety planted in OGB Bio- technology Research and Development Center, Emene Industrial Layout, Enugu at the ages of 8,10, 12 and 16 months. The tubers were processed into chips and the chips were sun dried and milled into flour using a manual grinding machine. After grinding, the flour was sieved with a muslin cloth and used for ethanol production as described above. However, the amount of *koji* was increased to 45 g/200 ml.

Statistical analysis: All the experiments were performed four times. The results were subjected to Analysis of Variance (ANOVA) and where there were significance differences, the means were separated using List Significance Difference (LSD).

Results

Some physical properties of cassava tubers: There were variations in the water contents of the cassava tubers harvested from the four cassava varieties. The water content in variety 98/0505 was significantly higher than the others. The starch content of variety 98/2101 was the highest among the four varieties investigated. Both in terms of percentage dry weight and wet weight, the starch content of variety 98/2101 was

Table 1: Composition of the four cassava varieties

Composition	Varieties			
	TMS 4 (2) 1425	TME 419	98/ 0505	98/ 2101
Water content (%)	61.43	62.43	65.71	62.86
Dry mater (%)	38.57	37.57	34.29	37.14
Starch content (% of dry mater)	62.75	62.78	64.25	70.16
Starch content (% of fresh tuber)	24.14	23.59	22.03	26.06
Thickness of the peel (cm)	0.15	0.21	0.19	0.20

significantly higher than those of the others. However, there was no significant difference in the starch contents of the other three varieties (98/2101 > (98/0505, TME 419, TMS 4(2) 1425). Table 1 also shows that there were slight variations in the thickness of the peels. Variety TMS 4(2) 1425 has very thin peel but there were no significance differences in the thickness of the peel from the other three varieties.

A comparison of the ratio of amylose to amylopectin of the four cassava varieties is shown in Figure 1. Variety TMS 4(2) 1425 has significantly ($p<0.5$) lower amylose content than the other three varieties. The amylose content of 98/2101 was the highest (29.5%) but it was not significantly higher than those of 98/0505 and TME 419 varieties.

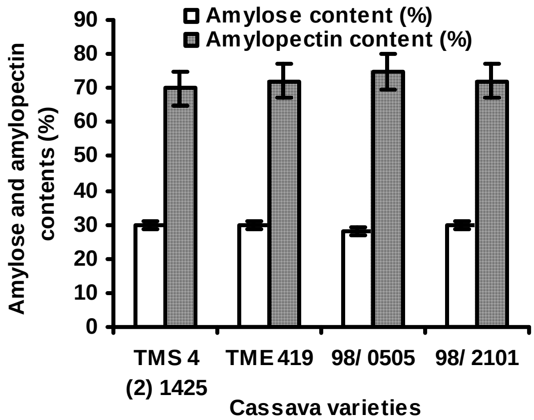

Fig: 1. Amylose and amylopectin contents of the cassava varieties

Ethanol production from the four cassava varieties: A comparison of ethanol production using cassava flour obtained from the four different cassava varieties are shown in Figure 2. Variety 98/2101, which gave the highest starch and amylose contents, also yielded the highest ethanol concentration. The final ethanol concentration obtained from the four varieties was ranked as (98/2101) > (98/0505) > (TME 419) > (TMS4 (2)/1425). It is interesting to note that the final ethanol concentration obtained with TMS 4(2) 1425 was only about 30% of the value obtained with variety 98/2101. As shown in Figure 3, variety 98/2101 still gave the highest ethanol yield. Statistically, the four cassava varieties were ranked in the following order in terms of their ethanol yield ((98/2101) > (TME419, 98/0505) > (TMS4 (2)1425)). Cassava variety 98/2101 was therefore selected for subsequent experiments.

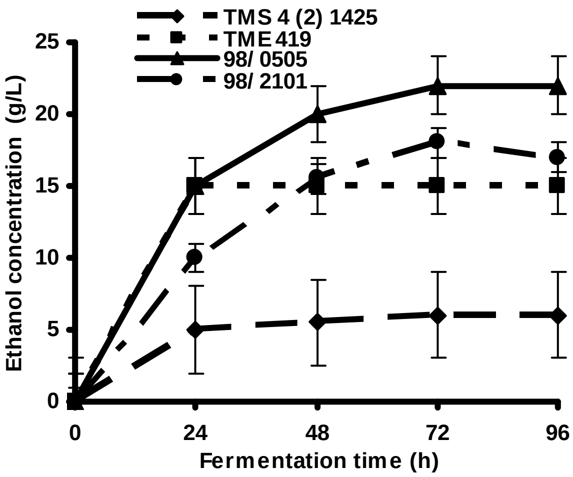

Fig. 2: Effects of cassava varieties on ethanol production from cassava koji. The quantity of cassava koji was 20 g per 200 mL of distilled water

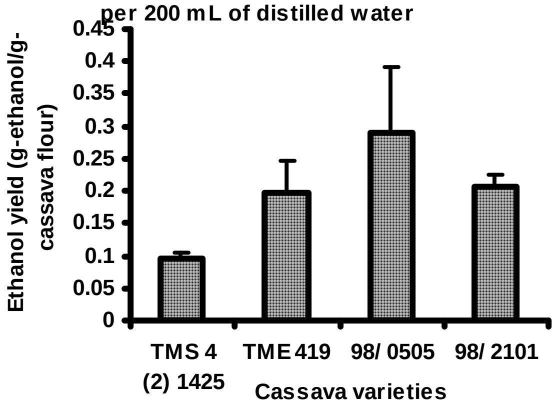

Fig. 3: Effect of cassava variety on ethanol yield from cassava flour

Effect of variety 98/2101 age on ethanol productivity: Ethanol productivity and yield were influenced by the age of the cassava. Cassava ages 8 and 10 months gave the same but slightly lower final ethanol concentrations than the values obtained from cassava of 12 and 16 months (Figure 4). As shown in Figure 5, ethanol yield tended to increase with the age of the cassava up to 12 months but the increase was not statistically significant. It is also worthy to note that above 12 months, there was no appreciable increase in the tuber yield, rather, some tubers already started deteriorating, and became very fibrous at the age of 16 months (data not shown).

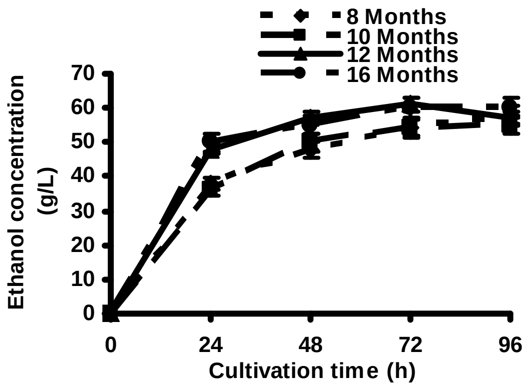

Fig. 4: Effects of variety 98/2101 cassava age on ethanol production from cassava flour

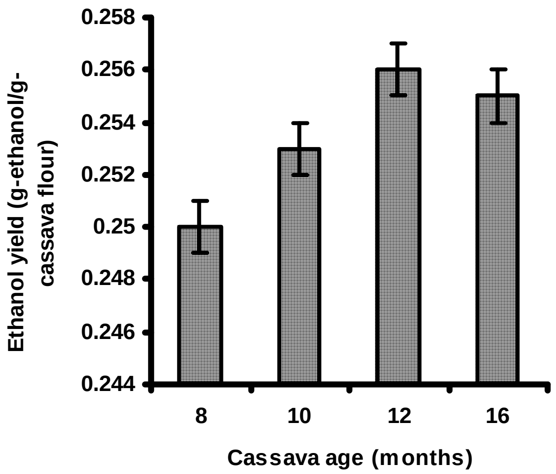

Fig. 5: Effect of the age of variety 98/2101 on ethanol yield from cassava flour

It is therefore recommended that variety 98/2101 cassava should be harvested at the age of 12 months for ethanol production.

Discussion

The cassava varieties used in this study were all developed by the International Institute for Tropical Agriculture and are cultivated in various parts of Nigeria. The purposes of cassava breeding in Nigeria include yield improvement, drought resistance and resistance to pests and diseases. The starch contents of all the four varieties were lower than the 32% (wet weight basis) or 75% (dry weight basis) reported for the varieties cultivated in China (Zhang *et al,* 2003; Dai, *et al,* 2006). Also an average yield of 11 ton/hectare in Nigeria is much lower than the 45 tons/hectare reported in China (Zhang *et al,* 2003). Since the cost of cassava represents more than 70% of the total ethanol production cost from cassava (Krishna et al, 2000; Zhang *et al,* 2003), efforts must be made to increase both cassava yield and starch contents of cassava varieties in Nigeria.

The amylose content of cassava starch is another important parameter for evaluating the suitability of cassava for ethanol production. It is known that amylose is much easier to be hydrolyzed than amylopectin. Unfortunately, the amylopectin contents of cassava are usually much higher than the amylose contents. The amylose contents of the four cassava varieties are within the range reported for other cassava varieties (Rickard *et al.,* 1991; Fernandez *et al.,* 1996; Defloor *et al.,* 1998; Moorthy, 2004). In the present study, it was found that the total starch contents of TME 419 and TMS 4(2) 1425 were comparable but TME 419 gave much higher ethanol yield because its amylose : amylopectin ratio is higher.

The results have shown that age of the cassava should be considered while harvesting tubers for ethanol production. The cassava tuber yield per hectare tends to increase with age but above certain age, deterioration of the tubers starts. Although 12 months is recommended for variety 98/2101, the optimum age would depend on the cassava variety.

Conclusion: Among the four varieties of cassava investigated, 98/2101 variety was the best for ethanol production. The total starch as well as the amylose contents was higher than those of the other three varieties. It also gave highest ethanol productivity and yield. The four varieties investigated are the popular varieties in Ebonyi State Agricultural Development Program (EBADEP) and are known to yield more tuber than the local varieties. The International Institute for Tropical Agriculture as well as the Tropical Root Crop Research Institute at Umudike have different programs on development of improved cassava varieties. On the whole, the cassava tuber yield per hectare of about 11 tons (personal communication from EBADEP) and their starch contents (as shown in the present investigation) are lower than the values reported for other cassava varieties cultivated in other parts of the world. Nigeria is currently the world largest producer of cassava and this production can only be sustained if their demand is sustained by developing other methods of processing cassava tubers into value-added products. Ethanol production is one of them but to produce ethanol from the cassava tubers at competitive prices, it is necessary to develop varieties with higher tuber yields, starch contents and amylose : amylopectin ratios.

References

Adetuyibi. A. (1989). Introduction to Biomedical Research. University Press LTD Ibadan 27

Bailey, J. E., and Olis, D. F.(1986). Biochemical engineering Fundamentals 2nd Edition McGrow –Hill 270.277.

Barnola, J. M., Raynaud, D., Korotkevich, Y. S., and Lorius, C. (1987). Vostok ice core provides 160,000 years record of atmospheric CO_2, *Nature* 329: 408-414.

Dai, D., Hu, Z., Pu, G.., Li, H. and Wang, C. (2006). Energy efficiency and Potentials of cassava fuel ethanol in Guangxi region of China *Energy Conversion and Management* 47: 1686-1699.

Defloor, I., Dehing, I. and Delcour, J. A. (1998).

Physico-chemical properties of cassava starch. Starch/Starke. 50: 58-64.

Fernandez, A., Wenham, J., Dufour,D. and Wheatley, C.C.(1996). The influence of variety and processing on the physicochemical and functional properties of cassava starch and flour. In Cassava flour and Starch: Progress in Research and Development; Dufour, D., O'Brien, G. M., Best, R. (Eds) CIRAD/ CIAT. Montpelier France Cali / Columbia 263-269.

Food and Agriculture Organization of the United Nations (2004) www.fao.org

Genthon, C., Barnola, J. M., Raynaud, D., Lorius, C., Jouzel, J., Barkov, N.I., Korotkevich, Y.S., and Kotlyakov, V. M. (1987). Votok ice core, climatic response to CO_2 and orbital forcing changes over the last climatic cycle, *Nature* 329: 414-418.

Krishnan, M. S., Taylor, F., Davison, B. H., and Nghiem, N. P., (2000). Economic analysis of Fuel ethanol production from corn starch using fluidized -bed bioreactors *Bioresource Technology* 75: 99 – 105.

Moorthy S. N. (2004). Tropical sources of starch in food. Eliasson, A.C. (Ed). C.R.C Press,. Boca Raton, Fl 321-359

Rickard, J. E., Asaoke, M., and Blandshard, J. M. V.(1991). The Physico –chemical properties of cassava starch. *Tropical Science* **31:**189-207.

Zhang, C., Han W., Jing, X., Pu, G.., and Wang, C. (2003). Life Cycle economic analysis of fuel ethanol derived from cassava in South West China *Renewable and Sustainable Energy Reviews* 7:353-366.