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Enzymatic Production of Ethanol from Cassava Starch Using Two Strains of *Saccharomyces cerevisiae*

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ABSTRACT

Cassava starch from TMS 30572 and *Idileru* were hydrolyzed with α-amylase and amylo-glucosidase before fermentation using two strains of *Saccharomyces cerevisiae* from palm wine and bakers' yeast. The per cent yield of sugars and total dissolved solids were 66 % and 26% respectively while pH was 7. Spectrophotometric measurement of the cell growth revealed steady but insignificant ($p \leq 0.05$) increase in cell concentrations up to 48 h fermentation time with a gradual decline by 72 h. *Saccharomyces cerevisiae* strain from palm wine grew best on TMS 30572 hydrolysate at 20% sugar concentration (optical density 0.663; fermentation time 48 h) while on Idileru hydrolysate it grew best at 25% (optical density 0.698; fermentation time 60 h). The pH values obtained from the fermenting hydrolysates for both yeast strains declined gradually as the fermentation progressed with the lowest pH values (3.01 for *S. cerevisiae* from palm wine; 3.06 for *S. cerevisiae* from bakers' yeast) obtained for TMS 30572 cassava variety at 25% sugar concentration. Changes in pH were significant ($p \leq 0.05$). The *Saccharomyces cerevisiae* strain from palm-wine had a higher conversion of available sugar into ethanol. The yield of ethanol was found to vary but the highest ethanol concentration obtained was 5.3% at 10% initial sugar concentration, which gave a sugar conversion efficiency of 37.3%. The results obtained suggest that *Saccharomyces cerevisiae* strains from sources other than those used conventionally can serve as good substitutes for bio-conversion processes in the industrial production of ethanol.

Keywords: Cassava, enzyme, ethanol, *Saccharomyces cerevisiae*, starch.

Introduction

Cassava *(Manihot esculenta Crantz)* is a tropical root crop that serves as a food security and income generation crop for millions of people in sub-Saharan Africa and other regions in the developing world (Scott *et al.*, 2002). In West Africa, cassava is processed mainly to some local traditional foods and its use for the production of industrially useful products is yet to be fully exploited. The potential of cassava for the production of confectionery products (Vuilleumier, 1993), industrial starch (Nduele, 1993) glue (Tonukari, 2004) and ethanol (Atthasampunna *et al.*, 1987) have been previously identified. Gu Bi and Ye Guozhen (2002) reported on a China facility which is capable of producing 3,000 tonnes of industrial grade ethanol from cassava pulp annually.

Ethanol is presently produced from molasses in a number of countries, but cassava, a starch-accumulating tuber crop with up to 30% of fermentables, appears to hold more benefits when used for industrial ethanol production. Firstly, cassava can adapt well to a wide range of growing conditions and requires minimal inputs. Secondly, unlike sugar-based distilleries that are seasonally operated, cassava-based ethanol plants can run year round, due to the crop's rapid growth and ease of harvesting, in addition to its capability to be stored

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as dried chips. Sukphisal (2005) reported that the total output for cassava ethanol was projected to reach about 3.4 million litres per day in Thailand. Atthasampunna *et al.* (1987) had earlier noted that while the basic procedures for obtaining ethanol from cassava have been well defined, there is need to improve on technologies relating to efficiency and increased yields when compared to other substrates.

The microorganisms and enzymes used for the production of ethanol from cassava are very critical to the production efficiency and output. Lack of industrially suitable microorganisms for converting biomass into fuel ethanol has traditionally been cited as a major technical roadblock to developing a bioethanol industry (Dien *et al.*, 2003). Currently, few bacterial and yeast species are known to ferment various sugars into ethanol. The potential of local microbial strains for ethanol production is yet to be verified. Information is not available on the suitability of local cassava varieties for ethanol production and there is still the need to improve the technology of production and define the yield of products.

Starch is a high yield feedstock for ethanol production, but its hydrolysis is required to produce ethanol by fermentation. Starch was traditionally hydrolyzed by acids, but the specificity of the enzymes, their inherent mild reaction conditions and the absence of secondary reactions have made the amylases to be the catalysts generally used for this process. Alpha-amylase obtained from thermo-resistant bacteria like *Bacillus licheniformis*, engineered strains of *Escherichia coli* or *Bacillus subtilis* is used during the first step of hydrolysis of starch suspensions. For amylases to attack starch, these suspensions should be brought to high temperatures (90 – 110°C) for the breakdown of starch kernels. Apar and Ozbek (2004) provide information about the effects of operating conditions on the enzymatic hydrolysis of corn starch using commercial α-amylase.

In the last few years, the possibility of hydrolyzing starch at low temperatures for achieving energy savings has been investigated (Robertson *et al.*, 2006). The product of this first step, called liquefaction, is a starch solution containing dextrines and small amounts of glucose. The liquefied starch is subject to saccharification at lower temperatures (60–70°C) through gluco-amylase obtained generally from *Aspergillus niger* or *Rhizopus species* (Pandey *et al.*, 2000; Shigechi *et al.*, 2004). This study was therefore aimed at investigating the ethanol producing abilities of two local varieties of cassava using indigenous yeast species from palm wine compared with commercial bakers' yeast after exogenous enzyme hydrolysis.

Materials and Methods

Sample collection

Freshly harvested cassava roots (10 to 12 months old) of two indigenous varieties, TMS 30572 and *"Idileru"* were obtained from the University of Agriculture, Abeokuta research farm. The enzymes used in this study were α-amylase and amylo-glucosidase which were obtained from the enzymology laboratory of the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi, Nigeria.

Preparation of cultures

The yeast strains used were *Saccharomyces cerevisiae* isolated from palm wine and commercial Baker's Yeast. The organisms were propagated (incubation conditions: 30°C for 72 h) on potato dextrose agar medium (Oxoid, UK) to which 0.14 g/l streptomycin sulphate for inhibition of bacterial growth. Recovery of the yeasts was confirmed by the methods of Collins and Lynes (1989). The yeast cultures were transferred into broth medium and incubated on a shaker for 24 h at 28°C after which they were ready to be used as inoculums in the cassava hydrolysates.

Starch extraction

Starch extraction was by the method of Oyewole and Obieze (1995). Fifty (50) kg of cassava roots were peeled, washed in water and grated with a commercial mechanical grater. The resultant pulp

was immediately sieved through a screen (25 mesh) and suspended in water. This separates the fibrous and other coarse root materials from the starch pulp. The starch pulp milk was allowed to sediment for 4 – 6 h before decanting the supernatant. The resultant thick starch cake at the bottom of the bowl was then pressed to remove the remaining water. The bright white coloured starch cake obtained was sun-dried for 72 h (Figure 1).

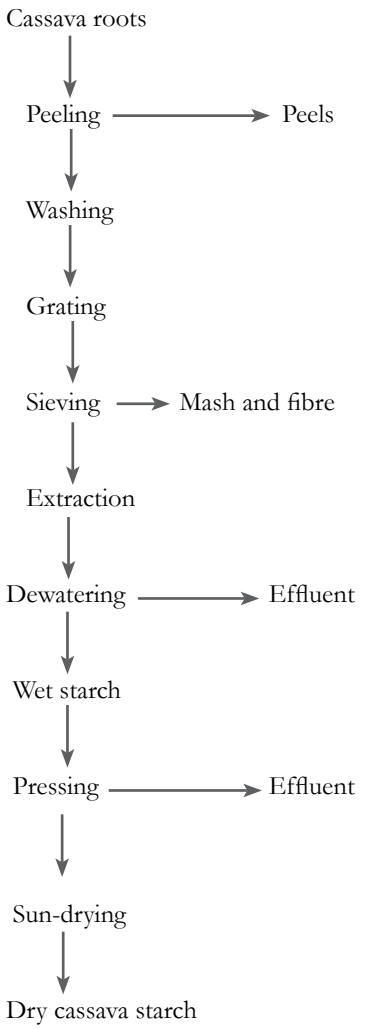

Fig. 1: Production of cassava starch (Adapted from Oyewole and Obieze, 1995)

Production of ethanol from cassava starch

Ethanol was produced from cassava starch by the method of Anyakorah *et al.* (1998), as presented in Figure 2. The steps involved in the production were: Gelatinization (50 kg cassava starch was stirred in 200 ml water at 80°C until smooth gel was formed); Liquefaction (4 ml of α-amylase was added to gelatinized starch at 80°C and incubated for 60 min); Saccharification (4 ml of aqueous solution of amylo-glucosidase was added to liquefied starch

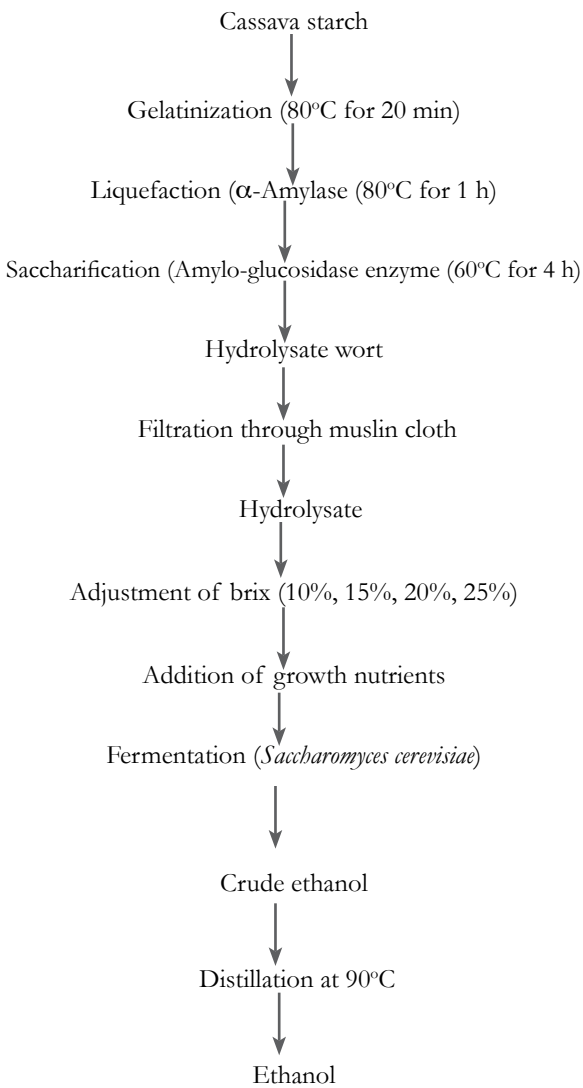

Fig. 2: Ethanol production from cassava starch (Anyakorah *et al.*, 1998)

at 60°C and incubated for 240 min); Filtration (the hydrolysate was filtered through muslin cloth); Fermentation (yeast inoculum was added to the filtrate before anaerobic incubation at 28°C) and Distillation (fermentation broth was filtered and the filtrate passed through the distillation unit twice at 90°C).

Analyses

Physic-chemical properties of samples: Moisture, ash and fibre contents of cassava starch samples were determined according to (AOAC, 1990).

Total titratable acidity and pH: Total titratable acidity (TTA) was determined using 10 g of sample homogenized with 90 ml distilled water and expressed as the amount (ml) of 0.1 M NaOH to get pH of 8.3. pH of the samples were determined with a combined glass electrode and a pH meter (Mettler-Toledo, Essex M3509 Type 340).

Total soluble sugars: The refractometer was used to determine the percentage total soluble sugar solids of the cassava hydrolysate after hydrolysis. This was carried out by placing a drop of cassava hydrolysate on the graduated hand refractometer glass slide and expressing the brix reading in percentage.

Dextrose equivalent analysis: The action of the enzymes on starch sample was determined by measuring the reducing sugar (calculated as dextrose equivalent) produced using the spectrophotometric (Spectonic 20D model) method of Bernfeld (1959).

Brix determination: The brix (%) was deter-mined using a hand refractometer according to AOAC (1990).

Yeast cell growth: The yeast growth deter-mination was carried out using spectro-photometer by the method of Olasupo *et al.* (1996).

Total reducing sugar determination: The total reducing sugar determination was carried out using spectrophotometer (Spectonic 20D model) at a wavelength of 540 nm against dinitrosalicylic acid reagent with concentrations of glucose as standard curve (Miller, 1959).

Determination of sugar consumption: The amounts of sugar consumed were determined after fermentation by AOAC (1990). The initial amounts of sugar before fermentation and sugar concentration after fermentation were recorded and used to calculate the amounts of sugar consumed.

Ethanol yield determination: The ethanol yield was estimated according to AOAC (1990) by calculation using the formula:

$$\text{Ethanol yield} = \frac{\text{Ethanol produced}}{\text{Sugar consumed}} \times 100$$

Fermentation efficiency determination: The fermentation efficiency was determined using AOAC (1990) method and the data obtained from the sugar consumed and the initial sugar x 100%:

$$\text{Fermentation efficiency} = \frac{\text{Sugar consumed}}{\text{Initial sugar}} \times 100$$

Sugar conversion efficiency: The ability of yeast to produce ethanol from available sugar is expressed as sugar conversion efficiency and it was determined by the method of De Macilla and Pearson (1984) and calculated using the conversion efficiency factor of 0.504.

Results and Discussion

Two cassava varieties, namely TMS 30572 and *Idileru* were used in this work. Cassava peel and starch yields were 12.5% and 29.5% for TMS 30572 respectively while for *Idileru*, peel and starch yields were 13% and 30% respectively. There were no significant differences in the yields of peel and starch by the two cassava varieties. The values, however, fall within the ranges earlier reported by Leaky and Wills (1977), FAO (1984) and Kocchar (1986). In addition, the direct extraction of starch from cassava roots permitted enhanced recovery compared to the steeping process.

Physico-chemical analysis of cassava starch

Table 1 shows the chemical analysis of the starches extracted from the two cassava varieties. The

moisture contents of the cassava starch samples recorded were 10% and 10.5% for TMS 30572 and *Idileru* respectively. The ash and fibre contents were all 0.2% for both varieties. No significant differences were observed for all values including pH (8.9 for TMS 30572 and 8.5 for *Idileru*) and titratable acidity (0.6% for TMS 30572 and 0.7% for *Idileru*). Apart from the time saving advantage of the process, the direct starch extraction procedure used also produced starch with generally high quality and good physico-chemical characteristics required for effective hydrolysis.

Composition of cassava hydrolysates from two cassava varieties

Cassava starch was hydrolyzed with α-amylase and amylo-glucosidase to yield cassava hydrolysate. Table 2 shows the analysis of the hydrolysates obtained from the two cassava varieties. The average yields of cassava hydrolysate for each sample at different enzyme concentrations were 66% for TMS 30572 and 66.3% for *Idileru*. The per cent total sugar soluble solid for the hydrolysates were 26% and 25% respectively. The pH of the hydrolysates from both varieties was 7.

Effect of enzyme treatment on cassava hydrolysates

The extents of hydrolysis on the cassava starch by the enzymes at different concentrations are presented in Figure 3. Increase in enzyme concentrate, temperature and time all affect the rate of hydrolyzing cassava starch to hydrolysates. There were variations in the values of dextrose equivalent produced by cassava starch samples at different reaction times with the enzymes. The highest value was recorded at 48 h after which a decrease was observed. The cassava starch samples generally yielded the highest dextrose equivalents (glucose concentration) at 40°C. In general, enzyme concentration of 1.0% v/v gave the highest dextrose equivalent.

The highest amount of reducing sugar was obtained at 40°C which falls within the range for optimum activity of the enzymes. It was at enzyme concentration of 1.0% v/v that the enzyme produced its maximum reducing sugar. Though enzyme activity increases with increase in enzyme concentration, when a peak is reached, addition of more enzyme does not necessarily increase to the activity of the enzyme.

The reaction time also affects the hydrolysis of cassava starch in that it was observed after 24 h of hydrolysis that there was an increase in the concentration of reducing sugars which could probably be as a result of a reversion process. The reversion process involves the conversion of glucose to iso-maltose by amylo-glucosidase, when there is high concentration of glucose in the hydrolysate (Padmanabham and Losame, 1993), as may be the case in cassava starch that appeared to be more readily hydrolyzed, probably because it contains

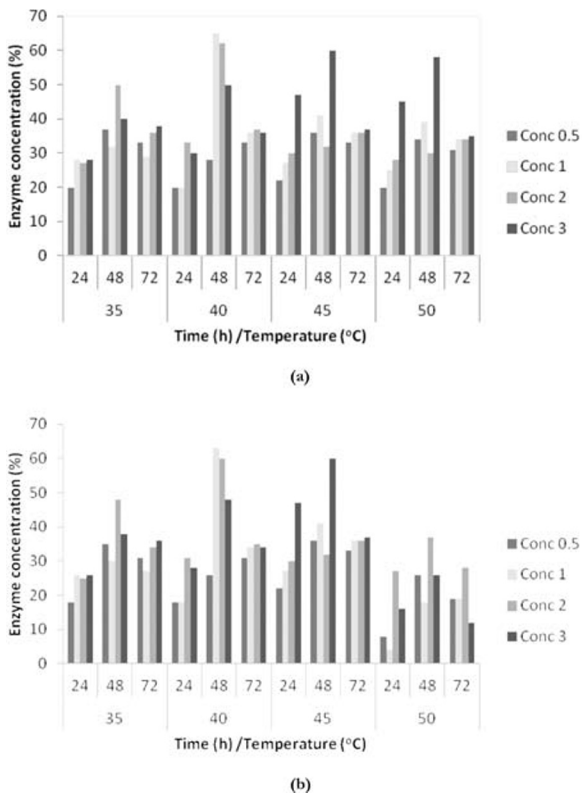

Fig. 3: Rate of hydrolysis in TMS 30572 (a) and *Idileru* (b) cassava hydrolysates at different temperatures using different enzymes concentrations

different proportions of low molecular weight carbohydrates.

Growth of yeast strains in cassava hydrolysates

The changes in the growth pattern of the *Saccharomyces cerevisiae* strains obtained from palm wine and baker's yeast are shown in Figure 4. The results reveal that there was a gradual increase in the cell growth of the yeast strains in both varieties of cassava used with increase in concentration of hydrolysate. However, the utilization of the different hydrolysates generally decreased after 48 h showing gradual decline at 72 h with different optical densities.

Various yeast strains from different sources have been used by previous workers in the production of ethanol (Morais *et al.*, 1996; Ezeogu and Emeruwa, 1993; Dhamija, *et al.*, 1996; Lucero *et al*, 2000). Saccharomyces species are generally tolerant to alcohol and can grow in the presence of 8 – 12% v/v alcohol, surviving exposure to up to 15% alcohol (Ingram and Buttke, 1984).

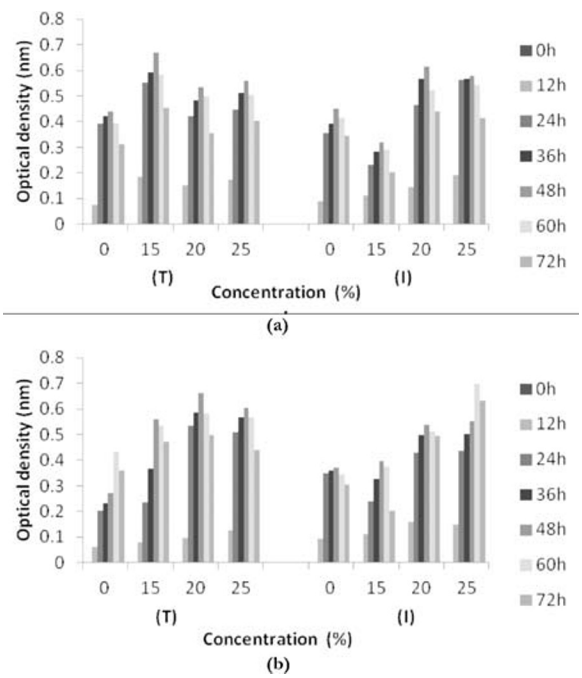

Fig. 4: Cell growth of *S. cerevisiae* from palm wine (a) and baker's yeast (b) using various concentrations of hydrolysate of TMS 30752 (T) and *Idileru* (I) cassava varieties

Sugar conversion and ethanol production from cassava

The amounts of reducing sugars in the hydrolysates of TMS 30572 and *Idileru* during fermentation were monitored. There was a gradual and consistent decrease in per cent brix for both hydrolysates (Figure 5). Although the amounts of sugars consumed during fermentation by baker's yeast in both cassava varieties were higher, the ethanol conversion values and amounts of ethanol produced were lower (Table 3). *S. cerevisiae* from palm wine was able to produce more ethanol from the two varieties more than the baker's yeast and TMS 30572 utilized more substrate for ethanol conversion during the fermentation more than *Idileru* variety.

The two yeast species used in this study were able to convert fermentable sugars into ethanol effectively. Ameh and Okagbue (1987) and Ezeogu and Emeruwa (1993) had observed that yeasts isolated from natural sources such as palm wine possess very high levels of ethanol and sucrose tolerance and may grow well in various substrates.

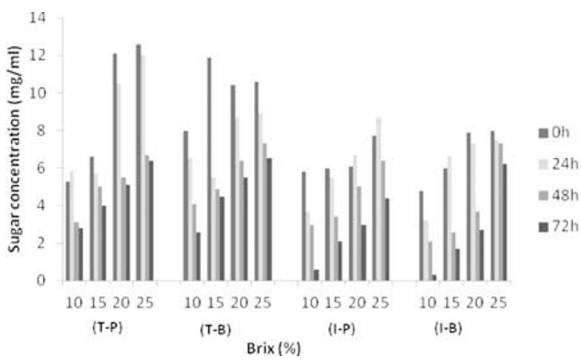

Fig. 5: Reducing sugar levels in hydrolysates of TMS 30572 and *Idileru* during fermentation with *S. cerevisiae*

T-P: TMS 30572 with palm wine yeast; T-B: TMS 30572 with baker's yeast; I-P: *Idileru* with palm wine yeast; I-B: *Idileru* with baker's yeast

Table 1: Chemical composition of starches from *Idileru* and TMS 30572 cassava varieties

Property	Starch from cassava varieties	
	TMS30572	Idileru
Moisture content (%)	10[a]	10.5[a]
Ash (%)	0.2[a]	0.2[a]
Crude fibre (%)	0.2[a]	0.2[a]
pH	8.9[a]	8.5[a]
TTA	0.6[a]	0.7[a]

Each value represents means of three replicates. Means in rows with the same superscripts (letters) are not significantly different by New Duncan's multiple range tests.

Table 2: Composition of hydrolysates from *Idileru* and TMS 30572 cassava varieties

Properties	Hydrolysates from cassava varieties	
	TMS30572	***Idileru***
Syrup yield (%)	66	66.3
Degree Brix (%)	26	25
pH	7	7

Table 3: Ethanol yield and sugar conversion by *Sacchromyces cerevisiae* from palm wine and baker's yeast in TMS 30572 and *Idileru*

Sample	Initial sugar concentration	Sugar content after fermentation	Fermentation efficiency (%)	Ethanol produced	Ethanol yield	Sugar conversion
T-P	12.6	6.4	49.2	2.34	5.85	16.11
T-B	12.6	4.4	65.1	1.73	4.33	11.98
I-P	11.9	4.5	62.2	2.00	5.00	23.81
I-B	11.9	1.7	85.7	1.53	3.83	18.12

T-P: TMS 30572 with palm wine yeast; T-B: TMS 30572 with baker's yeast; I-P: *Idileru* with palm wine yeast; I-B: *Idileru* with baker's yeast.

Conclusion

This study evaluated the bio-conversion of cassava to ethanol using two *Saccharomyces cerevisiae* strains from non-cassava origin. *Saccharomyces cerevisiae* strain from palm wine showed great potential for ethanol production from cassava, although more research is required to improve the efficiency of the process. The main contribution to knowledge is the establishment of the feasibility in using yeast strains from non-cassava niches for effective ethanol production.

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