Ethanol Production By Saccharomyces Cerevisiae From Cassava Peel Hydrolysate

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Citation

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Abstract

Fungal isolates from rotten Cassava wastes were identified and screened for amylolytic activity. Aspergillus niger (isolate A1), displaying the highest amylase activity on starch agar, was used to degrade a liquid suspension of milled Casssava peels under sterile, closed conditions. Sacharomyces cerevisae (isolate Y1) was subsequently used to ferment the degraded medium for ethanol production. The liquid suspension of milled Cassava peel was inoculated with A. niger and the production of simple sugar was monitored using the dinitrosalicylic method. The highest concentration of simple sugar (0.88 mg/ml) was produced by the seventh day, while the ethanol produced after seeding the cell-free extracts with S. cerevisae was 1.05% after three days.

INTRODUCTION

Cassava (Manihot esculenta crantz), which is cultivated extensively as a food crop in Africa, is the third largest source of carbohydrate in food for human consumption in the world ([1]). Cassava roots play an important role in the African diet and they are processed, using simple traditional methods, into products such as Gari, Fufu and Lafun flour ([2]). It is estimated that about ten million tonnes of Cassava is processed for Gari annually in Nigeria alone ([3]). In the processing of Cassava, the roots are normally peeled to rid them of two outer coverings, i.e. a thin brown outer covering and a thicker leathery parenchymatous inner covering. The peels constitute about 20-35% of the weight of the tuber, especially in the case of hand peeling ([4]). Concequently, a large amount of Cassava peel waste is generated annually ([5]).

In developing countries, there is a growing interest regarding the utilization of organic wastes generated by the food processing sector and through other human endeavours. This has led to a new policy of complete utilization of raw materials so that there will be little or no residue left that could pose pollution problems ([6]). The agriculture-based industries generate a significant amount of solid waste that, amongst other, include peels from Cassava, plantain, banana, oranges and straw from cereals. Rather than allowing these wastes to become solid municipal wastes, it is necessary to convert them to useful end-products. It is now realized that these wastes may be utilized as cheap raw materials for some industries or as cheap substrates for microbiological processes ([7]).

Much work has been carried out regarding the utilization of Cassava peels as substrates for microbial protein enrichment ([5]), increasing microorganism biomass ([8]) and on their use as food additives ([9]). However, the possibility of using Cassava peels for the production of ethanol has not been given much attention. This study was therefore initiated to explore the possibility of using Cassava peel as a substrate for producing ethanol.

MATERIALS AND METHODS

SAMPLE COLLECTION

Solid Cassava peel wastes were obtained from Gari processing industries located around the Oluorogbo area of Ile–Ife, Osun State, Nigeria. They were sorted and then washed under running tap water to remove sand and other dirt particles. Samples were sun-dried for about two weeks and then milled into a powder (flour) form.

MICROORGANISMS

Aspergillus niger (isolate A1) and Sacharomyces cerevisae (isolate Y1) used in this study were isolated from a Eba-(a Cassava preparation), stored in a tightly closed food flask for about six months at the Department of microbiology of

Obafemi Awolowo University. With a sterile loop, an inoculum was taken from the brown solution in the food flask and mixed with 10 ml sterile saline solution in MacCartney bottles in duplicate. The mixture was shaken vigorously after which 0.1-ml aliquots were spread on Malt Extract Agar (MEA) and nutrient agar. Aspergillus niger (A1) and Sacharomyces cerevisae (Y1) were isolated from the MEA agar plates. Pure cultures of the isolates were obtained by repeated transfers onto fresh culture medium and then stored as agar slants at 40°C. When required, stock cultures of the respective isolates were incubated in nutrient broth, harvested and standardized by Nephelometer. The freshly harvested cells were used to inoculate liquid suspensions of milled Cassava peels.

HYDROLYSIS OF CASSAVA PEEL

Twenty grams of Cassava peel flour was mixed with 500 ml of distilled water in two conical flasks and the mixtures were sterilized in an autoclave at 212 lb/sq for 15 min. Sterile distilled water was then added to each flask to a final volume of 1 liter and the flasks were plugged with sterile cotton wool to avoid contamination. After cooling, freshly harvested cells of Aspergillus niger (A1) was added to one of the flasks, while the second uninoculated flask served as control. A 10 ml aliquot from each of the flasks was transferred into sterile MacCartney bottles on a daily basis for nine days. The samples were centrifuged at 3500 rpm for 15 min, the cell-free supernatant recovered and transferred into clean MacCartney bottles and then used to determine the concentration of reducing simple sugars. The number of organisms in each sample was also estimated according to Ofuya and Nwajuba ($_{6}$). The percentage peel hydrolysis was calculated using the following formulae:

% Peel hydrolysis = [(Reducing sugars produced by growth – Reducing sugars in control) / Reducing Sugar in Control] x 100.

DETERMINATION OF MINERAL COMPOSITION OF CASSAVA PEEL WASTE AND OF FERMENTATION PARAMETERS

The proximate and mineral composition of Cassava peel waste was determined according to AOAC ($_{10}$). Fermentation parameters, i.e. ethanol, sugar and biomass yield, were determined as fermentation progressed, while single cell protein of the pooled biomass was determined at the end of fermentation. Ethanol (concentration) was measured using gas chromatography, as described by Konlani et al. ($_{11}$). The concentration of residual sugar in the medium was determined calorimetrically using 3, 6-dinitrosalicylic acid (DNS). For this purpose, 1 ml of diluted supernatant, obtained after removal of the biomass, was mixed with 1 ml of DNS and heated to 100°C for 5 min. The reaction was stopped by incubation on ice and the optical density at 540nm was read. The concentration of residual sugar was determined against a glucose standard graph.

RESULTS

Figure 1

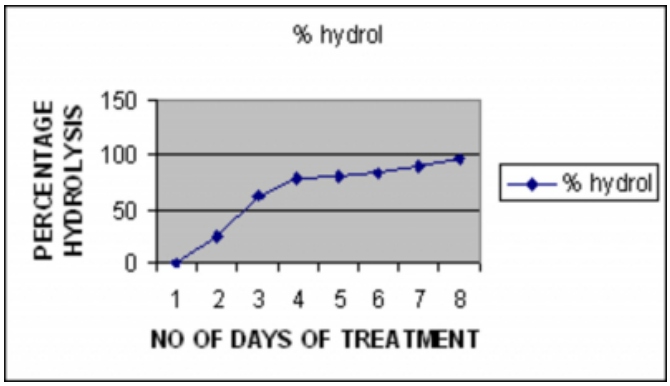
Table 1: Mineral composition of inoculated and uninoculated Cassava peel wastes

	Cassava peel media control	Degraded Cassava peel media inoculated with *Aspergillus niger*
Crude protein (%)	1.70	14.2
Crude fibre (%)	48.7	32.2
Fat (%)	1.74	5.12
Ash (%)	6.30	4.41

Table 1 shows the changes in the percentage of crude protein, crude fibre, fat and ash yield of the fermented peeled cassava wastes inoculated with Aspergilus niger. The fermentation of the wastes with Aspergilus niger caused an increases in the percentage of crude protein and fats,and a decrease in the percentage crude fibre and ash.

Figure 2

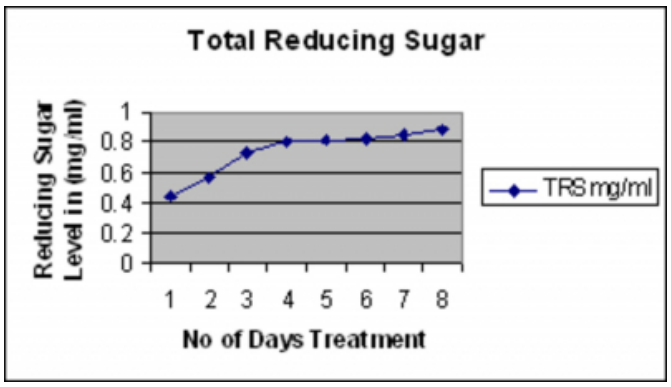
Figure 1: Percenatge hydrolysis of milled Cassava peels.



Percentage hydrolysis increases with time, with a steady increased observed between day 4-6.

Figure 3

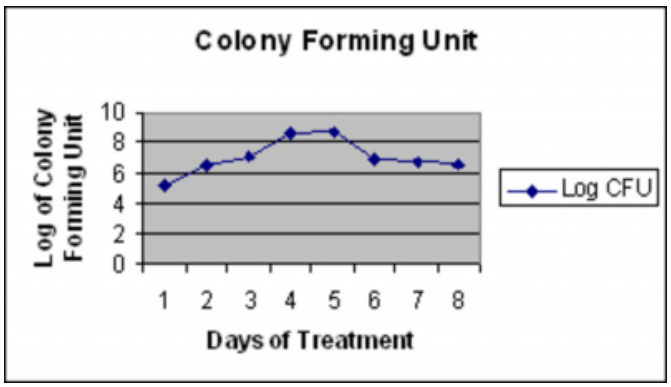
Figure 2: Reducing sugar concentration in a suspension of sugar milled Cassava peels.



The concentration reducing sugar of uninoculated milled suspension remains at 0.45mg/ml throughout experiment, maximum level of simple sugar produced in the inoculated was 0.88mg/ml.

Figure 4

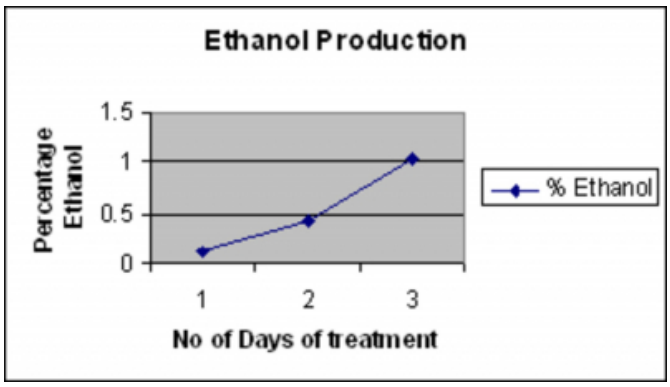
Figure 3: Rate of growth of cells in suspension of milled Cassava peels.



Average number of organism was estimated by multiplying count per plate by the dilution factor. The number of organisms increased in a logarithmic fashion reaching a maximum by the 4th day – 4.8 x 108 cells but gradually declined to 4.4 x 106 on the 7th day,which indicate degradative activity of the organism in milled Cassava.

Figure 5

Figure 4: Production of ethanol over three days following inoculation of the Cassava peel hydrolysate with .



The concentration of alcohol in control was 0.01% - 0.02%, the concentration of alcohol produced reached a value of 1.05 % by the 3rd day.

DISCUSSION

The Aspergilus niger (A1) isolated from Cassava peel wastes successfully hydrolysed the Cassava peel material as was evidenced by an increases in protein content and a decrease in fibre content (Table 1). It has been reported that the cyanogenic glucoside linamarin present in Cassava peel can be degraded and used as source of nitrogen by microorganisms ($_{12}$). Moreover, this study showed that Cassava peel starches can be readily degraded by an enzyme(s) produced by A. niger (A1). Thus, the successful degradation of Cassava peel by A. niger (A1) may be attributed to its amylolytic nature, as over 96% of the starchy component of the peel was transformed to simple reducing sugar during the wet-state fermentation. The highest concentration of reducing sugar (0.88 mg/ml) was obtained after incubation for seven days. This observation is in agreement with the earlier work of Okolo et al. ($_{13}$) and Omemu et al. ($_{14}$). Similar results have also been reported in instances where Rhizopus spp. and Trichoderma spp. were used to degrade Cassava peel ($_{6}$).

Inoculation of the cell-free Cassava peel hydrolysate with Saccharomyces cerevisiae (Y1) resulted in maximal ethanol production after incubation for three days, albeit that the concentration of ethanol produced was rather low (1.05%). Since yeasts are unable to synthesis amylase, the results obtained in this study indicated that hydrolysis of the Cassava peels by A. niger (A1) to yield simple reducing sugars was sufficient to allow S. cerevisiae (Y1) to produce ethanol by fermentation. Despite the low concentration of ethanol produced, the results are nevertheless comparable

with the data obtained in other studies in which it was reported that Cassava peel hydrolaytes prepared enzymatically yielded 2.3% ethanol, while an acid hydrolysate yielded 3% ethanol after inoculating with special strains of Saccharomyces cerevisiae ([15,16]).

Cassava peel is a source of cheap, degradable material for the production of simple sugars, which can be fermented by yeast to produce ethanol, as a cheap energy source for the use in our local communities. However, there is a need for further work to optimize the conditions for the production of simple sugar from the peels and to isolate a Saccharomyces cerevisiae strain that may result in improved yields of ethanol. From this study, it can be deduced that Cassava peel wastes from Cassava processing may serve as a good source of carbon for yeast fermentation to produce ethanol.

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