4

PRODUCTION OF ETHANOL FROM CASSAVA AND SWEET POTATOES USING YEAST EXTRACT FROM PALM WINE

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ABSTRACT

Cassava and sweet potatoe pulps and starches have been fermented using a strain of Saccharomyces cerevisiae isolated from palm wine. Analysis of the fermented products indicated a high yield of ethanol. Comparative studies conducted using the isolated strain and a similar strain contained in Baker's Yeast showed that the former is more efficient in fermenting blackstrap molasses than the latter.

Keywords *Ethanol, Saccharomyces cerevisiae, Hydrolysis, Fermentation, Yeast strains.*

INTRODUCTION

Ethanol is present in all alcoholic beverages in varying proportions. Apart from its importance in the beverage industry, it is a useful solvent for many organic compounds. It is also gaining popularity in its use as an automobile fuel especially as a substitute for gasoline.

There are two main sources of ethanol: the synthetic route using petroleum as raw material and the fermentation of sugar-containing biomass materials. Though the production of ethanol by fermentation has roots deep in antiquity, the abundance and expensiveness of petroleum, coupled with its easy use made the production of ethanol through synthesis more attractive. However, with the oil crisis and the fact that petroleum is not a readily renewable source of feedstock, attention is gradually being shifted to the fermentative route for ethanol production. Brazil, for example, which has engaged in a large-scale ethanol programme, is using the fermentation of such feedstocks as molasses, cassava etc.

NEW TECHNIQUES

With the renewed interest in the fermentative production of ethanol, many new techniques are being developed to make production more efficient. These include the use of immobilized microbial cells. (McGhee et al 1982 [1], Ghose and Bandyophadhyay, 1980[2], Wada et al 1981 [3] etc.); Cell recycle (Cycewski and Wilkie, 1971 [4], Ghose and Tyagi, 1979 [5]. Rogers et al 1980 [6] and vacuum fermentation (Ramalingan and Finn, 1977 [7]).

MICROOGANISMS

A number of microoganisms are capable of producing ethanol. However, ethanol is generally produced using Saccharomyces cerevisiae under anaerobic conditions (Rehm, 1980 [8] and efforts to increase the efficiency of the process have been made by the development of genetically modified strains (Panschal et al, 1983 [9] and recycling of flocculent cells (Herbert, 1961 [10, 11]).

Palm wine, which is one of the raw materials often used for the production of ethanol in Ghana contains some microorganism which are capable of alcoholic fermentations. Among them are the strains of Saccharomyces and Candida which belong to the yeast genera and Zymomonas mobilis Linder which is a bacterium. It has been shown that the most predominant yeast species in palm wine is of the genus Saccharomyces.

HYDROLYSIS OF STARCHY MATERIALS

In the production of ethanol from starchy raw materials, the starch has to be hydrolysed first in order to convert it into fermentable sugars. Yamamoto, Matsumura and Uenakai, 1982 [12] simultaneously applied pectic enzyme glucoamylase and yeast for hydrolysis and fermentation respectively of uncooked cassava, sweet and white potatoes. Tsujisaka et al, 1958 [13] identified the specificity of saccharogenic amylases of Rhizopus delemar and Aspergillus niger; these enzymes which were called glucoamylases, were found to digest various raw starches to liberate glucose. Nishi, 1969 [14] reported the saccharification of uncooked sweet potatoes using glucoamylase.

Aschengreen et al, 1979 [15] have shown that when tapioca and potato starches are saccharified, using Amyloglucosidase Novo 150L under the same conditions as in the experiment under discussion, high yields of glucose are obtained.

Manners, 1971 [16] has shown that during the degradation of starch before fermentation, maltose, maltotriose and maltotetrose are produced. Maltose is hydrolised to glucose by the yeast enzyme maltase. The efficiency of a yeast fermentation is enhanced when the strain of yeast can utilize maltotriose and maltotetrose to give fermentable sugars. Not all strains of Saccharomyces have this desirable property.

Another source of ethanol in Ghana is the fermentation of molasses and pure sucrose using Baker's Yeast. This is done on a commercial scale.

In this paper, the production of ethanol from non-conventional feedstocks, namely cassava and sweet potatoes using a strain of yeast isolated from palm wine is outlined. The efficiency of this yeast is producing ethanol from blackstrap molasses is compared with that of Baker's yeast.

EXPERIMENTAL

Hydrolysis of Cassava and Sweet Potato Pulps and Starches:

Cassava and Sweet Potatoe starches were first produced from the raw tubers. Slurries of both the cassava and sweet potato were prepared. These and the suspensions of starches from the two sources were separately gelatinised by cooking at a temperature of 98°C for 30 minutes. The starch-jellies obtained from these operations were hydrolysed using Amyloglusidase (AMG 200L) at a temperature of 60°C for 72 hours.

The dextrose equivalents of the hydrolysates were determined using both the DNS (Dinintro Salicylic acid) and Fehling's solution methods. The DMS method is a spectrophotometric technique and so a standard curve was produced using glucose solution and the usual reagents. The Fehling's solution method is titrimetric.

Isolation of Yeast From Palm Wine

Sediments from palm wine were poured onto YM agar contained in a petri dish and incubated for 48 hour. A combination of the pour plate and streak plate techniques were employed to obtain a pure culture of yeast which was identified by standard microbiological methods.

Determination of Optimum Conditions for Fermentation

The optimum pH, temperature and sugar concentrations for the fermentations using the palm wine yeast isolate were determined with blackstrap molasses. In the preparation of each mash, the desired pH was obtained by adjusting with lactic acid. Ammonium orthophosphate was also added with a dosage of 0.6% (w/w) to make up for any possible deficiencies in nitrogen and phosphorous. The mash was sterilized in an autoclave at a steam pressure of 15 pounds per square inch for 15 minutes.

In order to determine the optimum pH and temperature (which were done simultaneously) an arbitrary sugar concentration of 12% (w/w) was used. The pH values employed were 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0. Three different temperature ranges viz, 19 - 20°C, 25 - 26°C and 28 - 29°C were employed. Each mash was seeded with a starter which represented 5% of its total volume and contained enough yeast cells to give an initial yeast population of 20,000 cells per cm^3 of the mash. In each case, fermentation was monitored by observing the evolution of gas. The cessation of this gas evolution was taken as an indication of the end of fermentation. The beer so obtained was analysed for ethanol content using an alcoholimeter. The determinations were done several times until a constant ethanol concentration was obtained. Before these determinations were done, a standard curve was prepared using known ethanol concentration.

The optimum pH and temperature range obtained in this experiment were used in determining the optimum sugar concentration by using these conditions with different sugar concentrations. The sugar concentrations used were 10, 12, 14, 16, 18, 20 per cent of the total mash.

Use of Optimum Conditions for the Fermentation of Blackstrap Molasses, Cassava and Sweet Potato Sugars

In these determinations, the isolated palm wine yeast (Saccharomyces cerevisiae) and Baker's Yeast (Saccharomyces cerevisiae) were employed. The mashes were prepared according to the procedure outlined earlier and the beers analysed as before. All fermented mashes were distilled using standard methods.

ANALYSIS OF PRODUCTS

Apart from using the alcoholimeter to determine the ethanol content of the beers, the distilled products were analysed using an Abbe refractometer.

In this determination, the refractive index of each product was determined and compared with similar determinations made using standard ethanol solutions. From a calibration curve, the ethanol content of each distilled product was determined.

Standard chemical analysis was used to confirm that the product was ethanol.

RESULTS AND DISCUSSIONS

Dextrose Equivalent of Hydrolysates

Below is a summary of the results obtained

Table 1

Product	DNS Method/%	Fehling's Solution Method/%
Hydrolysed Cassava Pulp	20.09	21.33
Hydrolysed Cassava Starch	81.67	82.39
Hydrolysed Sweet Potato Pulp	26.33	25.78
Hydrolysed Sweet Potato Starch	88.37	87.02
Blackstrap Molasses	-	54.27

The results indicate that the hydrolysed cassava pulp contains an average of 20.71% dextrose. Literature indicates that the average carbohydrate content of cassava is between 30% and 35%. Out of this, 64 - 72% is starch with amylose and Amylopectin consisting of 99% or more of the dry starch [17]. If the upper limits of these figures are taken, then the starch content of cassava is about 26.2%. Since amylose and amylopectin, which are the principal constituents of starch, are made up of glucose units, when starch is completely hydrolysed, a glucose yield of about 26% is expected. Making room for the likelihood of incomplete hydrolysis and the fact that the upper limits were used in arriving at 26%, it could be concluded that the glucose yield obtained in the experiment is reasonable.

For peeled potatoes, Martha et al, 1978 [18] have indicated that the dextrose equivalent is about 30%. Also, Aschengreen et al, 1979 [15] have shown that when Amyloglucosidase Novo 150L is used to hydrolyse tapioca and potato starch dextrose equivalents as high as 97.3% could be obtained. The comparatively lower values obtained in this experiment may be due to incomplete hydrolysis.

Results for the Determination of Optimum Conditions for Fermentation

Table 2 **Data for Optimum pH and Temperature**

pH	% ETHANOL (v/v)		
	At 19-20°C	At 25-26°C	At 28-29°C
3.5	4.8	4.5	4.3
4.0	5.7	5.3	5.2
4.5	6.0	5.6	5.4
5.0	4.3	4.1	3.9
5.5	3.8	3.5	3.0
6.0	3.0	2.3	2.0

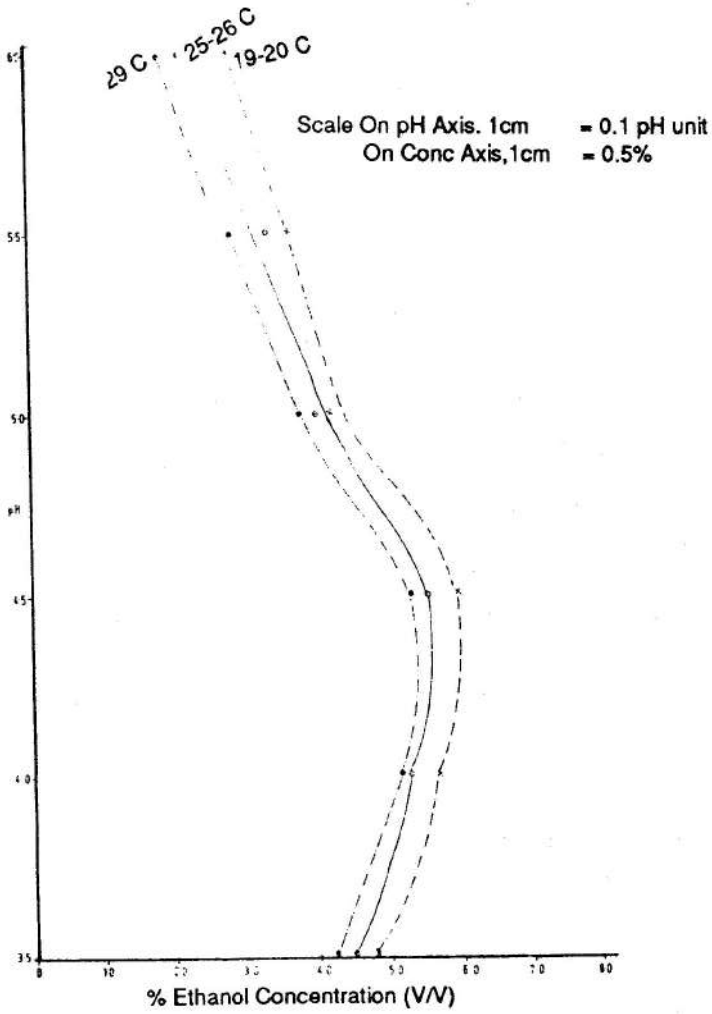

Fig.1 Graph of pH Against Ethanol Concentration at Different Temperatures.

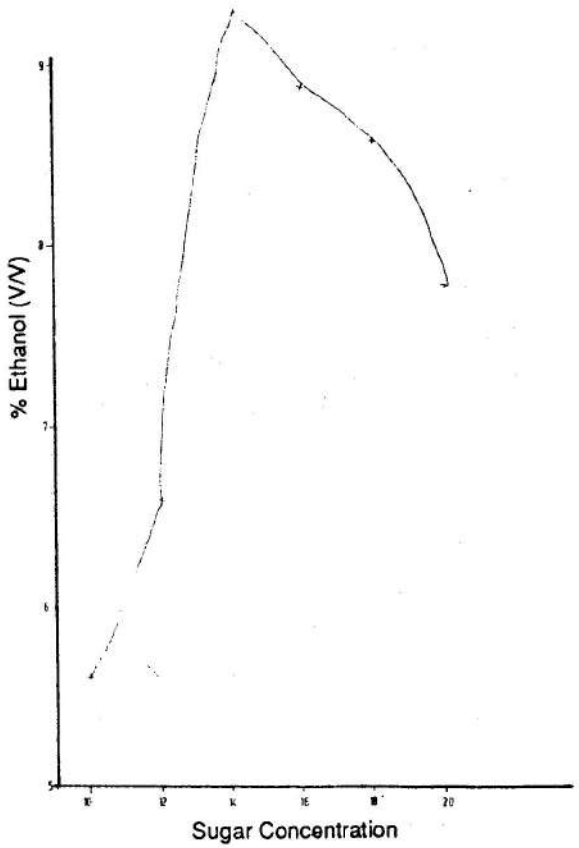

Fig.2: A Graph of % Ethanol Produced Against Sugar Concentration

Scale On Vertical Axis 1cm = 0.2 unit
On Horizontal Axis 1cm = 1 unit

Table 3 **Data For Optimum Sugar Concentration**

Sugar Concentration (w/w)	% Ethanol (v/v)
10.00	5.6
12.0	6.6
14.0	9.3
16.0	8.9
18.0	8.9
20.0	7.8

From these results, the optimum conditions for the production of ethanol using the isolated yeast are; pH 4.5 and sugar concentration 14% (w/w). Out of the three temperature ranges used, 19-20°C gave the best results. In the book "Industrial Microbiology", Prescott and Dunn, 1959 [19] gave the optimum conditions for the fermentation of blackstrap molasses using Saccharamyces cerevisiae as pH: 4.0 to 4.5, temperature: 21.1 - 26.7°C and sugar concentration 10 - 18 (w/w). The results obtained (except the temperature range) fall within the indicated values. It was also found from the results that the alcohol tolerance of the yeast is up to 9.3 (v/v).

If a high sugar concentration is used, it reacts adversely on the yeast, or the alcohol produced may inhibit the action of the yeast. The result is that the fermentation time is prolonged and some of the sugar may not be properly utilized. Too low a sugar concentration is uneconomical.

The optimum pH obtained above is favourable for yeast growth but is sufficiently low to inhibit the development of many types of bacteria. Maintaining the temperatures within a reasonable range is very important since at higher temperatures, ethanol evaporates rapidly and also bacterial growth is favoured.

Ismail and Ali, 1971 [20] have found that many strains of Saccharomyces have alcohol tolerance within the region of 8 - 9% (v/v) of alcohol and that a few strains can tolerate above 10% (v/v). Thus the alcohol tolerance of the isolated strain of yeast is very good.

Use of Optimum Conditions for Fermentations

The table below gives the results obtained when the optimum conditions were used for the fermentation.

Table 4

Source of Ethanol	Active Mass of Ethanol obtained/g	Theoretical Mass of Ethanol/g	Yield % of Theoretical
Blackstrap Molasses + Palm Wine Yeast 1st Determination	101.37	118.53	85.52
Blackstrap Molasses + Palm Wine Yeast 2nd Determination	64.38	73.13	88.04
Blackstrap Molasses + Baker's Yeast	56.85	73.13	77.74
Hydrolysed Cassava Pulp + Palm Wine Yeast	25.99	30.80	84.38
Hydrolysed Sweet Potato + Palm Wine Yeast	35.60	40.36	88.20
Hydrolysed Cassava starch + Palm Wine	103.94	125.20	83.01
Hydrolised Sweet Potatoe Starch + Palm Wine Yeast	111.64	135.47	83.89

The results are within the expected range. The low value for Baker's Yeast is understandable since the strains of Saccharomyces cerevisiae contained in it do not ferment sugar as much as those contained in other yeast sources e.g. distiller's yeast.

From literature, fermentation efficiencies exceeding 90% (v/v) are quite rare for the following reasons:

(a) conversion of part of the sugar into new cellular substance of the yeast.
(b) formation of glycerol, acetaldehyde and fusel oil as by-products of the ethanol fermentation;
(c) formation of volatile acids as products of bacterial metabolism and
(d) evaporation of ethanol and conversion into aldehyde and ethanoic acid.

In the light of these, the result obtained are quite good.

The results indicate that the maximum ethanol concentration which could be obtained from the distillation is 95%. This is due to the formulation of an azeotropic mixture between the ethanol and the water present.

CONCLUSIONS

The results show that instead of Baker's Yeast (which is imported) good quality yeast could be isolated from palm wine to be used for alcoholic fermentations. Hitherto, yeast flocks are thrown away by palm wine tappers and sellers. It is recommended that a project be initiated to use this otherwise waste product for large scale production of yeast.

ACKNOWLEDGEMENT

One of us (J.A.M.A.) wishes to acknowledge a grant received from the Ghana Government during the period this project was carried out.

Table 5 Data For Distilled Products

Source of Ethanol	Refractive Index	% Ethanol Content
Blackstrap molasses + Palm Wine Yeast (1st Determination)	1.3635	65
Blackstrap molasses + Palm Wine Yeast (2nd Determination0	1.3645	95
Blackstrap molasses + Baker's Yeast	1.3640	66
Hydrolysed Cassava Pulp + Palm Wine Yeast	1.3645	95
Hydrolysed Sweet Potato Pulp + Palm Wine Yeast	1.3643	80
Hydrolysed Cassava Starch + Palm Wine Yeast	1.3645	95
Hydrolysed Sweet Potato Starch + Palm Wine Yeast	1.3645	95

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