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Production of alcohol from cassava flour hydrolysate

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Alcohol was produced from cassava flour hydrolysate (CFH) with standard glucose and sucrose solutions used as controls. The conversion efficiency of sugar to alcohol, rate of fermentation and types of alcohol produced were determined. The effects of yeast concentrations (5, 15 and 20%) and fermentation time (24, 48, 72, 96 and 120 h) on yield of alcohol from CFH were also studied. Results showed that the maximum carbon dioxide evolved during fermentation was 8.57 g recorded by CFH. The conversion efficiency of sugars to alcohol was 248.4, 99.51 and 95.37% for CFH, standard glucose and sucrose solutions respectively. Alcohol produced was mostly ethanol with traces of methanol. Yeast concentrations were found not to have any significant effect ($p > 0.05$) on the alcohol yield, however, fermentation time was found to have had a significant effect ($p < 0.05$) on alcohol yield. The study suggests that high yield of alcohol could be produced from CFH.

Key words: Alcohol, cassava flour hydrolysate, fermentation, yield.

INTRODUCTION

Ethyl alcohol or ethanol ranks second only to water as the most widely used solvent in chemical industry and as these industries have expanded, so the demand for industrial alcohol has increased. Alcohol acts as a solvent for an immense range of industrial products, including paints, lacquers, dyes and oils. In addition, some are used as a raw material in chemical synthesis and a little in the form of fuel (Rose, 1961). Techniques for the recovery of ethanol from cassava flour were standardised at Central tuber crops research institute (CTCRI), Trivandrum and Central food technological research institute (CFTRI), Mysore (Vijayagopal and Balagopalan, 1978; Vijayagopal et al., 1989; Balagopalan and Hrishi, 1980). Ethanol production from fresh tubers, flour and starch of cassava was studied by simultaneous saccharification and fermentation procedures at CFTRI, Mysore (Balagopalan and Ray, 1992) . *In situ* saccharification and fermentation techniques for ethanol production from cassava flour were also attempted (Vijayagopal and Balagopalan, 1990). Attempts have also been made to modernize and improve the efficiency of fermentation by adopting novel techniques like immobilization of yeast cells on various carriers like alginate beads, Kieselghur, brick powder and silica gel (Balagopalan and Ray, 1992).

Large quantities of cassava are produced in Ghana but, despite a considerable effort, there is at present no commercially proven glucose syrup or alcohol from cassava industry. Cassava flour promises to be a good substrate for alcohol production due to its high content of fermentable sugars and stable shelf-life (Grace, 1977; Ocloo, 2002). Besides that, it has advantages such as complete and easier hydrolysis compared to other flours (Vijayagopal et al., 1981). The conversion efficiency of starch to fermentable sugar by starch hydrolyzing enzymes has been the focus of many research works. Attempts have also been made to produce ethanol from raw cassava starch in a single-step of combining liquefaction, saccharification and yeast fermentation without cooking and autoclaving (Ueda et al., 1981).

In Ghana, research has been done in the area of conversion of cassava flour or starch into fermentable sugars using malt. Rice, maize and sorghum malts have been used as sources of hydrolyzing enzymes. Hammond and Ayernor (2000) used various cereal malts in the hydrolysis of starch and observed that rice malt gave the highest yield of sugars. The use of rice malt in combination with amyloglucosidase in conversion of starch to sugars has also been investigated

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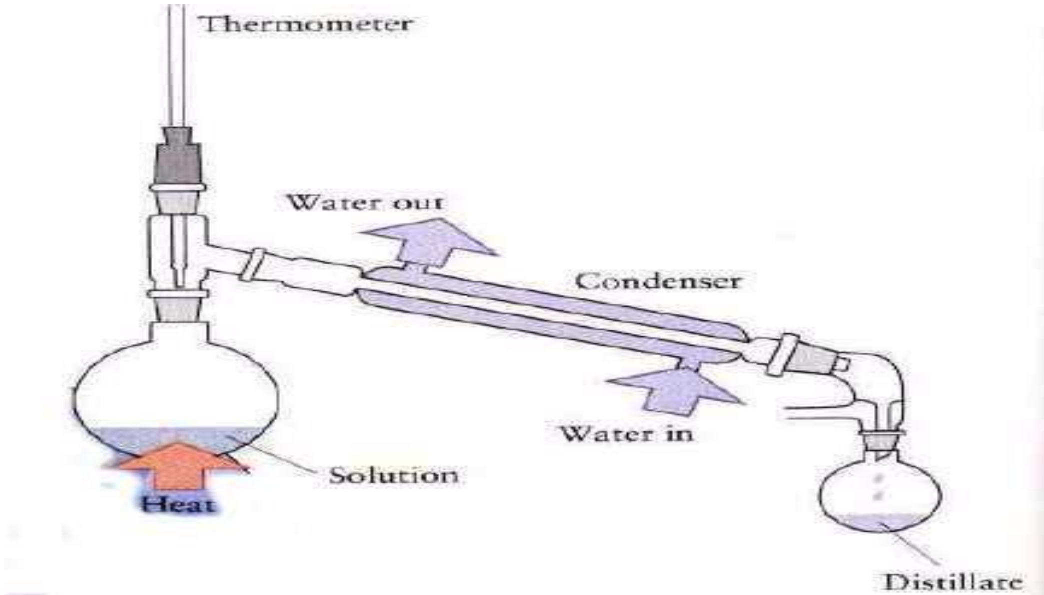

Figure 1. Schematic laboratory distillation equipment.

(Ayernor et al., 2002).

Generally, alcohol is produced in Ghana using sucrose, palm sap and sugar cane, however the cost of obtaining these raw materials is high compared to cassava which can be grown any where in the country. The technology of producing alcohol from cassava though not new, much has not been done to utilize this technology in the Country. There is therefore a need to investigate the suitability of converting sugar syrup produced from cassava flour into alcohol. The purpose of this study was to use cassava flour hydrolysate as a substrate for alcohol production.

MATERIALS AND METHODS

Cassava flour hydrolysate

The methodology described by Ocloo and Ayernor (2008) was used. Thousand grams of cassava flour was mixed with 5000 ml of water to form slurry. The mixture was allowed to boil until gelatinized at 70°C and allowed to cool. About 250 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 liquour was then heated to 70°C and the last batch of 250 g rice malt added to further convert the unhydrolysed starch to sugars. The mixture was boiled briefly and immediately filtered using cloth and a Laboratory test sieve of aperture 180 µm (Endecotts Limited, London, England). The sweet-wort produced was boiled again to arrest further enzyme action and then cooled. Cassava flour hydrolysate having a reducing sugar content of 8.72% was obtained.

Bakers yeast

Saf-levure bakers yeast (*Saccharomyces cereviseae*) manufactured by Lesaffre Group, France was purchased from a local market in Accra, Ghana.

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 3000 ml sugar syrup and 100 ml of 15% yeast inoculum (15 grams of dry baker's yeast rehydrated in 100 ml of distilled water at 37°C for 10 min). The bottles were topped with tubes to allow carbon dioxide (CO_2) to escape. Fermentation was done for 5 days at 28 - 30 °C (Ocloo and Ayernor, 2008) . At the end of the fermentation period, the alcohol was separated from the extract using simple distillation procedure at 78.3 - 80°C (Figure 1).

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.

Theoretical alcohol yield

Under anaerobic condition, glucose is converted to ethanol and carbon dioxide via glycolysis. The overall reaction produces two (2) moles of ethanol and carbon dioxide for every mole of glucose consumed.

$$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{Energy (stored as ATP)}$$

Every gram of glucose converted will yield 0.511 g of ethanol (Maiorella et al., 1981).
Thus,
1 g glucose 0.511 g ethanol

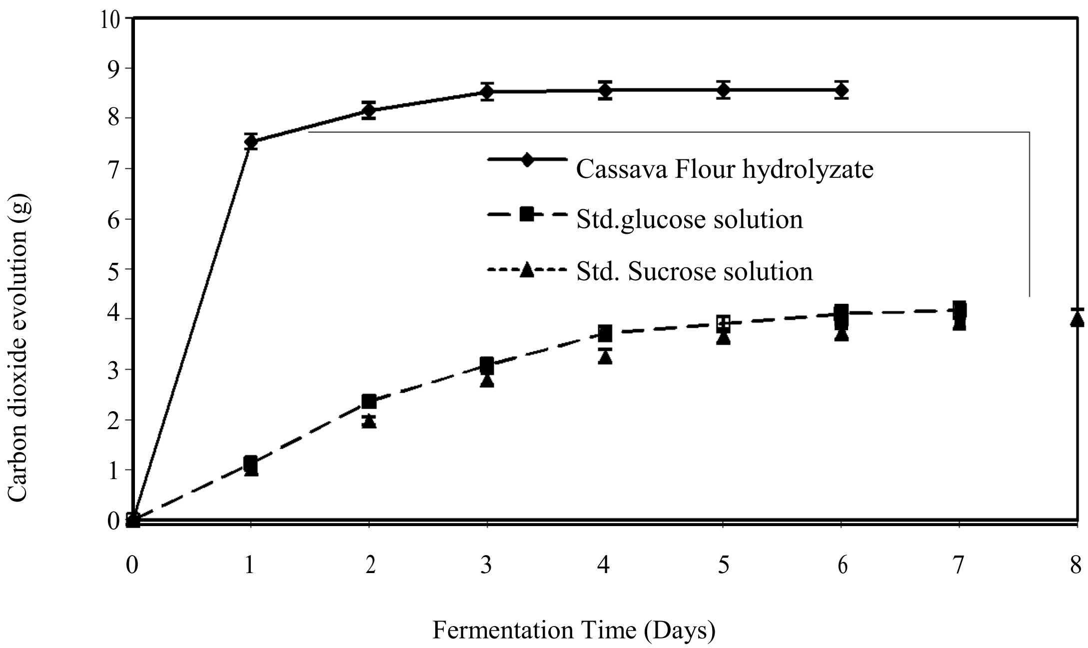

Figure 2. Time course of fermentation of CFH compared with standard glucose and sucrose solutions of the same concentration.

Analytical methods

The rate of fermentation was determined by weight loss of contents of fermentation. Alcohol content of distillate was determined by AOAC method 920.57 and 913.02 (1990) . Sugars were determined using AOAC methods 923.09 and 930.45 (1990).

Identification of Alcohols

This was done for distilled fermented cassava flour hydrolysate (CFH). Gas chromatography (GC) (Perkin elmer AutoXL connected to flame ionisation detector (FID), with a 3% Carbowax column) was used. The carrier gas used was nitrogen. Standard solutions of ethanol and methanol were prepared.

Procedure

Two to three micro litres of the standards were injected through an injection port with micro syringe, having injector temperature of 160°C, oven power of 80°C and attenuation of 8 to obtain good peaks. The samples mixed with carrier gas (nitrogen), enters the column and then to the detector at the flow rate of 15 ml/min at 200°C. The fermented and the distilled cassava flour hydrolysate were also injected as done with the standards and their peaks compared with the standard peaks.

Effect of yeast concentration and fermentation time on alcohol yield

Experimental design

A 3 x 5 factorial design was used with variables consisting of yeast concentrations (5, 15 and 20%) and fermentation time (24, 48, 72, 96 and 120 h). 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.

Statistical analysis

Statgraphics Computer Software (Statistical Graphics Corp., STST Inc; USA) and Microsoft excel were used for ANOVA and graphs respectively.

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 for standard glucose and sucrose solutions. These results were statistically significant ($p<0.05$) (Figure 2). Multiple tests on the data to ascertain where in particular the differences occurred showed that the difference only existed between CFH and the other 2 substrate used. After 3 days of fermentation at 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. Ueda et al. (1981) observed a similar trend. In their study on production of ethanol from raw cassava starch by a non-conventional fermentation method they reported that the rate of fermentation or carbon dioxide evolution ceased

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	8.72 ± 0.16	2.18 ± 0.14	8.30 ± 0.70	3.34 ± 0.01
Glucose	8.72 ± 0.16	0.84 ± 0.48	4.01 ± 0.15	4.03 ± 0.16
Sucrose	8.72 ± 0.16	1.00 ± 0.42	3.77 ± 0.29	3.95 ± 0.13

Determinations are averages of 5 batches of production.

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 (Briggs et al., 1981). 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 metabolised 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 limit-dextrins, which were not considered as reducing/ fermentable sugars.

Conversion efficiency of CFH, 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 6.54 ± 0.02, 7.88 ± 0.32 and 7.72 ± 0.26 for CFH, standard glucose and sucrose solutions respectively. These reducing sugars gave rise to alcohol contents of 8.30 ± 0.76, 4.01 ± 0.15 and 3.77 ± 0.29% v/v, respectively (Table 1 and Figure 3). However, the theoretical/ predicted alcohol production values were 3.34 ± 0.01% v/v, 4.03 ± 0.16% v/v and 3.95% 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 as the ratio of the actual alcohol produced to theoretical/predicted alcohol based on fermented sugar (×100). The values for CFH, standard glucose and sucrose solutions were 248.4 ± 22.0, 99.51 ± 0.33 and 95.37 ± 4.10%, respectively (Figure 3). 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. 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 -amylase to hydrolyse 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.

Identification of alcohols

Ethanol and methanol were used as standards during the analysis. The fermented cassava flour hydrolysate and the distilled product were found to consist mainly of

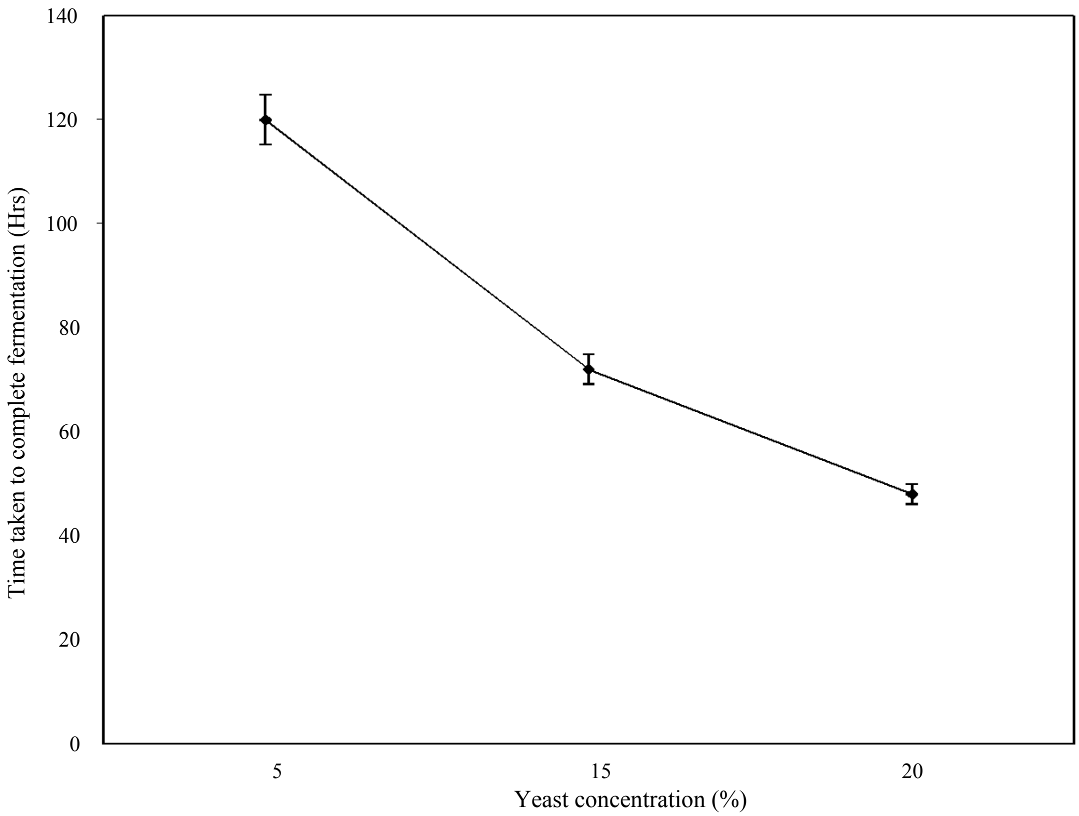

Figure 3. Efficiency of alcohol yield (%) from CFH compared with standard glucose and sucrose solutions of the same concentration.

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	7.90	8.30	8.31	8.31	8.31
15	7.63	7.89	8.30	8.31	8.31
5	7.35	7.67	8.06	8.29	8.30

About 100 ml of yeast suspension was added to 3000 ml of cassava flour hydrolyzate Initial sugar concentration = 8.72%

ethanol and traces of methanol (Figure 4). This observation showed that the fermentation was efficiently done and the products are safe for use as ethanol. Methanol is poisonous and therefore undesirable in fermented product. The concentration of alcohol in the distilled product using the GC was about 77.4% ethyl alcohol (ethanol).

Hang et al. (1982) also observed various alcohols in solid-state fermentation of apple pomace. In their study, alcohols such as ethyl alcohol, methyl alcohol, n-propyl alcohol, isobutyl alcohol and isoamyl were identified. Of the alcohols analysed, ethyl alcohol (ethanol) was present in the highest concentration; other alcohols were produced in much smaller quantities. Ayernor and Matthews (1971) also observed a similar trend in the production of palm wine.

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

Yeast concentration of 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 significantly ($p > 0.05$) did not affect the alcohol yield. The yeast concentration however significantly ($p < 0.05$) affected the time taken for the

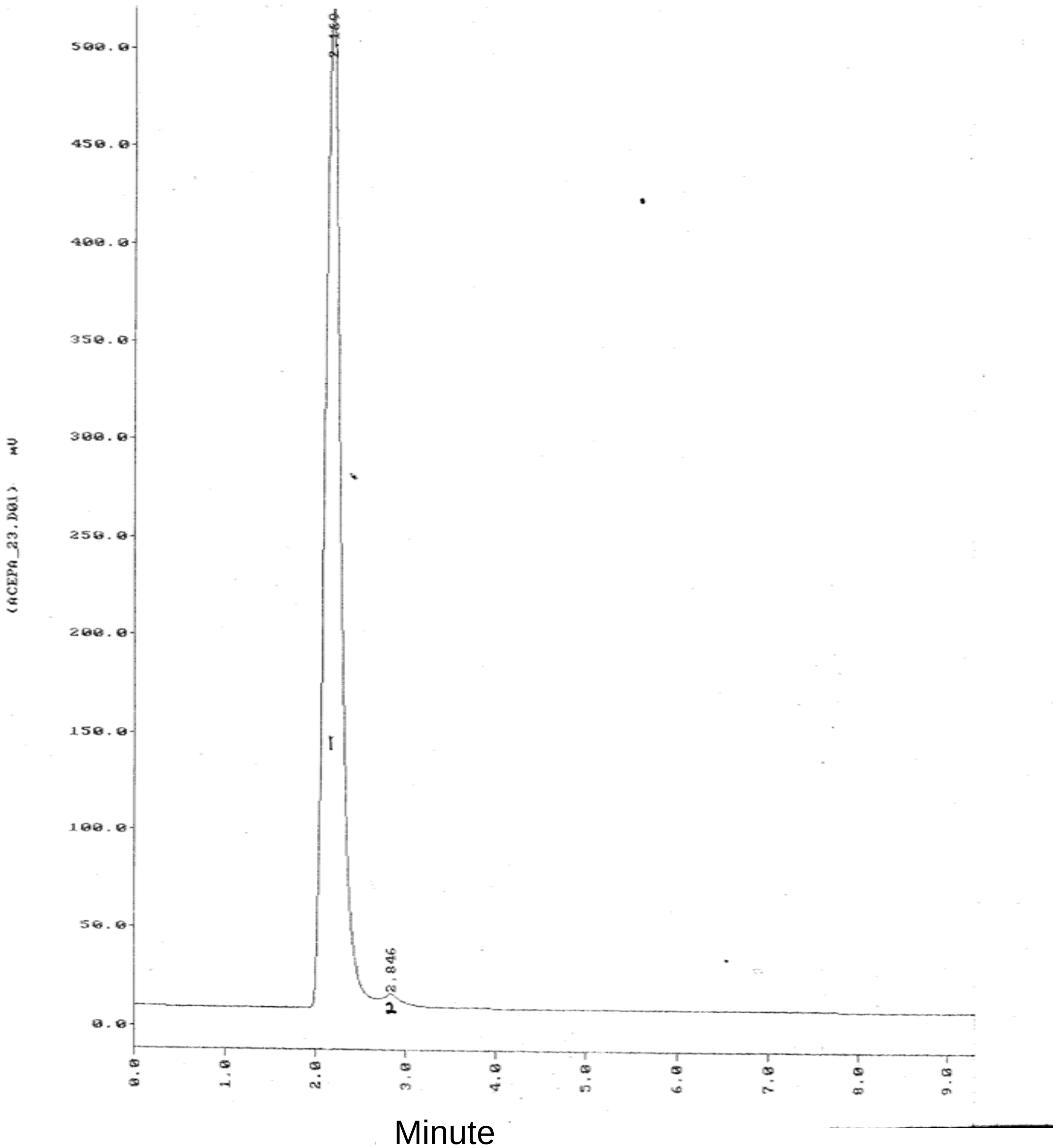

Figure 4. Gas chromatogram of distilled alcohols from fermented CFH (1) Ethyl alcohol; (2) Methyl alcohol. Column: 3% Carbowax, Oven temperature: 80°C, injector and detector temperatures: 160 and 200°C, respectively, Nitrogen flow rate: 15 ml/min, Attenuation: 8 and detector: FID.

fermentation to be completed, that is, to achieve maximum alcohol yield. Fermentation was completed over the period of 48, 72 and 120 h for 20, 15 and 5% yeast concentrations respectively (Figure 5). 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.

Conclusions

Alcohol of high yield was produced during the fermentation of Cassava Flour Hydrolysate. The conversion efficiency of sugars to alcohol was 248.4,

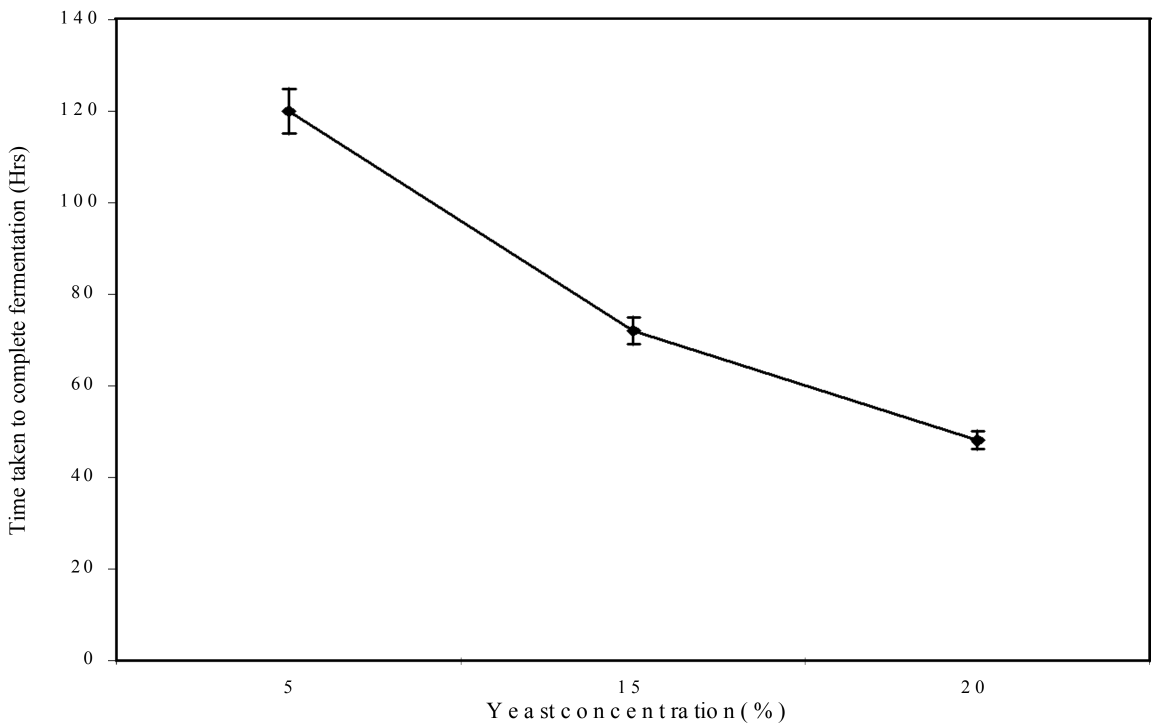

Figure 5. Effect of yeast concentration on time taken to complete fermentation of cassava flour hydrolysate.

99.5 and 95.4% for cassava flour hydrolysate, standard glucose and sucrose solutions, respectively. This suggested that the limit-dextrins in the cassava flour hydrolysate were converted by the yeast during the fermentation process. The type of alcohol produced was mainly ethanol with traces of methanol. The yeast concentrations used had no significant effect ($p > 0.05$) on the yield of alcohol.

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