

Full Length Research Paper

Physical, chemical and microbiological changes in alcoholic fermentation of sugar syrup from cassava flour

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Accepted 4 January, 2008

Changes in alcoholic fermentation of sugar syrup produced from cassava flour with *Saccharomyces cerevisiae* (baker's yeast) were investigated. Cassava flour paste was hydrolysed using rice malt to produce hydrolysate (sugar syrup), which was fermented at 28 – 30°C for 1, 2, 3, 4 and 5 days. The fermented sugar syrup was analysed for alcohol content, reducing sugars, specific gravity, soluble solids, pH, volatile acids and total acidity using standard analytical methods. Yeast growth was also monitored. Results showed that pH values decreased with increased total acidity with concomitant increase in yeast growth (biomass) and alcohol contents of the fermenting sugar syrup. There were decreases in soluble solid contents, refractive indices of the fermenting medium. Volatile acids (as acetic acids), increased with alcoholic fermentation. Fermentation of sugar syrup from cassava flour is associated with physical and chemical changes that occur in other form of fermentation alongside increased in biomass.

Key words: Fermentation, biomass, alcohol, sugar syrup, *Saccharomyces cerevisiae*.

INTRODUCTION

Cassava flour hydrolysate (sugar syrup) is the wort or the liquor obtained after the hydrolysis of cassava flour with enzymes. The hydrolysate is known to contain fermentable sugars such as glucose and maltose (Ayernor et al., 2002), which can be fermented to produce alcohol. Cecil (1995) also reported of maltose, glucose and higher polymers in sugar syrup from cassava using rice malt as enzymes source. During alcoholic fermentation, various changes are observed as a result of the metabolic activities of the yeasts. These changes, physical, chemical or microbiological are found to influence the fermentation process (Hough et al., 1971; Pearson, 1976).

The pH of fermented products is found to be important during fermentation and storage (Pearson, 1976). Sugar concentration is reported to affect the yield of alcohol produced (Maiorella et al., 1981). The alcoholic content of a fermented product, especially beer, is usually regarded

as a measure of its strength (Hough et al., 1971). Total acidity and volatile acids are known to influence the flavour and the aroma of the fermented product, and their levels can also be used as an index of shelf-life of the fermented product (Pearson, 1976). The presence of volatile acids is usually due to acetic acid bacteria contamination (Pearson, 1976). Hough et al. (1971) reported that the production of volatile constituents during fermentation of wort closely follows the attenuation of the wort and this is influenced by yeast strain, temperature, composition of wort and the method of fermentation employed.

The purpose of this study was to investigate physical, chemical and microbiological changes associated with alcoholic fermentation of sugar syrup produced from cassava flour.

MATERIALS AND METHODS

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Paddy rice (PSB Rc. 34) was purchased from the Irrigation Development Authority (IDA) at Ashaiman near Accra, Ghana. Fresh

cassava roots were purchased from the Centre for Scientific and Industrial Research (CSIR) farms at Pokuase near Accra, Ghana. Bakers yeast was obtained from a local market in Accra, Ghana.

Preparation of enzymes source (rice malt)

Paddy rice was cleaned, washed thoroughly and soaked in a volume of water 3 times the weight of seeds for 24 h. The soaked seeds were placed on a jute sack in a basket and kept under ambient temperature ($32 \pm 5^\circ\text{C}$) and watered 2 – 3 times a day. Germination was allowed to proceed for 9 days. The germinated seeds were then solar dried ($38 \pm 5^\circ\text{C}$) for about 20 – 25 h. The dried malt samples were milled using a disc attrition mill (Straub model 4E Grinding mill, Straub Co PHILA, USA). The milled rice malt samples were packaged and stored at cold room temperature (4°C).

Preparation of sugar syrup from cassava flour

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 $180 \mu\text{m}$ (Endecotts Limited, London, England). The sweet-wort produced was boiled again to arrest further enzyme action and then cooled.

Alcoholic fermentation of sugar syrup from cassava flour

Standard alcoholic fermentation experiments were conducted in aspirator bottles 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 allowed to continue for 5 days.

Physical and chemical analysis

pH was measured with a TOA pH meter (HM-30S, TOA Electronics Limited, Tokyo, Japan). Total and volatile acidity were determined by the methods described in ISI Handbook of Food Analysis, Part X (ISI, 1984). Soluble solids and refractive indices were measured using an Abbé refractometer (Mouri Industries Company Limited, Japan). Sugars were determined using AOAC methods 923.09 and 930.45 (1990). Specific gravity of the fermented sugar syrup was determined using a hydrometer. Alcohol content was determined using AOAC methods 920.57 and 913.02 (1990).

Yeast cell growth (biomass)

The biomass of the fermenting medium was determined using standard cell count procedure.

Statistical analysis

ANOVA and regression analyses were performed on the data using Statgraphics Computer Software (Statistical Graphics Corp., STST Inc; USA) with probability, $p < 0.05$. Microsoft excel was used for graphical representations.

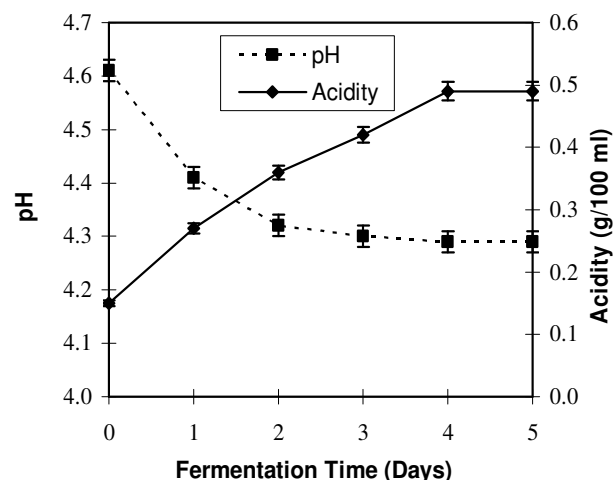


Figure 1. Changes in the pH and acidity of the sugar syrup over the fermentation period.

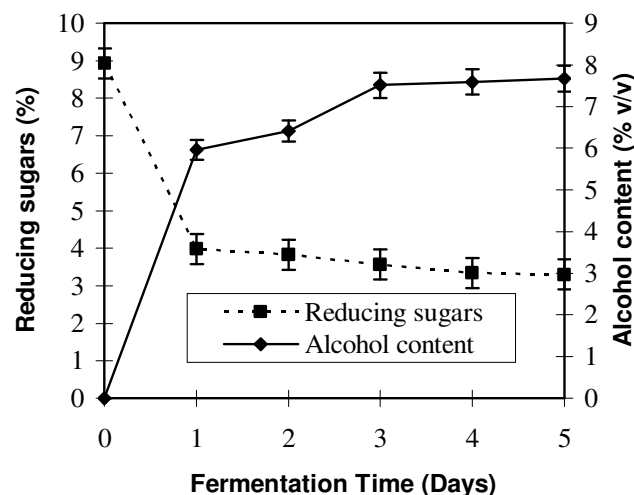


Figure 2. Changes in reducing sugar and alcohol contents of sugar syrup over the fermentation period.

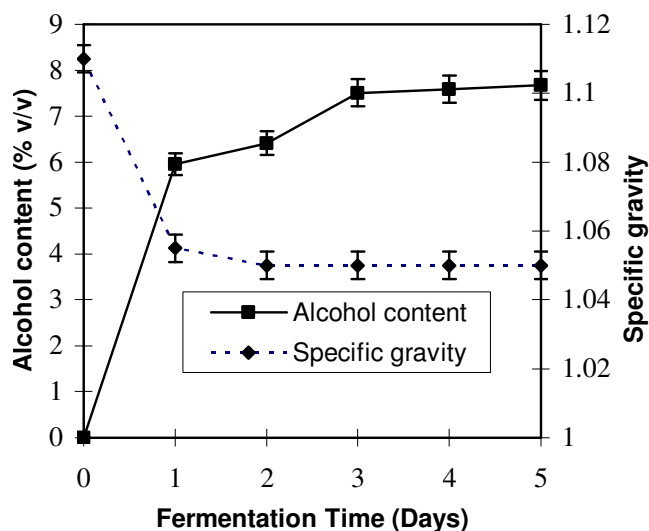
RESULTS AND DISCUSSION

pH and total acidity

Changes in pH and the total acidity of the sugar syrup during the fermentation are illustrated in Figure 1. The initial pH and total acidity of the sugar syrup were 4.61 and 0.15 g/100 ml respectively. There was a rapid decrease in the pH of the sugar syrup (wort) in the course of the fermentation at the initial 2 days. This decrease almost remained constant from day 3 to 5. Consequently, there was a corresponding steady and rapid increase in the total acidity of the fermenting medium till day 4, where it remained constant till day 5. The rise in the total acidity also corresponded to the fall in the reducing sugar content and increase in alcohol concentration (Figure 2).

Table 1. Some other properties associated with alcoholic fermentation of sugar syrup from cassava flour.

Property	Fermentation Time (Days)					
	0	1	2	3	4	5
Soluble solids (%)	27.0	17.5	17.0	16.5	16.5	16.5
Refractive index	1.376	1.360	1.359	1.358	1.358	1.358
Volatile acidity (as acetic acid) g/l	ND	0.0120	0.0132	0.0240	0.0276	0.028

**Figure 3.** Effects of fermentation on the specific gravity and alcohol content of fermented sugar syrup.

Though the acidity of the fermenting medium was rising gradually after day 3, the pH remained almost constant within the range 4.29 and 4.30. This phenomenon might probably be due to unionized weak acids such as acetic acid (Table 1) which caused buffering action in the fermenting medium. It could also be due to other buffering systems in the medium. According to Mark et al. (1963), the pH of a fermenting medium must be adjusted to 5.28 - 4.8 to inhibit bacterial development. They also reported that these pH ranges provide buffering action during the fermentation cycle, which is important, since secondary conversion will not take place if the fermenting pH drops below 4.1 in the intermediate stages of fermentation.

Soluble solids, refractive index and volatile acidity

Table 1 shows some other properties associated with alcoholic fermentation of the sugar syrup. Soluble solids decreased from 27 to 16% after the 3rd day of fermentation and then remained constant for the rest of the fermentation period. The decrease observed corresponded well with decreases in refractive index of the fermented sugar syrup, since refractive index is a measure of dissolved solids in the medium. The decrease in soluble and refractive index corresponded with decreases

in reducing sugar contents of the medium (Figures 2 and 5). The volatile acids component of the fermenting medium increased during the fermentation period (Table 1). This increase over the fermentation period corresponded well with increases in the total acidity observed (Figure 1).

Reducing sugar, alcohol content, specific gravity and cell growth (biomass)

Figure 2 shows changes in reducing sugar contents and alcohol content of sugar syrup during fermentation. There was a rapid decrease in the reducing sugars concentration (8.72 to 2.18%) with concomitant increase in the alcohol content of the sugar syrup over the fermentation period (0 to 8.30%, v/v). According to Webb (1964), it is evident that as the alcohol content of the fermenting medium increases, the sugar content decreases by a proportionate amount. The initial rapid decrease observed in the reducing sugar content was due to a rapid multiplication of yeast cells and rapid conversion of the sugars to alcohol via glucose metabolism. The conversion of reducing sugars ended within 3 days of fermentation, indicating maximum alcohol content at this stage. Similar trends were reported by Stark (1954) and Ueda et al. (1981). There was also a fall in specific gravity of the medium, which was expected (Figure 3).

There was a positive correlation between reducing sugar contents and specific gravity of the fermenting medium (Figure 4). Decrease in reducing sugar content resulted in decrease in specific gravity and vice-versa. The falls in specific gravity and reducing sugar levels were due to the disappearance of carbohydrates (sugars) in the fermenting medium. The following regression model (equation) was produced:

$$y = 89.299x - 90.194$$

Where y = reducing sugar content and x = specific gravity

The correlation coefficient (r) and the coefficient of determination (R^2) of this expression were 0.9972 and 99.45% respectively. The model indicated a significant ($p < 0.05$) effect (Table 2). The above equation could be used to predict the reducing sugar of fermented sugar syrup at a known specific gravity. The R^2 value obtained showed that about 99.45% of variability of reducing sugar

Table 2. Analysis of variance for the model.

Source	Sum of square	Df.	Means square	F-ratio	Prob. level
Model	23.81501	1	23.81501	531.3591	0.00002*
Error	0.1792762	4	0.0448190		
Total (Corr.)	23.994283	5			

*Significant at $p < 0.05$

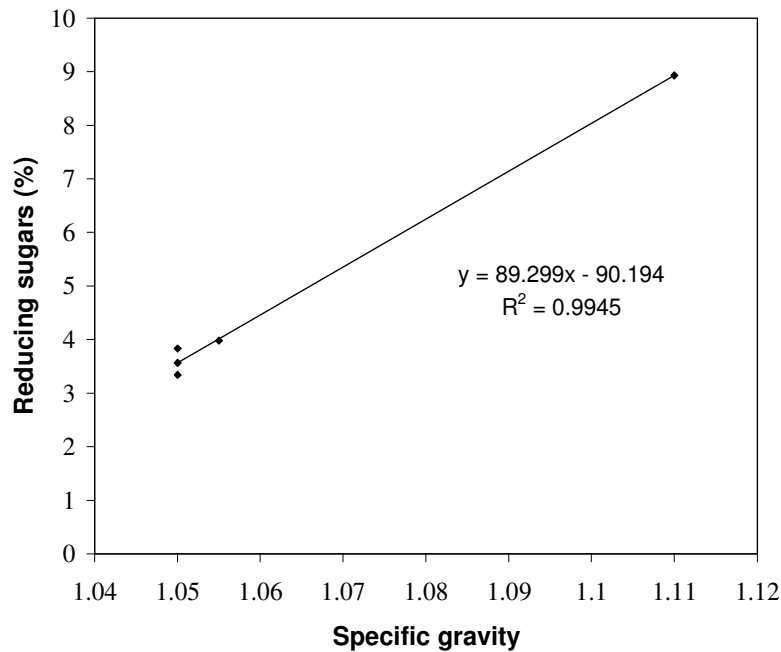


Figure 4. Correlation between reducing sugar contents and specific gravity of fermented sugar syrup.

content could be attributed to changes in the specific gravity of the fermented sugar syrup and vice-versa.

Figure 5 shows the changes in the reducing sugar content and yeast growth (biomass) associated with the fermentation of sugar syrup. The initial yeast population at time zero was 1.15×10^8 cfu/ml. This concentration increased during the fermentation process to 1.18×10^9 cfu/ml after 72 h (3 days) of fermentation. Generally, microbial growth is in four distinct phases, namely, the lag, log/exponential, stationary and death phases. The observed graph (Figure 5) shows only 2 phases, the exponential and the stationary phases. The Lag phase of the growth might have occurred within the h of 0 and 12, where samples were not taken for enumeration. The enumeration was done within 12 h interval. The exponential phase was observed within the period of 0 to 60 h of fermentation. There was a gradual increase in the biomass, indicating maximal growth and cell division within this phase of growth. The increase in biomass was accompanied by concomitant decrease in reducing sugar contents. A rapid decrease in reducing sugar content was

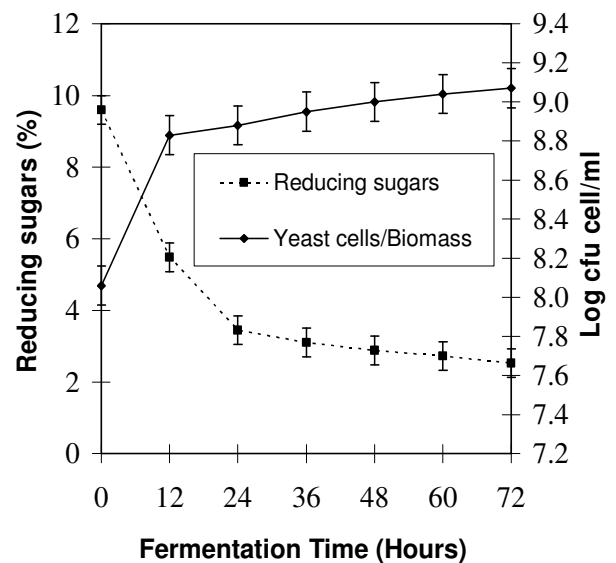


Figure 5. Changes in reducing sugar contents and biomass associated with fermentation of sugar syrup with time.

observed within 36 h and then slowed down. The changes observed were due to the metabolism of yeast cells. The sugars served as substrate for the yeast and thereby using them as energy source and for growth, resulting in the production of alcohol and carbon dioxide as end and by-products respectively. According to Prescott et al. (1993), the fermentation product (alcohol) is classified as primary metabolite, since it is formed in the growth phase or trophophase. This means that, alcohol was formed alongside the increase in the biomass. The stationary phase observed could be attributed to substrate (sugar) limitation and the alcohol content of the fermenting medium. This occurred within 60 – 72 h of fermentation. High alcohol content in a fermenting medium was reported to slow down or stop the growth of yeast (Gutcho, 1973).

Yeast multiplies by budding and a new cell is produced every 70 min (Mark et al., 1963). Cell growth during the logarithmic (exponential) phase is at a constant rate (Fellows, 2000). This follows the equation:

$$\ln C_b = \ln C_o + \mu t$$

Where C_o = original cell concentration; C_b = cell concentration after time t (biomass produced); μ (h^{-1}) = specific growth rate and t (h) = time of fermentation

The highest growth rate during the fermentation of the sugar syrup occurred in the logarithmic phase. The above equation was therefore used to estimate the specific growth rate of yeast during the fermentation process. The specific growth rate (μ) estimated was 0.032 h^{-1} . According to Aiyar and Luedeking (1966), the doubling time for cell mass = $0.693 / \mu$, as such the doubling or generation time estimated from the process was 21.65 h. This means that the time taken by the yeast cells to reach twice the initial yeast population was about 22 h. This favoured less cell production but high alcohol content in the fermenting medium. The maximum specific growth rate (μ) for yeast was reported as 0.6 h^{-1} , which give rise to 1.16 h generation time (Aiyar and Luedeking, 1966). In general, anaerobic fermentation results in high alcohol yield compared with yeast or cell mass yield.

Conclusion

Alcoholic fermentation of sugar syrup from cassava flour was observed to be associated with physical and chemical changes besides yeast growth. The process results in decrease pH, reducing sugars, soluble solids, specific gravity and refractive index with concomitant increases in the total and volatile acidity, alcohol content and biomass

over the fermentation period. This is due to metabolism of carbohydrates by yeast and other chemical reactions. The increase in acidity and the stabilization of pH was partly due to the buffering action caused by unionized weak acids during day 3 to 5 of the fermentation period.

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