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Full Length Research Paper

Production of ethanol from cocoyam (*Colocasia esculenta*)

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Cocoyam is an edible root crop belonging to the family Araceae. It makes significant contribution both as root crop and vegetable in the diet of mainly Africans people and Nigerians inparticularly. The high percentage of starch was exploited in the production of ethanol. Two hundred grams (200 g) of mashed *Colocasia esculenta*, gelatinized in a pressure cooker was allowed to undergo two stage enzyme hydrolysis using bacterial alpha-amylase (Amylitic-TS) and fungal alpha-amylase (AMG) to produce fermentable sugar (wort). The hydrolysed liquor was inoculated with viable yeast cells, *Saccharomyces uvarum*, and yielded 12.9% ethanol after 7 days of fermentation. The pH and brix level (total soluble solids) of the fermenting broth dropped significantly from 4.50 to 3.82 and 15.0 to 2.0 respectively. This suggested that the saccharification process was effective. The reduced pH provided an enabling environment for optimum activity of the yeast. The sugar level decreased appreciably as the ethanol content increased from 0 to 12.9%. The decrease in the specific gravity from 1.0000 to 0.9830 could be attributed to the decrease in the brix level as the sugar in the broth was converted to ethanol.

Key words: Cocoyam (*Colocasia esculenta*), ethanol production, enzyme hydrolysis, *Saccharomyces uvarum.*

INTRODUCTION

Alcohol fermentation is a biochemical process that is brought about by the action of yeast through a process that transforms the natural sugar present in any starchy material into alcohol with the evolution of carbon dioxide (CO_2) under controlled environmental conditions (Miller and Litsky, 1976; Okafor, 1987; Saraswati, 1988; Saucedo et al., 1992; Dubey, 2005; Okorondu et al., 2009). The process is an anaerobic fermentation in accordance with Embden-Meyerhoff Pathway (EMP) catalyzed by enzymes (Amylitic-TS and AMG) produced by bacteria and fungi respectively. Starchy materials are first hydrolysed to fermentable sugars, and subsequently fermented with the required yeast species to produce ethanol (Kuboye and Akinrele, 1971; Suraswati, 1988; Jaleel et al., 1988). During the fermentation process, part of the sugar is assimilated by the yeast cells and part is transformed into glycerol, acetaldehydes and lactic acid. The fermentable sugar level for the production of ethanol is in the range of 8 to 20%, with 13% as the optimum. Optimum temperature for the production of desirable ethanol is maintained at 23.9 to 26.7°C (Joslyn, 1970). Adams (1978) reported that pH, temperature and the nutrient level of the substrate influence the alcoholic fermentation process.

Cocoyam is very important, and served as a root crop and vegetable in the diet of many rural dwellers where they are freely available (Okigbo, 1987). The percentage composition of starch in cocoyam is 72% which makes it an excellent raw material for alcohol production (Baum, 1982). The demand for ethanol is high where as the substrate for its production is limited and competitive. Various investigations have been carried out (Okorondu et al., 2009) aimed at improving the processes employed in the production of ethanol from different feedstock (sugar, starch and cellulose based raw materials).

Cocoyam is cheap and available throughout the year. The high carbohydrate (10%w/v) level in cocoyam is yet to be fully harnessed in the industries (Nwufo and Fajola, 1998). Nwufo and Fajola (1998) reported that the sugars present in a healthy cocoyam are sucrose, maltose, glucose and fructose. This study reports on the production of ethanol from cocoyam. The brix level, pH, and specific gravity of the final product were also assessed to determine the alcoholic content.

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Table 1. Mean pH values of fermenting broth for 7 days.

Period of fermentation (days)	pH values (Mean ± SD)
1	4.50 ± 0.2
2	4.22 ± 0.2
3	4.18 ± 0.1
4	4.10 ± 0.2
5	4.06 ± 0.2
6	4.00 ± 0.4
7	3.82 ± 0.1

Table 2. Total soluble solids content of fermenting broth for 7 days (Mean ± SD).

Period of fermentation (days)	Brix Level (TSS)
1	15.0 ± 0.2
2	7.5 ± 0.4
3	6.0 ± 0.3
4	4.2 ± 0.2
5	2.5 ± 0.1
6	2.1 ± 0.2
7	2.0 ± 0.1

MATERIALS AND METHODS

Gelatinization of cocoyam

Corns of cocoyam cultivars were washed, peeled and grated. 200 g of the cocoyam mash were weighed into a beaker containing one hundred milliliters of water and the contents thoroughly homogenized by stirring. The beaker with its content was covered with aluminum foil and cooked in a pressure cooker for 30 min at a pressure of 10 psi and at a temperature of 108.9°C (A.O.A.C, 1990). The sample became gelatinized by this treatment.

Saccharification of gelatinized sample

The method of saccharification used was the enzyme hydrolysis which involved two stages. The first stage involved the use of bacterial alpha-amylase (a liquefying agent) which break down starch, while the second stage; fungal alpha-amylase (a saccharifying agent) completed the process. 900 ml of water was added to make slurry of the gelatinized sample to give 20% (w/v) solution. In the first stage, 1ml of 0.1N solution of bacterial alpha-amylase (Amylitic-TS) was added to the slurry and pH (controlled by addition of some drops of diluted sulphuric acid) and temperature was adjusted to 6.0 and 95 to 100°C respectively. A partially liquefied solution was obtained on continuous agitation for 45 min.

In the second stage, the solution was cooled to 60 to 64°C and the pH was adjusted to 5.4 to favour the activities of fungal alpha amylase. 2 ml of 0.1N solution of fungal alpha-amylase (AMG) was added to this slurry. The solution was agitated in a water bath for 45 min to obtain complete liquefaction of slurry (Miller and Litsky, 1976). In order to stop the action of the enzyme and to sterilize the wort, the slurry was further heated for 10 min at 100°C. The saccharified liquor was cooled to 28°C and the pH adjusted to 4.2 to 4.5. The brix level and specific gravity was determined by standard methods (A.O.A.C, 1990).

Production media and inoculation

500 ml of hydrolysed liquor was mixed with 700 m of deionized water. 5 g of glucose, 1 g of urea and 1 g of ammonium hydrogen phosphate were added to the mixture and autoclaved for 30 min at 121°C and at a pressure of 760 mmHg. The sterile liquor was cooled to 30°C and the pH was adjusted to 4.5. 100 ml of the sterile liquor was added to dissolve 4.8 g of bakers yeast in a sterile 250 ml uniscope beaker. 200 ml of yeast was allowed to grow for 1 h before pitching into the main inoculum broth, and aerated by shaking at a speed of 1000 rpm at room temperature for 48h (A.O.A.C, 1990).

Alcoholic fermentation of broth sample

400 ml of the inoculum was pitched into 4 L of hydrolysed liquor in a flask and aerated before closing with cotton wool. Fermentation was allowed to take place for 7 days at 30°C. The brix level, pH, specific gravity and percentage alcohol by volume produced were determined daily and recorded (A.O.A.C, 1990).

Chemical analysis of sample during alcoholic fermentation

Hydrogen ion concentration (pH) of the sample was determined with a uniscope pH meter. The method described in A.O.A.C (1990) was adopted. Specific gravity was determined according to the method 945.06 (A.O.A.C, 1990). Specific gravity was calculated by standard method. Brix level of the sample was determined by hand refractometer method (A.O.A.C, 1990). Simple distillation method described in A.O.A.C (1990) was used in the determination of percentage alcohol in the sample.

Determination of viability of starter culture

Brewers yeast used for pitching was obtained from consolidated breweries located in Awo-mamma in Imo State, Nigeria. Cultures were made on Saboraud dextrose agar (SDA) and incubated at ambient temperature. Colonial, microscopic and biochemical characteristics was determined by methods described in Cheesbrough (2000), Harrigan and McCance (1976) and Harrigan and McCance (1990).

RESULTS

Table 1 shows the pH values recorded for a period of 7 days. The table reveals that the pH of the broth decreased daily until the fermentation was completed. As it decreased, the fermenting broth became more acidic for optimum yeast activities. Table 2 shows that the total soluble solids (brix level) of the broth decreased with an increase in the period of fermentation until a constant value was obtained. That is, the brix level dropped as the yeast utilized the sugars and reduced its quantity in the medium. The specific gravity of the broth decreased with an increase in alcoholic content of the fermenting broth (Table 3). The table reveals that as the period of fermentation increased, the specific gravity of the broth decreased while the level of alcohol produced increased.

Table 3. Specific gravity and percentage alcohol produced (Mean ± SD).

Period of fermentation (days)	Specific gravity	% alcohol produced
1	1.0000 ± 0.1	0.00
2	0.9991 ± 0.1	0.60 ± 0.1
3	0.9963 ± 0.3	2.50 ± 0.1
4	0.9955 ± 0.1	3.00 ± 0.2
5	0.9901 ± 0.2	7.00 ± 0.1
6	0.9850 ± 0.1	11.10 ± 0.2
7	0.9830 ± 0.3	12.90 ±0.3

DISCUSSION

The yeast strain used in the fermentation process required a fairly acidic medium which is favourable to break down sugar effectively and to produce alcohol of an acceptable and wholesome quality. The pH is an indication of the strength of the medium, and the yeast requires an acidic (pH 4.5) medium to effect fermentation (Okafor, 1987). *S. uvarum* is acidophilic and thrives at low pH. The pH value of the medium decreases with fermentation (Table 1). The metabolic activity of the yeast was enhanced by the action of the enzymes (Amylitic TS and AGM). Gelatinization of the cocoyam cultivars increased the surface area for enzyme hydrolysis and subsequent yeast activities. The gradual lowering of the pH of the medium created an enabling environment for the conversion of the sugars present in the medium by the yeast (Adam, 1978).

The brix level is a measure of the amount sugar present in the medium. The brix level dropped as the yeast utilized the sugar and reduced its quantity in the medium. Fermentation stopped when the sugar present in the broth was exhausted (Table 2). Table 3 showed the tripartite relationship existing between the specific gravity, percentage alcohol produced and the period of fermentation. The result reveals that the yeast cells were dormant at the initial stage of the fermentation, presumed to be the lag phase. Within the next five days, depletion was very rapid and the rate of carbon dioxide evolution was vigorous with subsequent increase in alcohol production. This period (log phase) is characterized by rapid cell multiplication indicated by rapid fermentation (Amerine, 1988). The sugar level increased appreciably while the alcoholic content increased from 0 to 12.9% within 7 days. The specific gravity decreases from 1.0000 to 0.9830 (Table 3). This could be attributed to the decrease in the brix level as the sugar present in the broth was converted to alcohol.

This research effort therefore opens a gateway towards the development of an efficient method for ethanol production using cocoyam as a cheap source of raw material. Although cocoyam is one of the stable root crop in Nigeria, however over 20 million tonnes are lost yearly due to inadequate storage facilities (IITA, 2009). Since cocoyam is perishable after harvesting, speedy conversion of the surplus harvest will reduce wastage and improve economic gains. The use of cocoyam in the production should be encouraged because of its high ethanol yield. In addition, cocoyam can be successfully cultivated in poor soils with low capital and labour requirement.

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