

A Study Of Changes In Some Biochemical Parameters During Bacterial Fermentation Of *Dioscorea Esculenta* Tubers

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ABSTRACT: Changes in some biochemical parameters such as reducing and total sugar, alpha amylase activity, lactic acid and ethanol productivity were evaluated in this study. Randomly selected tubers of lesser yam (*Dioscorea esculenta*) were peeled, diced, washed and put into sterile beaker containing distilled water and left to ferment for 28 days. *Bacillus* species was isolated from the fermented steep liquor. This organism produces alpha amylase that is able to hydrolyse starch to sugar. Further hydrolysis of sugar resulted in the production of lactic acid and ethanol. The pH decreased initially from 5.89 to 4.70 and later to 5.26, triturable acidity increased from 0.09 to 0.35%, reducing and total sugars increased from 1.4 to 24.7% and 4.6 to 23.8% respectively, alcohol content increased from less than 0.41 to 5.6%, while crude protein increased from 0.04 to 1.45%. Alpha amylase activity decreased during the later days of fermentation. This could be attributed to the increasing alcohol content as well as lactic acid production which are inhibitory to alpha amylase activity. [Report and Opinion 2010;2(6):88-93]. (ISSN: 1553-9873).

Key words: *Dioscorea esculenta*, *Bacillus species*, fermentation, alpha amylase, starch.

INTRODUCTION

Yam of the genus *Dioscorea* is among the most valuable staple food sources for millions of people in tropical and sub-tropical countries. In Nigeria, especially, it is one of the most valued sources of food. Unfortunately however, despite the relatively high production level of this crop, it is still in short supply due primarily to the colossal amount of wastage of both the processed and unprocessed products. Wastage from unprocessed yam tubers results from chemical reactions after an injury in the tubers, excessive sprouting, shrinkage and spoilage due to bacterial and fungal attack (Paneerselvan and Abdul Jaleel, 2008). The storage of fresh yam tubers is justifiable not only from the economic point of view but also from the biochemical point of view. Biochemical studies have shown that the biological half life of the tuber is one year (Osuji, 1981).

Dioscorea esculenta is believed to have originated from China (Coursey, 1961). Unlike the majority of edible yams, the tubers of *D. esculenta* are small, ovoid in shape with a thin brownish skin, closely resembling sweet potatoes in appearance. About ten to fifteen tubers are produced on each plant. The period of dormancy is very short as tubers start to sprout after only a short period in storage (Paneerselvan and Abdul Jaleel, 2008). Oyenuga (1968) reported that *D. esculenta* is rich in calcium, phosphorus, and iron, ascorbic acid, thiamine, riboflavin and niacin. The amino acid constituent of the protein from this yam specie is rich in arginine and leucine (Ukpabi, 2010).

They are of good palatability as the texture is softer than that of bigger yams. The leaves are simple, carried alternately and smooth textured. The stems of the vine are usually cylindrical and spiny and twine in a clockwise direction (Olayemi and Ajaiyeoba, 2007).

Fermentation of yam has been described as a two-stage process (Uzogara et al, 1990). During the first phase, the yam tubers bacterium break down the starch in yam tubers to simple sugars, lactic acid and alcohol, leading to a drop in the pH of the fermenting mash. The fall in pH also encourage the growth of fungi which brings about further acidification and the characteristic aroma of the final product.

Amylases are enzymes that hydrolyse starch. They are classified in various ways depending on how they act on the starch molecules (Omonigbo 2000). The microbial source of this enzyme is from *Bacillus subtilis* and *Bacillus licheniformis* (Rose, 1980).

The objective of this research is to study the amylase producing strains of bacteria from *Dioscorea esculenta* and other biochemical parameters involved in the yam fermentation with a view to their exploitation to enhance productivity in industries.

MATERIALS AND METHODS

Dioscorea esculenta tubers were harvested from the biological garden of University of Lagos during harvest season of 2008/2009. *Dioscorea esculenta* steep liquor was prepared by dicing about 100g of the tubers. It was washed and put into sterile beaker and

600ml of distilled water was added and left at room temperature (28°C) for three days. On the third day 1ml of aliquot from the fermented mash was taken and serially diluted. 0.1ml of aliquot of 10^{-4} dilution was plated out on starch agar plates in duplicate and incubated overnight at 37°C.

Test for amylase producing isolates.

Each culture plate was flooded with Lugol's iodine and observed for the formation of clear zones which indicates presence of amylase.

Isolation of Amylase producing isolates.

Amylase producing isolates were picked with the aid of a sterile wire loop and streaked on starch agar plates and incubated at 37°C for 24 hours according to Akpata and Nwachukwu (1987). It was then stored in a refrigerator.

Identification of Amylase producing isolates

This was carried out using morphological parameters as well as biochemical tests.

Morphological characteristics.

Cultural Characteristics:

These include the shape, size, colour, margin, pigmentation and elevation of the colonies on agar.

Cellular characteristics:

Gram's stain test, Endospore stain test, Motility test, Catalase test, Indole test, Citrate utilization test, Vogues proskeaur test, Methyl red test, Hydrogen sulphide and Sugar utilization test were carried out as described by Akpata and Nwachukwu (1987).

Assay of Alpha-Amylase

10ml of steep liquor was centrifuged at 10,000rpm for 15 minutes. The supernatant was used as a source of crude enzyme. In one test tube, 0.5ml of the enzyme extract and 0.25ml of 1N HCl was simultaneously added to stop enzyme activity. This serves as the control.

In another test tube 0.5 ml of 1% starch solution was added 0.5ml of the enzyme extract, and was incubated at 37°C for 20 minutes, after which 0.25ml of 1N Hcl was added to stop enzyme activity. The two test tubes were thoroughly shaken. Into another two test tubes, 0.5ml of the mixture was added separately; 0.5ml of solution C was added to each test tube and was boiled in water-bath for 30 minutes. It was rapidly cooled in water and 0.25 ml of arsenomolybdate reagent was added to each tube. The solution in each tube was diluted to 10ml by adding 8.75ml of distilled water. The optical density

at 620nm filter was measured. The result was read off from standard graph for reducing sugar.

Quantitative Analysis of Fermented Yam Steep. Determination of the Total Sugar.

To 1 ml of steep liquor, 9ml of distilled water was added to give a diluted extract. In triplicate test tubes was added 0.1ml of the diluted extract. 0.9ml of distilled water was further added to the three test tubes. Total sugar was determined by adding 4ml of anthrone reagent to each test tube as described by Clegg (1956). The concentration of the total sugar equivalent to the average O.D present in the steep liquor was read from the standard curve obtained earlier.

Determination of Soluble Starch

To 0.1ml of steep liquor, in triplicate was added 0.9ml of distilled water. A blank tube contained 1ml of distilled water. To the four test tubes, 2 drops of iodine solution was added. Shaken vigorously and the O.D. was immediately read against the blank with a 660nm filter. The concentration corresponding to the average O.D was read from the standard graph and this was used in estimating the starch content of the steep liquor.

Determination of Protein

The protein content of the sample was determined as described by Lowry et al (1951), using Bovine Serum Albumin (B.S.A) as standard. The protein was then estimated from a standard curve.

Determination of pH

A 20 ml of the yam steep liquor was put in a 50ml beaker and pH read using a standard pH meter (model L. PUSL Munchen 15). This was repeated thrice and the average was calculated.

Determination of Lactic Acid Concentration

5 ml of 0.2 M sodium hydroxide was pipetted into 100ml conical flask. Two drops of Bromocresol green indicator was added and shaken thoroughly. The steep liquor was poured into a burette and titrated against the content of the flask. This was repeated thrice and average titre gave the equivalent volume of steep liquor required to neutralise the base.

Determination of Alcohol Content Produced.

100 ml of yam steep liquor was poured into a round bottom flask. This was connected to a distilling tube and distilled until there was no further distillate being collected. The volume of distillate as well as the weight was measured and used to calculate the percentage of alcohol produced.

RESULTS.

Bacterial Isolate

Amylase producing bacteria was isolated from *Dioscorea esculenta* steep and identified as *Bacillus* species.

Table 1. Morphological and biochemical characteristics of amylase producing bacterial isolates

Test	Result
Cultural morphology	Cream, flat, with round colonies.
Cellular morphology	rods
Gram stain reaction	positive
Catalase test	positive
Endospore test	negative
Indole test	Negative
Motility test	Negative
Methyl Red test	Negative
Vogues Proskauer test	Negative
Citrate test	Negative
Glucose	Produced acids and gases
Sucrose	Produced acids and gases
Lactose	Produced acids and gases
Mannitol	Produced acids and gases
Probable identification	Bacillus species

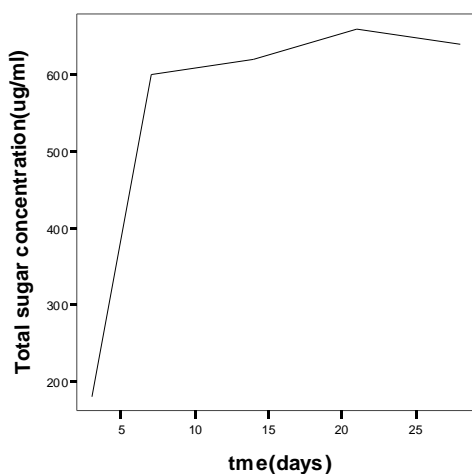


Figure 1. Changes in total sugar concentration during fermentation.

Total sugar concentration reached its peak at the 21st day and started to decrease with additional increases in fermentation time.

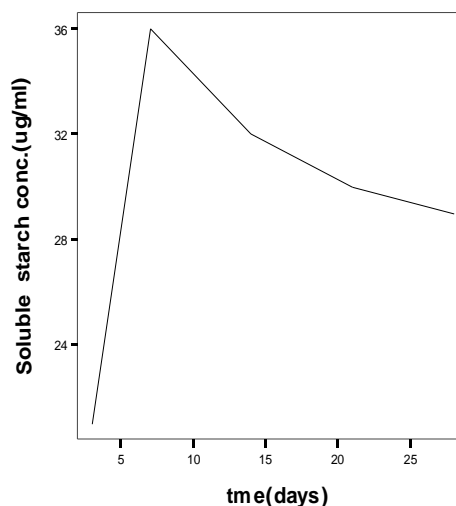


Figure 2. Changes in soluble starch concentration during fermentation.

Soluble starch reached its peak at the 7th day and started to decrease with further increase in fermentation time

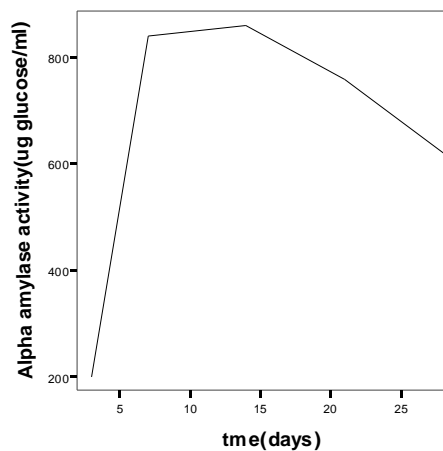


Figure 3. Changes in alpha amylase activity during fermentation.

Alpha amylase activity reached its peak at the 14th day and started to drop at further increase in fermentation time.

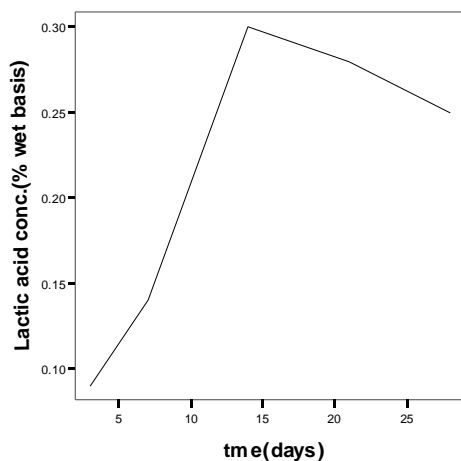


Figure 4. Changes in lactic acid concentration during fermentation.

There was a sharp increase in lactic acid concentration reaching its peak on the 14th day and thereafter dropped slightly during the rest of fermentation days.

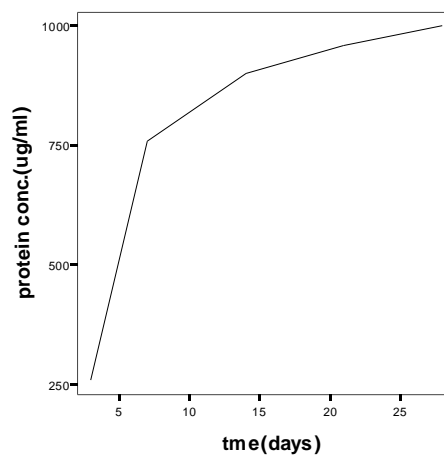


Figure 6. Changes in protein concentration during fermentation.

There was a steady increase in protein concentration as fermentation time increases.

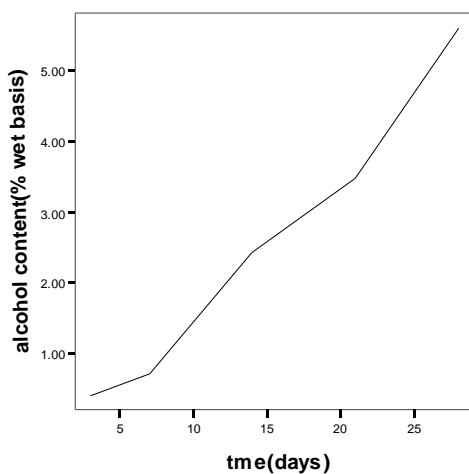


Figure 5. changes in alcohol content during fermentation.

There was a gradual increase in alcohol content during fermentation but the rate increased sharply from the 7th day of fermentation.

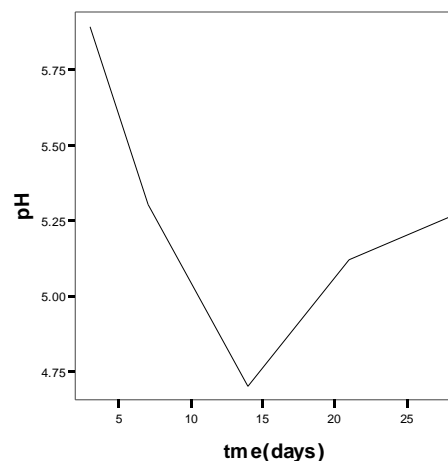


Figure 7. Changes in pH during fermentation.

The P^H decreased to about 4.75 on the 14th day and slightly increased with further increase in fermentation time.

DISCUSSION

Yam is predominantly carbohydrate and this is the largest replenishable source of carbon compound which is available for conversion by microorganism into biomass. The main carbohydrate content of yam is starch. Starch is a heterogeneous polysaccharide composed of two high molecular weight entities called amylose and amylopectin.

The fermentation of yam starch requires an initial hydrolytic step for the release of fermentable sugar (Hurng et al 1993). An amylolytic bacterial isolate that is capable of breaking down starch to simple sugar was isolated. Physiological and biochemical studies showed that the bacterial isolate was *Bacillus* species. The organism could have arisen during the steeping process.

Results as shown in figure 3 revealed that maximum accumulation of alpha-amylase occurred between the end of the growth phase and beginning of the phase of decline. This signifies that starch is a very good inducer of this enzyme (Hurng et al, 1993).

The decrease in pH (fig. 7) during yam fermentation till the 14th day could be as a result of the steady increase in lactic acid production and ethanol content in the steeping liquor (Kanoh et al, 1992).

The total sugar concentration, however, increase gradually during the first seven days of fermentation (fig.1), probably resulting from the action of amylolytic enzymes which hydrolysed the starch into sugar (Koussi et al 1990). Results showed that there was conversion of starch to sugar during fermentation. The decrease in total sugar after some days could have resulted from the conversion of sugar to lactic acid and ethanol during fermentation by the *Bacillus* species that were present in the steeping liquor. The reduction in total sugar coincided with a sharp rise in lactic acid concentration as well as ethanol production (figs.4 and 5). However, problems in the fermentation stage of ethanol production derive from the inhibitory effect of ethanol on microbial activity, so that the basic key economic factor of yield, productivity and conversion must be added product concentration (Wiseman, 1983). Ethanol concentration must be kept well below inhibitory level – about 4% to 9% to obtain realistic fermentation rates and reasonable productivity.

During the fermentation, there was a steady increase in crude protein (fig.6). This could have been as a result of proteolytic enzymes produced by the *Bacillus* species. Studies have shown that *Bacillus amyloliquefaciens* and *Bacillus Licheniformis* produce proteolytic enzymes as well as amylolytic enzymes (Rose, 1980).

In conclusion, this work has tried to study and isolate the bacteria associated with enzyme production during fermentation of *Dioscorea esculenta* tubers, with a view to their exploitation in industries to enhance productivity. With the increase demand for ethanol production and its exploitation to replace motor-fuel, an alternative source for generation of ethanol could be by microbial fermentation of many carbohydrate sources of which yam is one of them.

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