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Production and Chemical evaluation of Bioethanol derived From White Cocoyam (*Colocasia Antiquorium*) and Sweet Potatoes (*Ipomoea Batatas*) cultivars

Ogunsuyi H.O[1] and Adejoju Badiru[2]

[1,2]Department of Chemistry, School of Sciences, Federal University of Technology, P.M.B. 704, Akure, Nigeria

Abstract

The production and application of bioethanol from *Ipomoea Batatas* (sweet potatoes) and *colocasia antiquorium* (white cocoyam) for the synthesis of ethylbenzoate was investigated. 1kg each of the feedstock was washed peeled and grated using mechanical grater and gelatinized. The gelatinized sample was subjected to a two stage enzyme hydrolysis using Industrial enzymes namely alpha amylase for liquefaction and amylo-glucosidase for saccaharification to produce fermentable sugar. The hydrolysed sample was then fermented with industrial baker's yeast *S.cerevisea*. The fermented liquor was distilled with simple distillation set-up and further distilled with fractionating column to obtain purer liquor. The FT-IR spectra, specific gravity (0.8113, 0.8136), refractive index (1.3608, 1.3610) for cocoyam and sweet potatoes respectively showed that the alcohol produced was ethanol when compared with the standard values . The bioethanol products were then used to synthesize ethyl benzoate.

Keywords: *Ipomoea batatas, Colocasia antiquorium, ethyl benzoate, hydrolysis, fermentation*

1. Introduction

Ethanol or ethyl alcohol has existed since the beginning of recorded history. The ancient Egyptians produced alcohol by naturally fermenting vegetative materials. Also in ancient times, the Chinese discovered the art of distillation, which increases the concentration of alcohol in fermented solutions. Ethanol was first prepared synthetically in 1826, through the independent effort of Henry Hennel in Britain and S.G in France. Michael Faraday prepared ethanol by the acid-catalyzed hydration of ethylene in 1828, in a process similar to that used for industrial synthesis of ethanol today [Boullanger 1924.]

Bioethanol has a number of advantages over conventional fuels. It comes from renewable resources i.e. crops and not from finite resources and the crops it derives from can grow. Another benefit over fossil fuel is the mitigation of greenhouse gas emissions and through the use of bioethanol, some of this emission will be reduced as the fuel crops absorbs the CO_2 they emit through growing. By encouraging bioethanol use, the rural economy would receive a boost from growing necessary crops. Bioethanol is biodegradable and far less toxic than fossil fuels.

Also, it had been pointed out that it is being used as cooking fuel since the use of firewood, kerosene and charcoal in households have adverse effects on human health [Adelekan and Adelekan, 2004]. There are three principle methods of extracting sugars from biomass. These are concentrated acid hydrolysis, dilute acid hydrolysis and enzymatic hydrolysis. Hydrolysis process breaks down the cellulosic part of the biomass or corn into sugar solutions that can then be fermented into ethanol, and yeast is added to the

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solution. the yeast contains an enzyme called invertase, which acts as a catalyst and helps to convert the sucrose sugars into glucose and fructose (both C6H12O6). The fructose and glucose sugars then react with another enzyme called zymase, which is also contained in the yeast to produce ethanol and carbon dioxide.

The ethanol, which is produced from the fermentation process, still contains a significant quantity of water, which must be removed. This is achieved by using the fractional distillation process. The distillation process works by boiling the water and ethanol mixture. Since ethanol has a lower boiling point (78.3^0C) compared to that of water (100^0C), the ethanol vaporizes before water, which then condensed and recovered as distillates.

Cocoyam (*Colocasia* and *Xanthosoma* species), a member of the Aracea family of plants, is one of the oldest crops grown [Adelekan, 2012]. On a global scale, it ranks 14th as vegetable crop going by annual production figures of 10million tonnes (FAO, 2005). Cocoyam thrives in infertile or difficult terrains that are not well suited for large scale commercial agriculture for growing most conventional staple crops. [Williams and Haq, 2002]. Also Sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family Convolvulaceaehas It..has been considered a promising substrate for alcohol fermentation since it has a higher starch yield per unit land cultivated than grains [Duvernay et al. 2013; Lee et al. 2012; Srichuwong et al.2009; Ziska et al. 2009].Industrial sweet potatoes are not intended for use as a food crop as said earlier. They are bred to increase its starch content, significantly reducing its attractiveness as a food crop when compared to other conventional food cultivars (visual aspect, color, taste). Therefore, they offer potentially greater fermentable sugar yields. It has been reported that some industrial sweet potatoes breeding lines developed could produce ethanol yields of 4500–6500 L/ha compared to 2800–3800 L/ha for corn [Duvernay et al. 2013; Ziskaet al.2009].

2. Materials and Methods

2.1 MATERIALS

The cocoyam and sweet potatoes tubers used for this project work were obtained from Oja Oba market, Akure Ondo State, Nigeria.

The enzymes used are commercial enzymes from novozymes, namely alpha amylase (liquiflow) and amyloglucosidase (sacc enzyme) the enzymes and the yeast were kindly donated by ASTL distilleries in Sango Ota,Ogun State Nigeria. Other reagents used were of analytical grade and was purchased from Pascal chemical company Akure.

All glass wares used were properly washed, rinsed and dried in the oven to avoid contamination. The black drum used for fermentation was purchased from Isikan market Akure Ondo state. The GC-MS model used for this analysis was Agilent 7890A. The Spectrophotometer model was AJ-ICO3

Alpha amylase activity assay was done using analytical procedure as described by Demoraes et al, (1999). Amyloglucosidase assay was done using analytical procedure as described by Cereia et al, (2000).

Other properties were carried out using A.O.A.C official method 1990.

2.2 METHOD

2.2.1 Hydrolysis of Gelatinized Sample

A Two – stage enzyme hydrolysis procedure was adopted for liquefaction and saccharification of the gelatinized sample. The first stage involved the application of alpha-amylase a liquefying agent which liquefy the starch of the substrate, 0.2mL of the enzyme was added to the gelatinized substrate and then heated to 90^0C, after which 0.4mL of the same enzyme was added and allowed to cool down to 55^0C, the substrate was placed on a shaker for 1h at the pH of 5.5. 2mL of the substrate was taken and iodine solution was added to it to check if the starch has been completely broken down into simple sugar.

The second stage involved application of amyloglucosidase (AMG) a saccharifying agent to convert the liquefied starch into simple sugar. After the holding time of 1hour, 0.6mL of saccenzyme was added to the substrate still at 55^0C and was further shaken for 1hr at the pH of 5.5 .2mL of the substrate was taken and iodine solution was added to it to check if the starch have been completely broken down into

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simple sugar, and later cooled to 33^0C at the pH of 4.5.

2.2.2. **Alcoholic fermentation of the hydrolyzed sample**

The liquefied sample was fermented with 50g Industrial baker's yeast (*saccharomyces cerevisea*) along with 20g of glucose and 60g of urea. Fermentation process was allowed to take place for 3days at 33^0C, the fermented broth was distilled using a simple distillation set-up and then further purified using another distillation set-up consisting of a Liebig condenser and fractionating column.

2.2.3 Synthesis of Ethyl benzoate

5mL of benzoyl chloride was measured with a measuring cylinder into a round bottomed flask inside the fume cupboard, followed by addition of 5mL of pyridine and then 5 mL of the bioethanol product. The reaction was allowed for 10min and was cooled; it was then washed thrice with distilled water to remove the pyridinium chloride formed in the reaction. The mixture was poured into a separatory funnel, the ester product was extracted with chloroform and magnesium sulphate was added to remove the water completely. The chloroform was distilled off; the extract product was characterized using GC –MS.

3. Results and Discussion

3.1 Enzyme Activity Assay

One enzyme unit is equivalent to the amount of enzyme that releases one mol of glucose per minutes under assay condition.
Result shows that the activity of alpha amylase uder the assay condition used is 0.023U/min while the activity of amyloglucosidase is 0.097U/min.

3.2: Physico-chemical properties of the Derived Bioethanol and Conventional ethanol

Table 1 shows, the specific gravities of 0.8113 and 0.8136 for *ipomoea batatas* and *colocasia antiquorium* respectively when compared with the standard (0.7974) showed that the percentage of ethanol obtained from the samples *colocasia antiquorium* (92%) and *ipomoea batatas* (90%) were comparatively close to the standard (98%).. The Table shows that the physico-chemical properties of the bioethanol derived from the cultivars are comparable with those of the conventional ethanol, particularly, in properties such as the boiling point and refractive index

Table 1: Comparison between the Physico-chemical properties of the bioethanol of *Ipomoea batatas* and *colocasia antiquorium* and conventional ethanol

Properties	**Sample A(standard)**	**Sample B**	**Sample C**
	Conventional Ethanol	***(colocasia antiquorium)***	**(*Ipomoea batata*)**
Chemical formulae	C_2H_5OH	C_2H_5OH	C_2H_5OH
Molecular weight	46.7	46.7	46.7
Boiling point in ^{0}C	78	78	78
Specific gravity at 30^0C	0.7974	0.8113	0.8136
Refractive Index	1.36	1.3608	1.361
Centipoise at 30^0C	1.2	1.17	1.21
Flash point(open Cup)^{0}C	38	40	42

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3.3: Fourier Transform Infra-red Spectroscopy (FTIR) Analysis

Fig 1 of the functional groups present in the Standard ethanol, *Colocasia antiquorium* and *Ipomoea batatas* respectively. The spectra clearly show the presence of O-H bonds in the fermented product which absorbed IR radiation and showed dip between the frequencies of 3364 - 3365cm-1 on the spectra. The frequencies at which the fermented products of the two cultivars absorbed Ir radiation were very similar to that of the Standard sample

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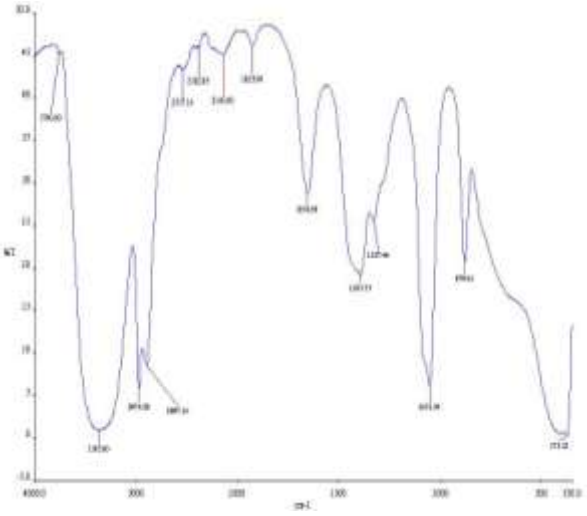

Fig 1 FT-IR OF THE STANDARD (98% Ethanol)

The spectrum above shows absorption in the following region:

2974.28cm-1 shows absorption peak for C-H strech of alkane

3365.00 cm-1 shows absorption peak for O-H stretch of alcohol

1051.59 cm-1 shows absorption peak for C-O Stretch

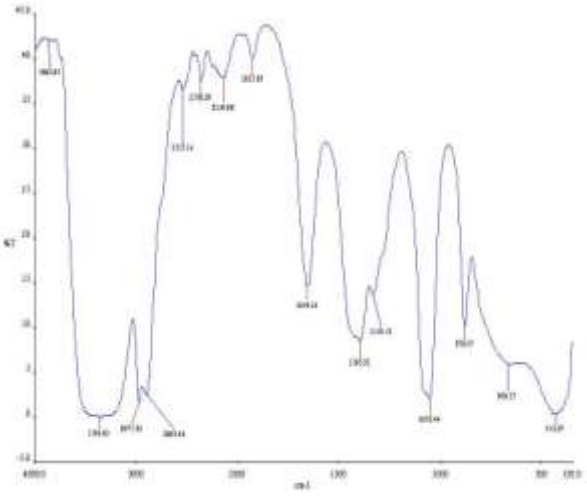

Fig 2 : FT-IR OF C*OLOCASIA ANTIQUORIUM*

The spectrum above shows absorption in the following region:

2973.52cm^{-1} shows absorption peak for C-H strech of alkane

3364.00 cm^{-1} shows absorption peak for O-H stretch of alcohol

1052.44 cm^{-1} shows absorption peak for C-O Stretch

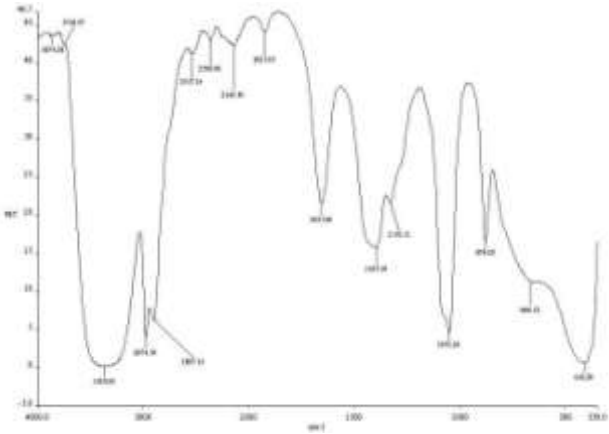

Fig 3 : SPECTRUM 3: FT-IR OF *IPOMOEABATATAS*

The spectrum above shows absorption in the following region:

2974.39cm^{-1} shows absorption peak for C-H strech of alkane

3365.00 cm^{-1} shows absorption peak for O-H stretch of alcohol

1051.24 cm^{-1} shows absorption peak for C-O Stretch

3.4:GCMS analysis of the fermented products

The GC-MS spectra shown in figures (4-6) show different fragmentation, retention time and total ion chromatogram of the new compound produced. The line produced by the heaviest ion passing through the machine represents the molecular weight of the compound to be analysed in Gas chromatography mass spectrometry. In these spectra the heaviest ion is 150 which proved that the compound produced from the standard ethanol and the bioethanol produced was ethyl benzoate, the instrument also confirmed the structure of this compound just with slight change in the total

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ion chromatogram of the compound in each sample in which that of the standard recorded 11.709, Colcasia antiquorium indicated 11.812 and ipomea batatas showed 11.835. These are the total ion chromatogram of the tallest peak i.e. the peak of the compound which indicated the new compound produced.

Fig 4 : GC-MS OF THE STANDARD (ETHYL BENZOATE SYNTHESIZE WITH CONVENTINAL ETHANOL)

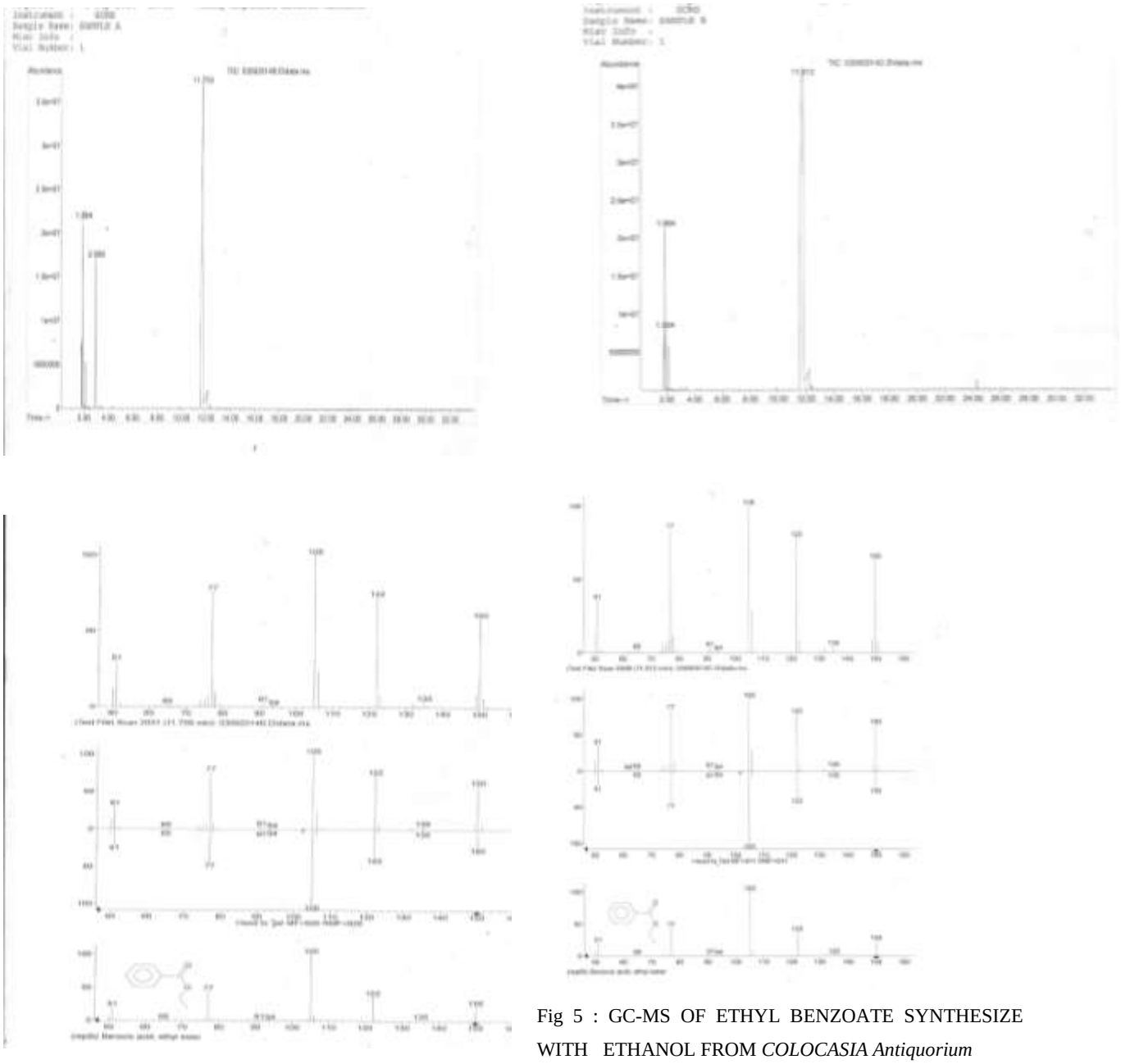
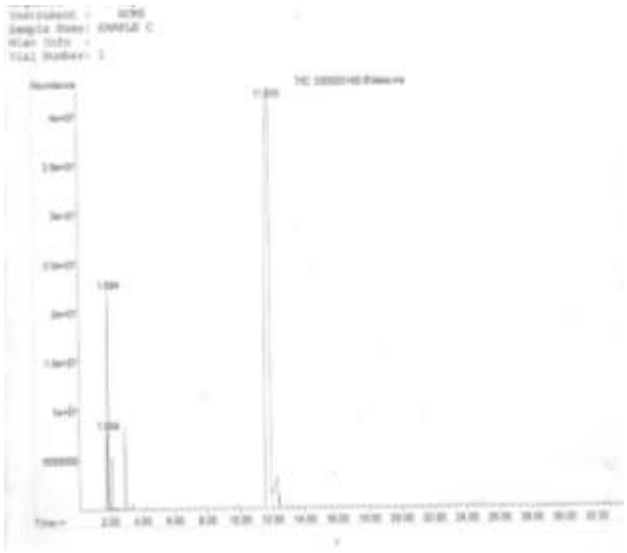


Fig 5 : GC-MS OF ETHYL BENZOATE SYNTHESIZE WITH ETHANOL FROM *COLOCASIA Antiquorium*



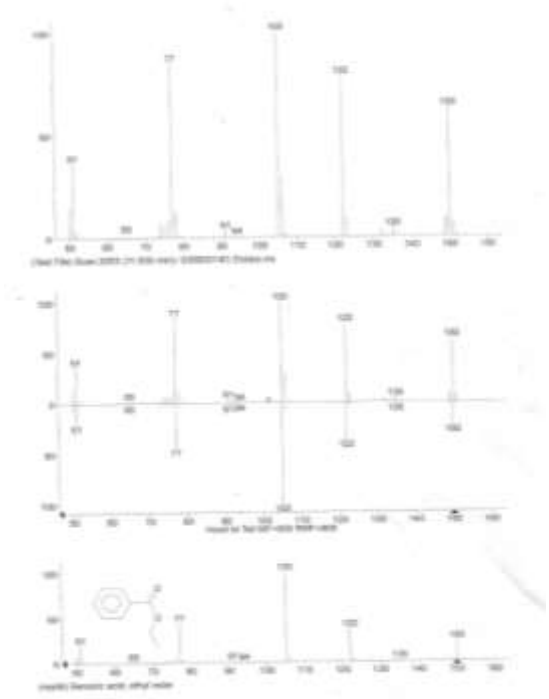


Fig 6 : GC-MS OF ETHYL BENZOATE SYNTHESIZE WITH ETHANOL FROM IPOMEA *Batatas*

This results from spectrum four (4), five (5) and six (6) shows different fragmentation, the retention time and total ion chromatogram of the new compound produced. The line produced by the heaviest ion passing through the machine represents the molecular weight of the compound to be analysed in gas chromatography mass spectrometry. In these spectra the heaviest ion is 150 which proves that the compound produced from the standard ethanol and the bioethanol produced is ethyl benzoate, the instrument also confirms the structure of this compound. just with slight change in the Total ion chromatogram of the compound in each sample in which that of the standard is 11.709,colcasia antiquorium 11.812 and ipomeabatatas 11.835. These are the total ion chromatogram of the tallest peak i.e. the peak of the compound which indicates the new compound produced.

4. Conclusions

Bioethanol produced from sweet potatoes (*ipomoea batatas*) and white cocoyams (*colocasia antiquorium*) are potential alternatives to conventional ethanol. Also the bioethanol produced from these tubers can also be used in the laboratory for esther synthesis, as there is no difference as such between the bioethanol produced and conventional ethanol.

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