

2 **BIOHYDROLYSIS OF BANANA AND PLANTAIN PEELS FOR THE PRODUCTION**
3 **OF BIOFUEL**

4
5 **ABSTRACT**

6 This study was carried out to assess the potentials of banana and plantain peel as feedstock for
7 biofuel production. Fungi were isolated from spoiled banana, burkutu and spoiled bread using
8 the standard microbiological method. The concentration of reducing sugar of the peels were
9 measured using DNS calorimetry method and biofuel were measured using chromium (VI)
10 reagent by Ultraviolet-Visible Spectrophotometer. *Aspergillus niger*, *Saccharomyces cerevisiae*
11 and *Mucor racemosus* were isolated. A reducing sugar concentration of 59.12 mg/g and 56.62
12 mg/g was observed for the banana and plantain peels. The highest concentration was found to be
13 0.35 mg/L for banana peels and 0.10 mg/L for plantain. The IR characterization of the banana
14 and plantain sample revealed an intense strong broad band of alcohol O-H and alkane C-H
15 stretching. The GC-MS result revealed the presence of benzaldehyde in all the biomass while
16 2,3-butanediol was only detected in the plantain peels biomass. This study showed the potential
17 of banana and plantain peels biomass for biofuel production.

18
19 **Keyword:** Biofuel, banana peels, plantain peels, hydrolysis, *Saccharomyces cerevisiae*, *mucor*
20 *racemosus*.

21
22 **INTRODUCTION**

23 Biofuel has been used in large scale since the implementation of Brazilian alcohol program
24 (Goldemberg, 2008). The production of biofuel from lignocellulosic materials also called
25 Second-generation is proposed as an alternative without adverse effects. These fuels include
26 Bioethanol, Biodiesel, Bioether, Biogas etc (Pimentel *et al.*, 2005). In Nigeria, all stages of the
27 fruit (from immature to over ripe) are used as a source of food in one form or the other. Banana
28 and plantain peels represent 40% of the total weight of fresh banana or plantain. *Musa* species
29 are rich in vitamin C, B₆, minerals and dietary fiber. They are also a rich energy source, with
30 carbohydrates accounting for 22% and 32% of fruit weight for banana and plantain
31 (Tchobanoglous *et al.*, 1993). Biofuels are considered as a replacement for fossil fuels and the
32 answer to poverty and even the climate crisis. They are presented as being both renewable and
33 environment friendly (Bassey, 2010). Increasing attention is being focused on the production of
34 biofuels as the alternatives that will contribute to global reduction in greenhouse gas emissions
35 (Oniemola and Sanusi, 2009). Currently, there is a growing interest for ecological sustainable
36 bio-fuels all over the world. The aim of this research is to consider the possibility of

37 biohydrolysis of banana and plantain peels for biofuel production, which are common
38 agricultural wastes in this part of the world. The objectives herein are to isolate and identify
39 *Saccharomyces cerevisiae* from burukutu, *Aspergillus niger* and *Mucor racemosus* from spoiled
40 banana and bread respectively. Biological pretreatment with *Mucor racemosus* and reducing
41 sugar contents were determine from banana and plantain peels sample and finally to identify the
42 biofuel produced in banana and plantain feedstock fermentation broth using Gas
43 Chromotography, Mass spectroscopy (GC-MS) and Fourier Transform Infrared (FT-IR).

44 **MATERIALS AND METHODS**

45 **Sample Collection**

46 Banana (*Musa acuminata* - colla) and plantain (*Musa paradisiaca* - linnaeus) peels were
47 collected from fruit vendors at Gawon-nama area, Sokoto metropolis. The banana and plantain
48 peels were washed with distilled water to remove dirt and other contaminants. The samples were
49 chopped into small pieces and allowed to air dried. It was grounded to powdered form using
50 mortar and pestle and collected in a transparent polythene bag.

51 **Isolation and identification of *Aspergillus niger***

52 Small portion of spoiled banana was aseptically picked with wire loop and inoculated at the
53 middle of the prepared media PDA (Potato dextrose agar) plate. The plate were incubated at
54 27°C temperature for 7 days for colonies development. Pure culture of *Aspergillus niger* was
55 obtained by streak plate method. All observed colonies were maintained on agar slants at 4°C
56 (Devanathan *et al.*, 2007).

57 **Isolation and identification of *Sacchararomyces cerevisiae***

58 The burukutu samples were serially diluted up to 10⁵ tubes. An Aliquot of 0.1 mL of burukutu
59 was spread on the surface of yeast potatoes dextrose agar (YPDA) plate and incubated at 30°C
60 for 3 days. Colonies suspected to be *Sacchararomyces cerevisiae* based on colonial
61 characteristics were sub-cultured on PDA. A smear of the isolate was examined microscopically
62 after staining. The isolates were identified by comparing their characteristics with those of
63 known taxa using the schemes of Domsch and Grams (1970), as described by Oyeleke and
64 Manga (2008) .

65 **Isolation and identification of *Mucor racemosus***

66 A small portion of the spoiled bread was aseptically picked with wire loop and inoculated at the
67 middle of prepared solidified media sabourand dextrose agar (SDA). It was incubated at 30 °C
68 for 5 days. Colonies suspected to be *Mucor racemosus* based on colonial characteristics were sub
69 cultured on PDA. For identification, One (1) drop of lectophenol blue was dropped on dried
70 grease free slide, little portion of the growth was picked with inoculated needle and emulsified.
71 Cover slide was used to cover and viewed at 10x objective and confirmed by 40x (David *et al.*,
72 2007).

73 **Biological pretreatment**

74 The biological pretreatment was carried out as described by Ekunsaumi (2006). Mandels culture
75 medium was prepared by adding (g^l⁻¹): Urea 0.3,(NH₄)₂SO₄ 1.4, KH₂PO₄ 2,CaCL₂ 0.3,
76 MgSO₄7H₂O 0.3, bacto peptone 0.75, and yeast extract 0.25. Trace elements was also added,
77 using a 1% (v/v) solution of salts (mll⁻¹):FeSO₄7H₂O 0.5, MNSO₄ 0.16, ZnSO₄ 0.14, CoCl₂ 2g at
78 pH of 5.5. The medium was measured as 100 mL, 150 mL and 200 mL and the substrate (banana
79 and plantain peels) were added as 5g, 10g and 15g into 250 mL Erlenmeyer conical flasks. The
80 medium was then sterilized by autoclaving at 121 °C at 15 minutes. All the flasks was inoculated
81 with 1 mL of the test organism (*Mucor racemuses*). The flasks were incubated at 28°C ambient
82 laboratory temperature on an orbital shaker at 200 rpm for 5 days. After 5 days the mixture was
83 separated by filtration through a Whatman filter paper no 1. The filtrate was then used for further
84 studies.

86 **Enzymatic Hydrolysis**

87 Enzymatic hydrolysis was carried out on the biological pretreated samples. Distilled water was
88 dispensed into the substrate across the labeled flask and heated gently on a hot plate to ensure
89 dissolution. The mixture was autoclaved at 121°C for 15 minutes for sterilization. After cooling
90 at room temperature of 30°C it was inoculated with *Aspergillus niger* suspension using
91 Mcfarland turbidity standard at 0.5, 1.0 and 1.5 mL using sterile syringe. The flasks were
92 incubated at 28°C for five (5) days for hydrolysis to take place. Then, Concentration of reducing
93 sugar was quantified from the hydrolysate obtained from enzymatic using dinitrosalicylic
94 colorimetric method (DNS) as described by (Oyeleke *et al.*, 2012).

95 **Determination of Reducing Sugar**

96 After hydrolysis the sample were filtered using Whatman's filter paper no 1 and presence of
97 reducing sugar in each sample was determine using dinitrosalicylic colorimetric DNS (Miller,
98 1959). One (1.0) mL of the sample was added to 1 mL of 3,5 dinitrosalicylic acid, followed by
99 boiling for 10 minutes. The absorbance was measured at 540 nm with a spectrophotometer
100 (Jenway 6100).

101 **Fermentation of Hydrolysate**

102 To obtain a broth after the hydrolysis step, the fermentation media was filtered using whatman
103 no 1 filter paper. The flasks containing the filtrate were covered with cotton wool wrapped in
104 aluminum foil then autoclaved at 121°C for 15 minutes. The sterile hydrolysate was allowed to
105 cool and *Saccharomyces cerevisiae* suspension were inoculated at 0.5, 1.0 & 1.5 mL and
106 incubated at 30°C ambient temperature for 5 days to monitor ethanol production.

107 **Distillation**

108 The fermented broth were filtered using Whatman no 1 filter paper. Each sample were weighted
109 into kjeldahl flasks and heated at 78°C (boiling point of ethanol) on the Microkjeldahl apparatus
110 until solution turns colorless.

111 **Determination of biofuel Quality**

112 Determination of biofuel quality was according to the method described by Patel *et al.*
113 (2007). This was carried out using UV-VIS quantitative analysis of alcohol using chromium VI
114 reagent. A quantity (1ml) of standard ethanol was diluted with 100 mL of distilled water to give
115 a concentration of 1%. Then, each of 0, 2, 4, 6 and 8 mL of the ethanol was diluted to 10 mL
116 with distilled water to produced 0, 0.2, 0.4, 0.6 and 0.8% of the ethanol. To each of the varying
117 ethanol concentrations 2 mL of chromium reagent was added and allowed to stand for an hour
118 for colour development. The absorbance of each concentration were measured at 588 nm using
119 UV -VIS spectrophotometer and the readings used to developed standard ethanol curve. Then
120 1ml of each biofuel samples were put into test tubes and treated with 2 mL of the chromium
121 reagent. The mixture was allowed to stand for an hour and the absorbance measured at 588 nm
122 using the Ultraviolet-Visible spectrophotometer (Rabah *et al.*, 2011).

123 **Determination of compounds present in the Biofuel produced**

124 Gas Chromatography and mass spectrometry (GC-MS) analysis of the produced bio-ethanol was
125 conducted at General Science Laboratory, Usmanu Danfodiyo University, Sokoto on Agilent
126 technologies 6890N Network GC System and Agilent technologies 5973 network mass selective
127 detector coupled with 7683B series injector. During the analysis, the oven temperature was
128 maintained at 50°C for 5 minutes. The injector and detector temperature were 120°C and 160°C
129 respectively. The flow rate for carrier gas (Helium) was set at 1.2ml/min and the injection
130 volume were 1NL (Mei-ling *et al.*, 2003). The inlet temperature was maintained at 230 °C. The
131 oven was programmed again to increase to 300°C at a rate of 10°C ending with 25 minutes and
132 this temperature was held for 15 minutes, total runtime were 45 minutes. The mass spectrometry
133 (MS) transfer line was maintained at a temperature of 250⁰ C. The source temperature was also
134 maintained at 230°C and Ms quad at 150°C. The ionisation mode to be used is electron ionization
135 mode at 70 Ev. Total Ion count (TIC) was used to evaluate for compound identification and
136 quantification. The spectrum of the separated compound were compared with the database of the
137 spectrum of known compound saved in the compared with the database of the spectrum of
138 known compound saved in the NISTO2 Reference Spectra Library. Agilent chemstation software
139 was used to carry out data analysis and peak area.

140 **Fourier Transform Infrared Analysis of the produced Biofuel**

141 The pellet technique as described by Gershon, *et al.*, (1981) was used. FT-IR spectra were taken
142 on a Kb 3000 series (Shimadzu, Kyoto, Japan) spectrophotometer using the KBr pressed disk
143 technique, ethanol was used to clean the KBr cells. The machine calibration was tested by
144 applying drops of nujol (liquid paraffin) solvent to a KBr cell and run its Infrared to ascertain its
145 conformity with existing spectra. A few drops of the sample was added and 3 drops of nujol
146 solvent were used to dissolve it in a mortar. The paste formed was pressed on the KBr cell and
147 inserted into the machine for 10 Mpa for 3 minutes, the spectra were recorded over the spectral
148 range between 4000 and 400 cm⁻¹ with a resolution of 4 cm⁻¹.

150 **Statistical Analysis**

151 All the work experiments were conducted in triplicates. All data obtained is expressed as mean
152 and standard deviation.

153

154 **RESULTS**

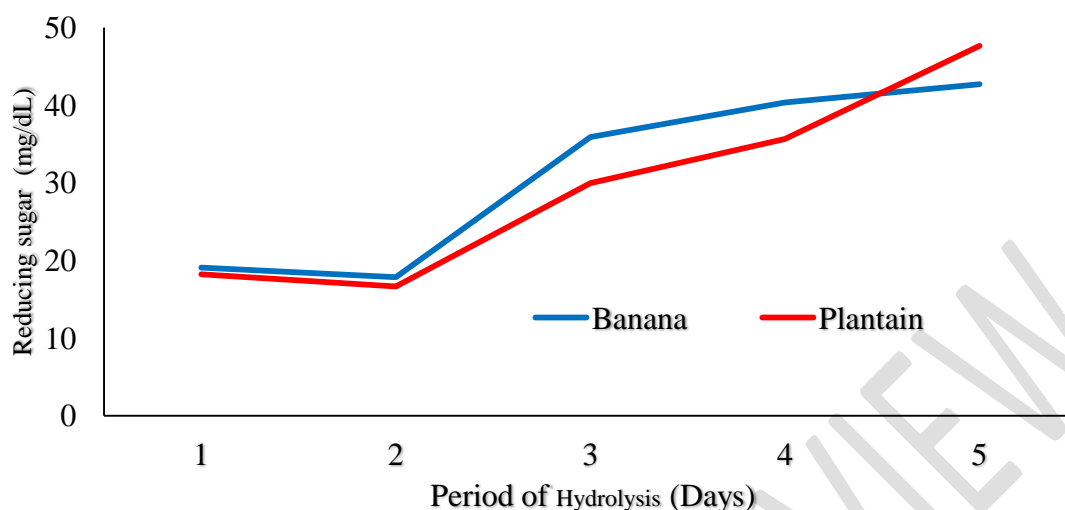
155 The results presented in Table 1 shows the phenotypic characteristics of *Aspergillus niger*
 156 isolated from spoiled banana and *Saccharomyces cerevisiae* isolated from 'burukutu' as well as
 157 *Mucor racemoses* isolated from spoiled bread. *Aspergillus niger* showed a black mycelium on
 158 the agar medium and presence of conidial head. *Saccharomyces cerevisiae* was identified as
 159 having creamish colonies with white buds forming cluster of cell.

160 Table 1: Phenotypic identification of fungi isolated from Burukutu, spoilt banana and bread

Sample	Macroscopic	Microscopic	Organism
Spoiled banana	White colonies with dense layer of dark brown Presence of black conidiophores	Presence of conidial heads Conidiophore stripe smooth walled Hyaline brown color	<i>Aspergillus niger</i>
Burukutu	White dense layer	White buds, forming cluster of cell.	<i>Saccharomyces cerevisiae</i>
Spoiled Bread	Colony white presences of tall and short Sporangiohores Sporangia hyaline becoming brownish to gray with age	Columella obovoid ellipsoidal Sporangiospore broadly ellipsoidal Smooth-walled grayish	<i>Mucor racemosus</i>

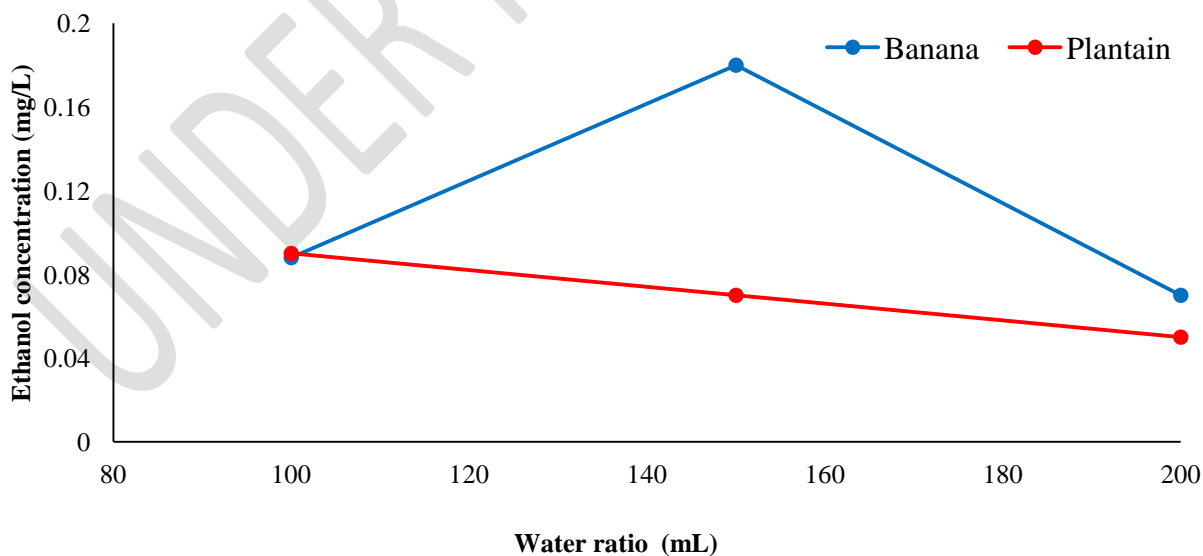
161
 162 The results of effect of hydrolysis on reducing sugar content from banana and plantain peels as
 163 substrate are presented in Figure 1. It was observed that at 5th day of hydrolysis the highest
 164 yields of reducing sugar of 59.12 and 56.62 mg/dL were obtained from banana and plantain
 165 peels respectively with water ratio of 200 mL, 15g of substrate ratio and 1.5 mL of inoculum
 166 size using *Aspergillus niger*. And 2nd day of hydrolysis, lowest concentration yield of reducing
 167 sugar (16.10 mg/dL) was obtained from plantain peels with water level of 150 mL, 10 g of
 168 substrate and 1.0 mL of inoculum of *Aspergillus niger*. While 1st day showed the lowest
 169 concentration yield of reducing sugar of 16.35 mg/dL obtained from banana peels with water
 170 level of 100 mL, 5 g of substrate and 0.5 mL of inoculums.

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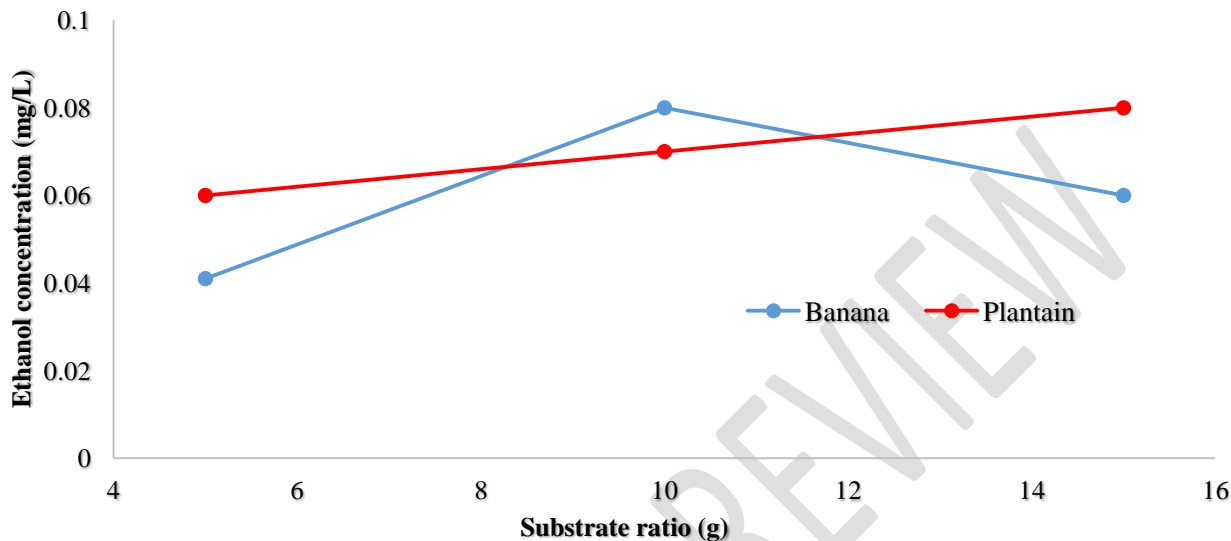
173
 174 **Figure 1: Effect of Hydrolysis on reducing sugar content from banana and plantain peels**
 175 **as substrate.**

176 The effect of water ratio on biofuel production from banana and plantain peels using
 177 *Saccharomyces cerevisiae* indicated a decreased in biofuel concentration with increase water
 178 ratio figure 2. It was observed that the plantain produced highest ethanol at 100 mL water ratio
 179 and gradually declined toward 200 mL, while the banana peel produced highest ethanol at 150
 180 mL.



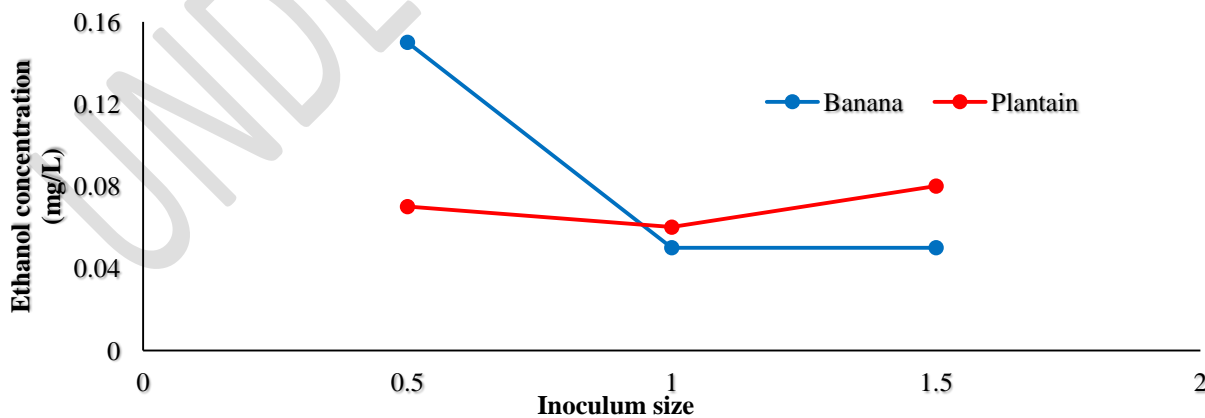
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 182 **Figure 2: Effect of water ratio on biofuel yield using banana and plantain peels as**
 183 **substrate at the 5th days of fermentation**

184 The result of effect of banana and plantain peels concentration on biofuel yield is presented in
 185 Figure 3. It was observed that substrate concentration increased from 5 to 15 g/L and biofuel
 186 yield increased to 0.08 mg/L.



187
 188 **Figure 3: Effect of substrate ratio on biofuel yield using Banana and plantain peels as a**
 189 **substrate at the 5th days of fermentation.**

191 The effect of inoculum size on biofuel production of banana and plantain peels using
 192 *Saccharomyces cerevisiae* is presented in Figure 4. It was observed that 0.5 mL inoculum size of
 193 *Saccharomyces cerevisiae* produced highest yield of 0.16 mg/mL ethanol on banana peels. While
 194 on plantain peels *Saccharomyces cerevisiae* produced moderate yield of 0.09 mg/mL at 1.5 mL
 195 of inoculum size.



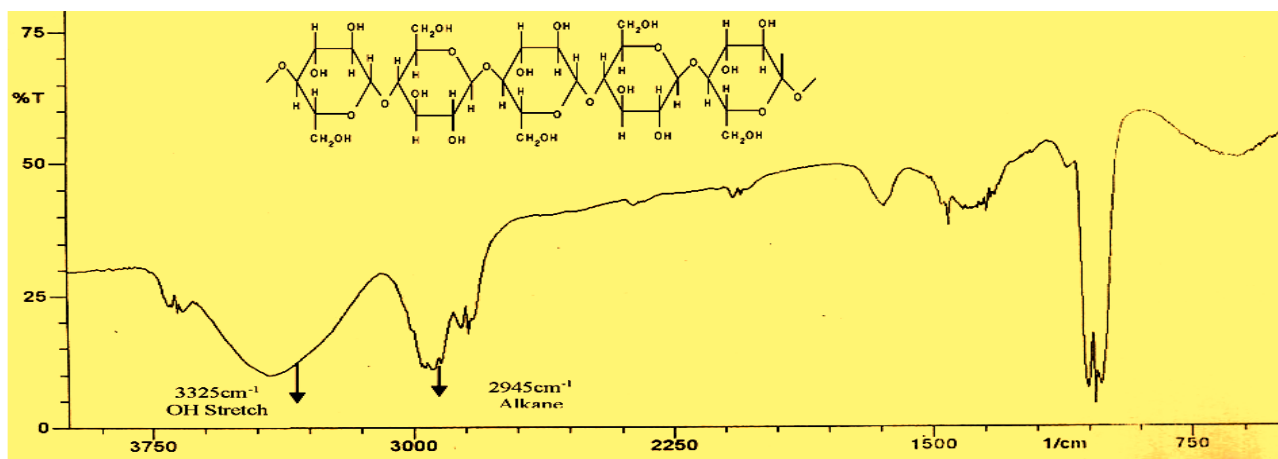
196
 197 **Figure 4: Effect of inoculum size on biofuel yield using banana and plantain peels as**
 198 **substrate at the 5th days of fermentation**

199 The volatile organic metabolites of banana and plantain peels were hydrolysed using *A. niger*
 200 and fermented for biofuel production. Banana peels had high abundance of benzaldehyde
 201 (22.20%), which was present in the plantain peel, and the plantain peels had the biofuel '2,3-
 202 butanediol' while 9-Octadecenoic, Methyl ester (18.80%) had the highest abundance. The results
 203 is presented in Table 3.

204
 205 **Table 3: Volatile organic metabolite of banana and plantain peels fermented for biofuel**
 206 **production potential**

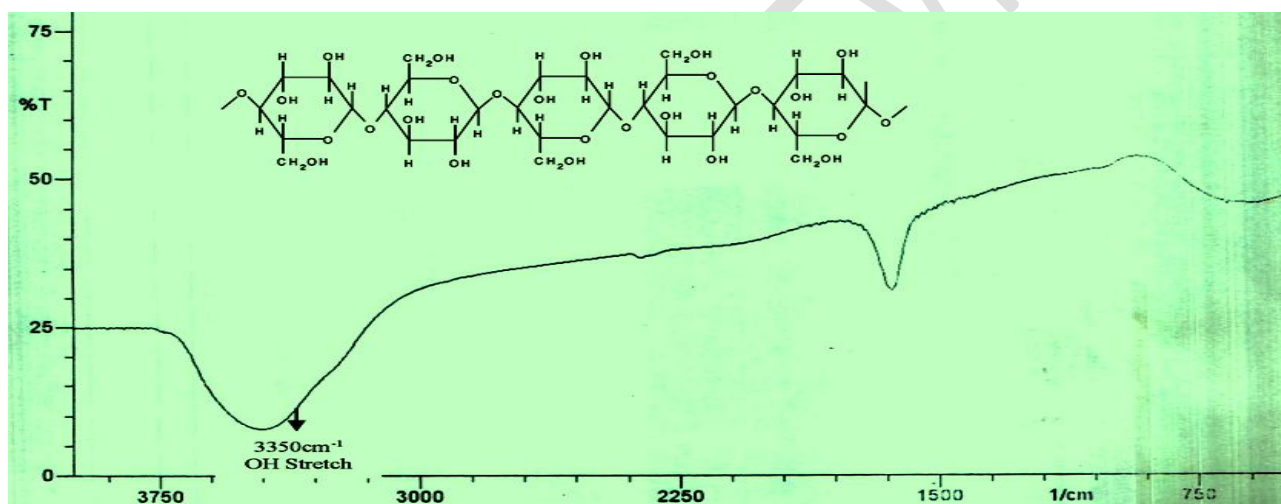
Retention Time(min.)	Organic metabolite profile	Abundance (%)	
		Banana	Plantain
2.315	2,3-Butanediol,	-	1.66
3.019	Benzaldehyde	22.20	8.63
6.705	Caprolactam	-	1.18
14.047	4-Benzylaniline	3.85	4.30
15.431	Hexadecanoic acid, methyl ester	4.84	5.36
16.856	9-Octadecenoic acid, methyl ester, (E)-	14.52	18.80
17.057	Methyl stearate	13.95	16.42
17.186	Oleic Acid	4.66	4.54
17.366	Octadecanoic acid	7.58	8.58
18.520	Eicosanoic acid, methyl ester	1.00	0.76
19.190	9-Octadecenoic acid (Z)-	0.43	1.27
19.358	Octadecanoic acid, 2,3-dihydroxypropyl ester	0.34	2.39
19.512	Z,Z-4,16-Octadecadien-1-ol acetate	2.08	2.39
19.675	Glycidol stearate	2.30	2.46
19.741	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2.32	1.40
20.136	Butyl 14-methylhexadecanoate	1.24	7.72
20.892	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	8.36	12.14
21.038	Octadecanoic acid, 2,3-dihydroxypropyl ester	10.34	4.54

207
 208
 209 The result of Infrared spectroscopy for characterization of banana and plantain peels from the
 210 produced biofuel at (figure 5 and 6) shows an O-H characteristics stretch with an intense and
 211 broad band of 3500cm^{-1} to 2200cm^{-1} and 1260cm^{-1} to 1050cm^{-1} respectively corresponding to
 212 alcohol.



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Figure 5 : FT-IR spectrum of the distillate obtained from fermented banana peels.



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Figure 6: FT-IR spectrum of the distillate obtained from fermented plantain peels.

221 DISCUSSION

222 The results from this study indicated *Aspergillus niger*, *Mucor racemosus* and *Sacchromyces*
 223 *cerevisiae* were isolated from spoiled banana, bread and burukutu. It is not surprising the
 224 organisms are found on the samples as genus *Aspergillus* are filamentous fungi which are
 225 ubiquitous and of great importance in medical and industrial microbiology. They comprise
 226 species and strains such as *Aspergillus niger* that secrete copious amounts of cellulolytic
 227 enzymes (Acharya *et al.*, 2008). Essien *et al.*, (2005) reported banana fruit peel could support
 228 microbial growth thus it could be used as a substrate for the production of variable micro fungal

229 biomass. The result is in conformity with Yahaya and Ado (2008) who reported the mycelial
230 protein production of *Aspergillus niger* using banana peels. *Saccharomyces cerevisiae* was
231 isolated from "burukutu" in this studies. *Saccharomyces cerevisiae* is the organism of choice as
232 the fermentation process biocatalyst in alcoholic beverages and fuel ethanol industry (Vallet *et*
233 *al.*, 1996). The organism was able to ferment glucose, fructose, Sucrose, maltose and galactose
234 producing acid and gas. This result agree with Elijah *et al.* (2010) who reported the isolation
235 *Saccharomyces cerevisiae* among other yeast from palm wine.

236 At day 5 the highest yield of reducing sugar of 59.12 and 56.62 mg/dL was obtained from
237 banana and plantain respectively with water ratio of 200 mL, 15g of substrate ratio and 1.5 mL
238 of inoculums size using *Aspergillus niger*. This result might be due to high lignocellulosic
239 biomass contained in the substrate that can be converted to reducing sugar which later
240 metabolize by yeast to ethanol, but it cannot produce cell enzymes which is required to degrade
241 the cellulose, present in the banana peels (Essien *et al.*, 2005). *Aspergillus* spp are able to
242 produce different kind of enzymes e.g. amylase, cellulase and protease which is responsible for
243 degradation of polysaccharide into monosaccharide (Auta *et al.*, 2012).

244 At day 2 lowest concentration yield of reducing sugar (16.10 mg/dL) was obtained from plantain
245 peels with water level of 150 mL, 10g of substrate and 1.0 mL of inoculum using *Aspergillus*
246 *niger* and day 1 showed the lowest concentration yield of reducing sugar of 16.35 mg/dL was
247 obtained from banana peels with water level of 100 mL, 5g of substrate and 0.5 mL of
248 inoculums. It is not surprising because at the lag phase cell synthesis enzymes that could convert
249 readily available sugar, whereby the organisms undergo cell repair by adapting to the
250 environment. This is in agreement with the results of Laopaiboon *et al.* (2010), they reported
251 that the hydrolysis of sugarcane baggasse (SCB) for lactic acid production using HCl is quite
252 slow compared to when using H₂SO₄, maximum total sugar and reducing sugar concentration of
253 49.95 and 21.33 g/c were obtained when using 5% (v/v) of H₂SO₄. While in contrary to Itelima
254 *et al.* (2013) the highest reducing concentration (0.94mg/cm³). *Trichoderma reesei* released
255 maximum reducing sugar of 22.30 mg/g in paddy straw, 25.56 mg/g in wheat straw, and 28.26
256 mg/g in sugarcane bagasse. *Phanerochaeta chrysosporium* recorded reducing sugars of 14.55
257 mg/g in paddy straw, 18.11 mg/g in wheat straw and 20.96 mg/g.

258 The effect of water ratio on biofuel production from banana and plantain peels sample using
259 *Saccharomyces cerevisiae* indicated a decrease in bio ethanol concentration with increase water
260 ratio. The decrease in ethanol concentration with increase in water ratio observed for plantain is
261 likely due to water activity. As previously stated that water activity is the key variable to ethanol
262 concentration for the description of some of the nonspecific inhibitory effects apparent in ethanol
263 fermentation (Jones and Greenfield, 1986). The peak ethanol concentration observed at water
264 ratio of 1:15 suggests that it is the peak at which the ethanol exerts the lowest water activity.
265 Ethanol induced water activity stress has been previously reported to exert stress on yeast
266 metabolism and cell wall integrity (Hallsworth, 1998). These findings are in conformity with the
267 work of Epstein *et al.* (2010) who reported an ethanol volume as low as 0.06 g/mL from apple
268 and grape juices.

269 The effect of banana and plantain peels concentration on biofuel yield indicated substrate
270 concentration increase from 5 to 15 g/L, ethanol yield increased to 0.08 mg/L. The decrease in
271 ethanol yield beyond the optimum concentration 0.05 mg/L could be as a result of product
272 inhibition. This implies that the ethanol produced inhibits the activity of the yeast, at low
273 substrate levels, an increase of substrate concentration normally results in an increase of the yield
274 and reaction rate of the hydrolysis (Cheung and Anderson, 1997). However, high substrate
275 concentration can cause substrate inhibition, which substantially lowers the rate of the
276 hydrolysis, and the extent of substrate inhibition depends on the ratio of total substrate to total
277 enzyme (Huang and Penner, 1991; Penner and Liaw, 1994). These findings are in conformity
278 with the work Reddy *et al.* (2010) that carried out an investigation on use of banana agro waste
279 for the ethanol production. They used cellulolytic thermophilic *Clostridium thermocellum* CT2
280 for the biological production of ethanol. This culture was isolated from elephant droppings. They
281 obtained the maximum ethanol yield of 0.41 g/g substrate used.

282 The increase in fuel yield may be due to continuous excretion of enzymes by the microorganism
283 in the solution. The decrease in fuel yield beyond the optimum concentration could be that at the
284 high substrate concentration, the active sites of the enzyme molecules at any given moment were
285 virtually saturated with substrate. Thus any extra substrate has to wait until the enzyme complex
286 has released the products before it may itself enter the active site of the enzyme (Wen, 2004).

287 The effect of inoculum size on biofuel production of banana and plantain peels sample using
288 *Saccharomyces cerevisiae* indicated an increase in ethanol production. A study done by
289 Mahamed and Reddy (1986) reported that the increasing *Saccharomyces cerevisiae* inoculums in
290 the co-cultures *Aspergillus niger* and *Saccharomyces cerevisiae* from 4% to 12% showed an
291 increase in the rate ethanol production from potato starch. Ocloo and Aywnor (2010) also
292 reported that the time taken for the fermentation to be completed was affected significantly by
293 the yeast concentration. The result obtained supported the fact that the speed of fermentation
294 depends on the yeast concentration and the shorter the fermentation period required to achieve
295 maximum alcohol yield (kordylas, 1990). Ueda *et al.*, (1981) reported 5days fermentation for
296 raw cassava root starch using 15% yeast suspension. Togarepi *et al.*, (2012) reported increase
297 production rate rapidly with the increase in the amount of yeast to the concentration of 8g/20g
298 fruit pulp. Beyond that point the rates no longer significantly increased. At this point the
299 substrate becomes limiting and increase the yeast amount does not increase the rate of reaction.

300 The FT-IR on the produced alcohol are shown on Fig. 5 have an alcohol characteristic IR
301 absorption associated with O-H and C-H stretching vibrations. When run as a liquid film the
302 region $3550-3200\text{cm}^{-1}$ region correspond to O-H stretching and C-H stretching region $2850-$
303 3000cm^{-1} . The spectra of the banana peels show the band at around 3325cm^{-1} and 2945cm^{-1} for
304 phenol/alcohol O-H stretch and alkane C-H stretching respectively with a very intense and strong
305 broad band. The broad O-H correspond to peak for O-H from alcohol. This might be the alcohol
306 group of 2,3-butanediol. The C-H stretch that corresponds to alkane might represent be the body
307 compound such as Benzaldehyde. This is likely as a result of the various vibration modes that
308 are found in carbohydrate and Lignin (Bodirlau *et al.*, 2008). The spectra of the plantain peels
309 indicated the presence (OH) alcohol stretching at 3350cm^{-1} and intense strong broad. Although
310 all samples were similar slight changes were observed possibly indicating that equivalent
311 strategies should be employed for releasing fermentable sugar from these lignocellulosics. And
312 also the broad O-H peak was due to the interaction (hydrogen bonding) and the water content.
313 The samples consist of alkene, alcohol and alkenyl indicating the presence of hydrocarbons in
314 the biofuel.

315 The biofuel 2,3-butanediol (2,3-BD) was detected in the distillate of the plantain biomass. 2,3-
316 butanediol is a potential valuable fuel additive that has 27.2KJ/g heat value. The heat value of

317 2,3-BD is comparable to those of other liquid fuel such as ethanol which has 29.055KJ/g and
318 methanol which has 22.081KJ/g (Flickinger,1980).The presences of 2,3-BD is not surprising as
319 the yeast *S. cerevisiae* used for fermentation in this study has been previously shown to posses
320 three different 2,3-BD biosynthetic pathway (Ng *et al.*, 2012).

321 **Conclusion**

322 This study concludes that banana and plantain peels has potential as a sustainable and low-cost
323 biomass for the production of biofuel such as 2,3-butanediol (2,3-BD). *Aspergillus niger*,
324 *Sacchromyces cerevisiae* and *Mucor racemus* were Identified. A reducing sugar concentration
325 was observed for the banana and plantain peels. The biofuel concentration was found to be
326 highest in banana and plantain peels. The IR characterization of the sample revealed with intense
327 strong broad band of alcohol O-H and alkane C-H stretching respectively. GC-MS result
328 revealed the presence of benzaldehyde in all the biomass while 2,3-butanediol was only detected
329 in the plantain peels biomass. Burukutu can be exploited for sufficient *Saccharomyces cerevisiae*
330 for biofuel production. This study shows the potential of bioconversion of banana and plantain
331 peels biomass for biofuel production.

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