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Bioethanol Potential of Cassava (*Manihot esculenta cranz*) and Sweet Potato (*Ipomoea batatas*) Leaves via Hydrolysis and Fermentation Processes

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Bioethanol is an alternative and renewable source of sustainable transportation fuels. It is generally considered an environmentally benign source of energy. This study investigated the feasibility of leaves of both Cassava (*Manihot esculenta cranz*) and Sweet potato (*Ipomoea batatas*) for bioethanol production using acid pretreatment and hydrolysis and *S. cerevisiae* as fermenting agent. FTIR spectrum of bioethanol produced confirms the presence of alcohol in the sample while UV-visible spectroscopy was used to determine the concentration of the bioethanol produced. The percentage yield of bioethanol from cassava was 5.09%, 3.50% and 2.31% for 10%, 5% and 2% H_2SO_4 respectively while sweet potato leaves gave percentage yield of 1.12%, 0.90% and 0.44% for 10%, 5% and 2% H_2SO_4 respectively. The results supported the bioethanol potential of both samples with the highest yield obtained at 10% sample concentration.

Keywords: Bioethanol, Cassava, Potato, Leaves, Fermentation.

1. Introduction

The world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. These are being used to produce fuel, electricity and other goods (Uihlein and Schbek, 2009). Excessive consumption of fossil fuels, particularly in large urban areas, has resulted in generation of high levels of pollution during the last few decades. The level of greenhouse gasses in the atmosphere has drastically increased (Ballesteros *et al.,* 2006). With the expansion of human population and increase of industrial prosperity, global energy consumption also has increased gradually. Import of transport fuel is affected by limited reserves of fossil fuel and annual global oil production will begin to decline within the near future (Boboyeand Dayo, 2009). In this scenario, renewable sources might serve as an alternative. Wind, water, sun, biomass, geothermal heat can be the renewable sources for the energy industry whereas fuel production and the chemical industry may depend on biomass as an alternative source of their feedstock in the near future (Lynd and Wang, 2003). All petroleum-based fuels can be replaced by renewable biomass fuels (such as bioethanol, bio-diesel, bio-hydrogen, etc.), derived from cassava, sweet potato, sugarcane, corn, switch grass, algae (Nibedita*et al., 2012*).

Kanagaraj *(*2013) carried out an experiment on bioethanol production from cassava by fermentation process using saccharomyces cerevisiae. The objective of his experiment was to find out the effect of agitation speed at 0, 150, 200 and 250 rpm, at temperatures of 20, 30, 40 and 50^{o}C and substrate concentrations of 25, 50, 75, 100 and 150 g. As for the optimum condition, temperature 30^{o}C maintained, agitation speed fixed at 200 rpm, pH maintained at 4.5 and substrate concentration fixed about 100 g. At the end of this experiment, agitation speed of 200 rpm, substrate concentration of 150 g and temperature of 40^{o}C gave the highest bioethanol production, highest cell growth and highest glucose concentration. Anbuselvi and Balamurugan (2013) carried out a similar study on ethanol production from cassava leaves and Pulp using *S. cerevisiae*. The study examined the optimization of ethanol production using cassava pulp and leaves. The enzymatic hydrolysis was carried out by barley and α-amylase and a maximum yield of ethanol (5.89%) was observed in cassava leaves treated with enzymatic hydrolysis of barley. The cassava pulp also showed ethanol production (2.56%) in barley using a fermentation medium.

Fangzhong*et al.* (2016) presented a paper on an environmentally friendly and productive process

for bioethanol production from potato waste. He studied the swelling behavior of cellulose that causes high-gravity sweet potato residues to be recalcitrant to enzymatic hydrolysis using cellulose and pectinase. Cellulose plays a major role in viscosity reduction and glucose production. In contrast, pectinase has a minor role in viscosity reduction but acts as a "helper protein" to assist cellulase in liberating glucose, especially at low cellulase activity levels. In total, 153.46 g/L and 168.13 g/L glucose were produced from high-gravity sweet potato residues (SPRs) with cellulase and a mixture of cellulase and pectinase, respectively. These hydrolysates were fermented to form 73.37 and 79.00 g/L ethanol, respectively. Each kilogram of dry sweet potato residues (SPR) was converted to form 209.62 and 225.71 g of ethanol, respectively.

Lareo *et al.* (2013) worked on evaluation of sweet potato for fuel bioethanol production from hydrolysis and fermentation. The enzymatic starch hydrolysis and bioethanol production from a variety of sweet potato developed for bioenergy purposes (K 9807.1) on the basis of its high starch yields, was studied. He reported that drying at 55°C and 95°C of sweet potato neither affected the sugar content nor the starch enzymatic hydrolysis efficiency. Simultaneous scarification and ethanol fermentations for dry matter ratio of sweet potato to water from 1:8 to 1:2 (w/v) were studied. Fresh sweet potato and dried at 55°C (flour) were assayed. At ratios of 1:8, similar results for fresh sweet potato and flour in terms of ethanol concentration (38-45 g/L), fermentation time (16h) and sugar conversion (~ 100%) were found. At higher dry matter content, faster full conversion was observed using flour. A higher ratio than that of fresh sweet potato (1:2.2) did not improve the final ethanol concentration (100 g/L) and yields. High ethanol yields were found for very high gravity (VHG) conditions. The sweet potato used is an attractive raw matter for fuel ethanol, since up to 4800 L ethanol per hectare can be obtained.

The current research is aimed at assessing the potential of cassava (*Manihot esculenta cranz*) and sweet potato (*Ipomoea batatas*) leaves for bioethanol production using acid hydrolysis and *Saccharomyces cerevisiae* (dry baker's yeast) as fermenting agent.

2. Materials and Methods

2.1 Sample Collection

The Cassava and Sweet potato leaves were collected in polythene bags from Asare, Wamakko local government of Sokoto State, dried in a room for three days and taken to laboratory for analysis at Sokoto State University, Sokoto. The *Saccharomyces cerevisiae* was obtained at meat and vegetable market Sokoto. The dried cassava and potato leaves were grinded to powder form, using mortar and pestle and then sieved through 36 µm mesh.

2.2 Sample Pretreatment

Thirty grams (30 g) of each powdered leaf of cassava and sweet potato sample were taken into three conical flasks and then 300 mL of 10% H_2SO_4 was added. They were plugged with cotton wool and wrapped in aluminum foil. The samples were heated at a temperature of 120^oC for 6h and allowed to cool. The later procedure was repeated using 5% and 2% H_2SO_4. The content of each flask was filtered (Pramanik, 2005).

2.3 Hydrolysis

10% H_2SO_4 was prepared and mixed with the biomass of cassava leaves and sweet potato leaves produced from the pretreatment processes. The flasks were plugged with cotton wool, wrapped in aluminum foil and sterilized at 121^oC for 15 mins. They were then allowed to cool and hydrolyse for three days. The later procedure was repeated using 5% and 2% H_2SO_4. The content of each flask was filtered and pH value were then adjusted to 4.5 before fermentation (Pramanik, 2005). Glucose assay was carried out using Benedict's test to confirm the presence of reducing sugar and non-reducing sugar prior to the fermentation processes. There was changed of color to brownish which indicates the presence of glucose (Gaddafi*et al.,* 2016).

2.4 Reducing Sugar Determination

Total reducing sugar produced from the hydrolysis of cassava leaves and sweet potato leaves was estimated using Benedict's test prior to the fermentation. The Benedict's test was conducted at 0, 24, 48, and 72 h. Color changes were monitored from the samples after adding 3 drops of Benedict's solution and heating in a water bath for 5 mins. The color change from Benedict's reaction gives a semi-quantitative or a rough estimate of the reducing sugars present within a sample. The amount of reducing sugar present in a sample can be quantified using the following color change Blue (no sugar), Green (0.5 % sugar), Yellow (1 % sugar), Orange (1.5 % sugar), Red (2 % sugar), Brown (highest level of sugar) (Gaddafi *et al,* 2016).

2.5 Fermentation Process

In this analysis, conical flasks containing the hydrolyzed sample filtrate were covered with cotton wool and wrapped in aluminum foil and sterilized at 121°C. After cooling of the flasks at room temperature, the pH of each flask was adjusted to 4.5 with NaOH. The samples were inoculated with 3 g of *Saccharomyces cerevisiae* (dry baker's yeast) and incubated aerobically at 37°C for five days. After that, the broth obtained was distilled at 78.3°C. The collected distillate was analyzed using UV-visible instrument (Uduak*et al.,* 2008).

2.6 Quantitative Test for Ethanol

Two (2) drops of acidified 0.1M $K_2Cr_2O_7$ was added to the 2 mL of distillate produced and heated for 30 mins on a water bath. The content of the test tube changed to green color indicating the presence of ethanol (Shanmugam*et al,* 2009). However, concentration of bioethanol produced was determined using UV-visible spectrometer using $KMNO_4$ as an oxidizing reagent whereby the ethanol will be oxidized to ethanoic acid. Absolute ethanol (98 % v/v) was used to prepare series of standard solution. The content of each test tube was then heated in water bath for 5 mins, for color development. The absorbance of each concentration was measured at 585 nm and the readings were used to plot the standard ethanol curve. Consequently, 5 mL of each of the sample were taken in the test tube in water bath, and then 2 mL of dichromate reagent was added to each. The content of each test tube was then heated in water bath for 5 mins, for color development. The absorbance of each concentration was measured at 585 nm using UV-visible spectrophotometer (Miller, 1959).

2.7 Fourier-Transform Infrared Spectroscopy (FT-IR)

The sample of bioethanol produced was analyzed using MB3000 FTIR spectroscopy machine to determine the vibration frequencies of the bioethanol produced.

3. Results and Discussion

3.1 Estimation of Reducing Sugar from Cassava Leaves

The green bars in Figure 1 indicate the amount of reducing sugars obtained from the hydrolysis of the 2% H_2SO_4 acid pretreated samples of cassava leaves. The samples taken at 0 h and 24 h of hydrolysis both showed small amount of reducing sugar (yellow color or 1% from the rating scale). The concentration of reducing sugar increased significantly after 48 h of sampling which showed a dark coloration (orange-red coloration or 1.4 % of the total sugars). At 72 h, increase in the amount of reducing sugar was observed (red color or 2% from the rating scale).

The blue bars indicate the amount of reducing sugar obtained from the hydrolysis of 5% H_2SO_4 acid pretreated samples of cassava leaves. The sample taken at 0 h showed amount of reducing (yellow color or 1 % from the rating scale). At 24 h the sample showed orange color (1.5 % from the rating scale) while the highest level of reducing sugar (red color or 2%) was observed at 48 h and 72 h of hydrolysis.

The yellow bars indicate the amount of reducing sugar obtained from the hydrolysis of 10% H_2SO_4 acid pretreated samples of cassava leaves. The sample taken at 0 h showed orange color (1.5 % from the rating scale). While the samples taken at 24 h, 48 h and 72 h showed the highest level of reducing sugar (red color or 2 % from the rating scale). The scale of colors was in accordance with the scale given by Gaddafi *et al.* (2016) for rough estimate of reducing sugar present in banana peel samples during hydrolysis, where the blue colour signifies (no sugars) < green (0.5% sugars) < yellow (1% sugar) <orange (1.5% sugar) <red (2% sugar) < brown signifying highest level of sugars (Gaddafi *et al.,* 2016). The highest concentration of reducing sugars were observed after hydrolysis from the 10% H_2SO_4 acid pretreated samples of cassava leaves. The amount of reducing sugars produced from different concentrations of the acid hydrolysis of sweet potato leaves between 0 –72 h is shown in Figure 2.

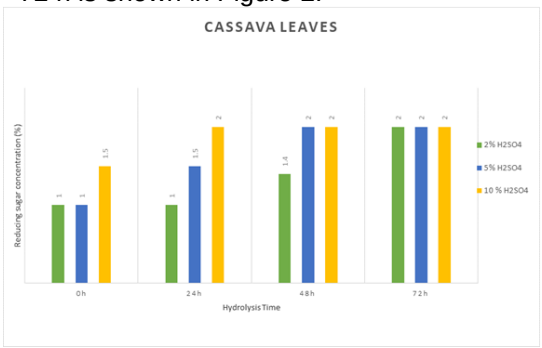

Figure 1. Reducing sugars from hydrolysis of cassava leaves.

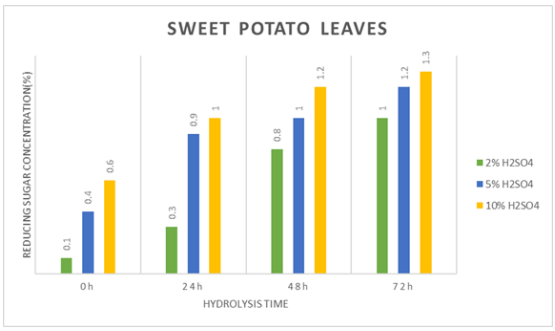

Figure 2. Reducing sugars produced hydrolysis of sweet potato leaves.

3.2 Estimation of Reducing Sugar from Sweet Potato Leaves

The green bars in Figure 2 indicate the amount of reducing sugars obtained from the hydrolysis of the 2% H_2SO_4 acid pretreated samples of sweet potato leaves. The result was in agreement with what was reported by Gaddafi, *et al* (2016), where he noticed changed of color to brownish which indicated the presence of glucose after hydrolysis of banana peel sample. The samples tested at 0 h and 24 h showed a blue coloration which signifies the absence of reducing sugar (0.1% and 0.3 respectively). At 48 h the sample showed a pale green coloration which signifies the presence of little reducing sugar (0.8% from the rating scale). At 72 h the tested sample showed the presence of reducing sugar (yellow color or 1% from the rating scale).

The blue bars indicate the amount of reducing sugar obtained from the hydrolysis of 5% H_2SO_4 acid pretreated samples of sweet potato leaves. The sample taken at 0 h showed the absence of reducing sugar (blue color or 0.4% from the rating scale), at 24 h the sample showed presence of reducing sugar (pale green coloration or 0.9% from the rating scale) and at 48h and 72h of hydrolysis, both showed yellow coloration which signifies the presence of reducing sugar (1% and 1.2 % respectively).

The yellow bars indicate the amount of reducing sugar obtained from the hydrolysis of 10% H_2SO_4 acid pretreated samples of sweet potato leave. The sample taken at 0h showed green color (0.6% from the rating scale). While both samples taken at 24 h and 48 h showed yellow color (1% and 1.2% respectively from the rating scale). At 72 h the sample tested showed pale yellow color (1.3% from the rating scale). The scale of colors was in accordance with the scale given by Gaddafi *et al.* (2013), in the rough estimate of reducing sugar present in banana peel samples during hydrolysis, where the blue signifying (no sugars) < green (0.5% sugars) < yellow (1% sugar) < orange (1.5% sugar) < red (2% sugar) < brown signifying highest level of sugars (Gaddafi *et al., 2013*). The results obtained indicates that the highest concentration of reducing sugars after hydrolysis can be obtained from the 10% H_2SO_4 acid pretreated samples of sweet potato leaves.

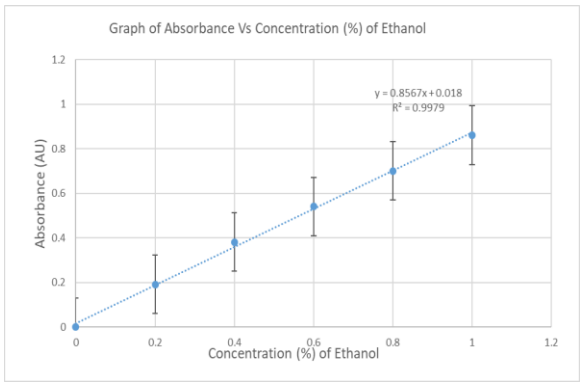

Figure 3. Standard ethanol curve for reducing sugar estimation

3.3 Bioethanol Produced

The result of bioethanol produced from cassava leaves is shown in Table 1, where sample A1, A2 and A3 represent hydrolysate obtained from hydrolysis of cassava using 10% H_2SO_4, 5% H_2SO_4 and 2% H_2SO_4 respectively. The amount of ethanol increases with increase in concentrations of sulphuric acid. The highest percentage of ethanol was noted from A1 which gave 5.09%, followed by the A2 which was 3.50%. A percentage of 2.31% gave the lowest level of ethanol produced in cassava leaves (samples by A3). The highest percentage of ethanol obtained also agrees with that reported by Rajendran and Saravana (2013), where 4% yield of bioethanol obtained using *S. Cerevisiae*. Anbuselvi and Balamurugan (2013) also reported 5.89% yield bioethanol obtained from cassava leaves using *S. Crevisiae* which is closed to the yield obtained in present study.

Table 1 also contain the result of bioethanol produced from sweet potato leaves. Samples B1, B2 and B3 represent hydrolysate obtained from hydrolysis of potato leaves using 10% H_2SO_4. 5% H_2SO_4 and 2% H_2SO_4 respectively. The highest percentage of ethanol was obtained in sample B1 which was 1.12%, followed by B2 that gives 0.90% and the lowest yield was recorded from B3 which was 0.44%. Kumar *et al.* (2014) reported that maximum ethanol produced from tuberous plant (sweet potato) using *S. cerevisiae* was 7.93%. He attributed this trend of low bioethanol production on sweet potato leaves samples to the accumulation of other toxic compounds or secondary metabolites that were produced during fermentation. The results (Table 1) obtained from the sample were extrapolated

with the standard ethanol curve (Figure 3) to calculate the percentage of ethanol obtained.

Table 1: Total bioethanol produce in each of the sample in percentage.

Samples	Absorbance	Amount of ethanol produced (%)
A1	4.384	5.09
A2	3.022	3.50
A3	1.99	2.31
B1	0.998	1.12
B2	0.791	0.90
B3	0.599	0.44

3.4 FT-IR Spectroscopy

Figure 4 gives IR spectra of products from cassava leaves. A1, A2 and A3 represent samples with 10% H_2SO_4, 5% H_2SO_4 and 2% H_2SO_4 of cassava leaves respectively. All the samples show absorption of strong broad peak at 3450 – 2850 cm^{-1}, therefore indicating $–CH_2$ and $–CH_3$ stretching vibration and well resolved peaks around 3416 cm^{-1} which are assigned to alcoholic –OH vibrations. These values correlate with results obtained by Yusuf *et al.* (2016) research of bioethanol production from Neem tree leaves (*Azadarichta indica*). These values also are in agreement with values obtained according to spectra (2017), free –OH stretching absorption normally occurs at 3550 – 3200 cm^{-1}, while C-H stretching occurs at 3000 – 2840 cm^{-1}. Therefore, the production of ethanol in all the cassava leaves samples was successful.

IR spectra of products from the sweet potato leaves samples are presented in Figure 5. B1, B2 and B3 samples represent samples with 10% H_2SO_4, 5% H_2SO_4 and 2% H_2SO_4 of sweet potato leaves respectively. The sample B1 which is 10% H_2SO_4 shows all the absorption stated in the sample A1, A2 and A3, that is absorption of strong broad peak at 3450 – 2850 cm^{-1}, which indicates $–CH_2$ and $–CH_3$ stretching vibration and well resolved peak around 3416 cm^{-1} which are assigned to alcoholic –OH vibrations. Samples B2 and B3 also shows the same absorption. These values are also in agreement with values obtained according to spectra (2017), free –OH stretching absorption normally occurs at 3550 – 3200 cm^{-1}, while C-H stretching occurs at 3000 – 2840 cm^{-1}. Therefore, the production of ethanol in all the sweet potato leaves samples was successful.

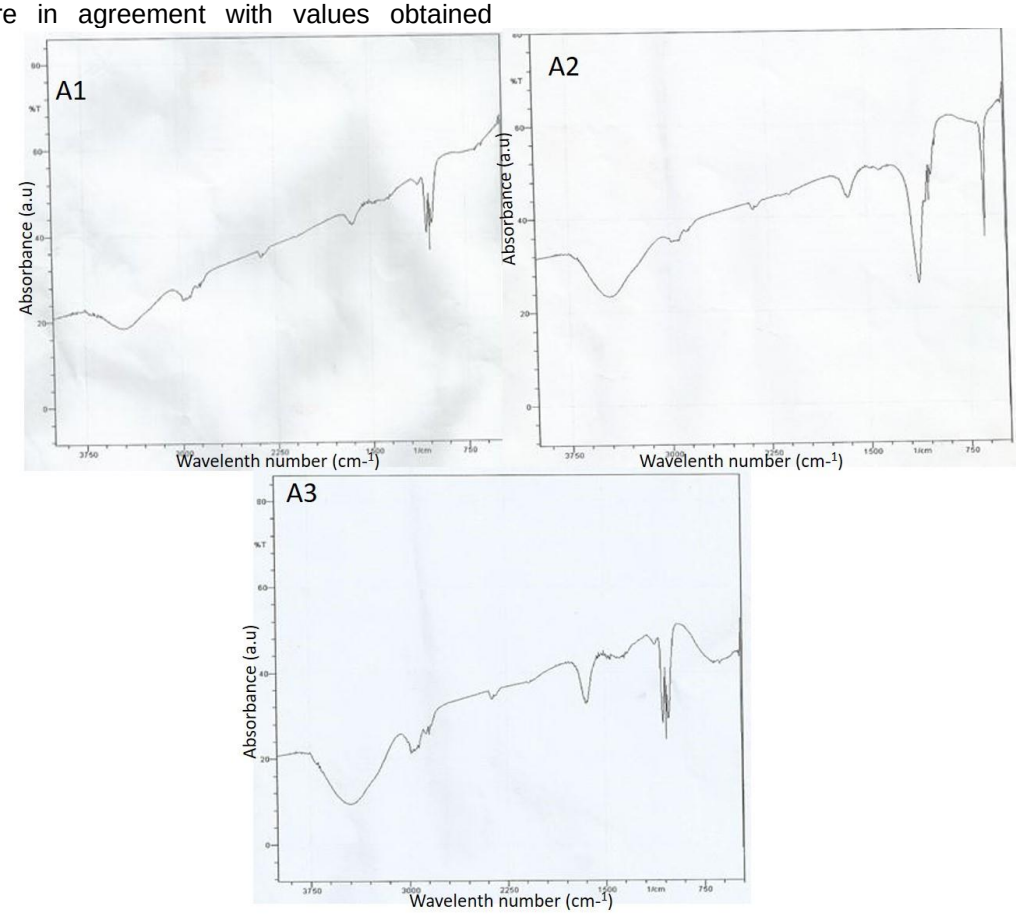

Figure 4. FTIR spectrum of sample A1, A2 and A3 samples of cassava leaves

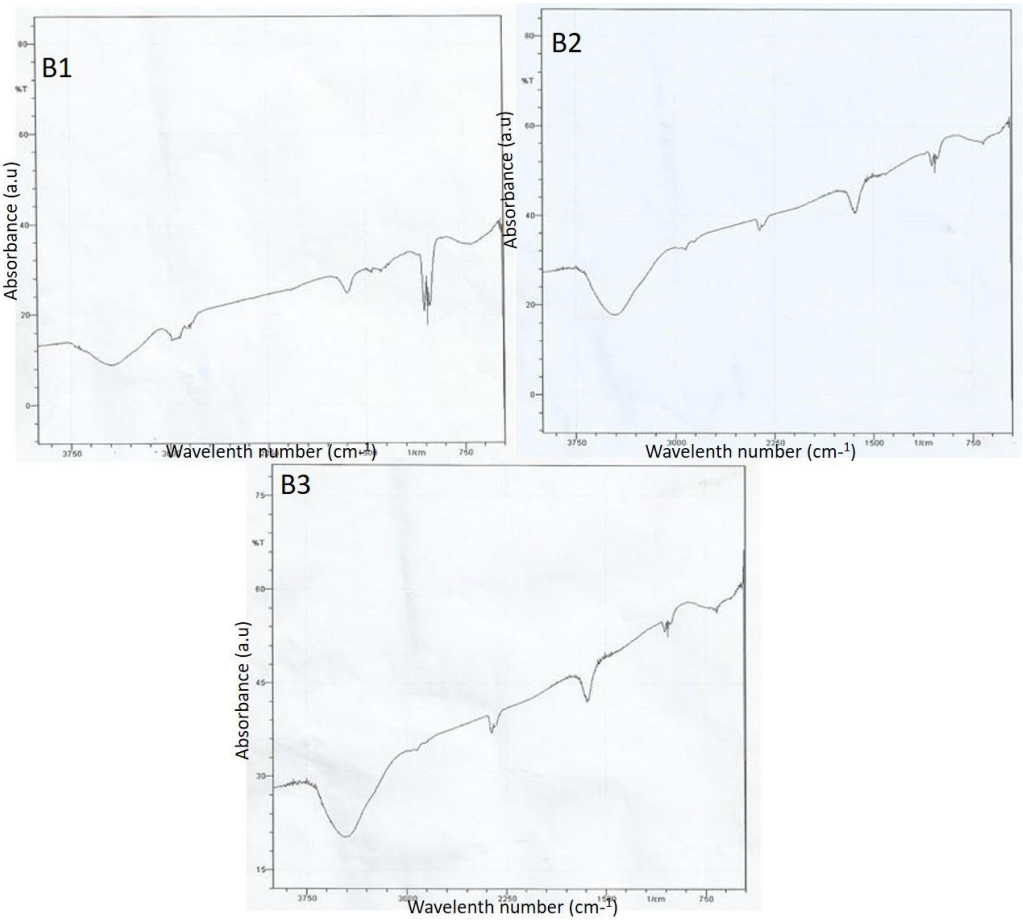

Figure 5. FTIR spectrum of sample B1, B2 and B3 samples of potato leaves.

4. Conclusion

Production of bioethanol from agricultural waste requires the development of an efficient pretreatment method which will maximize the delignification as well as enhances the hydrolysis processes. The present study showed that cassava and sweet potato leaves both can produce bioethanol, however, cassava leaves yielded higher percentage of bioethanol. The research has successfully demonstrated that waste biomass such as cassava and sweet potato leaves are potential source of *S. cerevisiae* and could be used to produce bioethanol.

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Conflict of interest

The authors declare no conflict of interest.

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