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Bioproduction of Ethanol by Saccharification-Fermentation Process using Sweet Potato (*ipomoea batatas*) and Irish Potato (*solanum tuberosum*) as Substrate

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ABSTRACT: Ethanol generally are renewable liquids usually produced from sources which can be easily replenished, therefore, the production of ethanol by fermentation using substrate such as carbohydrate crops may provide an economically competitive source of energy by its incorporation into gasoline. Hence, the objective of this paper is the bioproduction of ethanol by saccharification-fermentation process using sweet potato (*Ipomoea Batatas*) and Irish potato (*Solanum Tuberosum*) with their peels as substrate with standard techniques for evaluation. The infrared (IR) spectroscopic data of potato extracts revealed that the absorption band of hydroxyl (OH) group of an alcohol ranges from 3747.03 - 3444.40 cm^{-1} in all samples analyzed. The ^1H-NMR in $CDCl_3$ (60 MHz), the proton at δ_H 4.09-4.69 appearing as singlet, integrating for one proton which indicates an hydroxyl group, another proton signal at δ_H 3.46-3.69, appearing as quartet with coupling constant of 7.2 Hz and each integrating for 2 protons, assigned to the methylene group next to the methyl group. The peak observed at δ_H 0.95-1.96 ppm, integrating for three protons with coupling constant of 6.6 Hz, which was assigned to the methyl group on the ethanol structure. The ^{13}C-NMR spectrum of all the samples, showed an intense peak at 57.1-57.9 ppm which accounts for methylene peak on ethanol and an upfield carbon signal at 17.4-18.0, which was assigned to the methyl carbon in ethanol structure. The Nuclear Magnetic Resonance (NMR) results showed the presence of ethanol in the samples analyzed. Consequently, the refractive index result showed that all the extracts have a significant level of alcohol content.

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Sweet potato, *Ipomoea batatas* is one of the important starchy crops having a short growth cycle of (90-120 days) and capable of growing in agro- climatic conditions (Komiljon and Dilnoza, 2021). It is a dicotyledonous plant belonging to Convolvulaceae family, it is an important crop mainly seen in tropical countries (Hayati *et al.*, 2019). It is a storehouse of many important pigments like β-carotene, anthocyanin etc which act as a good antioxidant (Dwiyanti *et al.*, 2018). In addition to its nutritional benefits, the crop's adaptability to tropical climate and minimal growth requirements makes it a crop of high commercial importance most especially for the production of ethanol (Low *et al.*, 2020). Starch occurs in plants as granules consisting of amylose and amylopectin (Pfister and Zeeman 2016). Amylose is a straight chain of glucose molecules bound to each other by oxygen links while amylopectin is a water-soluble polysaccharide and highly branched polymer of α-glucose unit found in plant (Pfister and Zeeman 2016). Irish potato (*Solanum tuberosum*) is the root and tuber crop that have emerged as the fourth important crop in

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the World after maize, rice and wheat (Ogheneruemu and Dominic 2020). It is one of the important agricultural crops with a corresponding increase in consumption (Mengui *et al.*, 2019). Related words for Irish potato are white potato, potato, spud, tater and Murphy. Irish potato requires high altitude of 1000-1800 meters above sea level and low temperature of 15°C. In Nigeria the crop is grown in Jos and Manbilla in Plateau, with altitude of at least 1400 meters above sea level and temperature of 10°- 20°C (Daniel Beatrice 2015).

Irish potato provides employment opportunities, sustaining food security and improves the livelihood for majority of small-scale farmers in the rural areas (Hudu *et al.*, 2018). *Saccharomyces cerevisiae* are microorganisms that cannot be seen with naked eye, they convert sugar to ethanol during fermentation process (Ojewumi *et al.*, 2018). In breweries, *Saccharomyces cerevisiae* is used in alcohol production, this occurs by converting sugar to energy (Parapouli *et al.*, 2020). *Saccharomyces cerevisiae* is used in bakeries for raising dough. Crops such as corn, rice, wheat, potato, cassava, yam, plantain and sorghum are the main carbon source for ethanol production (Yang *et al*., 2016). Alpha and beta amylases are important enzymes in food industry (Ozcan and Sipahioglu 2020).

Like other enzymes, they are proteins with ability to catalyze specific chemical reactions. Alpha-amylase occurs in animals, plants, and microbial kingdoms with important industrial sources being cereal (Far *et al*., 2020). All alpha-amylases have 1-10 atoms of calcium per molecule of enzyme; the binding strength of protein is dependent on the enzyme source (Liao *et al*., 2019). Therefore, the objective of this paper is the bioproduction of ethanol by saccharification-fermentation process using sweet potato (Ipomoea Batatas) and irish potato (Solanum Tuberosum) with their peels as substrate with standard techniques for evaluation

MATERIALS AND METHOD

Collection of Samples: Fresh and uninfected sweet potato tubers and Irish potato were bought from New Sabo market. Sweet potato and Irish potato tubers were washed under tap water to remove soil and dirt. The tubers were peeled; the peelings and the tubers were sun dried separately. The dry peelings and tubers were grinded separately.

The flour was mixed with water in the ratio 1:3 for sweet potato tuber and Irish potato together with their peels, the mixture was left in a warm place for 3days. The mixture was placed in water bath at 80 °C for 3 hours and then cools to 50-55°C. Exactly 5 g of alpha-amylase enzyme was measured to the mixture and the enzyme was allowed to dissolve. Yeast was sprinkled, the mixture was allowed to stand in a warm place for 4 days in order to ferment. The product was filtered using a clean cotton cloth, the residue was collected for drying and the filtrate was distilled.

To obtain accurate alcohol content, ABBE Refractometer was used to know the refractive index of all the extract, FT-IR spectroscopy analysis, ^{1}H and ^{13}C NMR spectroscopy was carried out after fermentation.

RESULTS AND DISCUSSION

Table 1 contains strong, intense and broad peaks at 3747.03-3646.40cm^{-1} indicating the presence of –OH stretching vibration. A medium and broad peak at 2074.00 cm^{-1} indicating a –C-H vibrational frequency, also other peaks at 1061.70 and 1044.00 cm^{-1}which shows the presence of –C-O bending vibration and a medium and broad peak at 716.82 cm^{-1}representing C-H rocking vibration.

Characterization of Sweet Potato: The ^{1}H-NMR in $CDCl_3$ (60 MHz), the proton at δ_H4.69 (s, 1H) was integrated for only one proton which indicates a hydroxyl proton, the proton at δ_H 3.66 (q, J = 7.2 Hz, 2H) was integrated for the proton assigned to the methylene group next to the to the methyl group. The last peak was observed at 1.19 ppm, integrating for three protons (J = 6.6 Hz) which was assigned to the methyl group on ethanol structure.

The ^{13}C-NMR spectra showed an intense peak at 57.8 ppm which accounts for a methylene peak on ethanol and an up-field carbon signal at 18.0, assigned to the methyl on ethanol structure. All these spectra data and information confirm the formation of ethanol as a product of fermentation.

The Refractive Index of sweet potato: The results of the refractive index of sweet potato as shown in Table 5 showed sweet potato to have a refractive index of 1.35 at a temperature of 27.2 ^{o}C.

Table 1: Fourier Transform Infrared Analysis of Sweet Potato

S/N	Wave number (cm^{-1})	Characteristics	Interpretation
1	3747.03, 3646.40	Strong/intense and broad	Free –OH of alcohol
2	2074.00	Medium and broad	-C-H stretching vibration
3	1061.70, 1044.00	Strong and sharp	-C-O bending vibration
4	716.82	Medium and broad	$-[CH_2]_n$

Table 2 contains a strong, intense and broad peak at 3444.40 cm^{-1}, which indicates the presence of –OH stretching vibration in a sample. Medium and broad peaks at 2979.43, 2901.70 cm^{-1}, representing -C-H stretching, a weak and broad peak at 2094.50cm^{-1} indicating a –C-H vibrational frequency, also another peak at 1452.50cm^{-1} which shows the presence of –C-H bending vibration. Other weak and sharp peaks at 1081.49, 1045.36 cm^{-1}, representing –C-O bending vibration and a medium and sharp vibration at 878.62cm^{-1}.

Characterization of Sweet Potato Peel: The ^{1}H-NMR in $CDCl_3$ (60 MHz), the proton at δ_H4.09 (s, 1H) was integrated for only one proton which indicates a hydroxyl proton, the proton at δ_H 3.46 (q, J = 6.6 Hz, 2H) was integrated for proton assigned to the methylene group next to the to the methyl group. The last peak was observed at 0.95 ppm, integrating for three protons (J = 6.6 Hz) which was assigned to the methyl group on ethanol structure.

The ^{13}C-NMR spectrum of Extract 5, showed an intense peak at 57.1 ppm which accounts for a methylene peak on ethanol and an up-field carbon signal at 17.4 ppm, which was assigned to the methyl carbon on ethanol structure. All these spectra data and information confirm the formation of ethanol as a product of fermentation in sweet potato peel.

The Refractive Index of Sweet Potato peel: The results of the refractive index of sweet potato peel as shown in Table 5 showed sweet potato to have a refractive index of 1.36 at a temperature of 27.1 ^oC.

Table 2: Fourier Transform Infrared Analysis of Sweet Potato peel

S/N	Wave number (cm^{-1})	Characteristics	Interpretation
1	3444.40	Strong/intense and broad	Free –OH of alcohol
2	2979.43, 2901.70	Medium and broad	-C-H stretching vibration
3	2094.50	Weak and broad	-C-H vibrational frequency
4	1452.50	Weak and sharp	-C-H bending vibration
5	1081.49, 1045.36	Weak and sharp	-C-O bending vibration
6	878.62	Medium and sharp	$-[CH_2]_n$

Table 3 contains strong, intense and broad peaks at 3473.63cm^{-1} indicating the presence of –OH stretching vibration. A medium and broad peak at 2067.00cm^{-1} indicating a –C-H vibrational frequency, also other peaks at 1085.20 and 1047.40 cm^{-1}which shows the presence of –C-O bending vibration and a medium and broad peak at 704.60 cm^{-1}representing C-H rocking vibration.

Characterization of Irish Potato: The ^{1}H-NMR in $CDCl_3$ (60 MHz), the proton at δ_H4.66 (s, 1H) was integrated for only one proton which indicates a hydroxyl proton, the proton at δ_H 3.69 (q, J = 6.6 Hz, 2H) was integrated for proton assigned to the methylene group next to the to the methyl group. The last peak was observed at 1.15 ppm, integrating for three protons (J = 7.2 Hz) which was assigned to the methyl group on the ethanol structure.

The ^{13}C-NMR spectrum of Extract 5, showed an intense peak at 57.9 ppm which accounts for a methylene peak on ethanol and an upfield carbon signal at 17.9, which was assigned to the methyl carbon on an ethanol structure. All these spectra data and information confirm the formation of ethanol as a product of fermentation in an irish potato.

The Refractive Index of Irish potato: The results of the refractive index of Irish potato as shown in Table 5 showed Irish potato to have a refractive index of 1.35 at a temperature of 27.2 ^oC.

Table 3: Fourier Transform Infrared Analysis of Irish Potato

S/N	Wave number (cm^{-1})	Characteristics	Interpretation
1	3473.63	Strong/intense and broad	Free –OH of alcohol
2	2067.00	Medium and broad	-C-H stretching vibration
3	1085.20, 1047.40	Weak and sharp	-C-O bending vibration
4	704.60	Medium and broad	$-[CH_2]_n$

Table 4 contains strong, intense and broad peaks at 3624.77 cm^{-1} indicate the presence of –OH stretching vibration. A medium broad peak at 2079.73cm^{-1} indicating a –C-H vibrational frequency, also other peak at 1044.92 cm^{-1}which shows the presence of –C-O bending vibration and a medium and broad peak at 700.25cm^{-1}representing C-H rocking vibration.

Characterization of Irish Potato Peel: The ^{1}H-NMR in $CDCl_3$ (60 MHz), the proton at δ_H4.55 (s, 1H) was integrated for only one proton which indicates a hydroxyl proton, the proton at δ_H 3.47 (q, J = 7.2 Hz, 2H) was integrated for the proton assigned to the methylene group next to the to the methyl group. The last peak was observed at 1.96 ppm, integrating for three protons (J = 6.6 Hz) which was assigned to the methyl group on the ethanol structure. The ^{13}C-NMR spectrum of Extract 5, showed an intense peak at 57.8 ppm which accounts for a methylene peak on ethanol and an up field carbon signal at 18.0, which was assigned to the methyl carbon on ethanol structure. The observed peaks in the extract of an Irish potato peel, are not as sharp but showing weak signals in the proton NMR spectrum.

The Refractive Index of Irish potato peel: The results of the refractive index of Irish potato peel as shown in Table 5 showed Irish potato peel to have a refractive index of 1.34 at a temperature of 27.1^{o}C

Table 4: Fourier Transform Infrared Analysis of Irish Potato peel

S/N	Wave number (cm^{-1})	Characteristics	Interpretation
1	3624.77	Strong/intense and broad	Free –OH of alcohol
2	2079.73	Medium and broad	-C-H stretching vibration
3	1044.92	Weak and sharp	-C-O bending vibration
4	700.25	Medium and broad	$-[CH_2]_n$

Table 5: Result of Refractive Index for all studied sample of potatoes

S/N	Potato extract	Refractive index	Temperature (^{O}C)
1	Sweet potato 1:3	1.35	27.2
2	Irish potato 1:3	1.35	27.2
3	Sweet potato peel 1:3	1.36	27.1
4	Irish potato peel 1:3	1.34	27.1

Conclusion: The results of this current research showed that both the edible and waste peels of both potato species can be utilized in the production of ethanol as biofuel as a form of alternative energy generation. The study also established the fact that the waste peels from both potato species also exceptional potentials as sources of ethanol production. This could serve as a measure of curbing challenges associated with environmental pollution as a result of improper disposal of these waste peels.

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