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An Investigation into the Possibility of Producing Fuel Ethanol from Forest Anchomanes (Anchomanes Difformis (Bl.) ENGL.)

Oyelayo Ajamu Oyedele* Olusegun Adeoti Opeyemi Oluremi Adeyanju
Department of Agricultural and Bio-Environmental Engineering
The Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria

Abstract
One of the factors responsible for the slow growth of the Nigerian Biofuel Programme is the concern trailing the use of cassava, a crop that has food, feed and industrial value in Nigeria, for fuel production. As an alternative feedstock, this study investigates the possibility of producing fuel ethanol from forest anchomanes (FA), *Anchomanes difformis* (Bl.) Engl., a plant that has no food and feed value in Nigeria. Results showed that FA tubers were rich in carbohydrate, between 70 and Sarkar, 2011 %. The stoichiometric ethanol content of FA varied from 0.032 to 0.054 kg kg^{-1} of fresh tubers, while the crude FA ethanol had a mean density of Nwachukwu and Simonyan, 2015 1.2 kg/m^3 at 20 °C. This suggests that FA has some potential for fuel ethanol production. This finding is useful to countries in the West African sub-region where FA is found.
Keywords: Forest anchomanes tuber; Forest anchomanes starch; Ethanol content; Ethanol density; Nigeria biofuel programme
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1. Introduction

Multiple factors explain why biofuel production is gaining global attention. These include rising crude oil prices which imposes foreign exchange burdens, the desire to be energy secured and eliminate oil dependency, climate change threats, increasing rural poverty, to improve urban air quality, to increase the share of renewable energies in the total energy mix, the realisation that crude oil is finite, and to achieve sustainable development, job as well as wealth creation.

To ensure that Nigeria becomes a biofuel producer and user, the Nigerian Biofuel Policy and Incentives (NBPI) was released in 2007 following a Biofuel Programme that started in 2004. It was not until 2008 that the country joined the league of biofuel users through e-blend fuel import when the then Federal Government agreed to start with 5 % ethanol addition (E5) to Premium Motor Spirit (PMS) for automotive use (Adeoti, 2010). The policy document has articulated the production of biofuel from cassava, sugarcane, cellulose-based biomass, and any other crops as may be approved by the Biofuel Energy Commission. Its target is to reduce the country's dependence on imported PMS, cut environmental pollution resulting from fossil fuel use, and create jobs. The Nigerian Biofuel Programme creates a deliberate action plan to link the downstream petroleum sector with the agricultural sector of the economy. The NBPI approved fuel ethanol consumption in Nigeria at 10 % blend ratio of fuel ethanol (E10) with PMS. At 10 % blend ratio, the NBPI estimated that the Nigerian market requires 1.3 x 10^6 m^3 of fuel ethanol annually, projected to increase to 2.0 x 10^6 m^3 per year by 2020. While both E5 and E10 PMS are suitable for use without any engine modifications (İçöz *et al.,* 2008), the concern in Nigeria is that higher levels could harm existing or conventional automobile engines. Consistent with the NBPI and for clarity purposes, fuel ethanol is defined as hydrous or anhydrous bioethanol denatured for use as a motor fuel, and biofuels as fuels obtained from biomaterials.

At the global level, over 80 % of the primary energy being consumed is derived from fossil fuels, and roughly 58 % of it is being consumed in the transportation sector (Escobar *et al.,* 2008). At present Nigeria is a major crude oil producing (Table 1) and exporting country and has enormous potentials of becoming a major player in the global biofuels production and trade (Abila, 2010). The oil sector provides 95 % of Nigeria foreign exchange earnings and 80 % of its budgetary revenues (Global Edge, 2017). Proceeds from oil accounted for 8.60 % of the aggregate real GDP in 2018 and non-oil for the balance (National Bureau of Statistics (NBS), 2019). As illustrated in Table 2, Nigeria depends largely on refined PMS importation. Notwithstanding this, the Federal Government has emphasised its determination to reduce the importation of refined petroleum products by Arshad *et al*., 2018 % by December 2018 and stop its importation by 2019 (Kachikwu,2017; Eboh, 2017, NNPC, 2018). With Nigeria being a crude oil producer, rising unemployment, increasing cost of refined petroleum products import, and mounting public criticisms are some of the factors pressing the federal government to stop refined petroleum products importation. However, an implication of this ban is that the E10 component of the PMS will have to be sourced locally. Nigeria, which has entered the second phase of the Biofuel Production Programme implementation, intends to achieve a 100 % domestic production of biofuels by 2020 (Federal Republic of Nigeria, 2007). As at 2017, the annual ethanol demand in Nigeria was 1.7 x 10^6 m^3 (Table 3), while local production was 9.0 x 10^3 m^3 per year (Table 3). As shown in Table 3, the amount of ethanol produced in Nigeria was barely able to meet 3 to 4 % of the requirements of the manufacturing sector alone. At present the Nigerian National Petroleum Corporation

(NNPC) is the sole importer of refined petroleum products in Nigeria, importing fuel ethanol to blend locally produced PMS (Ben-Iwo *et al.,* 2016).

Twelve years after making the NBPI public, one of the factors responsible for the slow growth of the Nigerian Biofuel Programme is the concern trailing the use of agricultural crops that have food, feed and industrial value for fuel production. The concern is that this could cause ripple effects on food security, exacerbating hunger and poverty. Cassava mentioned in the NBPI has food, feed and industrial value in Nigeria and also to a lesser extent sugarcane. An analysis of the proposed fuel ethanol projects in Nigeria revealed that ten of those projects were cassava-based, eight sugarcane-based and the remaining two sweet sorghum-based (Ohimain, 2010). Besides this, in 2017, the NNPC signed a Memorandum of Understanding (MoU) with the Government of Ondo State to establish a 65 x 10^3 m^3 per annum cassava-based biofuel plant in Okeluse, Ondo State (Nnodim, 2017). These projects are yet to commence. Traditionally, sugarcane is grown by smallholder farmers for chewing as juice and for livestock feed (Ben-Iwo *et al.,* 2016). In Nigeria, sugarcane-based fuel ethanol production poses very little threat to the local economy, as the crop does not serve as a daily food like sorghum and cassava (Galadima *et al.,* 2011). Although the Nigerian Sugar Master Plan seeks to make Nigeria achieve self-sufficiency in its sugar requirements and reduce dependency on sugar imports, the country still commits between 450 and US$600 x 10^6 annually on sugar importation. The influx of cheaper refined sugar has contributed to the death of many sugarcane plantations in Nigeria (Abila, 2010). This situation has encouraged the much attention being shifted to cassava for fuel ethanol production. There is no doubt that if all the cassava and sugarcane produced in Nigeria is diverted to ethanol production (Table 4), this is capable of meeting the country's ethanol requirements (Table 3). But this is unrealistic. Experiences from the 2007 – 08 global food crisis have shown that biofuels production from food crops altered the food and feed markets by way of increasing demand for crops and placing rising prices on crops (Naylor *et al.,* 2007; Stein, 2007; Dong, 2007; Dale, 2008; de Fraiture *et al.*, 2008; Ewing and Msangi, 2008; Elliott, 2008; Sugrue, 2008; Timilsina and Shrestha, 2010; Sorda *et al.,* 2010). It is therefore likely

Table 1: Crude oil and condensate production in Nigeria

Year	2018	2017	2016	2015	2014	2013	2012	2011	2010
Average daily production (x 10^6 barrels/day)	1.92	1.89	1.83	2.12	2.19	2.19	2.27	2.37	2.45
Annual production (x 10^6 barrels/year)	699.51	690.01	670.00	773.46	798.54	800.49	852.78	866.25	896.04

Sources: (NNPC, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2018 and NNP, 2019)

Table 2: PMS situation in Nigeria

Year	2018	2017	2016	2015	2014	2013	2012	2011	2010
E10 PMS* consumption (x 10^6 m^3)	18.69	13.31	11.00	8.06	6.91	7.82	8.39	8.04	9.09
Importation (%)	96.6	90.1	89.6	95.8	90.0	78.0	83.8	27.6	87.1
Local production** (%)	3.4	9.9	10.4	4.2	10.0	22.0	16.2	72.4	12.9
Average daily consumption (x 10^3 m^3)	51.19	36.48	30.05	22.08	18.94	21.43	22.93	22.03	24.91

*Indicates Premium Motor Spirit (or Gasoline) with 10 % fuel ethanol by volume
**Nigeria has a total installed refining capacity of 445,000 barrels per day. As at the end of 2015, the country had 37 x 10^9 barrels of proven crude oil reserves, making it the second largest in Africa after Libya.
Sources: (NNPC, 2016; 2017, 2019 and U.S. Energy Information Administration, 2016)

Table 3: Ethanol demand in Nigeria

Sector	Description	Annual demand (x 10^6 m^3)	Reference(s)	Implementation status**
Transportation*	E10 PMS blend	1.3	(FGN, 2007)	On-going
Household	Ethanol-based household cooking and lighting fuel to replace kerosene fuel	3.75	[Ben-Iwo *et al.,* 2016 and Ohimain, 2010)	The programme is yet to commence
Manufacturing	Industrial use/products	0.4***	(Essiet, 2017)	On-going
Total		1.7		

*Includes stationary users such as private electricity generators, etc.
** Authors' contribution
***Between 96 and 97 % were imported into Nigeria from various sources

Table 4: Ethanol production from cassava and sugarcane in Nigeria

Crops	Production (tonnes, t) (average: 2010 - 2017)*	Area harvested (hectares, ha) (average: 2010 - 2017)*	Ethanol yield (x 10^6 m^3)	Conversion factor
Cassava	52,850,513	5,795,394.25	7.24	7.3 kg per 10^{-3} m^3**
Sugarcane	1,226,873	71,261.13	0.09	14.3 kg per 10^{-3} m^3***
Total			7.33	

*(FAO, 2019), **(Naylor, *et al.,* 2007), ***(Oshewolo, 2012), 1 t = 10^3 kg, 1 ha = 10^4 m^2

that the prices of gari and other cassava cassava-based derivatives may increase as a result of increased cassava prices. Estimates are that the cost to the gari consumers might increase by about 10 to 15 % due to higher cassava prices. Roughly two-thirds of fresh cassava produced in Nigeria are converted to gari (Amoah *et al., 2010*, Adeoti *et al*, 2009), with the remaining one-third going into industrial and other applications. Besides this, a federal government policy has also pushed for a 10 per cent inclusion of cassava in bread to reduce wheat flour import. Food prices volatility has the greatest effect on poor households (Naylor *et al*, 2007) and net-food-purchasing households (Dongl, 2007). However, while expansion may be considered an option, it is important to highlight that Nigeria's arable land is limited and the country does not even have the climate to grow cassava and sugarcane in all the agro-ecological zones due to soil, land, water, and ecosystem requirements.

In the case of Nigeria where governance structures are weak and inadequate, crop-based biofuels production may alter the prices of agricultural food crops through direct and indirect channels, exacerbating hunger and poverty. Since the use of cassava for fuel ethanol production may threaten the food security of Nigeria, looking for a replacement becomes very vital in promoting the Nigerian Biofuel Programme. This study therefore investigates the possibility of producing fuel ethanol from forest anchomanes (FA), *Anchomanes difformis (Bl.) Engl*. Being a less researched plant, the study takes a detailed look at the proximate, phytochemical and elemental properties of FA tubers with a view to suggest its use for fuel ethanol production. FA is a plant that grows in the southern part of Nigeria which at present has no food and feed value. The finding of this study is also relevant to countries in the West African sub-region where FA is found.

1.1 Existing use of FA tubers

While the medicinal use of FA (leaves, stems, and tubers) has been well reported in the literature, there is a paucity of information on the use of this plant for fuel ethanol production. FA has been used to treat cough, ulcer, dysentery, and as an eyes drop for the treatment of river blindness (Okpo *et al*., 2011). An aqueous extract of the tubers has been used to treat oedemas, kidney pains, as diuretic for the treatments of jaundice, urethral discharge, as an antidote for poison (Akinkurolere, 2007; *Olawale et al.,* 2013; Ovuakporie *et al.,* 2015; Agyare *et al.,* 2016), and has also been reported in the cure of dysentery (Oyetayo, 2007). Traditionally, FA has also been used to treat diabetes, tuberculosis, malaria, and as an oral and anal lesions (Bero *et al*., 2009, Jacob and MacDonald, 2015). Also, Jacob and MacDonald (2015) found FA to reduce serum concentrations of some sex hormones linked to pathogenesis of uterine fibroids, suggesting its possible use in uterine myomata management. As antimicrobial (Ovuakporie *et al*., 2015), powder from FA tubers has also been found to be toxic on storage beetles (Akinkurolere, 2007). Agyare *et al*. (2016) found that FA had some antioxidant and anti-inflammatory properties. The presence of phytochemicals has been held responsible for plants medicinal use (Ifemeje *et al.,* 2014 and Egwurugwu *et al.,* 2017). However, no fuel application has been reported.

1.2 Ethanol as a Fuel

Ethanol production as beverages is not new in Nigeria. It has been part of the livelihood systems of people living in the Niger Delta and those in the riverine areas of the southwestern states of Nigeria. The ethanol produced by various small-scale processors are used by local industries and consumed as liquor (Abila, 2010). However, there is no history of its use for mobility in Nigeria before 2008 when the country joined biofuel users through e-blend fuel import. Using ethanol as a fuel is potentially renewable and carbon neutral (because biofuels recycle atmospheric carbon dioxide), and can serve as a petroleum alternative that provides large-scale economic, national security and environmental benefits to Nigeria. The use of ethanol in the automobile industries is not new as it has been designated for use as a fuel from the early days (İçöz *et al.,* 2008). Globally, ethanol is the most utilised non-fossil fuel for mobility (Oke *et al.,* 2016). Between 2000 and 2017 global fuel ethanol production rose from 28 x 10^6 m^3 in 2000 *(*İçöz *et al.,* 2008*)* to 101.49 x 10^6 m^3 in 2017 (Renewable Fuels Association, 2019). In 2018, total global fuel ethanol production was 108.13 x 10^6 m^3 (Renewable Fuels Association, 2019), with Brazilian sugarcane-based fuel ethanol contributing 27.7 %, 56.2 % from the corn-based fuel ethanol in the US, 3.7 % from the corn and wheat-based fuel ethanol in China, and 5.0 % from the EU grains, molasses and sugarbeet-based fuel

ethanol (Renewable Fuels Association, 2019). Global fuel ethanol production is expected to hit 134.5 x 10^6 m^3 by 2024 (OECD/FAO, 2015).

Compared with that of PMS of 80 , ethanol has a motor octane number of 98 and has a lower vapour pressure than PMS (Adeoti, 2010). 1 m^3 of ethanol contains roughly two-thirds the energy of a 1 m^3 of PMS (Naylor *et al*, 2007; Timilsina and Shrestha, 2010; Crago and Khanna, 2013). In the PMS additives market, ethanol and methyl-tertiary butyl ether (MTBE) are close substitutes (Anderson and Elzinga, 2014). With the phasing out of MTBE (an octane booster which replaced lead) as a fuel additive due to environmental concerns, ethanol provides a good substitute for MTBE and also serves as a PMS extender and octane enhancer. Ethanol can be blended with PMS at the rates of 5, 10, and 85 % (İçöz *et al.,* 2008), as well as at 20 to 25 % (Goldemberg, 2008). There are flexible fuel vehicles which are capable of running with blends from E0 to E100 (Elliott, 2008, Goldemberg, 2008). These vehicles are common in some countries, for example, US and Brazil. Ethanol has a significant higher heat of vapourisation, which helps to provide a cooling effect, thereby lessening engine knock (Renewable Fuels Association, 2018). As noted by Goldemberg (Goldemberg, 2008), ethanol-fuelled engines are about 15 % more efficient than gasoline-fuelled engines. Adding ethanol to PMS increases the octane property of the fuel blend, thereby protecting against engine knock (Larsen, 2009). Ethanol has also been reported in the literature as capable of contributing to decrease in brake specific energy consumption of spark-ignition engines using the electronic fuel injection system (Barakat *et al*., 2015). Because ethanol contains oxygen, PMS-ethanol blends produce less carbon monoxide when compared with the conventional PMS (Ohimain, 2010). Despite these merits, ethanol also has some drawbacks. The ethanol itself could cause skin and eye irritation (Arshad *et al*., 2018), and can induce dry-corrosion of aluminum alloys (Japan Automobile Manufacturers Association, 2009). Breathing vapours of ethanol can cause headaches, dizziness and nausea (Renewable Fuels Association, 2011). Although less harmful to the environment and human health compared with formaldehyde when PMS is burnt, acetaldehydes are produced from engines using neat or blended ethanol (Goldemberg, 2008). With increasing global demand for fuel ethanol and the need to replace cassava with a plant that has no food and feed value, investigating the possibility of producing fuel ethanol from FA tubers becomes very vital to support the growth and sustainability of the Nigerian Biofuel Programme.

1.3 About the Plant, FA, Anchomanes difformis (Bl.) ENGL

The narrations presented below on FA plant were obtained from the literature and field observations conducted between September and June 2018.

FA, *Anchomanes difformis* (Bl.) ENGL, of the family of Araceae, is an herbaceous plant with a huge divided spathe leaf and prickly stem that grows from a horizontal underground tuber. The plant, which is annual, is naturally occurring in the forests of West Africa, especially in the southern guinea savanna and the humid forests. The plant is adapted to temperatures ranging from 30 to 35 °C, and to rainfall of 1.0 to over 2.0 m annually. Stem diameter, which tapers upward, can be up to 0.05 m at 0.5 m aboveground. Vertical growth (or plant height) is slow in the first 3 weeks after sprouting (between 0.05 and 0.15 m per week), and can be rapid in the last 3 to 4 weeks (between 0.4 and 1.0 m per week). Vertical growth stops when the leaves are fully spread, although an incremental vertical growth can still occur (between 0.01 and 0.03 m per week) before it finally stops after two to three weeks. It is assumed that starch accumulation begins after the full spread of leaves. From sprouting to when leaves are fully spread could vary from 6 to 8 weeks. Depending on field conditions and the number of shoots, aboveground plant height to where the stem first branched could reach 2.19 m, or more. Vertical height ranging from 0.8 to 2.0 m has also been reported (Olanlokun *et al*., 2017).

Similar to cassava (Adeoti, 2010, Akinoso and Olatunde, 2014), the fresh tubers of FA (Figure 1) do not store for long before they rot within 3 to 5 days after harvest. An important attribute of FA is the production of large horizontal underground storage tubers, which continuously accumulate without decaying. These horizontal tubers have diameters varying from 0.04 to 0.12 m, extending over 0.6 m, and weighing up to Nwachukwu and Simonyan, 2015 kg. Olanlokun *et al*. (2017) and Jacob and MacDonald (2015) have reported that the tubers could also reach 0.5 to 0.8 m long and 0.1 to 0.2 m in diameter. Depending on the methods used, peels of fresh FA could amount to 26.5 % of the fresh tubers, higher than that of cassava, 15 % (Adeoti, 2010). The stored carbohydrates in the tubers support the growth of FA plant in the beginning of raining season, usually March or April following break in dormancy. No commercial use of FA has been reported, except the traditional use of the plant to cure ailments as reported earlier. Because the plant is less researched, data on soil requirements and biomass yield (belowground and aboveground) is lacking. Information on the population of FA that have produced an appreciable stock of carbohydrates or when it begins storing starch and other fermentable carbohydrates in the tubers after sprouting is also unknown. Nonetheless, FA tubers may be harvested in 5 to 6 months, usually as from September. At present the massive underground tubers mostly cause obstruction to disc ploughs during tillage operations and hoes during heaping or ridging operations.

In order to suggest the possibility of FA tubers for fuel ethanol production, this study takes a detailed look at the elemental, proximate, phytochemical, and starch contents of FA tubers. It also examines the ethanol content

and the density of the crude FA ethanol. The need to quantitatively understand these properties of FA tubers has informed the materials and methods adopted by this study.

2. Materials and Methods

2.1 Material Sourcing and Sample Preparation

The tubers of FA used in this study were sourced from the forests around the departmental farm. The first round of tubers used was collected from three locations in September 2017 following leaf senescence. The sites for the plant were located and carefully dug out manually in its natural state in the soil to avoid damages. Tubers were harvested to a depth of 0.3 m. All tubers in each excavation were collected and weighed. The uprooted tubers were first sifted by hand and then brought to the laboratory where they were washed with water to remove attached soils and roots. It was further rinsed with water in order to properly remove any other plant and soil materials. Weight and measurement of other physical parameters were taken and recorded. To test whether the elemental, proximate, phytochemical, and starch contents varied across the horizontal length of the tubers, three sampling areas were carefully located on the tubers to represent the three stages of tuber storage development. This is because, as explained earlier, FA plants do continuously store in their tubers without decay which seems common to the Araceae family. Sample one (S1) represented the part of the tubers that was very recent, say between 1 and 2 years old. This part had yellow colour. Sample two (S2) represented the intermediate part, say between 3 and 5 years old. This part was light pink in colour, while sample three (S3) represented the part of the tubers assumed to be older than 5 years. This part was pink in colour. The age classification is rough, because of a dearth of scientific information on FA plants. However, one point to note is that the colour differences along the horizontal dimension of the tubers had only helped to suggest possible variations in the age of storage in the tubers. Since this may have implications for starch parameters, this study examines data from the three different storage areas in the tubers and links these data to when tubers were harvested.

Fig. 1: Fresh FA tubers

2.2 Elemental and Proximate Analyses of FA Tubers

The fresh samples collected in September 2017 were sliced into tiny pieces and oven dried at 65 °C to a constant weight, milled into powder and kept in air tight nylon to prevent moisture re-absorption. The samples were assayed for elemental and proximate contents. The elemental analysis was carried out at the 5SDH Tandem Accelerator Laboratory, Centre for Energy Research and Development (CERD), Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria, while the proximate analysis was carried out at the Central Research Laboratory (CRL) of the Federal Polytechnic, Ado Ekiti, Nigeria.

2.2.1 Elemental analysis

The Proton-Induced X-ray Emission (PIXE) technique was used to determine the concentration of elements in FA tuber samples. The analytical facility used a 3.0 MeV proton beam. Details of the procedure followed are as described by (Alatise *et al*., 2009).

2.2.2 Proximate analysis

Moisture content, ash, crude fat, crude fibre, protein and carbohydrate were determined using the test methods of the (Association of Official Analytical Chemists (AOAC), 1990).

2.2.2.1 pH, Moisture, Density, Starch Content, And Phytochemical Screening Of Fa Tubers

Another round of fresh FA tubers was harvested in March 2018 to carry out the pH, moisture, tuber density determination, starch content, and phytochemical screening. These analyses were carried out in the Processing Laboratory of the Department of Agricultural and Bio-Environmental Engineering (ABE), the Federal Polytechnic, Ado Ekiti, Nigeria.

pH analysis

5 g (1 g = 10^{-3} kg) of fresh FA tubers was weighed, milled and added to 20 mL (1 mL = 10^{-6} m^3) of deionised water in a test tube. The mixture was shaken for 5 min using an electric shaker, and centrifuged (G-Bosch Centrifuge model Nwachukwu and Simonyan, 2015 0D) at 4000 rpm for 5 min. The supernatant was tested for acidity and alkalinity using a digital pH meter tester (Cyber Tech PH-107 digital meter tester). pH 4.0 and pH 9.2 buffer solutions were used to calibrate the pH meter tester.

Moisture content (MC) analysis

The gravimetric method of Nwabanne (2009) was used to determine the moisture content of fresh FA tubers. The moisture content (MC) on a wet basis was obtained from Equation 1.

$$MC_{\text{wet basis}} = \frac{m_i - m_f}{m_i}\ x\ 100\% \quad (1)$$

Where, m_i is the initial mass (kg), and m_f is the final mass (kg).

Tuber density determination:

Various sections of unpeeled FA tubers were cut and weighed using an Electronic Balance (MP10001) and the volume determined using the water displacement method. The density of the unpeeled fresh FA tubers was obtained from Equation 2.

$$\text{Density} = \frac{Mass}{Volume}\ (kg/m^3) \quad (2)$$

2.3 Theoretical starch content:

The perchloric acid – anthrone reagent method as described by Hodge and Hofreiter (1962) was used to determine the starch contents of FA tuber samples. The values obtained were assumed as the maximum (or theoretical) starch content of FA tubers.

2.3.1 Phytochemical (or antinutrient) screening

Using quantitative approaches, the phytochemical (or antinutrient) properties of fresh FA tubers evaluated include total phenols, tannins, total flavonoids, phytates, oxalates, cardiac glucosides, alkaloids, and total saponins.

The Folin-Ciocalteu spectrophotometric method as described by Singleton *et al*. (1999) was used to measure the amount of phenols. The tannin content was determined according to the Folin-Ciocalteu method of Tambe and Bhambar (2014). The absorbance for test and standard solutions was measured against the blank at 725 nm using an Ultraviolet (UV)/Visible spectrophotometer. The aluminium chloride colorimetric assay described by Tambe and Bhambar (2014) was used to measure the total flavonoid content. The colorimetric method of Lolas and Markakis (1975) was used to determine the phytate content. The percentage phytic acid was calculated using Equation 3.

$$\%\ \text{Phytic acid} = \frac{Titre\ Value\ x\ 0.001 \text{Shajeela } et\ al\ 2011\ x\ 1.19}{weight\ of\ test\ sample}\ x\ 100 \quad (3)$$

The titrimetric method, which involved three major steps: digestion, oxalate precipitation, and permanganate titration, as described by Ifemeje *et al*. (2014) was used to determine the oxalate content. The amount of cardiac glycoside was determined using the aqueous sodium hydroxide - dinitro salicylic acid method of Ifemeje *et al.* (2014). Equation 4 was used to calculate the percentage cardiac glycoside.

$$\%\ Glycoside = \frac{(wt\ of\ filter\ paper + Residue) - Wt\ of\ empty\ filter\ paper}{wt\ of\ sample}\ x\ 100 \quad (4)$$

The alkaloid and total saponin contents were measured using the acetic in ethanol – ammonium hydroxide method as described by Ifemeje *et al*. (2014). The percentage alkaloid was calculated using Equation 5, while the percentage saponin was calculated using Equation 6.

$$\%\ Alkaloid = \frac{W_2 - W_1}{Weight\ of\ sample}\ x\ 100 \quad (5)$$

Where, W_1 is the weight of empty filter paper and W_2 is the weight of empty filter paper + Alkaloid

$$\%\ Saponin = \frac{W_2 - W_1}{Weight\ of\ sample}\ x\ 100 \quad (6)$$

Where, W_1 is the weight of empty filter paper, and W_2 is the weight of the filter paper + residue

2.4 Starch extraction

Starch was extracted from another round of fresh FA tubers harvested in March 2018. After cleaning as described

earlier, the fresh tubers were peeled and weighed. 500 g of peeled sample was milled into pulp using a hand-held grater. Leal, 2014 mL of water was added to the pulp, agitated for 5 min using an electric shaker, and sieved using muslin cloth. Trial experiments carried out in September 2017 revealed that the starch granules would not settle quickly in water, therefore the mixture was centrifuged (using G-Bosch Centrifuge model 800D) at 4,000 rpm for 10 min. The supernatant was decanted and the mucilage manually scrapped off. The resulting starch was dried in an oven at 50 °C until constant weight, weighed, and stored for ethanol content determination. Equation 7 was used to calculate the mean starch recovery ratio.

$$\text{Mean starch recovery ratio (\%)} = \frac{\text{ϰ}}{\text{ϒ}}\ x\ 100 \quad (7)$$

Where, ϰ is the starch obtained (kg), and ϒ is the theoretical starch content (kg)

2.5 Ethanol content

Starch is a polymer of glucose. Although the starch content in tuber crops varies, it nevertheless serves as a predictor of ethanol yield (Li *et al*., 2015). To produce ethanol, the starch present in tuber crops would have to be hydrolysed into sugar, and the sugar fermented by yeasts. Hydrolysis of starch may be done by acid or enzyme, in a continuous or batch process (Sánchez and Cardona, 2007, Leal, 2014). Since there is yet no documented process on how to produce ethanol from FA starch, a modified procedure of Ajibola *et al*. (2012), based on the batch - enzymatic process, was used. At the experimental stage, the batch process seems reasonable to use, while the continuous process may be appropriate for use at the industrial scale. Compared with acid hydrolysis, enzymatic hydrolysis consumes less energy, requires mild environmental conditions, and is less corrosive (Sarkar, 2011). The steps involved in the batch - enzymatic production of the crude FA ethanol are:

- Cooking and liquefaction (50g dry mass of FA starch was mixed with 100 mL of distilled water at room temperature to obtain slurries with 50 g solids per 150 g slurry. Slurry pH was adjusted to 6.5 using 5.0 N H_2SO_4 solution. After this, the slurry was mixed with 4 mL of α-amylase and 0.84 g of $CaCl_2$, and then liquefied in a water bath at 60 °C for 1 h with continuous stirring. After 1 h, the slurry temperature was decreased to 30 °C, and its pH adjusted to 5.0 with 5.0 N H_2SO_4 solution);
- Saccharification (6 mL aqueous solution of amylo-glucosidase was added to the liquefied starch, stirred, and incubated at 55 °C for 4 h. After 4 h, the slurry temperature was decreased to 30 °C);
- Fermentation (a culture of *Saccharomyces cerevisiae* yeast was prepared by mixing 5 g of yeast in 20 mL of distilled water at 30 °C for 20 min with continuous shaking. 5 mL was added to the filtrate before it was anaerobically incubated at 30 °C for 72 h. The commercial strain S. cerevisiae is widely used in fuel ethanol production (Quintero *et al*., 2015); and
- Distillation (although Pimentel and Patzek (2005) submitted that 3 distillations were required to obtain 95.0 % ethanol, the fermented broth was filtered using muslin cloth and the filtrate passed through the distillation unit thrice at 80 °C and once at 78 °C, since ethanol boils at 78 °C).

The two enzymes used in this study, α-amylase and amylo-glucosidase, were obtained from the Enzymology Laboratory, Department of Biotechnology, Federal Institute of Industrial Research, Oshodi, Nigeria in June 2018. The yeast, *Saccharomyces cerevisiae*, from Guangdong Guanghua Sci-Tech Co. Ltd, China, JHD®, was obtained from a chemical supplier in Nigeria. It expires on 22 August 2020. The specific gravity of the crude ethanol was measured after distillation. The value of the specific gravity was used to determine the ethanol concentration from a standard curve prepared from known concentrations of ethanol (Ado *et al*., 2009). The process performance was evaluated based on crude ethanol recovery per mass of substrate (kg-ethanol/kg-substrate). Ethanol recovery ratio was determined using Equation 8 after Li *et al*. (Li *et al*., 2015). The basic fermentation equation is as stated in Equation 9.

$$\text{Ethanol recovery ratio} = \frac{\sigma}{\eta}\ x\ 100\% \quad (8)$$

Where, σ is the crude ethanol obtained (kg), and η is the theoretical (or stoichiometric) ethanol content (kg)

$$C_6H_{12}O_6\ \text{(Glucose)} \xrightarrow{\text{Yeast}} 2C_2H_5OH\ \text{(Ethanol)} + 2CO_2\ \text{(Carbon dioxide)} + \text{Heat} \quad (9)$$

2.5.1 Density of crude FA ethanol

Apart from being an important fuel quality indicator, an accurate determination of density is necessary for the conversion of measured mass to volume at a standard reference temperature. The International Organization for Standardization (ISO) 758 test method was adopted to measure the density of the crude FA ethanol. The measurement was done at 20 °C using a 10 mL pycnometer. The density was calculated from the difference in mass between the full and the empty pycnometer and its known volume.

3. Results and Discussion

3.1 pH, moisture content, and density of fresh FA tubers

The pH values of fresh FA tubers were within the range of 6 and 6.5 (Table 5), which are comparable with most starches used in ethanol production. Similar to other tuber crops (Adeoti, 2010 Nwabanne, 2009, Nwachukwu and

Simonyan, 2015 , Adepoju and Adejumo, 2015), fresh FA tubers were also rich in moisture. This varied from 81 to Chao *et al*., 2017 per cent (Table 5). The unpeeled fresh tubers mean density, which is slightly higher than those of cassava, 10Ocloo and Ayernor, 2010 kg/m^3 (Nwachukwu and Simonyan, 2015) and sweet potato, 1104 kg/m^3 (Teye and Abano, 2012), varied from 1200 to 1400 kg/m^3 (Table 5). The dry matter contents of FA tubers ranged from 11.97 to 17.04 %. The dry matter contents of FA tubers increased from S1 to S3.

3.2 Proximate, theoretical starch content, and starch recovery

FA tubers were rich in carbohydrate (Table 6). This is quite appreciable as the dry matter of most tubers is made up of between 60 and 90 % carbohydrate. However, the low amounts of ash, crude fat, fibre and protein (Table 6) are typical of tuber crops. As illustrated in Table 7, S2 had the highest amount of starch. The theoretical starch content of FA tubers was substantial when compared with starches from other sources and higher than that of cassava (Table 8). In quantitative terms, drawing on the data in Table 7, the theoretical starch content of FA tubers varied from 0.056 to 0.095 kg kg^{-1} of fresh tubers (mean ≈ 0.074 kg kg^{-1} of fresh tubers). Table 9 illustrates the results of starch

Table 5: pH, moisture content, and density of FA tubers harvested in March 2018

Parameter	Sample		
	S1	S2	S3
pH*	6.38 ± 0.08	6.18 ± 0.26	5.88 ± 0.05
Moisture content* (%)	86.80 ± 1.76	84.00 ± 2.00	81.60 ± 1.67
Density** (kg/m^3)	1168.19 ± 21.52	1246.49 ± 21.18	1398.48 ± 20.77

*Number of replicates (N) = 5; **N = 3; Mean ± standard deviation

Table 6: Proximate composition of FA tubers harvested in September 2017 (%) (N = 3)

Sample	Moisture content	Ash	Crude fat	Fibre	Protein	Carbohydrate
S1	9.34 ± 0.07	4.39 ± 0.02	3.01 ± 0.01	3.78 ± 0.02	5.33 ± 0.03	74.15 ± 0.05
S2	8.66 ± 0.05	5.03 ± 0.03	3.72 ± 0.02	4.06 ± 0.05	3.86 ± 0.02	74.67 ± 0.04
S3	7.40 ± 0.01	7.23 ± 0.03	5.11 ± 0.02	6.31 ± 0.01	3.79 ± 0.07	70.16 ± 0.05

Differences between the carbohydrate values were non-significant at $p < 0.05$ (by one-way ANOVA and a Tukey's means test)

Table 7: Carbohydrate and starch content of FA tubers harvested in March 2018 (%) (N = 5)

Sample	Carbohydrate	Starch content
S1	74.34	62.50 ± 0.35
S2	76.46	72.80 ± 0.12
S3	72.24	70.98 ± 0.20

Differences between the starch values (S1 – S3) were significant at $p < 0.05$ (by one-way ANOVA and a Tukey's means test)

Table 8: Starch content of some agricultural crops or biomaterials

Crop	Starch content (%)	Reference(s)
Sweet potato (*Ipomoea batatas*)	20.1	(Leal, 2014)
Cocoyam *(Xanthosoma sagittifolium)*/ Taro *(Colocasia esculenta)*	25-35	(Leal, 2014 and O'Hair, 1990)
Water yam (*Dioscorea alata*)	16.7	(Leal, 2014)
Cassava (*Manihot esculenta*)	25-40	(O'Hair, 1990)
Corn (*Zea mays*)	70-72	(Shapouri *et al*., 2006)
Sorghum (*Sorghum bicolor*)	68-70	(Shapouri *et al*., 2006)
Yams (*Dioscorea spp*)	15-40	(O'Hair, 1990)
Wheat (*Triticum aestivum*)	62.0	(das Neves, *et al.,* 2006)
Brown rice (*Oryza sativa*)	60.0	(das Neves, *et al.,* 2006)
Breadfruit (*Artocarpus communis)*	67.9	(Loos *et al.,* 1981)
Acorn (or oak nut)	61.8	(Chao *et al.,* 2017)

Table 9: Starch recovery from FA tubers harvested in March 2018 (N = 5)

Sample	S1	S2	S3
Amount (kg kg^{-1} of fresh tubers)	0.046	0.055	0.039
Std dev	0.98	1.02	0.99

recovery. The obtained dry starch granules were slightly off white in colour, with no smell. Starch recovery was highest in S2 (Table 9), amounting to 0.055 kg kg^{-1} of fresh tubers, translating to starch yield of 6.5 %. The percentage starch content of S1 was the lowest possibly because of starch mobilisation for new shoot formation, while there was the possibility of starch conversion to fibre in S3 (Table 7).

As illustrated in Table 9, starch recovery from S3 was the lowest, possibly because starch granules were trapped in the fibre. As shown in Table 6, S3 had the highest fibre content. The results of starch extraction in Table 9 should be considered as indicative. It however affirms the availability of starch in FA tubers. The mean starch recovery ratio was 63.5 %. Starch recovery might have been affected by (i) grater/grating performance, (ii) sieving efficiency, and (iii) the manual removal of mucilage. These constituted activities that led to starch loss.

3.3 Theoretical (or stoichiometric) ethanol content and ethanol recovery

Drawing on the theoretical starch content data therefore, the stoichiometric ethanol content of FA varied from 0.032 to 0.054 kg kg^{-1} of fresh tubers (mean ≈ 0.042 kg kg^{-1} fresh tubers). This conversion was based on the assumption that 1 kg of starch completely hydrolysed would give 1.11 kg of glucose, and 1 kg of glucose would theoretically yield 0.511kg of crude ethanol (Li *et al*., 2015, Ocloo and Ayernor, 2010), resulting into a maximum conversion efficiency of glucose to ethanol of 51.0 % on a weight basis. The value 1.11 is the theoretical conversion factor of starch to glucose (Li *et al*., 2015, Borglum, 1980). On the average, 1 t of fresh FA tubers would yield 74.2 kg of starch or 82.4 kg of glucose. Therefore, the mean maximum ethanol yield translates to 42.11 kg t^{-1} of fresh FA tubers. The mean maximum (or stoichiometric) value does not take into account ethanol loss due to un-hydrolysed starch and glucose utilised by yeast.

The mean ethanol recovery was 0.34 kg kg^{-1} of FA starch at 96.1 % ethanol concentration. This translates to 0.34 kg per 9.93 kg of fresh FA tubers or 1 kg of fresh FA tubers yielding 34.25 x 10^{-3} kg of crude ethanol. Compared with cassava tubers (1 kg ≈ 0.08 kg of crude ethanol (Adeoti, 2010) at an average of 809.3 kg/m^3 at 20 °C), the crude ethanol from FA tubers was lower. Ethanol recovery ratio was about 60 %. Although there was a disparity between the stoichiometric ethanol content and ethanol recovery value, the recovery of ethanol indicates that the starch of FA was susceptible to enzymatic hydrolysis. The lack of agreement could be attributed to a number of factors which included the possibility of starch not getting converted into ethanol, possible variations in fermentation conditions, such as enzymes and yeast activity, and the presence of phytochemicals (for example, tannin) retarding microbial activities. Tannins are known to inhibit microbial activities (Chao *et al*., 2017, Sage *et al*., 2008), and their presence is capable of retarding the liquefaction (Li *et al*., 2015) as well as the fermentation process (Chao *et al*., 2017), thereby affecting ethanol yield. As shown in Table 10, FA tubers contained tannins and other phytochemicals (with oxalates constituting the major), some of which may get into the hydrolysis and fermentation process through the amounts contained in the starch granules. Also, enzymes concentration, temperature and time might have affected the rate of hydrolysing starch to hydrolysates (Ajibola *et al*., 2012), while some glucose might have been utilised by the yeast for the manufacture and maintenance of cell mass and in the formation of small amounts of glycerol as well as lactic acid (Borglum, 1980). However, while not a major consideration for this explorative study, understanding the optimum conditions for high ethanol production from FA tubers will require further research. At this stage, this study affirms the possibility of obtaining ethanol from FA tubers which is the aim of this study.

3.4 Density of FA ethanol

The quality of the ethanol to be blended with PMS is very important. Density is a key fuel property, which directly impacts engine performance. Fuel properties such as heating value and cetane number are related to density (Alptekin and Canakci, 2008). The injection systems of vehicles measure the fuel by volume, therefore any changes in fuel density may affect engine output power as a result of different mass of fuel injected (Alptekin and Canakci, 2008). From the analysis, the mean density of the crude FA ethanol was 801.2 kg/m^3 at 20 °C. For anhydrous ethanol, the Brazilian Ethanol Specifications Resolution ANP #19 – 2015 has specified a maximum density limit of 791.5 kg/m^3 at 20 °C. Since the value obtained was higher, the presence of water and other elements might have influenced the density of the FA ethanol. Although the ethanol obtained was not dehydrated (being outside the scope of the present study), as illustrated in Table 11, FA tubers contained some appreciable amounts of elements, though low in vanadium, nickel, and copper. Potassium, followed by calcium, was the major constituent of all samples. How much of these elements (including the phytochemicals) were contained in the crude FA ethanol is difficult to explain at this stage. However, to be used as fuel grade, apart from the need to ensure that the amounts of sulphur, phosphorus, iron, sodium, and copper in the fuel ethanol are within limits, water also needs to be removed from the crude ethanol to meet the specification limits of 1.0 % (maximum) by ASTM D 4806 or 0.3 %

(m/m) (maximum) by EN 15376 in ethanol.

3.5 FA for fuel grade ethanol production programme in Nigeria

As this study has illustrated, starch from FA offers some important opportunities. Considering the data in Tables 6 and 7, a substantial amount of carbohydrate in FA tubers was available during the dormancy period from October to March. No studies have indicated the peak period for FA carbohydrate content. This study also observed some reasonable amounts of carbohydrate in samples collected in March (Table 7), suggesting harvests could last until plant sprouts. The dormancy period had little effect on the carbohydrate contents of tubers (Tables 6 and 7). Starch from all parts of the existing stock of FA tubers appears to be useable, although S2 had the highest amount (Table 7). As a rough indication of the potential contribution of FA to fuel ethanol production in Nigeria, it is assumed one third of the currently available FA tubers could be harvested. Assuming an average conservative starch yield of 2 t ha^{-1}, conversion efficiency of 0.4 m^3 t^{-1} of FA starch, the potential ethanol production from FA would be almost 0.8 x 10^6 m^3. To become fuel grade, FA ethanol must meet the EN 15376 or the ASTM D4806 specifications. When these specifications are met, the FA ethanol can be used in most automotive spark-ignition engines without any modifications while maintaining engine performance. To realise this, future studies will need to analyse the constituents of anhydrous FA ethanol.

Despite the above, an unknown issue is whether FA can be economically exploited. This is because cultivating FA may require input costs in terms of planting, pesticides, fertilizers, and crop management. Harvesting may be expensive, because of the need to excavate the tubers. An important concern is whether existing farm and ethanol processing equipment can be easily deployed to harvest and process FA tubers, thereby escaping large investment capital. Although the complexity of fuel ethanol production process depends on the feedstock (Sánchez and Cardona, 2007), no techniques exist for processing FA tubers to fuel ethanol, making it difficult to guess these costs as well as the cost of producing 1 m^3 of crude ethanol from FA tubers. Since the properties of starch are influenced by its biological origin (Afolayan *et al*., 2012), compared with corn, potato, and cassava starches, FA starch may be problematic to extract as it does not settle quickly in water. With fuel ethanol from cellulosic biomaterials becoming increasingly popular (Abbas and Ansumali, 2010), the 3 to 6 % fibre content in the tubers (Table 6) could be available for cellulosic fuel ethanol production, thereby increasing the overall ethanol yield from FA. While it may be cheaper to produce fuel ethanol from whole FA tubers, the issue of phytochemicals (or antinutrients) becomes a factor for consideration. Similar to other tuber or root crops, such as, sweet potato (Mitiku and Teka, 2017), yam (Shajeela *et al.,* 2011), and cassava (Shajeela *et al*., 2011), FA tubers were also rich in antinutrients (Table 10). Since details about the antinutrient contents of FA starch are less clear, this suggests that future research should explore the antinutrient contents of FA starch as well as the elemental contents. For example, according to Chao *et al*. (2017), the effect of tannins on ethanol yield becomes insignificant when tannin concentration is lower than 1 kg/m^3.

Table 10: Some phytochemical properties of FA tubers harvested in March 2018 (N = 3)

Sample	Phenols (mg/mL)	Tannins (mg/mL)	Flavonoids (mg/mL)	Oxalates (mg/100g)	Phytates (%)	Saponins (%)	Alkaloids (%)	Glycoside (%)
S1	6.85 ± 0.01	3.05 ± 0.01	0.97 ± 0.01	1755.00 ± 83.11	0.53 ± 0.03	2.71 ± 0.06	7.02 ± 0.11	1.63 ± 0.11
S2	19.10 ± 0.01	4.67 ± 0.02	3.96 ± 0.01	1210.74 ± 41.65	0.34 ± 0.01	3.46 ± 0.03	6.33 ± 0.09	1.09 ± 0.05
S3	23.50 ± 0.02	4.94 ± 0.01	1.12 ± 0.01	1445.61 ± 89.90	0.41 ± 0.02	3.23 ± 0.07	5.20 ± 0.08	1.54 ± 0.06

1 mg/mL = 1 kg/m^3

Table 11: Elemental composition (ppm) of FA tubers harvested in September 2017 (N = 3)

Mineral	Sample		
	S1	S2	S3
Na	6.4 ± 1.5	4.4 ± 1.5	18.6 ± 2.8
Mg	536.3 ± 14.8	441.2 ± 14.6	2864.7 ± 30.4
Al	64.8 ± 3.6	64.7 ± 3.3	158.9 ± 7.6
Si	227.3 ± 4.1	268.8 ± 4.5	885.4 ± 8.1
P	380.8 ± 6.5	212.1 ± 6.6	610.8 ± 11.8
S	274.1 ± 4.9	190.1 ± 5.1	587.3 ± 8.0
Cl	253.3 ± 4.2	70.4 ± 3.7	1527.9 ± 8.7
K	7213.7 ± 16.6	1661.2 ± 8.6	28996.6 ± 31.9
Ca	1939.6 ± 25.6	1781.3 ± 11.2	18070.6 ± 77.7
Ti	18.7 ± 3.2	9.0 ± 2.6	53.6 ± 8.8
V	BDL	BDL	BDL
Cr	21.1 ± 3.0	17.4 ± 2.5	19.9 ± 8.1
Mn	6.9 ± 3.3	16.0 ± 2.7	112.2 ± 9.7
Fe	76.6 ± 3.2	49.6 ± 2.6	128.4 ± 6.9
Zn	3.0 ± 1.8	9.7 ± 2.7	9.1 ± 4.0
Ni	BDL	4.8 ± 1.4	BDL
Cu	BDL	1.2 ± 1.5	BDL
Sr	BDL	BDL	51.3 ± 15.2

ppm indicates parts per million
BDL indicates below detection level

Another important uncertainty is the sustainability of FA exploitation for fuel ethanol production. The existing stock of FA has accrued over years, and may possibly sustain an appreciable amount of fuel ethanol production in the short term. This standing stock however will likely be consumed within one or two years. A sustained FA production will require plant breeding and cultivation, but it seems too premature to calculate production values as the agronomic behaviour of FA plants under cultivated farming is still unknown. As a first step, research would be needed to estimate plant yield in the first year and its fermentable carbohydrate (starch, glucose, fructose, maltose, and sucrose) contents, its fuel properties (in line with EN 15376 or ASTM 4808 specifications), the climate value of fuel grade ethanol production from FA in Nigeria, and whether FA can be cultivated on marginal lands which are not suitable for agricultural food crops. A preliminary investigation has shown that FA, like yams, could be cultivated from the tubers (from plot observations made up till September 2018). Similar to the ethanol programmes in Brazil and in the US (Elliott, 2008, Goldemberg, 2008, Ohimain, 2012), to build fuel ethanol industries around FA would also require substantial subsidies to get it off the ground. This may entail government providing soft loans to processors, moving the blend wall from E10 to E15, and the development of flexible fuel vehicles to further encourage fuel ethanol use as well as removing subsidies from fossil fuels.

4. Conclusion and recommendation

The paper has shown that FA could be a useful source of starch for fuel ethanol production, capable of replacing cassava mentioned in the 2007 NBPI which has food, feed and industrial value in Nigeria. To be exploited, additional studies are required on, for example, a detailed examination of the elemental and phytochemical properties of FA starch granules, how much fuel ethanol could be obtained from a cultivated hectare of FA tubers in the first year, and whether FA could be environmentally and economically cultivated, harvested, and processed. New planting, harvesting and processing techniques may have to be introduced, and the 2007 NBPI amended, especially if FA is expected to replace cassava. Because currently available FA plants have already amassed a large stock of carbohydrates, there seems to be reasonable justification to motivate the establishment of pilot schemes to explore the production of fuel grade ethanol from FA tubers in Nigeria. This challenge can be addressed by the NNPC under its renewable energy programmes (since the Biofuel Energy Commission is yet to be established) in collaboration with relevant research institutes in Nigeria. The intensification of biofuel programmes will help Nigeria in meeting some of its international environmental obligations by cutting CO_2 emissions. In addition to this, producing fuel grade ethanol locally in Nigeria to mix with PMS will help to decrease the pump price of E10 PMS in Nigeria, currently between 1Akinkurolere, 2007 and 1Ovuakporie *et al*., 2015 x 10^3 Naira/m^3. This study is also relevant to countries in the West African sub-region where FA is found.

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