



Extraction and Characterization of Forest Anchomanes (*Anchomanes difformis* (Bl.) ENGL.) Starch for Fuel Production

Adeosun Yetunde Mayowa^{1,a}, Adeoti Olusegun^{1,b}, Adeyanju Oluremi Opeyemi^{1,c}, Fatoye Abiodun Olaniyi^{2,d}
Ogunnaike Aderoju Funmilayo^{1,e}, Oyedele Oyelayo Ajamu^{1,f,*}

¹Department of Agricultural and Bio-Environmental Engineering, The Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria

²Department of Science Laboratory Technology, The Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria

*Corresponding author

ARTICLE INFO ABSTRACT

Research Article

Received : 16/03/2022
Accepted : 09/11/2022

Keywords:

Forest anchomanes starch
Fuel ethanol
Starch extraction
Physicochemical
Elemental property

The use of cassava for fuel ethanol production in Nigeria is supported by the Nigerian Biofuel Policy and Incentives (NBPI) of 2007. Because of its food, feed and industrial value, the need to replace cassava with crops/plants that are food and feeds neutral has motivated this research. Starch was extracted from forest anchomanes (FA) (*Anchomanes difformis* (Bl.) ENGL.) tubers and some of its physicochemical and elemental properties were determined. At present, the plant is uncultivated in Nigeria and other parts of Africa where it is found. Results showed that the starch content in FA tubers varied from 72.12 to 75.83%. Starch granules from all parts of the FA tubers had similar proximate, antinutrients and elemental properties and appeared usable for fuel ethanol production. However, to suggest its potential to sustainably replace cassava, further investigations are needed beyond these initial results.

^a major4glory13@yahoo.com <https://orcid.org/0000-0003-1456-3907>
^c yemiadeyanju1@gmail.com <https://orcid.org/0000-0002-5216-2525>
^e ogunnaikeaderoju@gmail.com <https://orcid.org/0000-0001-5259-2501>

^b olusegunadeoti@yahoo.co.uk <https://orcid.org/0000-0001-6692-9064>
^d fatoye_ao@fedpolyado.edu.ng <https://orcid.org/0000-0002-0301-2942>
^f segilayo4real@yahoo.com <https://orcid.org/0000-0002-4863-5983>



This work is licensed under Creative Commons Attribution 4.0 International License

Introduction

Deciding on the choice of crop or plant for fuel ethanol production is a process involving chemistry, economics and impacts assessment. Because of its potential food security impacts in Nigeria, the need to replace cassava, a crop that has food, feed and industrial value, has necessitated research into crops/plants that are food and feeds neutral. In Nigeria, the use of cassava for fuel ethanol production is being promoted by the Nigerian Biofuel Policy and Incentives (NBPI) of 2007. Although corn-for-ethanol is not common in Nigeria due to limited corn production, at the local level cassava is still a common crop being used for ethanol production. The ethanol produced from cassava by the various small-scale processors are used by local industries and consumed as liquor.

Extracting starch from tuber and non-tuber crops is not new. For example, cassava and corn are two important crops being used for ethanol production. Starch recovery from cassava using the wet milling method varies from 15 to 25% (Fakir et al., 2012; Kaur et al., 2016; Hasmadi et al., 2021). In the case of corn, extraction yield from freshly harvested corn could be up to 18% (Wangmo et al., 2020). However, starch yield from corn treated with 0.1% sodium bisulfite (NaHSO₃)

solution could reach 59% (Paraginski et al., 2013), while those treated with 1% sodium metabisulfite solution could vary from 45.0 to 64.0% (Ji et al., 2004).

Forest anchomanes (FA) (*Anchomanes difformis* (Bl.) ENGL.), a tuber plant of the *Araceae* family, is native to the African continent (Adebayo et al., 2014; Ataman and Idu, 2015; Egwurugwu et al., 2017). The plant, which grows in the southern guinea savanna and the rain forest agro-ecological zones, can be harvested in Nigeria as from September till dormancy ends in March. Preliminary field studies revealed that the tuber, sometimes branched, could be round or ovoid, 0.04 to 0.12 m in diameter and over 0.6 m long. Tuber diameter of up to 0.20 m has also been reported in the literature (Olanlokun et al., 2017). Depending on how long it has been left on the field, FA tubers could weigh up to 80 kg. The carbohydrate composition of FA tubers can vary from 64.0 to 76.0% (Oyedele et al., 2020; Doyinsola et al., 2012). The plant has a stout, tapered upward prickly stem and can grow up to 2 m high. In Nigeria and in other parts of Africa where it is found the plant remains largely uncultivated. Being a poorly researched plant, data on plant density, yield behaviour, water use as well as general and species are sparse.

Although the plant has some important applications in traditional medicine (Ahmed, 2018; Egwurugwu et al., 2017; Oyetayo, 2007), its current usage is limited because the tuber begins to rot within 3 to 5 days after harvest. Being a tuber plant, extracting the starch after harvest will provide a more stable storage form as well as enhance its use in fuel ethanol production. The use of isolated starch may help increase fermentation efficiency and ethanol yield. However, a better understanding of the properties of FA starch is a prerequisite for suggesting its use for fuel ethanol production. Therefore, this study was undertaken to extract and characterise the properties of FA starch in order to suggest its suitability for fuel ethanol production through commercial first-generation, enzymatic hydrolysis production processes. The majority of industrially-produced ethanol from starch still uses the enzymatic hydrolysis production processes.

Methodology

Flour Preparation for Fermentable Carbohydrates

The first batch of FA tubers was excavated from farms around the Federal Polytechnic, Ado Ekiti, Ekiti State, Nigeria in March 2019. The tubers weighed 14.2 kg after washing. Cutting through a transverse section of the tubers, it was discovered that each tuber had three storage sections, differentiated by colours (Figure 1). Therefore, 1 kg each was removed from the three different storage areas found in the tubers, labelled as Sample (S) 1, S2 and S3. The portions where S1 was obtained had yellow colour, S2 light pink, and S3 pink. Because of a dearth of scientific data, S1 was reasonably assumed as that part of the tuber with recent storage, S2 as older, and S3 as the oldest storage part of the tuber. While this classification is rough, this tuber property is less reported in the literature. The 1 kg sample was peeled, cut into pieces, dried at 50°C in an oven until constant weight, and manually ground into flour for fermentable carbohydrate analysis using a laboratory mortar and pestle.

Starch Extraction

The remaining fresh tubers were separated into S1, S2, and S3 based on the colour difference highlighted above. After peeling, starch was extracted from the peeled fresh tubers the same day they were harvested as illustrated in Figure 2. The resulting dry starch cake (dried at 50 °C in an oven until constant weight) was pulverised into a fine powder. Because the starch obtained from the first round looked insufficient for the required analysis, a second round of excavation was also carried out in March 2019. The tubers obtained weighed 18.1 kg after washing. The

starch extraction procedure illustrated in Figure 2 was also followed. The dry, pulverised starch powders of S1, S2 and S3 were stored in sealed nylon bags for proximate, antinutrients and elemental analyses.

The dry starch recovery ratio was calculated using the following equation:

$$\text{Dry starch recovery ratio (\%)} = \frac{\text{recovered dry starch (kg)}}{\text{original product (kg)}} \times 100$$

Analyses

Fermentable carbohydrate contents analyses were performed on each of the flour samples obtained from the fresh FA tubers, while proximate, antinutrients and elemental contents analyses were carried out on each of the starch samples obtained from the fresh FA tubers.

Fermentable carbohydrates

Starch and free sugar contents in FA flour were measured according to the Association of Official Analytical Chemists (AOAC) test methods of 2009. These fermentable carbohydrates were selected because they are important substrates for fuel ethanol production when using the commercial first generation fuel ethanol production processes.

Proximate and antinutrient properties

The proximate (moisture content, ash, crude fat, fibre, and protein) and the antinutrient properties (hydrocyanide acid (HCN), tannins, oxalates, saponins, and phytates) of the extracted starch were determined according to the AOAC test methods of 2009.

Elemental contents

The elemental concentration analysis was performed using the Proton-Induced X-ray Emission (PIXE) technique. The ion beam analytical facility used a 3.0 MeV proton beam to determine the concentration of each of the elements present in the FA starch. The evaluation procedure followed by the facility is described in Alatisse et al. (2009). The ion beam analytical facility was obtained from the Centre for Energy Research and Development (CERD), Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria,

Statistical analysis

Laboratory analyses for the samples were carried out in triplicate, and standard deviations were reported, except for fermentable carbohydrates analysis, which was performed in duplicate. Analysis of Variance (ANOVA) and Tukey's means test were used to compare mean values between samples using SPSS version 16.0. The statistical significance level was set at 95% confidence interval (α -value = 0.05).



Figure 1a. Washed, unpeeled fresh FA tuber

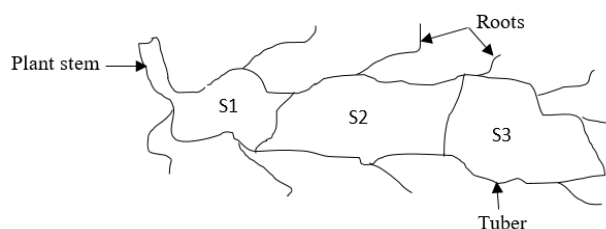


Figure 1b. Sketch illustrating where the samples were obtained in the tubers

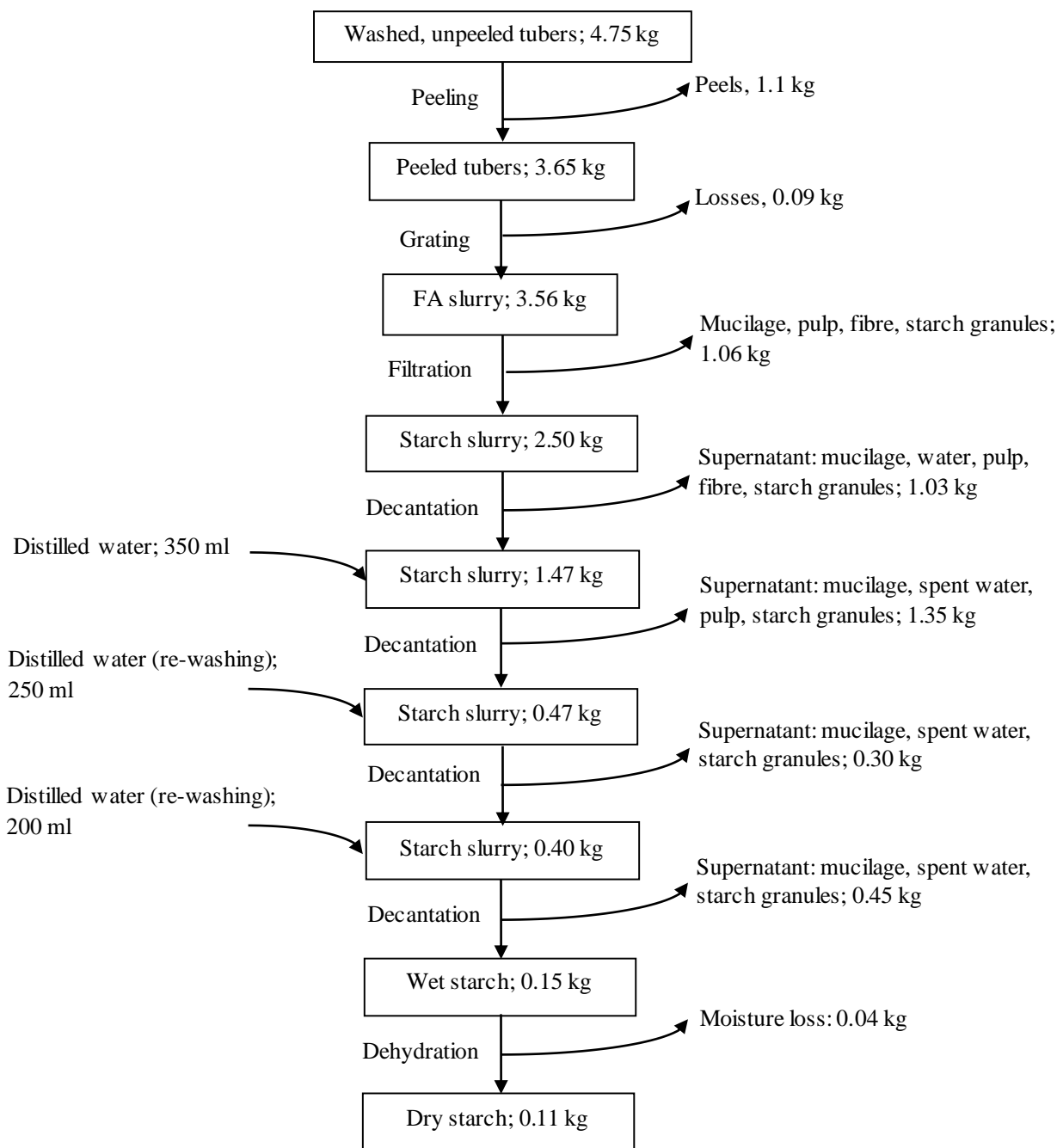


Figure 2. FA starch extraction flow diagram for S1. The extraction of starch from S2 and S3 followed the same procedure

Results and Discussion

Fermentable Carbohydrates

Carbohydrates essentially consist of both fermentable and non-fermentable carbohydrates. Fermentable carbohydrates are those capable of being processed into ethanol through a separate or simultaneous process of saccharification and fermentation effected by yeast. While simple sugars can be fermented directly, the starch needs to be broken down to simple sugars, using the acids or enzymes hydrolysis process, before it can be fermented.

The results of the fermentable carbohydrates composition of the fresh FA tubers are shown in Table 1. The fresh FA tubers contained a high amount of starch but were low in free sugars. The starch content varied between 72.12 and 75.83%, while the free sugars content varied from 5.42 to 6.92%. The starch content in FA tubers was

higher than that of cassava (*Manihot esculenta*), 25-40% (O'Hair, 1990) but comparable with that of corn (*Zea mays*), 70-72% (Shapouri et al., 2006). As illustrated in Table 1, there was no significant difference between the obtained starch values at $p > 0.05$ (by one-way ANOVA and a Tukey's means test), suggesting no difference in the amount of starch stored in the three locations found in the tubers.

Table 1. Starch and free sugar contents in FA tubers excavated in the first round (%)

Parameter	S1	S2	S3
Starch	74.56	72.12	75.83
Free sugars	6.92	5.42	6.40

Values are means of two replicates, n = 2

Starch Recovery from FA Tubers

Starch recovery was highest in S2, representing 4.95% of the unpeeled fresh tubers. This was followed by S3, 4.20%, and S1, 3.51% (Table 2). Although starch is insoluble in cold water, its recovery was influenced by the procedure used (see Figure 2) which resulted in starch loss. While the procedure further affirmed the presence of starch in FA tubers, it suggests that a more efficient extraction method should be put in place to recover starch from FA tubers. As also noted by Otegbayo et al. (2013), starches with very small granules ($< 5 \mu\text{m}$ in diameter) do not settle quickly in water during starch extraction when compared with starches with large granules. However, compared with FA tubers, starch recovery from cassava using the wet milling method could vary from 15 to 25% (Fakir et al., 2012; Kaur et al., 2016; Hasmedi et al., 2021), while 17.9% extraction yield has been reported for freshly harvested corn (Wangmo et al., 2020).

As a function of peeled fresh tubers, starch recovery in S2 amounted to 5.7%. The hexagonal FA starch granules, measuring 1 - 5 μm (Ameen et al., 2018), were smaller than those of corn, 2.3 - 19.5 μm (Mir et al., 2017) and cassava, 5 - 40 μm (Ceballos et al., 2007); the two important starch crops being used in ethanol production. As noted in this study, granule size has an impact on starch recovery if the wet milling method is followed (Figure 2). Besides this, it can also affect the gelatinisation temperature (Abdullah, 2018) as well as influence solubility, water absorption, and swelling (Chisenga et al., 2019). Small starch granule sizes are more prone to enzymatic hydrolysis due to their higher surface areas (Agyepong and Barimah, 2018).

Starch is a natural polymer of glucose. The starch content, according to Zhao et al. (2009), is a better predictor of ethanol yield. However, amylose and amylopectin are the two major constituents of starch (Garrido et al., 2012; Fallahi et al., 2016; Wu et al., 2006). The amylose/amylopectin ratio of starch has a significant impact on starch digestibility and ethanol yield. Similar to cassava starch, FA starch also exhibits an A-type diffraction pattern. A-type diffraction starch has inferior crystallinity and more susceptible to digestion by α -amylose (Ameen et al., 2018). The amylose/amylopectin ratio of FA starch (FA starch has 10.30% amylose and 90.70% amylopectin (Ameen et al., 2018) (0.11) is lower than that of cassava starch (0.2) and corn starch (0.38) (Fallahi et al., 2016; Mali et al., 2004). Since ethanol yield increases with decreasing amylose/amylopectin ratio

(Pradyawong et al., 2018; Sharma et al., 2007), the low amylose/amylopectin ratio of FA starch indicates the possibility of FA starch producing ethanol at a higher yield.

In this study, the extracted starch granules were without smell, both in wet and in dry forms and slightly off white in colour. Among the three samples, S2 had the highest dry starch yield ratio (Table 2). The reasons for this remain unknown. However, drawing on the data in Table 2, to obtain 1 kg of dry FA starch, about 23.2 kg of unpeeled, fresh FA tubers will be needed. While starch content is a key factor in suggesting the suitability of a particular plant/crop for fuel ethanol production, a more critical factor in fuel ethanol production is the efficient conversion of starch granules into ethanol. Because of its low amylose/amylopectin ratio and high starch content, FA starch can serve as a good substrate for fuel ethanol production.

Proximate Properties of FA Starch

Several factors including variety, growth, climate, soil condition, age, time of harvest, and processing methods can influence the physicochemical properties of a biomaterial. The proximate composition of the extracted FA starch is shown in Table 3. The FA starch granules contained a high amount of protein but were low in ash and crude fat. Compared with corn starch, 8.4% (Wu et al., 2006) and cassava starch, 3.03% (Gbadamosi and Oladeji, 2013), FA starch had a higher amount of protein than cassava but lower than that of corn. Provided that the yeast extract has substantial free amino nitrogen, the protein (The addition of proteases can help break down the protein to free amino acids (Bothast and Schlicher, 2004)) in the starch granules can serve as a source of free).

in the starch granules can serve as a source of free amino acids to meet yeast's growth and other fermentation requirements. The study of Zhao et al. (2009) has shown that significant relationships exist between ethanol yield and protein content.

In the case of starch moisture content, the maximum allowable limit is 15% (Achor et al., 2015). As indicated in Table 3, the obtained FA starch moisture contents were below this limit and within an acceptable range for effective starch storage without the risk of microbial contamination (Ojo et al., 2017). High moisture content is capable of reducing the shelf life of starch and encouraging microbial growth which directly affects other important starch qualities such as protein, colour, and amylose contents (Agyepong and Barimah, 2018).

Table 2. Starch recovery*

Parameter	Sample		
	S1	S2	S3
Washed, unpeeled fresh FA tubers** : mean value (kg)	3.70	5.45	5.48
Dry starch recovery from tubers: mean value (kg)	0.13	0.27	0.23
Dry starch recovery ratio (%)	3.51	4.95	4.20

*From the processing steps illustrated in Figure 2, **On the average, peels accounted for about 22.85% of unpeeled fresh tubers

Table 3. Proximate properties of the extracted starch (%)

Sample	Moisture content	Ash	Crude fat	Fibre	Protein
S1	10.56 \pm 0.12	2.29 \pm 0.07	2.28 \pm 0.06	2.62 \pm 0.03	5.31 \pm 0.02
S2	9.87 \pm 0.05	2.46 \pm 0.05	1.80 \pm 0.10	2.89 \pm 0.06	6.28 \pm 0.01
S3	10.31 \pm 0.06	2.01 \pm 0.16	2.42 \pm 0.09	2.66 \pm 0.14	5.88 \pm 0.03

Means \pm Standard deviation (SD), n = 3

In this study, the obtained values of FA starch moisture contents (Table 3) were lower than 12.94% reported by Gbadamosi and Oladeji (2013) for cassava starch and 12.6% reported by Horstmann et al. (2017) for corn starch. However, since the starch will be converted to glucose, and the glucose fermented by yeast to produce ethanol, starch granules moisture content (Table 3) has little impact on the hydrolysis and fermentation process and the resulting fuel ethanol yield. The ash content is the non-volatile inorganic matter remaining after decomposing the samples under high temperatures. The ash content in FA starch (Table 3) was higher than that of cassava, 0.32% (Ojo et al., 2017) and corn, 1.5% (Wu et al., 2006). This indicates that the FA starch had a higher amount of mineral elements in their granules than cassava and corn starches. This may influence the quality of the resulting fuel ethanol. As noted by Wu et al. (2006), fibre content has a limited effect on starch-ethanol conversion efficiency. The fibre content in FA starch (Table 3) was higher than those of cassava, 0.31% (Chisenga et al., 2019) and corn, 2.0% (Wu et al., 2006).

As revealed in Table 3, there was no significant difference between the samples at p -value > 0.05 (by one-way ANOVA and a Tukey's means test). This suggests that starch granules from all parts of the FA tubers appeared to possess similar proximate properties.

Antinutrients Properties of FA Starch

Antinutrients are microbial inhibitors, capable of inhibiting the hydrolysis and fermentation process. The results of the antinutrients screening of FA starch granules are presented in Table 4. The amounts of tannins and phytates were higher in all the samples, followed by HCN. The HCN content in FA starch was lower than the value reported for cassava starch, 76.3 mg/100g (Cuvaca et al., 2015), while the tannins content in FA starch was higher than that of cassava starch, 1.3 mg/kg (Oladebeye, 2007). However, according to Chao et al. (2017), for tannins not to inhibit the hydrolysis and fermentation process, its

concentration should not exceed 1.0 mg/L. For HCN, Cuzin and Labat (1992) report that its concentration should not exceed 6 mg/L. Considering the foregoing information and the data in Table 4 together, this suggests that measures would have to be put in place to especially lower the antinutrient concentrations in FA starch granules. However, as shown in Table 4, there was no significant difference between the antinutrients properties of the FA starch samples since $P > 0.05$ (by one-way ANOVA and a Tukey's means test). Therefore, because of the similarities in their antinutrients properties, starch granules found in any parts of the FA tubers may require some additional processing to enhance their suitability for fuel ethanol production. As revealed by Afolayan et al. (2012), FA starch gelatinises at 72 °C; the temperature at which the starch granules begin to swell irreversibly. However, with antinutrients having lower boiling points [for example, HCN evaporates at 26 °C (Montagnac et al., 2008)], this suggests that cooking during the process of starch hydrolysis may help reduce FA starch antinutrients concentrations. Thermal processing has been reported in the literature to reduce antinutrient contents (Akhtar et al., 2011). However, by how much are the antinutrients in FA starch may be reduced during cooking (or heat treatment) requires further investigations.

Elemental Contents of FA Starch

Another important consideration is the quality of the dehydrated ethanol to be blended with Premium Motor Spirit (PMS) or gasoline. After the fermentation - distillation process, it is possible that some elements in FA starch may remain in the dehydrated fuel ethanol in small quantities. Depending on their concentrations, this may impact the quality and the density of the resulting dehydrated fuel ethanol. However, at low concentrations, their effect may be minimal. Drawing upon relevant guidelines (Table 5), the presence of sulphur, phosphorus, and metallic ions such as sulfate, chloride, iron, sodium, and copper requires careful consideration when suggesting FA starch for fuel ethanol production.

Table 4. Antinutrients composition of the extracted FA starch (mg/kg)

Sample	HCN	Tannins	Saponins	Oxalates	Phytates
S1	8.16 ± 0.01	21.92 ± 0.12	3.75 ± 0.10	6.94 ± 0.39	21.68 ± 0.34
S2	6.48 ± 0.03	32.78 ± 0.06	2.92 ± 0.17	6.00 ± 0.08	32.85 ± 0.40
S3	10.14 ± 0.00	21.59 ± 0.03	3.55 ± 0.07	6.91 ± 0.04	31.59 ± 0.54

Means ± SD, n = 3

Table 5. Elements and maximum limits in fuel ethanol imposed by various guidelines

S/No.	Maximum limit/Element	Guideline
A	10.0 mg/kg for sulphur	EN 15376: 2014; EPA Tier 3 Regulations
B	1.3 mg/L for phosphorus	CAN/CGSB 3.516-2017
	0.15 mg/L for phosphorus	EN 15376: 2014
c	5 mg/kg for iron	ANP #19-2015
d	2.0 mg/kg for sodium	ANP #19-2015
e	0.1 mg/kg (or 0.08 mg/L) for copper	ASTM D4806-16a; EN 15376: 2014
f	1.5 mg/kg for inorganic chloride	EN 15376: 2014
	6.7 mg/kg (or 5.0 mg/L) for inorganic chloride	ASTM D4806-16a
	4.0 mg/kg (or 3.2 mg/L) for existent sulfate	ASTM D4806-16a
	3.0 mg/kg for existent sulfate	EN 15376: 2014
g	0.7915 g/mL at 20 °C for anhydrous ethanol	ANP #19-2015
	0.8076 - 0.8110 g/mL at 20 °C for hydrated ethanol	ANP #19-2015

Table 6. Some elemental contents in the extracted FA starch (ppm)

Element	Sample S1	S2	S3
Na	13.6 ± 3.8	-	96.9 ± 16.3
Mg	58.7 ± 11.8	59.4 ± 11.7	309.5 ± 14.5
Al	10.4 ± 0.2	6.3 ± 0.2	38.4 ± 0.32
Si	38.3 ± 0.4	21.9 ± 0.3	140.3 ± 0.6
P	214.6 ± 7.3	236.4 ± 7.5	239.6 ± 10.1
S	192.5 ± 8.5	118.3 ± 9.5	171.5 ± 8.7
Cl	31.1 ± 4.4	43.8 ± 4.7	67.5 ± 3.8
K	443.3 ± 4.3	358.9 ± 4.4	467.4 ± 4.0
Ca	415.8 ± 2.8	227.4 ± 2.3	2944.5 ± 5.3
Sc	0 ± 0.0	5.5 ± 1.9	33.4 ± 17.2
Ti	3.6 ± 1.1	4.3 ± 1.2	297.8 ± 3.7
V	-	-	0 ± 0.0
Cr	3.0 ± 1.1	-	-
Mn	10.9 ± 1.8	-	-
Fe	22.6 ± 0.8	14.1 ± 0.8	38.4 ± 1.2
Zn	-	-	-
Ni	-	-	-
Cu	-	-	-
Sr	-	-	-

Means ± SD, n = 3; - indicates Below Detection Limit

The data in Table 6 revealed that FA starch granules contained some amounts of sulphur, phosphorus, and chloride, but were low in copper and other metallic ions such as sodium and iron. More importantly, the burning of fuel containing sulphur may lead to the emissions of sulphur oxides and the poisoning of post-treatment devices (Schinas et al., 2008). As a potent catalyst, phosphorus can cause exhaust emissions to rise (Worldwide Fuel Charter Committee (WFCC), 2009), while the presence of metallic ions can promote corrosion, cause injector deposits as well as failure in the vehicle fuel line (WFCC, 2009). Another important fuel property is density, which directly affects engine performance. Cetane number and heating value are fuel properties that are directly related to density (Alpekin and Canakci, 2008). For vehicles using the fuel injection systems, any changes in fuel density may impact engine output power as a result of the different masses of fuel injected (Alpekin and Canakci, 2008). For these reasons, various guidelines have imposed maximum limits in the fuel ethanol to be blended with PMS as illustrated in Table 5.

As shown in Table 6, potassium and calcium were the dominant elements in all the samples, while the concentrations of zinc and copper were below the detection limit of the test method used. Compared with corn (For yellow corn: phosphorus (130.00 mg/kg), sodium (90.00 mg/kg), and iron (4.7 mg/kg), and copper (0.5 mg/kg) (Gwirtz and Garcia-Casal, 2013) and cassava (For cassava: phosphorus (434.40 mg/kg), sodium (103.40 mg/kg), and iron (1.10 mg/kg) (Note: data on corn and cassava starch sulphur contents were hard to find), and copper (not detected)) (Oladebeye, 2007), the sodium content in FA starch was lower, while the iron content was higher. The phosphorus content in FA starch was higher than that of corn but lower than that of cassava starch. With mixed results, it can be reasonably argued that ethanol derived from FA starch may require additional processing to ensure its safe use in automotive spark-ignition engines. This is because how much elements in FA starch will be retained in the dehydrated fuel ethanol remains unknown. As noted,

literature detailing information on how much antinutrients and elements in corn and cassava starches are retained in the dehydrated fuel ethanol is scarce. The absence of this information has made it difficult to gauge at this initial stage how much antinutrients and elements in FA starch from existing stocks may remain in the resulting dehydrated fuel ethanol. However, being rich in starch (Table 2) if the plant is cultivated yearly like corn and cassava this may likely have a significant reduction effect on the elemental concentrations in FA starch. According to Oladebeye (2007), age and soils on which plants are cultivated have important influence on the concentrations of elements and antinutrients in plants/crops. Except for sulphur and to some extent iron, the concentrations of sodium and phosphorous were lower in S1 than in other samples (Table 6). However, as shown in Table 6, there was no significant difference between the samples at P value > 0.05 (by one-way ANOVA and Tukey's means test). This suggests that the location of starch granules in the tubers had little influence on its elemental contents.

Therefore, considering the data in Tables 1 to 6 together, this study observed that: one, because of its low amylose/amylopectin ratio starch found in FA tubers offered some important opportunities for fuel ethanol production through commercial first-generation ethanol production processes; two, starch from all parts of the FA tubers appeared to have similar physicochemical and elemental properties and usable for fuel ethanol production; and three, the starch granules in FA tubers may require additional processing in light of the maximum limits imposed by various guidelines vis-à-vis the elemental and antinutrients contents in FA starch. This additional processing will help to minimise the impact of the resulting fuel ethanol on engine performance, emissions, fuel economy, and driveability. At this initial stage, information on tuber production after a year of planting and its physicochemical and elemental properties remains thin.

Study Limitation

Notwithstanding its merits, this study has two important limitations that worth pointing out to readers. One, the wet milling starch extraction method used in this study resulted into very low starch recovery from the FA tubers. Although some other studies have reported extraction yields of 12.25% (Ameen et al., 2018) and 16% (Abe and Lajide, 2014), this suggests that more efficient methods leading to a higher starch yield may be required. For example, starch yield of 21% from fresh FA tubers treated 1% w/v sodium metabisulphite has been reported (Afolayan et al., 2012). Two, for reasons of a lack of resources and laboratory equipment, the age of the excavated tubers was not measured. This may have important implications for the proximal composition of the tubers. According to Gomez and Valdivieso (1985), fibre and protein are the two proximal properties that vary the most with plant age.

Conclusions

Despite its limitations, it may be concluded that: one, FA tubers were rich in starch, between 72.12 and 75.83%; and two, starch granules from all parts of the FA tubers possessed similar physicochemical and elemental properties at the statistical level of $p > 0.05$. Drawing on the data obtained, the FA starch appeared usable for fuel ethanol production. This is the initial recommendation that can be made to intending fuel ethanol processors as well as to policymakers in Nigeria and elsewhere where FA is found. To increase starch suitability for use as fuel ethanol, plant breeders will have to develop new and improved FA hybrids with lower antinutrients and elemental contents, and higher extractable starch contents. Since the available stock of FA tubers remains largely uncultivated, future research should target investigating plant yield per hectare, water use under cultivated agriculture as well as the properties of the resulting fuel ethanol. At this stage, to suggest whether FA starch can sustainably replace cassava starch in the Nigerian fuel ethanol industry will benefit from a detailed comparative analysis beyond this initial recommendation.

Acknowledgements

Funding from TETFund, Nigeria is gratefully acknowledged. The technical assistance of Adebisi D. Adenifosi, Serah O. Olasehinde, Nelson O. Jegede, and Tosin M. Akinsuyi is gratefully acknowledged. Huge thanks to CERD, OAU, Ile-Ife, Osun State, Nigeria for the PIXE analysis and Banji S. Awe for the statistical analysis.

References

Abdullah AHD, Chalimah S, Primadona I and Hanantyo MHG. 2018. Physical and chemical properties of corn, cassava, and potato starch. IOP Conference Series: Earth and Environmental Science 160, 012003, doi:10.1088/1755-1315/160/1/012003

Abe TO and Lajide L 2014. Characterization of starch from hot water treated and untreated *Anchomanes difformis* rhizome. IOSR j. appl. chem 7 (6): 50-57

Achor M, Oyeniya JY, Musa M and Gwarzo MS 2015. Physicochemical properties of cassava starch retrograded in alcohol. J App Pharm Sci, 5 (10): 126-131, doi:10.7324/JAPS.2015.501021

Adebayo AH, John-Africa LB, Agbafor AG, Omotosho OE and Mosaku TO. 2014. Anti-nociceptive and anti-inflammatory activities of extract of *Anchomanes difformis* in rats. Pak J Pharm Sci, 27 (2): 265-270

Afolayan MO, Omojola MO, Onwualu AP and Thomas S. 2012. Further physicochemical characterization of *Anchomanes difformis* starch. Agric Biol J N Am, 3 (1): 31-38

Agyepong JK and Barimah J. 2018. Physicochemical properties of starches extracted from local cassava varieties with the aid of crude pectolytic enzymes from *Saccharomyces cerevisiae* (ATCC 52712). Afr J Food Sci, 12 (7): 151-164, doi:10.5897/AJFS2018.1701

Ahmed HA. 2018. *Anchomanes difformis*: A multipurpose phytomedicine. IOSR j. pharm. biol. sci 13 (2/111): 62-65

Akhtar MS, Israr B, Bhatti N and Ali A. 2011. Effect of cooking on soluble and insoluble oxalate contents in selected Pakistani vegetables and beans. Int J Food Prop, 14 (1): 241-249, doi:10.1080/10942910903326056

Alatise OI, Obiajunwa EI, Lawal OO and Adesunkanmi ARK. 2009. Particle-Induced X-ray Emission (PIXE) analysis of minor and trace elements in gallstones of Nigerian patients. Biol Trace Elem Res, 134:13-24

Alptekin E and Canakci M. 2008. Determination of the density and viscosities of bio-diesel-diesel fuel blends. Renew Energy, 33: 2623-2630

Ameen OM, Olatunji GA, Abdulrahman AA, Adenusi BF, Folorunsho OF, Okeola OF and Samoh FT. 2018. Physicochemical properties of starch obtained from tubers of *Anchomanes difformis* and *Tacca involucrate*. Centrepoint Journal 24 (2): 67-80

Ataman JE and Idu M. 2015. Renal effects of *Anchomanes difformis* crude extract in wistar rats. Avicenna J Phytomed, 5 (1): 17-25

Bothast RJ and Schlicher MA. 2004. Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biotechnol, 67: 19-25, doi: 10.1007/s00253-004-1819-8

Ceballos H, Saánchez T, Morante N, Fregene M, Dufour D, Smith AM, Denyer K, Peáñez JC, Calle F and Mestres C. 2007. Discovery of an amylose-free starch mutant in cassava (*Manihot esculenta* Crantz). J Agric Food Chem, 55: 7469-7476, doi:10.1021/jf070633y

Chao B, Liu R, Zhang X, Zhang X and Tan T. 2017. Tannin extraction pretreatment and very high gravity fermentation of acorn starch for bioethanol production. Bioresour 241: 900-907

Chisenga SM, Workneh TS, Bultosa G and Laing M. 2019. Characterization of physicochemical properties of starches from improved cassava varieties grown in Zambia. AIMS Agric. Food 4 (4): 939-966, doi:10.3934/agrfood.2019.4.939

Cuvaca IB, Eash NS, Zivanovic S, Lambert DM, Walker F and Rustrick B. 2015. Cassava (*Manihot esculenta* Crantz) tuber quality as measured by starch and cyanide (HCN) affected by nitrogen, phosphorus, and potassium fertilizer rates. J. Agric. Sci 7 (6): 36-49, doi:10.5539/jas.v7n6p36

Cuzin N and Labat M. 1992. Reduction of cyanide levels during anaerobic digestion of cassava. Int. J. Food Sci. Technol 27: 329-336

Doyinsola I, Adedayo A, Olutayo O, Michael A, Abayomi O, Moses O and Thomas S. 2012. Phytochemical, antioxidant and cytotoxicity properties of *Anchomanes Difformis* (Bl.) Engl. tuber extract. J. Appl. Chem 8 (3): 173-181

Egwurugwu JN, Ohamaeme MC, Chinko BC, Ebuanyi MC, Akunneh-Wariso CC, Ngwu EE, Ugwuezumba PC and Ezekwe SR. 2017. Effects of extracts of anchomanes difformis on haematological parameters of albino wistar rats. Int Res J Medical Sci, 5 (3): 1-6

- Fakir MSA, Jannat M, Mostafa MG and Seal H. 2012. Starch and flour extraction and nutrient composition of tuber in seven cassava accessions. *J Bangladesh Agril Univ*, 10 (2): 217-222
- Fallahi P, Muthukumarappan K and Rosentrater KA. 2016. Functional and structural properties of corn, potato, and cassava starches as affected by a single-screw extruder. *Int. J. Food Prop* 19 (4): 768-788, doi:10.1080/10942912.2015.1042112
- Garrido LH, Schnitzler E, Zortea MEB, de Souza Rocha T and Demiate IM. 2012. Physicochemical properties of cassava starch oxidized by sodium hypochlorite. *J Food Sci Technol*, doi:10.1007/s13197-012-0794-9
- Gbadamosi SO and Oladeji BS. 2013. Comparative studies of the functional and physico-chemical properties of isolated Cassava, Cocoyam and Breadfruit starches. *Int. Food Res. J* 20 (5): 2273-2277
- Gwartz JA and Garcia-Casal MN. 2013. Processing maize flour and corn meal food products. *Ann NY Acad Sci*, 1312: 66–75, doi:10.1111/nyas.12299
- Gómez G and Valdivieso M. 1985. Cassava foliage: Chemical composition, cyanide content and effect of drying on cyanide elimination. *J Sci Food Agric*, 36: 433-441
- Hasmadi M, Harlina L, Jau-Shya L, Mansoor AH, Jahurul MHA and Zainol MK. 2021. Extraction and characterisation of cassava starch cultivated in different locations in Sabah, Malaysia. *Food Res.* 5 (3): 44-52, doi:10.26656/fr.2017.5(3).550
- Horstmann SW, Lynch KM and Arendt EK. 2017. Starch characteristics linked to gluten-free products. *Foods* 6, 29, doi:10.3390/foods6040029
- Ji Y, Seetharaman K and White PJ. 2004. Optimizing a small-scale corn-starch extraction method for use in the laboratory. *Cereal Chem*, 81 (1): 55-58
- Kaur K, Ahluwalia P and Singh H. 2016. Cassava: Extraction of starch and utilization of flour in bakery products. *Intl J Food Ferment Technol*, 6 (2): 351-355, doi:10.5958/2277-9396.2016.00059.3
- Mali S, Karam LB, Ramos LP, and Grossmann MVE. 2004. Relationships among the composition and physicochemical properties of starches with the characteristics of their films. *J. Agric. Food Chem.* 52 (25): 7720–7725
- Mir SA, Bosco SJD, Bashir M, Shah MA and Mir MM. 2017. Physicochemical and structural properties of starches isolated from corn cultivars grown in Indian temperate climate. *Int. J. Food Prop.* 20 (4): 821–832
- Montagnac JA, Davis CR and Tanumihardjo SA. 2008. Processing techniques to reduce toxicity and antinutrients of cassava for use as a staple food. *Compr. Rev. Food Sci. Food Saf.* 8: 17-27
- O'Hair SK. 1990. Tropical root and tuber crops. <https://hort.purdue.edu/newcrop/proceedings1990/v1-424.html> (accessed 24 July 2018)
- Ojo MO, Ariahu CC and Chinma EC. 2017. Proximate, functional and pasting properties of cassava starch and mushroom (*Pleurotus Pulmonarius*) flour blends. *Am. J. Food Technol.* 5 (1): 11-18, doi:10.12691/ajfst-5-1-3
- Oladebeye AO. 2007. Physicochemical properties of starches of some tropical tubers. M.Tech thesis in Analytical Chemistry. The Federal University of Technology, Akure, Ondo State, Nigeria.
- Olanlokun JO, Babarinde CO and Olorunsogo OO. 2017. Toxicity of *Anchomanes difformis*, an antimalarial herb in murine models. *European J Med Plants* 20 (3): 1-13
- Otegbayo B, Oguniyan D and Akinwumi O. 2013. Physicochemical and functional characterization of yam starch for potential industrial applications. *Starch/Stärke* 65: 1-16, doi:10.1002/star.201300056
- Oyedele OA, Adeoti O and Adeyanju OO. 2020. An investigation into the possibility of producing fuel ethanol from Forest *Anchomanes (Anchomanes Difformis (Bl.) ENGL.)*. *Int. J. Energy Technol. Policy.* 10 (1): 21-35, doi:10.7176/JETP/10-1-03
- Oyetayo VO. 2007. Comparative studies of the phytochemical and antimicrobial properties of the leaf, stem and tuber of *Anchomanes difformis*. *Pharmacol Toxicol*, 2 (4): 407-410, doi:10.3923/jpt.2007.407.410
- Paraginski RT, Vanier NL, Moomand K, de Oliveira M, da Rosa Zavareze E, e Silva RM, Ferreira CD and Elias MC. 2013. Characteristics of starch isolated from maize as a function of grain storage temperature. *Carbohydr. Polym.* 102: 88-94, doi:10.1016/j.carbpol.2013.11.019
- Pradyawong S, Juneja A, Sadiq MB, Noomhorm A and Singh V. 2018. Comparison of cassava starch with corn as a feedstock for bioethanol production. *Energies* 11, 3476; doi:10.3390/en11123476
- Schinas P, Karavalakis G, Davaris C, Anastopoulos G, Karonis D, Zannikos F, Stournas S and Lois E. 2008. Pumpkin (*Cucurbita pepo L.*) seed oil as an alternative feedstock for the production of biodiesel in Greece. *Biomass and Bioenergy* 33: 44-49
- Shapouri H, Salassi M and Fairbanks JN. 2006. The economic feasibility of ethanol production from sugar in the United States. U.S. Department of Agriculture, Washington.
- Sharma V, Rausch KD, Tumbleson ME and Singh V. 2007. Comparison between granular starch hydrolyzing enzyme and conventional enzymes for ethanol production from maize starch with different amylose:amylopectin ratios. *Starch/Stärke* 59: 549–556
- Wangmo K, Tshering D, Lhamo S and Wangdi T. 2020. Determination of starch content in green maize cobs and its product development. *Bhutanese Journal of Agriculture* 3 (1): 30-39
- WFCC. 2009. Ethanol guidelines, First edition. <http://oica.net/wp-content/uploads/ethanol-guideline-final-26mar09.pdf> (accessed 21 September 2018)
- Wu X, Zhao R, Wang D, Bean SR, Seib PA, Tuinstra MR, Campbell M. and O'Brien A.. 2006. Effects of amylose, corn protein, and corn fiber contents on production of ethanol from starch-rich media. *Cereal Chem*, 83 (5): 569-575, doi:10.1094/CC-83-0569
- Zhao R, Wu X, Seabourn BW, Bean SR, Guan L, Shi Y-C, Wilson JD, Madl R and Wang D. 2009. Comparison of waxy vs. nonwaxy wheats in fuel ethanol fermentation. *Cereal Chem*, 86 (2): 145–156, doi:10.1094/CCHEM-86-2-0145