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(RESEARCH ARTICLE)

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Comparative evaluation of glucose and ethanol yield from *Icacina trichantha oliv* and *Anchomanes difformis blumel* as non-edible feedstocks

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Abstract

This work examined the comparative potential of starch and flour obtained from two non-edible tuberous plants Anchomanes difformis blume (ADB) and Icacina trichantha oliv (ITO) for glucose and bioethanol production. Acid hydrolysis using sulfuric and hydrochloric acids, alongside enzymatic hydrolysis using α-amylase and amyloglucosidase, was employed to convert the biomass into fermentable sugars. The optimal glucose yield from acid hydrolysis was recorded with 0.25 M sulfuric acid at 120 °C for 45 minutes, with ADB starch yielding 28.41 g/L, the flour yielded 20.69 g/L and ITO starch yielding 22.09 g/L while flour yielded 20.42g/L. Fermentation using Saccharomyces cerevisiae showed maximum ethanol yields of 9.6 g/L and 8.92 g/L from ADB and ITO, respectively. While starch samples consistently yielded higher glucose and ethanol concentrations, flour samples demonstrated greater economic efficiency due to reduced processing requirements and higher net output per gram of raw biomass. Enzymatic hydrolysis further enhanced yields, with ADB and ITO achieving up to 26.09 g/L and 23.62 g/L ethanol, respectively. Overall, both ADB and ITO, particularly in flour form, offer viable alternatives to conventional starch feedstocks for sustainable bioethanol production.

Keywords: *Icacina Trichantha Oliv* (ITO) And *Anchomanes Difformis Blumel* (ADB); Non-Edible Starches; Acid Hydrolysis; Enzymatic Hydrolysis; Bioethanol

1. Introduction

Though Climate change and energy security are key issues necessary for large scale replacement of petroleum-based fuels with renewable once, the use of biofuels improve vehicle efficiencies such as the octane number [1-3]. Bioethanol is largely used as an oxygenate fuel additive. The addition of bioethanol in conventional fuel enhances the octane number and lessens the use of toxic, octane enhancing additives like methyl tertiary butyl ether [3-4]. The oxygen content in bioethanol molecules helps in reducing CO_2 emission and non-combusted hydrocarbons. Predictions have been made that bioethanol is 15% higher in efficiency than fossil fuel (gasoline) in optimized spark-ignition engines and almost equal to diesel in overall transport efficiency of compression-ignition engines [3]. It is also predicted that a given volume of ethanol can make available enough energy to drive almost 75-80% of the distance equivalent that of gasoline, even though bioethanol only contains around two-thirds of the energy content [5]. The high cost associated with bioethanol production has been an issue restraining the intensive use of ethanol as an oxygenate in gasoline. In this regard, governments have made several subsidies to encourage the continuous production of bioethanol in order to meet the global mandate of 10% bioethanol blend in gasoline [6]. Hydrolysis of biomass for the release of sugars can be carried out using either acid or enzyme process. Acid conversion technologies are energy intensive due to high heat requirement at temperatures in the range of100 ºC - 200 ºC and large amount of water usage; in addition, the conversion route gave rise to inhibitors that are toxic to the bio-refining processes. The use of enzymes in conversion processes

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often eliminate the high energy consumption, toxic chemicals and extremes of pH, while at the same time offers increased reaction specificity, product purity and reduced environmental impact [7].

Corn, cassava and wheat are the most utilized starch-containing feedstocks in the production of bioethanol worldwide. The enormous starch content (60-90% DM) of these biomasses makes them potential raw materials for ethanol production [8]. Several challenges have been encountered in biofuels (bioethanol) production from different food crops. These challenges are in the area of (a) food security (b) edible crops utilization (c) production cost (d) conversion technologies. To make bioethanol a sustainable and cost-effective alternative to gasoline, it is crucial to source for non-edible crops with little or no economic value but have high carbohydrate content and used as feedstock for ethanol production [3].

Icacina trichantha oliv (ITO) from the family of Icacinacea is a small perennial shrub with alternate leave that grow up to 2-3m above the ground. The plant is indigenous to central and west Africa and is predicted to have high resistance to drought where it can survive at least four years without rain. It produces a large underground tuber which can weigh up to 3kg in southern part of Nigeria. The tuber is not edible, the plant is reported as weed of rice paddies in Edo state, Nigeria. ITO is known by different tribes in the southern part of Nigeria, it is called gbe-gbe which translate into "throw away" by the Yorubas in south-west Nigeria, Urumbia, ofo-ala and Eriagbo (which means eat and vomit) by the Igbo speaking tribe in eastern Nigeria. ITO tuber is yet to be included as a conventional source of starch, but studies has shown that it contains a high carbohydrate content in the range of 71.13-91.93% [9-10].

Anchomanes difformis (blume) Engl (ADB) is a wild herbaceous plant that belong to the family of Araceae. It is characterized with prickly stem with large divided leaves and spathe that sprout from a horizontal tuber. The plant grows about 2meters from the ground surface with its tuber measuring about 80cm long and 20cm wide. The tuber is slightly off white in color and has high carbohydrate content of 88.9%. The tuber is not edible in Nigeria especially in southern region where it grows as a wild plant because it causes irritation to the mouth, tongue and throat if eaten. The plant is known as abrisoko by the yorubas in south west Nigeria [11].

During the present study, the flour and starch extracted from tubers of Icacina trichantha oliv (ITO) and Anchomanes difformis Blume (ADB), which are not desired for food in Nigeria would be used as a biomass to evaluate their potential for bioethanol production.

2. Materials and Methods

The tubers of ITO and ADB as shown in Figure 1 were gathered from a farm in University of Port Harcourt, Choba, Rivers State, Nigeria. The tubers were peeled, washed, cut in pieces and milled for starch extraction t. Every reagent that was used was analytical grade. The enzymes were acquired from Sigma-Aldrich Germany: dry active yeast (saccharomyces cerevisiae), amyloglucosidase (10115-1G-F; with amyloglucosidase activity of 70U/mg), and α-amylase (10065-10G; with alpha amylase activity of 30 U/mg). Analytical-grade sulfuric acid (95–97%), potassium dichromate (99.5%), and other regents were purchased from BDH Chemicals in England.

Figure 1 (a) ADB tuber (b)ITO tuber

2.1. Starch Extraction and Characterization

ITO and ADB starch extractions were carried out in accordance with the procedures described by [11]. The Association of Official Analytical Chemists [12] standard was used to calculate the starches' proximate content. The calorimetric iodine affinity technique was used to assess the starches' amylose concentration [11].

2.2. Experimental Design

The experimental design was carried out by adopting the method described by [13].

2.2.1. Acid hydrolysis procedure

Acid hydrolysis of the flour and starch was carried out in a hydrolysing vessel (autoclave model TT-280A, Techmel, U.S.A). Starch slurry of 1:20 substrates to liquid ratio was made in a 250 ml apparatus at an acid (H_2SO_4) concentration of 0.25-0.75 moldm-3 and the mixture transferred into the autoclave for hydrolysis at 121oC for the period of 15-60 minutes. The resultant solution was analysed for total reducing sugar (TRS) concentration using DNS method [13].

2.2.2. Enzymatic Hydrolysis procedure

The conditions of the hydrolysis reaction of the starch samples are: α-amylase (60-180 units/g starch), amyloglucosidase (140-420 units/g starch), and substrate concentration (15%W/V). A water bath with a shaker (Shz-88 Thermostatic Digital Shaking Water Bath) was used for the hydrolysis. At a substrate to liquid ratio of 1:10, the starches were first gelatinized for 10 minutes at 90ºC. After that, the mixture was liquefied for one hour at 75ºC using 2 ml of various α-amylase activity (60–180 units/g starch). At the conclusion of the procedure, 1% HCl was used to bring the pH down to 5.0 and lower the medium temperature to 55ºC. In addition, 2 ml of various amyloglucosidase activity (140–420 units/g starch), was added and saccharification was continued for an additional three hours at 55ºC. The enzyme was deactivated by raising the medium temperature to 100 ºC. At this point, the solution was allowed to cool and the residue removed by filtration [13].

2.2.3. Fermentation and Ethanol determination

In a media consisting of yeast extract (2 g/L), glucose (2 g/L), peptone (3 g/L), and distilled water (1000 ml), the hydrolysate was fermented with Saccharomyces cerevisiae (1 g/L). $MgSO_4.7H_2O$ (1 g/L), NH_4Cl (1 g/L), KH_2PO_4 (2 g/L), and $CaCl_2$ (0.1 g/L) were added to the media as supplements. The broth was then exposed to anaerobic conditions for 72 hours at 30 ºC and a pH of 5.0. To estimate the ethanol content in the broth, the potassium dichromate approach of [13] was adopted. A Metesh UV-5200 spectrophotometer at a wavelength of 600 nm was used to evaluate the ethanol yield.

3. Result and Discussions

3.1. Proximate and Physicochemical Analysis of ITO and ADB

The flour and starch obtained from the tubers of ITO and ADB were analyzed for their proximate composition as using the method described by A.O.A.C (1990) as shown in Table 1. The moisture and carbohydrate content of the starches were observed to be higher than their flour counterparts. Conversely, the protein, ash, fiber and fat content of the starches were found to be lower than those of flour. Most edible crops have shown to have high carbohydrate content such as rice 89.78 g/100g, corn 90.48 g/100g, potato 90.75 g/100g, cocoyam 80.94g/100g and cassava 79.38-83.10 g/100g [14] and found use in pharmaceutical, cosmetics, food and bio-refining industries. The present study shows a carbohydrate content of 80.89 g/100g for ITO and 85.27 g/100g for ADB compared favorably to those of other conventional starch above. Ogunwa et al [9] obtained 88.91g/100g and 71.13g/100g for ITO starch respectively.

Table 1 Proximate composition of the flour and starch of ITO and ADB

PARAMTERS (%)	ITO		ADB	
	Flour	Starch	Flour	Starch
Moisture	9.20	10.40	5.20	8.10
Ash	3.50	0.80	2.40	0.30
Protein	6.51	4.38	5.25	4.30
Fat	5.60	2.80	4.10	1.50
Carbohydrate	71.43	80.89	78.57	85.27
Fibre	3.71	0.64	4.48	0.56

On the other hand, ITO produced the highest starch yield while ADB produced the least (Table 2) which is attributed to the formation of colloidal particles that refuses to settle down after starch extract. The starch of ADB was collected after centrifuging at 1500rpm. Conversely, ADB was found to have a higher amylose content than ITO.

Table 2 Physicochemical properties of ITO and ADB starches

PARAMETERS	ITO	ADB
Appearance/Colour	Off white	Off white
Odour	Odourless	Odourless
Starch Yield (%)	64.9 ±0.2	27.3±0.2
pH	6.2±0.1	5.8±0.1
Gelatinization Temperature (^oC)	78	73
Amylose Content (%)	11.2±0.01	15.0±0.02

3.2. Effects of acid type and acid concentration on the reducing sugar (glucose) yield of starch and flour of the biomass

In the process of producing bioethanol from cellulosic and starchy crops, diverse conversion technologies exist. Thermo-chemical conversion encompasses the use of acid (organic or inorganic) while biochemical processes encompass usage of enzymes under mild temperatures. Figures 2 and 3 compared the glucose yield of starch and flour samples hydrolysed with H_2SO_4 and HCl at different concentrations.

In acid hydrolysis process for producing reducing sugars, conditions which include: acid type, acid concentration, temperature and reaction time have been assessed and optimum sugar yield was identified to be reliant on the type of biomass used [7]. Different acids at different conditions of concentrations, time and temperatures have been reported to have different hydrolysis efficiencies on a particular biomass [7]. To assess the best acid type (H_2SO_4, HCl,) that would offer optimum glucose yield, an experimental design was employed and executed by varying acid concentration (0.25-0.75 M), time (45 mins) at a constant temperature of 120^oC.

The result shows that in almost all conditions, the optimum glucose yield (gl^{-1}) was obtained from sulphuric acid when compared with HCl. It was evident that the optimum glucose yield for each biomass: (ITO (24.89 gl^{-1}), ADB (32.43 gl^{-1}) was attained between 0.25- 0.5 M concentration of sulphuric acid at 45 mins hydrolysis time. On assessing the impact of acid concentration and time on the optimum glucose yield on each acid type, it was observed that both variables have different effect on each acid type. At 45 mins hydrolysis, an increase in glucose yield from 0.25 to 0.5 M concentration was attained but decreased as concentration increased to 0.75 M for both sulphuric and hydrochloric acids. Optimum glucose yield was attained at 0.25 M concentration for sulphuric acid and yield decreased as concentration increased to 0.75 M, while optimum sugar yield was attained at 0.5 M concentration for hydrochloric acids. This observation is not contrary to expectation. According to the work of Melo *et al*,[15], the most efficient condition for optimum sugar yield (30 gl^{-1}) was achieved at lowest acid concentration (0.25 M) and the highest temperature (120^oC), at 30 mins hydrolysis. This is also in line with the work of Gupta *et al*. [16] in which optimum glucose yield (45.69 gl^{-1}) was obtained at 1.5%

of HCl (range 1-3%), 30 mins (15-60 mins) at 120°C. Further increase in either the acid concentration or time beyond these optimal conditions resulted in decrease in sugar yield. Higher acid concentration and time at higher temperature (120°C) may have enhanced the degradation of sugars produced to degradation products such as 5-hydroxymethylfurfural and organic acids.

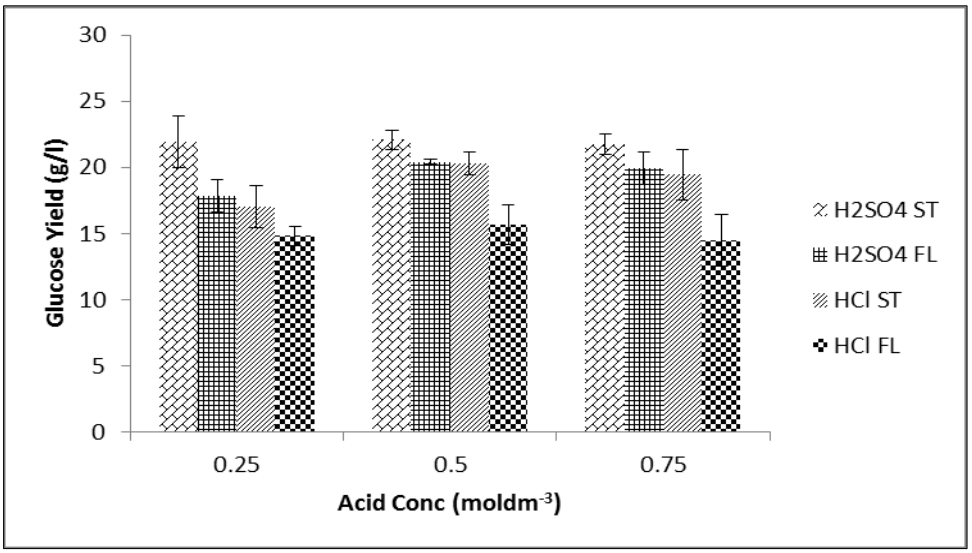

Figure 2 Glucose yield (g/L) from starch and flour of ITO samples using two acid types (H_2SO_4 and HCl) at different concentrations

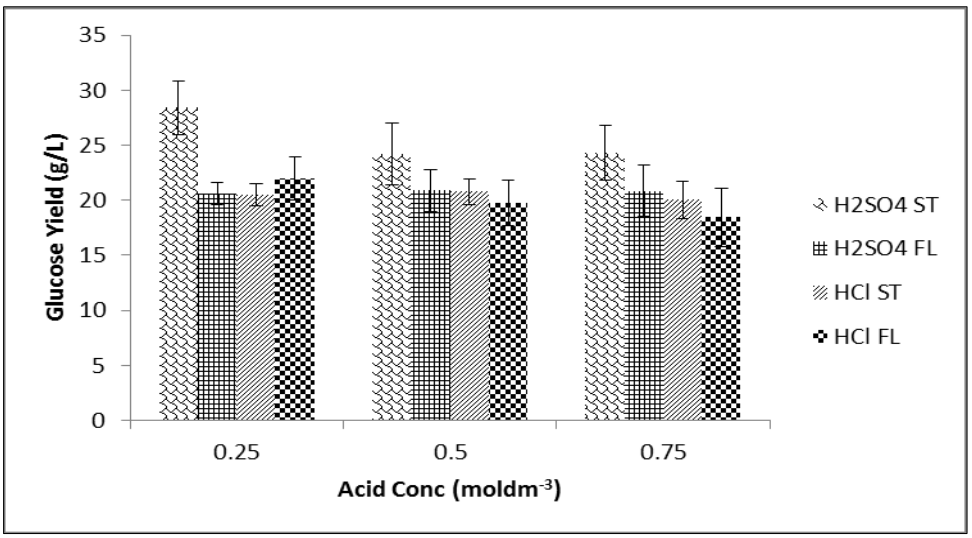

Figure 3 Glucose yield (g/L) from starch and flour of ADB samples using two acid types (H_2SO_4 and HCl) at different concentrations

3.3. Comparison of the glucose yield of the flour and starch samples

In Figure 1 and 2 the reducing sugar (glucose) yield between the starch and flour of all the biomass when hydrolyzed with two acid types (sulphuric and nitric acids) was compared. In almost all conditions, the glucose yield of the starch was higher than that of the flour samples for all the biomass. This may be attributed to higher carbohydrate content (Table 1) of the starch than the flour.

In many bioethanol producing industries, starch is a preferred raw material than flour, due to the high starch content while high fiber content from flour samples especially when using corn flour as raw material reduces the sugar yield. In bioethanol production, the economics of production (which factors in raw material, production and logistic cost as well

as the price of ethanol in market places) is an essential factor that is considered when selecting the conversion technique or feedstock. In 2012, the price of starches and their derivatives stood at US$ 51.2 billion and was further anticipated to increase to US$ 77.4 billion by 2018 [3]. The cost associated to raw material (starch) cover the entire process of harvesting, transportation, pealing, milling, starch extraction and drying. In starch extraction process, energy, time and manpower are required thereby raising the overall cost of the whole process. Though the starch content of most conventional crops is high: corn (60-80), cassava (60-85), barley (60-70), their yield from the flour (during extraction process) are sometimes relatively low [14]. The result obtained from this work showed that ADB flour with 78.57% carbohydrate content gave the least starch yield of 27.3% while ITO with 71.43 carbohydrate content gave the highest starch yield of 64.9% (Table 1). The low starch yield of most crops is attributed to their morphology [17]. Though the reducing sugar yield (glucose yield) of the flour is lower than that of starch samples, the use of ADB and ITO flour samples for producing bioethanol would be more economical and profitable since their starch yields are lower than 50% of an entire flour input (g/g). The use of flour from these samples may be profitable because, the total ethanol yield that would be obtained from using 100 g flour would be higher than that obtained from 27.3% starch/100 g of flour and 64.9% starch/100 g flour for ADB and ITO respectively.

3.4. Comparison of the ethanol yield of the Flour and Starch samples in acid process.

This produce bioethanol from two non-food biomass were accessed via their yield. The percentage (v/v), actual (g/l) and fermentation efficiency (%) of the ethanol produced from each biomass was determined from the absorbance readings, ethanol density formula and the initial glucose concentration in the hydrolysate before fermentation respectively. The ethanol yield of the starch and flour of each non-biomass was compared also using two acid types (Figures 4-5).

The ethanol yield obtained differs from each other according to acid type. This illustrates that acid type has an overall effect on the fermentation process and amount of ethanol produced. The result showed that the maximum ethanol yield (gl^{-1}) was obtained from hydrochloric acid. This is unlike the trend observed during acid hydrolysis where the optimum glucose yield (gl^{-1}) was obtained from sulphuric acid. Although, HCl produced the maximum ethanol yield,

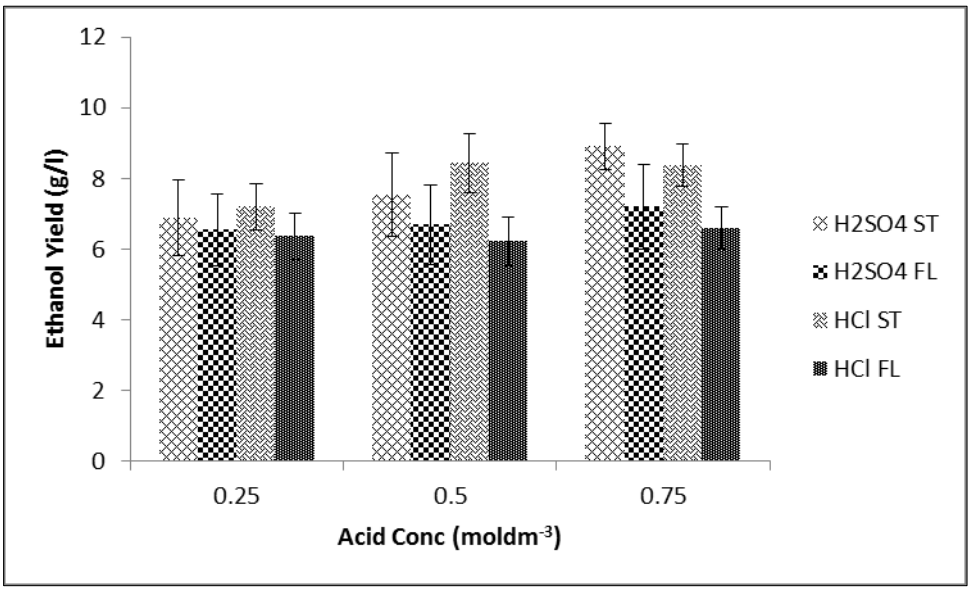

Figure 4 Ethanol yield (g/l) of ITO starch and flour samples using two acid types (H_2SO_4 and HCl) at different concentrations

The variation in the yield between ethanol and glucose prompted further investigation and it was found that salt concentration (Na_2SO_4, NaCl, and $NaNO_3$) formed during neutralization process with NaOH affected yeast cell growth [18]. Salts concentration was reported to display two impacts: osmotic and ion toxicity stress to yeast. Omori *et al*. [18] reported that high salt concentration (above 120 Psu) caused rapid inhibition of cell (yeast) growth and ethanol output in the fermentation process. Thus, the lower ethanol yield form H_2SO_4 hydrolyzed samples could be attributed to excess sodium sulphate in the medium (the amount formed with NaOH during neutralization, and the amount added as a nutrient for the medium). In addition, higher glucose concentration could also be likened to lower ethanol output observed with H_2SO_4 hydrolyzed samples. This is because higher substrate concentration (glucose) was reported to

cause osmotic shock to yeast cells thereby slowing down heat and mass transfer in the fermentation medium and thus the ethanol yield [13].

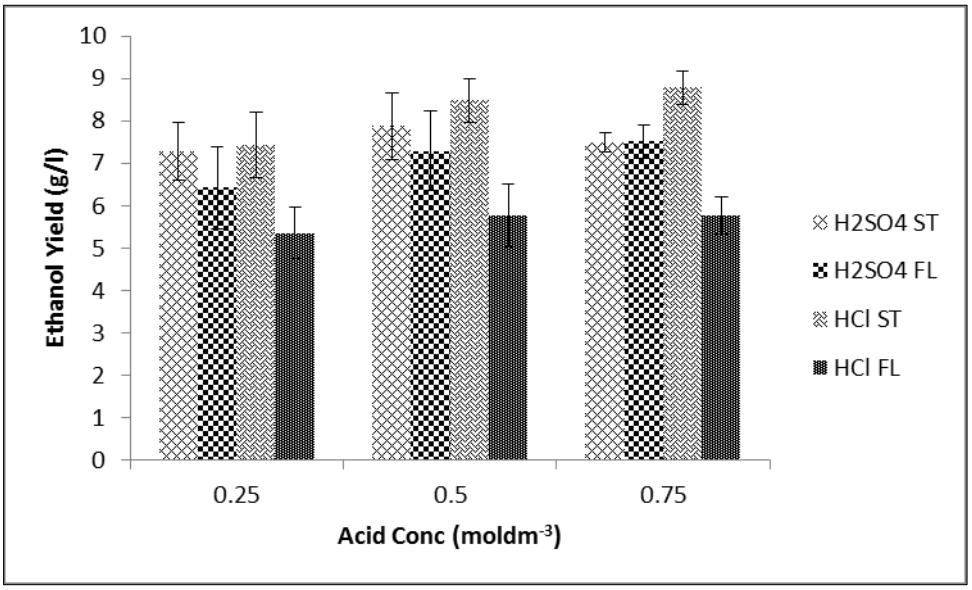

Figure 5 Ethanol yield (g/l) of ADB starch and flour samples using two acid types (H_2SO_4 and HCl) at different concentrations

The ethanol yield (Figure 3-4) of the starch and flour of the samples were evaluated using two acid types H_2SO_4 and HCl. In both conditions of hydrolysis, the ethanol yield of the flour samples was noticed to be less than those of the starch samples. As expected, ethanol yield of both the starch and flour of the samples was observed to follow similar order with that of the glucose yield (Figures 2-3)

Residues have remained a challenge in the exploitation of flour samples as feedstock for bioethanol production due to the existence of unhydrolysable substances (fibre). In the starch extraction process, some percentages of starch (Table 1) are left in residue thereby reducing the number of hydrolysable substances and the amount of ethanol yield that could be produced from the overall starch containing sample. Moreover, it is difficult to extract starch from some edible and non-edible starch containing samples such as cocoyam, yam, and non-edible ADB where the starch form colloidal particles rather than settling down. Thus, the ethanol yield obtained from the flour samples in both hydrolysis procedures have shown that more ethanol would be produced if flour was utilized than the amount that would be produced from the starch extracted from that same quantity of flour which therefore enhances the economics of bioethanol production.

3.5. Comparison of the ethanol yield of the Flour and Starch samples in enzymatic process.

To further compare the glucose and ethanol yield of the non-food biomass enzymatic hydrolysis condition (60-180 amylose unit and 280 amyloglucosidase unit) that offers optimum glucose yield was carried out. The result (Figure 6-7) shows that the ethanol yield of the non-food biomass produced very high glucose and ethanol yield when compared to their acid process yield. Within the hydrolysis conditions, maximum ethanol yield (with fermentation efficiency) for each biomass as observed was 23.62 gl-1 (68.58%), 26.09 gl-1 (81.91%) with initial glucose concentrations of 68.88 gl-1, 58.04 gl-1 for ADB and ITO samples respectively. The range of ethanol yield and conversion efficiency obtained in this study from the non-food biomass also compares very favorably with other starchy crops reported in recent literature: 11.0-34.5 gl^{-1} (65-88 %) from caster bean cake [15], 31.45 -41.28 gl^{-1} (67-88 %), from de-oiled Pongomia pinnata seed [7] all from non-food crops.

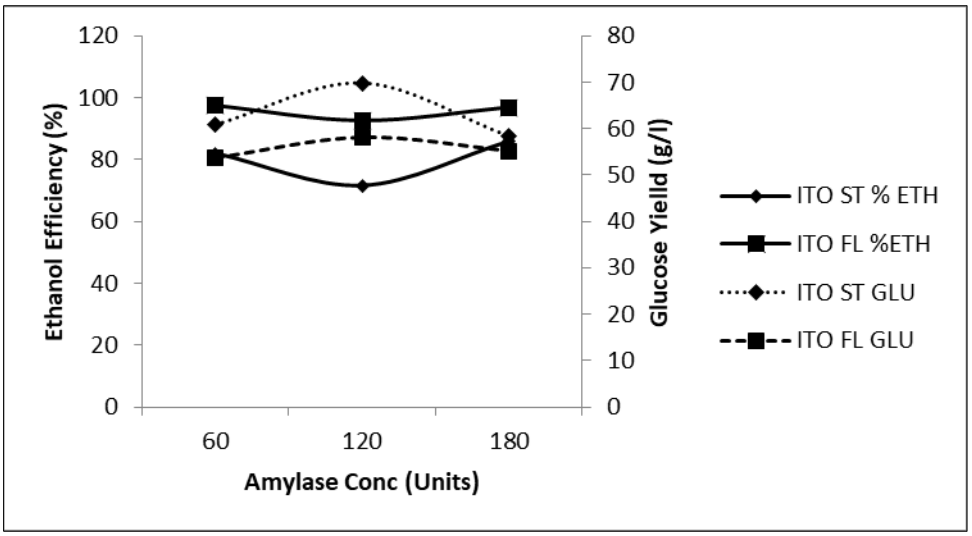

Figure 6 Glucose and Ethanol efficiency of ITO at different amylase concentrations and 280 amyloglucosidase unit

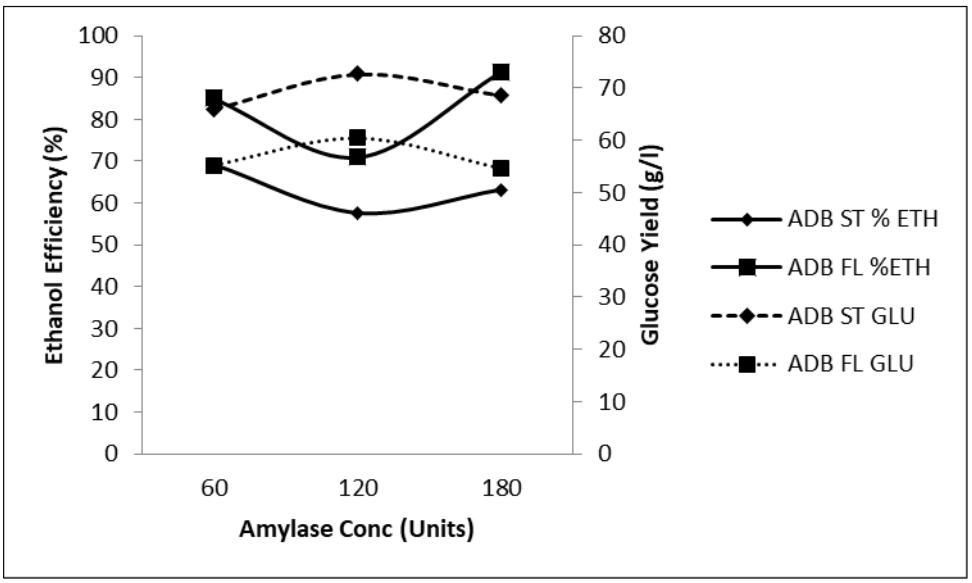

Figure 7 Glucose and Ethanol efficiency of ADB at different amylase concentrations and 280 amyloglucosidase unit

The low ethanol yield obtained from the acid process could be attributed to the existence of inhibitors (Phenolics, hydromethylfurfural and carboxylic acids) generated during acid hydrolysis at severe conditions. These compounds have been found to inhibit fermentation by inducing precipitation of enzymes [13].

Hence the ethanol output (yield) of ITO and ADB mainly from the enzymatic process demonstrated the potential of the non-food crops as feedstock for producing bioethanol, thus they can serve as substantial alternatives to cassava for bioethanol production in Nigeria.

4. Conclusion

The study establishes Anchomanes difformis blume (ADB) and Icacina trichantha oliv (ITO) as promising non-edible biomass feedstocks for bioethanol production, with distinct advantages and limitations when used in starch versus flour forms. Starch samples exhibited superior glucose and ethanol yields under both acid and enzymatic hydrolysis due to their higher carbohydrate content and lower levels of fermentation inhibitors. ADB starch, with 85.27% carbohydrate content and 15.0% amylose, demonstrated the highest overall yield potential. However, the starch extraction process,

particularly for ADB, was limited by low starch recovery (27.3%) and processing inefficiencies due to colloidal starch properties.

Conversely, flour samples though yielding less fermentable sugar per unit offered more practical and economical pathways, especially for ADB, by bypassing energy intensive extraction steps and yielding more ethanol per 100 g of input material. Enzymatic hydrolysis significantly improved overall efficiency, yielding up to 81.91% fermentation efficiency in ITO and 68.58% in ADB.

In summary, both starch and flour of ITO and ADB are viable for bioethanol production, but flour, particularly from ITO due to its higher starch yield and ease of processing, may present a more scalable and economically sustainable approach for industrial biofuel applications in Nigeria and similar regions.

Declaration

- The authors declare no conflict of interest in respect to financial or personal relationships with other people or organizations that could inappropriately influence this work.
- The authors can also confirm that this manuscript has been read by all and approved for onward submission to this journal and if accepted would not be published elsewhere.
- The authors declare that we did not receive any grant from any funding source

Compliance with ethical standards

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Disclosure of conflict of interest

The authors report there is no competing interest to declare.

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