COMPARISON OF BIOETHANOL YIELD FROM FOUR COCOYAM SPECIES IN NIGERIA

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Abstract
This work was undertaken to compare the yield of bioethanol from four species of Cocoyam: Edeofe (Colocasia esculenta Nce 003), Edeanambe (Colocasia esculenta Nce 001) and Edeuhie (Xanthosoma sagittofolium Nxs 002), Edeocha (Xanthosoma sagittofolium Nxs 001). Two hydrolysis steps was carried out in the work (acid hydrolysis was carried out using two acid types, sulphuric and hydrochloric acids while enzymatic hydrolysis was carried out using alpha-amylase and glucoamylase). Fermentation of the hydrolysates was done using commercial baker's yeast (Saccharomyces cerevisiae) at pH 5.0 for four days. The result shows that the highest ethanol yield of 4.89ml/20g (0.00024Lg^{-1}) with 19.32% yield was obtained from enzymatic hydrolysis as compared to 3.86ml/20g (0.00019Lg^{-1}) with 15.26% yield from acid hydrolysis with H_2SO_4. "Edeofe" produced the highest yield of ethanol when compared with other species: from enzymatic hydrolysis, Edeofe produced 0.00024Lg^{-1} when compared to 0.00021Lg^{-1}, 0.00020Lg^{-1} and 0.00022Lg^{-1} for Edeanambe, Edeuhie and Edeocha respectively. The same trend occurred in acid hydrolysis. Though enzymatic hydrolysis produced higher ethanol yield than acid hydrolysis, hydrolysis using H_2SO_4 was a better option than that of HCl: Edeofe produced 0.00019Lg^{-1} with 15.26% yield with H_2SO_4 hydrolysis when compared to 0.00015Lg^{-1} with 12.0% yield from HCl hydrolysis. Thus cocoyam, especially Edeofe specie, has shown to be a promising source of biomass for bioethanol production. Thus farmers should be encouraged to cultivate more of the specie in order to boost the bioethanol industry in Nigeria.

Key words: Cocoyam, Bioethanol, Biofuel Production, *Colocasia esculenta*, *Xanthosoma sagittofolium*

Introduction

Global warming and climate change are effects that have generated so much attention in the world, and immediate actions are required to combat the effect [1]. The continuous use of the world's crude oil reserves and the dwindling on its price together with the limited coal reserves has stimulated the hunt for renewable source of energy. It has been observed that with world reserves of petroleum fast depleting, bioethanol has emerged as one of the most important alternative source of liquid fuel and has generated a great deal of research interest in ethanol fermentation [2,3]. The development of biofuel crop best suited to each region of the world helps overcome the problems of energy crises as well as mitigation of global warming; for instance corn is a major biofuel crop in United States of America while sugar cane is to Brazil. Cassava and Cocoyam are well suited for tropical rainforest region like Nigeria [4, 5]. Cocoyam a member of the Aracea family of plants is one of the oldest crops grown, largely in the tropics, for its edible corms and leaves and as an ornamental plant [6]. On a global scale, it ranks 14th as a vegetable crop going by annual production figures of 10million tones [7]. Its production estimates vary. Africa accounts for at least 60% of world production [8] and coastal West Africa accounts for 90% output of the crop with Nigeria accounting for 50% of this [9]. Two species of cocoyam are widely cultivated in Africa, they are *Colocasia esculenta* (Taro) and *Xanthosoma sagittofolium* (Tania). In Nigeria different cultivar of these species exist and are locally called by the Igbo speaking people as "Edeofe", "Edecocoindia" and "Edeanambe" (Colocasia species) while "EdeUhie" and "Edeocha" (xanthosoma species) [10,11]. Cocoyam is one of the crops that have received inadequate attention, the reason is attributed to the presence of calcium oxalate raphide, the irritant which does not have any effect in its utilization for bioethanol production. The potential of cocoyam has been evaluated for the production of ethanol and methane for use as energy sources [6]. It was found that ethanol yield at the rate of 139 L/tonne of cocoyam [6].

The high percentage of carbohydrate in Cocoyam offers an advantage which when exploited in the production of ethanol would bring about an industrial bloom as well as help mitigate greenhouse gases and global warming. The optimal production of bioethanol requires several conditions which depends on type, variety and cultivation area of the biomass used [12.,13]. It has been shown that cocoyam is a good source of carbohydrate with composition of 80-90% total carbohydrate (14,15]. Though few works have been done with cocoyam for ethanol production, it is yet to be determined which specie would give the highest yield of ethanol and the condition under which an optimum yield can be achieved. Thus, the knowledge of the specie with the highest yield of bioethanol and data on the optimal conditions in the various processes would provide information required in the industrial production process design. In this

study, the ethanol production potential of four species of Cocoyam (Edeofe, Edeocha, Edeuhie and Edeanambe would be determined and compared. The information on the specie with the highest ethanol yield will offer farmers an option to select the best cultivar for cultivation, and the feasibility of high ethanol yield will enhance its use for full-scale bioethanol production in Nigeria.

Materials and Methods

Sample collection.

Four species of Cocoyam "Edeofe", "Edeanambe" (Colocasia species), "Edeocha", "Edeuhie", (Xanthosoma species) of at least 1000g were obtained from a local market in Port Harcourt and was identified at the department of crop science, faculty of Agricultural sciences, University of Port Harcourt. The samples were peeled, rinsed, cut into pieces and sun dried for one week before grinding into fine powder. Two commercial enzymes (bacterial α-amylase and glucoamylase) and 3Å molecular sieve used in this study for the dehydration process were obtained from Sigma-Aldrich, Germany. While Commercial baker's yeast (*Saccharomyces cerevisiae*) strain was purchased from a baker's shop in Port Harcourt

Bioethanol Production

The methods used for Bioethanol production includes; acid hydrolysis, enzyme hydrolysis, fermentation and distillation processes. Each species of cocoyam [20g] was used for the production and the process was repeated three times so the mean value was obtained.

Hydrolysis

Gelatinization: Each species of Cocoyam flour was dispersed in water in the ratio of 1: 5, in a conical flask. The mixtures were then gelatinized in an autoclave for 15min. the water to substrate ratio were estimated using the very high gravity method (VHG) of Kakuoon 2011[16]

Acid hydrolyis: Acid hydrolysis was carried out with two different acids H_2SO_4 and HCl at 2% concentration using an Autoclave at 121^{o}C and 0.15MPa pressure for 45mins.

Procedure: Each of the gelatinized samples [20g] were dissolved in 100ml of 2% acid each in flask and hydrolyzed for 45mins. The hydrolysate recovered after acid hydrolysis was filtered using a muslin cloth after subsequently fermented. The hydrolyzing or penetrating power of each acid was studied to determine which acid would yield more sugars and thus more volume of ethanol.

Enzymatic Hydrolysis:

Liquefaction: one percent (1%) alpha-amylase was added to 20g each of the gelatinized sample and enough distilled water was added to bring the volume to 200ml. Liquefaction was then carried out at 90^{o}C for 2hrs at pH 5.0 (using NaOH solution for the adjustment of the solution pH). After 2hrs the temperature was reduced to 60^{o}C.

Saccharification: To the liquefied samples was added 0.1% glucoamylase at 60^{o}C for 16hrs with shaking at intervals. After 16hrs of hydrolysis the temperature of the solution was raised to 99^{0}C for 30mins so as to deactivate the enzymes. This followed the method used by Kakuoon 2011 [16]. The solutions were then filtered and tested for both starch and sugar using standard test method.

Fermentation

Hydrolysate from the hydrolysis processes were transferred into another set of labelled conical flasks and supplemented with 0.2g NH_4Cl, 0.40g KH_2PO_4, 0.20g $MgSO_4.7H_2O$ and 0.05g $CaCl_2.2H_2O$. The pH of each mixture was then adjusted to 5.0 with dilute NaOH solution, each flask was covered, autoclaved at 121^{0}C for 15 minutes and allowed to cool. To each set of hydrolysed supernatant was added 2g of *Saccharomyces cerevisiae*. The flasks were covered and incubated at 30^{o}C for 4 days with shaking at intervals. [17]

Distillation and Dehydration

The ethanol content of the fermentation broth was distilled by simple distillation. A two-step distillation method was used in order to achieve an ethanol concentration up to the azeotropic point (94.5% from ≤ 12%) in the fermentation broth.[18].

The final purification (dehydration) above the azeotropic point was achieved with the use of Type 3Å molecular sieve using a liquid phase dehydration method at 79^{o}C. [19].

Determination of Ethanol Concentration

Ethanol concentration was determined by comparing the density of the ethanol produced with a standard ethanol density curve. [20,21].

The percentage conversion of the ethanol produced was obtained by the following equation

$$\% \text{ conversion} = \frac{\text{weight of ethanol obtained}}{\text{Weight of the initial biomass}} \times 100$$

Results and Discussion

The properties determined for the ethanol produced from the Cocoyam are as follows: The liquid boiled at 78.5^{0}C and had a relative density in the range of 0.791-0.804. The liquid was clear and colourless. It had a very sharp alcoholic taste, as well as the typical ethanol odour. When tested on a blue piece of cloth, it readily bleached it to almost white colour.

Bioethanol Produced from Cocoyam at Different Hydrolysis Conditions.

Table 1 shows the volume (mean ± SD), weight (mean ± SD) and percentage yield (%) of bioethanol produced from cocoyam species when hydrolyzed with 2% acids and enzymes and subsequently fermented with *Saccharomyces cerevisiae*.

TABLE 1: ETHANOL YIELD FROM 20g SAMPLES USING DIFFERENT HYDROLYSIS METHODS AT pH 5.0

SAMPLES	VOLUME OF ETHANOL (ml)	WEIGHT OF ETHANOL (g)	ETHANOL PRODUCTIVITY (L/g)	PERCENTAGE YIELD (%)
ACID HYDROLYSIS WITH 2% H_2SO_4				
EDEOFE	3.86 ±0.18	3.0513 ±0.17	0.00019	15.25 ±0.51
EDEANAMBE	3.30 ±0.20	2.6079 ±0.18	0.00016	13.03 ±0.48
EDEUHIE	3.06 ±0.18	2.4186 ±0.16	0.00015	12.09 ±0.48
EDEOCHA	3.26 ±0.20	2.5770 ±0.17	0.00016	12.88 ±0.52
ACID HYDROLYSIS WITH 2% HCl				
EDEOFE	3.00±0.18	2.3712±0.20	0.00015	12.00 ±0.48
EDEANAMBE	2.90 ±0.17	2.2918 ±0.20	0.00014	11.45 ±0.44
EDEUHIE	2.70 ±0.20	2.1341±0.18	0.00013	10.67 ±0.50
EDEOCHA	2.40 ±0.20	1.8969±0.17	0.00012	09.48 ±0.48
ENZYMATIC HYDROLYSIS				
EDEOFE	4.89 ±0.20	3.8641±0.20	0.00024	19.32 ±0.55
EDEANAMBE	4.16±0.17	3.2287±0.18	0.00021	16.44 ±0.54
EDEUHIE	4.06 ±0.20	3.2090±0.18	0.00020	16.04 ±0.52
EDEOCHA	4.42 ±0.20	3.4931±0.20	0.00022	17.46 ±0.56

Effect of Hydrolysis Method on Ethanol Production

Tables 1 shows the results obtained from acid hydrolysis of the cocoyam species by acid type (sulfuric and hydrochloric acids) at hydrolyzing temperature and time of 121ºC and 45min and that of enzymatic hydrolysis. The highest yield of ethanol of 4.89ml/20g (0.00024Lg^{-1}) with 19.32% conversion was obtained from enzymatic hydrolysis as compared to 3.86ml/20g (0.00019Lg^{-1}) with 15.26% conversion from acid hydrolysis (2% H_2SO_4). This suggests enzymatic hydrolysis to be a more suitable method than acid hydrolysis under the conditions of the study. This has been attributed to the decrease of sugar content by degradation of monomeric sugar like glucose to furfural and HMF during acid hydrolysis or conversion of glucose to levulinic, acetic or formic acids [17,22]. These substances are toxic to microorganisms such as yeast and can thus inhibit its growth by decreasing the intracellular pH of the fermenting medium and thus death to the organism [23.,24]; while high conversion of glucose by *Saccharomyces cerevisiae* during ethanol production provides evidence that enzymatic hydrolysate constitutes a suitable medium for the yeast growth. Gelatinization of the cocoyam species increases the surface area of a biomass [25]. It is reported that gelatinization affects digestibility and texture of starch containing foods, [26] thus leaching amylase enhances susceptibility of starch to enzyme attack. The increased enzyme active sites create more substrates for yeast activities which enhances an increase in ethanol production.

An ethanol yield of 3.86ml/20g (0.00019Lg^{-1}) with 15.26% conversion using H_2SO_4 as compared to 3.00ml/20g (0.00015Lg^{-1}) with 12.0% conversion using HCl for the hydrolysis suggests that the hydrolyzing or penetrating power of sulfuric acid is higher than that of hydrochloric acid. This might be attributed to the diffusivity of acids into the biomass where H_2SO_4 has higher penetrating power than HCl. This agrees with the work of Kim & Lee [27, 28]

Ethanol Production from Different Species of Cocoyam

Tables 1 also show that the specie Edeofe produced the highest yield of ethanol under all the conditions of the study more than other species with yield of 0.00024Lg^{-1} (19.32% conversion) when compared with 0.00021Lg^{-1}, 0.00020Lg^{-1} and 0.00022Lg^{-1} (16.44%, 16.04% and 17.46% conversions) for Edeanambe, Edeuhie and Edeocha respectively from enzymatic hydrolysis while in acid hydrolysis a yield of 0.00019L/g (15.26%), 0.00016L/g (13.03%) , 0.00015L/g (12.09%) and 0.00016L/g (12.88%) was obtained for Edeofe, Anambe, Edeuhie and Edeocha respectively. Though the yield of ethanol from other species of cocoyam were lower than that of Edeofe, their yield of ethanol were satisfactory when compared with those reported in the literature. For instance, Braide & Nwaoguikpe obtained a maximum of 12.9% ethanol from their work on Cocoyam [25]. This also compares favourably with the work of Adelekan [6] who obtained 0.00014Lg^{-1} of ethanol.

The carbohydrate value of the four species as reported in literature ranges from 82.83- 84.61 [10, 11] with Edeofe having the least value of 82.83 while Edeuhie

has the highest value. Edeofe have the highest water absorption capacity than other species while Edeuhie have the least value [10]. The high absorption capacity of Edeofe allows for easy penetration of both acids and enzymes into the biomass during hydrolysis, thereby enabling the breakage of more glycosidic linkage and production of more simple sugar than other species. Thus, the more the production of simple sugars, the more the conversion to ethanol. Therefore the high yield of ethanol by Edeofe may be attributed to this factor. High absorption capacity is associated with the particle size of the substance. Report shows that Edeofe has a high particle size (that is highest particle retention at sieve of 90μm) [10] and large particle size has greater bonding force on particle surfaces than smaller sizes. Ayenor [29] also stated that the degree of disintegration of the native starch granules influences the water binding ability of the starch system. Therefore the more the binding ability of water on starch the more the hydrolysis.

The biofuel production potential from different crops are commonly compared by their yield per hectare or liter per gram. For instance, the production per unit hectare of maize-ethanol is roughly half that of sugar cane ($3880Lha^{-1}$for corn and $6195Lha^{-1}$ for sugar cane) [30]. However, the data in [31] shows that maize has a better conversion rate of 410L/ton ($0.00041L\ g^{-1}$) than sugar cane 81L/ton ($0.00008Lg^{-1}$). Among the crops listed, sugar cane has the least ethanol conversion rate, but with sugar cane as a biomass feedstock, Brazil has the most successful biofuel program in the world and biofuel sustainable economy where there is no longer light vehicles operating on pure gasoline.

Given the Cocoyam-ethanol conversion of an average range of $0.00020L\ g^{-1}$ from the present study, it therefore compares very favourably with other biomass and more than either cassava, sweet potato or sugar cane.

Conclusion

The high yield of bioethanol from Cocoyam especially through enzymatic hydrolysis shows that it has a very good potential for bioethanol production and compares more favourably with other biomass feedstock like Cassava and Corn. Its choice as a biomass feedstock in the tropical region of the world would help improve fuel/energy mix without the problem of food crisis. The higher yield of bioethanol from Edeofe (*Colocasia esculenta Nce 003)* in almost all the study shows that among all the species of Cocoyam it has a better potential for bioethanol production and its use for full scale bioethanol production may not have any adverse effect on food security since it is mainly used as thickener for soup in Nigeria.

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