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Potential of Some Tuber Peels in Bioethanol Production Using *Candida Tropicalis*

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ABSTRACT
Cassava and sweet potato peels are two potential agro-wastes with high possibility for biofuel production due to the possession of high polysaccharides. Studies were conducted to evaluate the chemical compositions of these wastes as well as determine their potential as feedstock for bioethanol production using indigenously sourced yeast strain; *Candida tropicalis* (IMI 398401). Cashew nut shell extract was used to hydrolyze and saccharify the gelatinized mashed agro-waste. The results indicates that moisture content (14.16 %), ash content (2.25 %), fibre (5.10 %) and cellulose (734 mg/g) were higher in cassava peels and lower; 13.04 %, 1.12 %, 1.81 % and 498 mg/g respectively in sweet potato peels. Pretreatment of the peels with organic acid extract of *Anacardium occidentale* nut shell showed maximum reducing sugar yield of 300 mg/g and 471 mg/g in sweet potato peel and cassava peel. The amount of bioethanol (g/L) produced after 72 h of fermentation was higher; 23.90 ± 0.60 in sweet potato peel. The study shows that these peels may be useful for biofuel production after a proper pretreatment.
Keywords: Bioethanol; Candida tropicalis; Pretreatment; Proximate composition; Tuber peels.

INTRODUCTION
Plant biomasses are the targets for the supply of a sustainable energy resource because of their abundance and regenerative nature (Chaudhary and Qazi, 2011). Recent environmental problems arising from of the use of non-renewable fuels all over the world have brought about renewed interest in developing biomass energy technology to reduce the demand for fossil fuel and protect the environment from the effects of serious global warming (Bhatia *et al.,* 2012). The beneficial effects of using biofuel cannot be over emphasized as they include environmental friendliness, high energy efficiency, ability to curb greenhouse gas emission, reduced government budget deficit, support for crop prices, and strengthening of farm economy (Agba *et al.*, 2010; Demirbas, 2011). Cassava (*Manihotesculenta crantz*) is the third largest source of carbohydrate food for human consumption in the world (Adesanya *et al.*, 2008). It is deficient in protein, fat, some minerals and vitamins (Olsen and Schaal, 1999). Cassava is a major staple food in Nigeria which also serves as raw materials for the ethanol, pharmaceutical, confectionery, textile, beverages and packing industries (Henk *et al.,* 2007). Sweet potato (*Ipomoea batatas* Lam.) contains about 20.1 % carbohydrate, 1.6 % protein, 0.6 % oil. Besides simple starches, sweet potatoes are rich in β-carotene, complex carbohydrate, dietary fiber, vitamins B_6 and C (Mei *et al.,* 2010). These crops are indispensible to the diet of the common man. However, the residues from processing them generate annual dispersal challenges (Ledward *et al*., 2003). The objective of this study was to produce bioethanol from cassava and sweet potato peels after pretreatment, saccharification with organic acid extract and fermentation using *Candida tropicalis*.

MATERIALS AND METHODS
Collection and Processing of Agro-wastes
Cassava peel were collected from "Garri" and "Fufu" processing industries at Elere, Ifo Local Government area of Ogun State, Nigeria while sweet potato peels were gathered from household wastes. They were transported to the Botany Research laboratory (University of Lagos), sorted and then washed under running tap to remove sand and other dirt particles. Samples were sun-dried for two weeks and then

milled into flour. All powdered samples were sieved to obtain average particle sizes of 300 µm in diameter.

Proximate analysis of the agro-wastes

Determination of moisture content and total ash quantification were carried out using the method of Chinedu and Nwinyi (2012). The crude protein and lipid contents were determined by the standard AOAC (1990) method. The modified method of Updegraff (1969) was used for cellulose content. Two milligram (2 mg) of each ground sample was weighed into tubes and 100 µl of inositol solution was added. The tubes were capped tightly and incubated for 90 min at 12ºC. The tubes were allowed to cool and then centrifuged at 10 000 rpm for 10 min. The supernatant was transferred to glass screwed cap vials by ensuring that the pellet was not disturbed. One millilitre of Updegraff reagent (acetic acid, nitric acid, water, 8:1:2 v/v) was added to the pellets, the tubes were capped, vortexed and heated in a water bath at 100°C for 30 min. The samples were cooled on ice at room temperature and centrifuged at 10 000 rpm for 15 min, the supernatant was discarded. Distilled water (1.5 ml) was added to the pellets, mixed thoroughly, centrifuged and the supernatant discarded. This was repeated using 1.5 ml acetone instead of water. The pellets were carefully air dried. Then 175 µl of 75% H_2SO_4 was added and incubated at room temperature for 30 min. The content was vortexed and incubated for another 15 minutes after which 825 µl of water was added, vortexed and centrifuged at 10 000 rpm for 5 minutes. To 10 µl of the mixture, 90 ml of water was added and the glucose content of the supernatant was assayed using colorimetric anthrone assay by adding 200 µl of freshly prepared anthrone reagent. The mixture was heated on a microtiter plate for 30 min at 80°C in an oven. The plates were cooled to room temperature and shaken thoroughly. The absorbance of the pellets was read at 625 nm using a microtiter plate reader. The glucose was calculated based on absorbance compared to the standard curve prepared with varying concentrations of glucose.

Isolation and characterization of microorganism

The isolation and characterization of *Candida tropicalis* from kolanut had previously been described in Ebabhi *et al*. (2013b).

Gelatinization and hydrolysis of agro-wastes with cashew nut extract

A 10% (w/v) of powdered peels each was made into distilled water, heated to about 70°C to gelatinize and allowed to cool at room temperature (Ocloo and Ayernor, 2010). The cashew nut shell extract was used for saccharification obtained by soaking mashed cashew nut shell in ethanol at the ratio of 5:6 (w/v) for 72 h (Sofowora, 1993).The mixture was filtered through No. 1 Whatman filter paper. The filtrate was concentrated through the rotary evaporator under reduced pressure and controlled temperature and the pH was measured as 3.2 using a pH meter. The cashew nut shell extract was used to hydrolyze and saccharify the gelatinized mash as previously described by Ebabhi *et al.* (2013a). The mixture was autoclaved after an hour at 121°C for 15 min and immediately filtered using muslin cloth. The reducing sugar content in the hydrolysates was determined by Dinitrosalysaclic method (Miller, 1959).

Identification of specific simple sugars in the agro-waste hydrolysates

The hydrolysates were analyzed on an HP 6890 Series GC powered with an HP ChemStation Rev. A 09.01 (1206) and a flame ionization detector (FID). Sample (2-3 µl) was injected from slit injector. The carrier gas was hydrogen set at the flow rate of 1.0 ml/min. The fractionation was carried out in an isothermal temperature of 210°C. The injector and detector temperature were 250°C and 325°C respectively. Typical coefficient of correlation for standard curve was 0.95-0.99. Peaks were identified by comparison of retention times with those of standard glucose,

xylose, arabinose, maltose, rhamnose, lactose, sucrose, ribose and fructose as previously reported by Ebabhi *et al*. (2013a).

Fermentation of hydrolysates and quantification of ethanol produced

The fermentation studies were carried out on the agro-waste hydrolysates in three replicates using *C. tropicalis* (IMI 398401) isolated from *Cola acuminata*. The yeasts were added at the rate of 1ml yeast broth/50ml of mixture. Fermentation was allowed to proceed for 72 h at 30ºC. Replicate for each hydrolysates was setup to serve as control without the addition of the *C. tropicalis.* The volume of the distillate obtained after distillation of the fermented broth was measured and the quantity of ethanol produced expressed in g/L was determined by multiplying the volume of the distillate by the density of ethanol (0.8033 g/ml) (Oyeleke and Jibrin, 2009).

Statistical analysis

The data were expressed as mean ± S.D and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan multiple range test. Values were considered significant at $p< 0.05$.

RESULTS

The proximate composition of agro-wastes, cassava peels and sweet potato peels were determined. The result obtained showed that the moisture, ash, fibre and cellulose content were significantly higher $p< 0.05$ in cassava compared to sweet potato peel (Table 1). Also, protein and lipid content were highest in sweet potato and cassava peels respectively.

Table 1: Proximate analysis of the agro-wastes

Waste used	Moisture	Ash	Protein (%)	Lipid	Fibre	Cellulose
Cassava peel	14.16±0.056	2.25±0.026	5.23±0.015	7.20±0.032	5.10±0.031	73.43±0.070
Sweet potato peel	13.04±0.012	1.12±0.021	7.70±0.042	4.66±0.050	1.81±0.040	49.8±0.473

Values are expressed as means±SEM for three replicates

The reducing sugar obtained after pretreatment of agro-wastes was higher in cassava peels, compared to sweet potato peels (Figure 1).

The hydrolysates were subjected to Gas Chromatographic analysis in order to obtain the type and concentration of sugars present. The standards used were glucose, fructose, sucrose, arabinose, xylose, lactose, rhamnose, ribose and maltose. The correlation co-efficient of each selected standard is 0.99 g/L. Attenuation condition: Column: 0.0038 x 0.25µm, Oven temperature: Isothermal at 210°C, injector and detector temperatures: 250 and 325°C respectively, Hydrogen flow rate: 1.0ml/min, Detector: FID. The results showed the presence of the test sugars in varying concentrations as presented in Table 2.

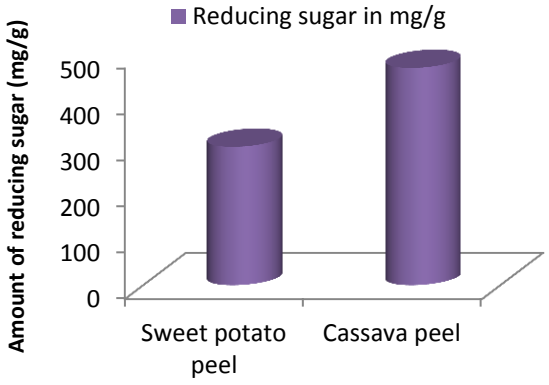

Figure 1: Reducing sugar in hydrolysates of agro-waste used in study

Table 2: GC-FID sugar analyses of Cassava and Sweet Potato Peels hydrolysates

Sugar component	Substrate (mg/g)	
	Cassava peel	Sweet potato peel
Arabinose	5.38×10^{-5}	2.07×10^{-4}
Fructose	1.21×10^{-5}	12.68
Glucose	38.19	15.7
Lactose	5.13×10^{-5}	1.67×10^{-4}
Maltose	5.68×10^{-5}	2.07×10^{-5}
Rhamnose	4.87	5.55×10^{-5}
Ribose	1.10×10^{-4}	2.06×10^{-4}
Sucrose	$1.10 \times 10^{-2\backslash}$	23.76
Xylose	3.93	1.28×10^{-4}

The amount of bioethanol produced varied according to the type of agro-waste fermented. It was significantly ($p<0.05$) higherin sweet potato peel compared to cassava peel.

DISCUSSION

Wastes from tubers are potential sources of biofuel (Adesanya *et al*., 2008; Ocloo *et al.,* 2010). However, these wastes are usually avoided for commercial usage because of the challenges in hydrolyzing them. The potential of tuber peels in the production of bioethanol was evaluated in this study. From this study the hydrolyzed cassava and sweet potato peels generated high amount of reducing sugars which could have resulted from the effective hydrolysis of the lignocellulose present in the peels of these tubers. Arumugam and Manikandan (2011) in their study obtained high amount of reducing sugars from banana and mango peels pretreated with dilute acid and further went to state that the initial pretreatment of the fibrous peel (banana and mango) residues breakdown its structure to make it more susceptible to enzymatic reactions. Likewise, significant concentration of sugar was obtained after the pretreatment and hydrolysis of the waste used in our study which showed that the cellulases and hemicellulases present in the hydrolyzing agent are potent enough for saccharification (Ebabhi *et al*., 2013a). This also correlates with the works of Ohmiya *et al*. (1995) and Hammond and Ayernor (2000) who reported that poplar and rice extracts rich in cellulases were ideal for saccharification.

Table 3: Amount of Bioethanol distilled from fermented Hydrolysates of Cassava and Sweet Potato Peels (Mean±SEM) in g/L

Agro-wastes	*C. tropicalis*	Control	% efficiency
Cassava peel (g/L)	7.47±0.60	0.00±0.00	23.8
Sweet potato peel (g/L)	23.90±0.60	0.00±0.00	47.99

Values are expressed as means±SEM for triplicate determinations

Yeast are generally known for their fermentative capability. The *C. tropicalis* (IMI 398401) used in this study was able to utilize the hydrolysates for the production of bioethanol as evidenced in the distillation results (Table 3). Kathiresan and Saravanakumar (2011) also demonstrated the potential of *C. tropicalis* in bioethanol production using pretreated sawdust as feed stock. The concentration of bioethanol produced from sweet potato peel was higher compared with the cassava peels in the present study. It could be

inferred that the organism effectively utilized the simple sugars present as higher amount of glucose was produced from the sweet potato peels. Using sawdust at 120 h of fermentation, Kathiresan and Saravanakumar (2011) were also able to produce 12.3g/L bioethanol with *C. tropicalis*as the fermenting agent. Previously, Ebabhi *et al*. (2013a) obtained 16.47±1.21g/L and 09.04±0.61g/L of bioethanol from sweet potato peels respectively using *Kluyveromyces marxianus* and *Pichia caribbica* which suggest that tuber peels could be used to produce bioethanol by yeast species if properly hydrolysed.

CONCLUSION

This study demonstrates the potential of cassava and sweet potato peels which are rich sources of lignocellulose in the production of bioethanol. The hydrolysis of these wastes to produce simple sugar for easy fermentation was actualized. The utilization of agro-waste such as cassava and sweet potato peels as demonstrated in this study for bioethanol production is a feasible economical venture, a source of financial empowerment and eco-friendly method of agro-waste management.

REFERENCE

Adesanya, O.A., Oluyemi, K.A., Josiah, S.J., Adesanya, R.A., Ofusori, D.A., Bankole, M.A. and Babalola, G.B. (2008). Ethanol production by *Saccharomyces cerevisiae* from cassava peel hydrolysate. *The Internet Journal of Microbiology,* **5**(1).

Agba, A.M.O., Ushie, M.E., Abam, F.I., Michael, S.A. and Okoro, J. (2010). Developing the biofuel industry for Effective Rural Transformation in Nigeria. *European Journal of Science Research,* **40**(3):441-449.

Arumugam, R. and Manikandan, M. (2011). Fermentation of pretreated hydrolysates of banana and mango fruit wastes for ethanol production. *Asian Journal of Experimental Biological Sciences,* **2**(2):246-256.

AOAC (Association of Official Analytical Chemist). Official methods of analysis (15th Edition) (1990).Washington, VA:AOAC.

Bhatia, L., Johri, S. and Ahmad R. (2012). An economic and ecological perspective of ethanol production from renewable agro waste: A Review. *AMB Express*, **2**:65.

Chaudhary, N. and Qazi, J.I. (2011). Lignocellulose for ethanol production: A review of issues relating to bagasse as a source material.*African Journal of Biotechnology,* **10**(8): 1270-1274.

Chinedu, S.N. and Nwinyi, C.O. (2012). Proximate analysis of *Sphenostylisstenocarpa* and *Voadzeia subterranean* consumed in South–Eastern Nigeria. *Journal of Agricultural Extension and Rural Development,* **4(3)**: 57 – 62.

Demirbas, M.F. 2011. Biofuels from algae for sustainable development. *Applied Energy,* **88**(10): 3473-3480.

Ebabhi, A.M., Adekunle, A.A., Osuntoki, A.A. and Okunowo, W.O. (2013a). Production of bioethanol from agro-waste hydrolyzed with cashew nut shell extract. *International Research Journal of Biotechnology,* **4**(3):40-46.

Ebabhi, A.M., Adekunle, A.A., Okunowo, W.O. and Osuntoki, A.A. (2013b). Isolation and characterization of yeast strains from local food crops. *Journal of Yeast and Fungal Research,* **4**(4): 38-43.

Hammond, T.K. and Ayernor, G.S. (2000). Combination of malted cereals and cassava starch in the production of sugar syrup. *Journal of Ghana Science Association,* **2**(1): 87-92.

Henk, K., Ezedinma, C., Kormawa, P., Asumugha, G., Makinde, K., Okechukwu, R. and Dixon, A. (2007). *Opportunities in the Industrial Cassava Marketing in Nigeria.* International Institute for Tropical Agriculture (IITA), Ibadan. 47pp.

Ledward, D.A, Taylor, A.J. and Lawrive, R.A. (2003). Upgrading waste for food and

feeds (3rdEdition).USA: Butter Orth. 321pp.

Kathiresan, K. and Saravanakumar, S.K. (2011). Bio-ethanol production by marine yeasts isolated from coastal mangrove sediment. *International Multidisciplinary Research Journal,* 1(1):19-24.

Mei, X., Mu, T. H. and Han, J. J. (2010). Composition and physicochemical properties of dietary fiber extracted from residues of 10 varieties of sweet potato by a sieving method. *Journal of Agriculture and Food Chemistry,* **58**(12): 7305-7310.

Miller, G.L. 1959. Use of dinitosalicyclic acid reagent for determination of reducing sugar. *Analytical Chemistry,* **31**(3): 426-428.

Ocloo, F.C.K. and Ayernor, G.S. (2010). Production of alcohol from cassava flour hydrolysate. *Journal of Brewing and Distillation*, **1**(2): 15-21.

Ohmiya, Y., Takeda, T., Nakamura, S., Sakai, F., Hayashi, T. (1995). Purification and bound endo-1,4-beta-glucanase from suspension cultured poplar cells. *Plant Cell Physiology*, **36**(4): 607-614.

Olsen, K. M. and Schaal, B. A. (1999). Evidence on the origin of cassava. *Phylogeography of Manihotesculenta In: Proceedings of the National Academy of Sciences of the United States of America.* **96**(10): 5587-5590.

Oyeleke, S.B. and Jibrin, N.M.(2009).Production of bioethanol from guinea corn husk and millet husk. *African Journal Microbiology Research,* **3**(4):147-152.

Patle, S. and Lal, B. (2007). Ethanol production from hydrolyzed agricultural wastes using mixed culture of *Zymomonasmobilis* and *Candida tropicalis. Biotechnology Letters* **29**:1839-1843.

Sofowora, A. E. 1993. Medicinal Plants and Traditional Medicine in Africa.Ibadan: Spectrum Books Limited. 288pp.

Updegraff, D.M. (1969). Semimicro determination of cellulose in biological materials. *Analytical Biochemistry* **32**(3): 420-424.