Potentials of Chemical and Biological Hydrolysis of Agricultural Wastes in the Production of Ethanol, Single Cell Protein (SCP) and Vinegar

C.U. Ogunka-Nnoka*, B.W. Abbey

Department of Biochemistry, University of Port Harcourt, Nigeria

Copyright©2017 by authors, all rights reserved. Authors agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Abstract The potentials of Chemical (acid) and Biological (*Aspergillus niger* and *Rhizopus oryzae*) hydrolysis on some agricultural wastes, namely: peels from ripe plantain (RPP), unripe plantain (UPP), red cocoyam (RCP), white cocoyam (WCP), yam (YP), cassava (CP) and garri processing chaff (GPC) into beneficial by products (Ethanol, Single Cell Protein and Vinegar) were investigated using standard methods. Glucose yield ranged from 4.68-17.24mg/g, 2.41-13.84mg/g and 1.28-5.22mg/g for *A. niger*, *R. oryzae* and Acid hydrolysis of the various wastes. Inoculating the hydrolysates with *Saccharomyces cerevisiae* yielded significant ($p < 0.05$) amount of ethanol after 120hrs, with significant ($p < 0.05$) reduction in glucose contents. Ethanol contents ranged from 3.82-6.89%, 3.74-6.44% and 3.28-6.25%, while the amount of single cell protein (SCP) obtained from the biomass after ethanol fermentation ranged from 9.10-15.80%, 7.50-13.11% and 6.00-10.20% for *A. niger*, *R. oryzae* and Acid hydrolysates respectively. The vinegar obtained after seven days fermentation of the alcoholic product had acetic acid concentrations ranging from 0.14-1.10%, 0.13-0.99% and 0.11-0.95% for *A. niger*, *R. oryzae* and acid treatments respectively. This study has shown usefulness of microbial methods of hydrolysis over the chemical treatment method, for improved yield of fermentation by products.

Keywords Agricultural Wastes, Hydrolysis, Ethanol, Vinegar, Single Cell Protein

1. Introduction

Efforts made for the expansion of food and energy resources through the conversion of glucose and other complex carbohydrates in wastes into ethanol, vinegar and single cell protein have heightened the interests of

researchers in the exploitation of several sources of hydrolysis [1]. Hydrolysis is an essential step in the conversion of waste to food and energy since it is through this process that complex carbohydrates in agricultural waste are degraded into their constituent sugars thus becoming readily available for fermentation by microorganisms responsible for the production of ethanol vinegar and single cell protein (SCP). Carbohydrate based agricultural products are important staple foods in the diet of people in most developing countries of the tropics. They consist of renewable resource of great variety of biotechnological potential. However, the generation of waste materials (peels) resulting from their processing pose pollution problems [2]. Notwithstanding this, in many cases, these peels might have potentials for conversion into useful products of higher value after biological treatment [3, 4]. The fermentable sugar produced from the hydrolysis of cellulosic fraction of the waste feed stock can serve as carbon source for microbes, especially *Saccharomyces cerevisiae* which is capable of fermenting sugar to ethanol. Ethanol production by fermentation faces competition with ethanol production from petroleum-based products as feed stocks and in this regard, the use of renewable materials would be more economical, since they are cheaper and easily available [5]. Ethanol serves as substrate for *Acetobacter aceti* for vinegar (acetic acid) production and presently, almost all acetic acid is produced synthetically [6] with a major disadvantage of the need of high cost catalyst and the dependence on non-renewable sources of raw materials such as crude oil. Hence acetic acid production by biological means become more attractive. The food and agricultural organization (FAO) of the United Nations established that vinegar is allowed for human consumption and that it must be produced from raw materials of agricultural origin by fermentation; by first converting glucose into ethanol followed by oxidation of the ethanol to acetic acid the main product of vinegar [7]. Another advantageous approach brought about by

fermentation, is the increased accessibility of protein of agricultural waste products in single-cell proteins (SCPs). Single cell proteins (SCP) are the dried cells of microorganism or microbial biomass obtained after fermentation which serve as protein supplement in human foods or animal feeds. With increase in population and worldwide protein shortage, the use of microbial protein as food or feed is more highlighted and such protein from microorganisms is said to be cheap and competes well with other sources of protein with good nutritive value [2,8,9]. Base on this foregoing, to exploit the potentials of non-conventional food sources such as peels and chaff which are by products of the stable foods in the developing countries as potential alternatives for novel foods using chemical and biological methods of hydrolysis as a primary step. Therefore, this study was carried out to evaluate the potentials of peels from ripe and unripe plantain, red and white cocoyam, yam, cassava, and garri processing chaff to produce ethanol, acetic acid, and single cell proteins.

2. Materials and Methods

Sample Collection

Peels of ripe plantain (RPP) and unripe plantain (UPP) (*Musa paradisiaca*), red cocoyam (RCP), white cocoyam (WCP) (*Colocasia esculenta*), yam (YP) (*Dioscorea rotundata*), cassava (CP) (*Manihot esculenta*) and garri processing chaff (GPC) were obtained from farmers and road side food vendors in Obio/Akpor Local Government Area of Rivers State of Nigeria, and identified at the Department of Plant Science and Biotechnology, University of Port Harcourt Choba, Rivers State Nigeria.

Sample Preparation

These seven agricultural wastes samples mentioned above were washed and oven-dried along with the garri processing chaff (GPC) at 60°C for 24 hours, then pulverized, sieved to 60µm particle size and packaged for further analysis.

3. Hydrolysis of Complex Carbohydrate

Acid Hydrolysis

Processed and stored samples of the Agricultural wastes were separately hydrolyzed following the method of Zainab *et al.*, (2011) as previously reported by Ogunka-Nnoka *et al.*, (2011). Briefly, samples (20g) of each peel were slurried in 120ml of 0.25M H₂SO₄ and extracted in water bath at 70-80°C for 90mins. Samples were cooled, neutralized then filtered through No. 1 Watman filter paper. Glucose content of filtrate was determined following the dinitrosalicylic acid method as described by Amadi and Ifeanchi, [5].

4. Microbial Hydrolysis

Source of Culture

Pure cultures of *Aspergillus niger* and *Rhizopus oryzae* were obtained from the Microbiology Department of the University of Port Harcourt, Rivers State, Nigeria. The Microbial cultures were maintained on potato dextrose Agar (PDA) slant.

Preparation of Fungal Inocula

Mineralized media consisting of (g/100ml), peptone 0.1, malt extract 0.1g, yeast extract 0.2g, MgCL 0.2g, (NH₄)₂SO₄ 0.2g, KH₂PO₄ 0.1g and FeSO₄.7H₂O 0.1g was prepared and sterilized then inoculated with 2.0ml of fungal culture. Cultures were incubated at room temperature for 5 days.

Hydrolysis

The method of Abouzied and Reddy (1986) was used. Briefly, 7.5g of each pregelatinized sample waste was suspended in a 250ml conical flask containing 200ml of mineralized media and autoclaved at 121°C for 30mins. The samples were cooled and a 5% (v/v) inoculation of *A. niger* or *R. oryzae* was used. Inoculation lasted for 72hrs and 10ml aliquot was centrifuged for 20min at 5,000xG. The supernatant was used for reducing sugar analysis following the Dinitrosalicylic acid method as described by Amadi and Ifeanchi, [5].

Determination of Ethanol, Single Cell Protein, and Acetic Acid

The hydrolysates from the Acid, *A. niger* and *R. oryzae* were inoculated each with 5% (v/v) *S. cerevisiae* under sterile condition. The fermentation lasted for 120hr under room temperature at pH 5.0. Aliquot of 20ml each of the sample waste was collected and centrifuged for 20min at 5,000xG and the supernatant was used for ethanol estimation using Gas Chromatography (HP6890 powered with chemstation Rev. A09.01 1206 Software). The single cell protein content was estimated using the biomass residue obtained after ethanol fermentation [10]. While the acetic acid (vinegar) was estimated using 33ml of the centrifuged alcoholic products inoculated with *Acetobacter aceti* inoculum [11].

Statistical Analysis

All data were analyzed using the analysis of variance. When analysis of variance revealed a significant effect, means were separated using Duncan's New Multiple Range Test as explained by [12].

5. Results and Discussion

Figures 1-7 shows the effect of various hydrolysis on glucose yield and also the residual glucose content after ethanol fermentation.

Figure 1. Glucose yield before and after fermentation of Ripe Plantain Peels. Bars bearing similar letters (a-d) are not significantly different ($p>0.05$)

Figure 2. Glucose yield before and after fermentation of Unripe Plantain Peels. Bars bearing similar letters (a-e) are not significantly different ($p>0.05$)

Figure 3. Glucose yield before and after fermentation of Red Cocoyam Peels. Bars bearing similar letters (a-c) are not significantly different ($p>0.05$)

Figure 4. Glucose yield before and after fermentation of White Cocoyam Peels. Bars bearing similar letters (a-c) are not significantly different ($p>0.05$)

Figure 5. Glucose yield before and after fermentation of yam Peels. Bars bearing similar letters (a-e) are not significantly different ($p>0.05$)

Figure 6. Glucose yield before and after fermentation of Cassava Peels. Bars bearing similar letters (a-f) are not significantly different ($p > 0.05$)

Figure 7. Glucose yield before and after fermentation of Garri Processing Chaff. Bars bearing similar letters (a-d) are not significantly different ($p > 0.05$)

Glucose yield ranged from 4.68-17.24mg/g, 2.41-13.84mg/g and 1.28-5.22mg/g for *A. niger*, *R. oryzae* and acid hydrolysis respectively. There was significant ($p < 0.05$) reduction in the glucose content after fermentation of all the hydrolysates. Ripe plantain peel, had the highest glucose content irrespective of the treatment used; while, low glucose yield was observed in the cocoyam variety (using microbial hydrolysis) and garri processing chaff (using acid hydrolysis). The effect of different hydrolysates on glucose yield showed that these organic wastes had appreciable glucose yield, with RPP ranging highest. The high content of glucose obtained in RPP from the various hydrolysates could be deduced from the fact that during ripening, starch molecules are hydrolyzed to sugar [7]. From the result, it suggests that the cocoyam peels were more resistant to microbial hydrolysis. The result of Figure 4 corroborates the study of Omemu *et al.*, [13] on cocoyam tuber. They stated that the reason for the variation could be as a result of some phytoconstituents present in the cocoyam cultivars that resist microbial attack. While for samples that had better glucose

yield with microbial hydrolysis, it was suggested that it could be due to the fact that the complex carbohydrate was pregelatinized prior to hydrolysis [14, 15].

Table 1. Ethanol yield of different hydrolysates

Sample	Hydrolysates (%)		
	Acid	<i>A. niger</i>	<i>R. oryzae</i>
RPP	3.28±0.11 ^b	3.82±0.25 ^a	3.74±0.16 ^a
UPP	3.83±0.14 ^b	5.08±0.18 ^a	4.88±0.22 ^a
RCP	3.36±0.09 ^b	4.11±0.30 ^a	4.01±0.15 ^a
WCP	3.50±0.18 ^a	5.16±0.21 ^b	4.60±0.13 ^c
YP	5.06±0.27 ^b	6.18±0.18 ^a	6.02±0.13 ^a
CP	4.17±0.33 ^b	5.70±0.25 ^a	5.60±0.16 ^a
GPC	6.25±0.29 ^a	6.89±0.31 ^a	6.44±0.20 ^a

Values are means ± SD of triplicate determinations. Means on the same row not followed by same superscript differ significantly ($p < 0.05$)

Table 1 shows the data on the ethanol yield from the various hydrolysate. Ethanol yield ranged from 3.28-6.25%, 3.82-6.89%, and 3.74-6.44% for Acid hydrolysate, *A. niger*, and *R. oryzae* hydrolysates respectively. Results obtained based on the different treatments showed that microbial hydrolysates had significantly ($p < 0.05$) higher values for the fermentation/products compared to acid hydrolysate. However, apart from the results shown for white cocoyam peels, the ethanol yields from both of the microbial hydrolysates of all the other substrates were comparable. Based on the sample performance, GPC had the highest ethanol yield and RPP had the lowest from the three hydrolysates. According to Omemu *et al.* [13], Akpan *et al.* [16], and Adesanya *et al.* [17], ethanol yield is expected to be directly proportional to glucose yield, however, a contrary result was obtained in this study for GPC and RPP. Ripe plantain peel had the highest glucose yield (Figure 1) with the lowest ethanol yield (Table 1) while the reverse was the case for GPC. This observation could have resulted from the presence of other products of hydrolysis; for instance, other sugars like sucrose and xylose which are not metabolized by the yeast (*S. cerevisiae*) may have been produced [18]. *S. cerevisiae* is specific for glucose, mannose and galactose, while other strains of yeast ferment sucrose, maltose and raffinose. Again, there might be an inhibitory effect of fermentation by-products like furfural, which has been reported to inhibit the activities of some glycolytic enzymes particularly dehydrogenases present in *S. cerevisiae* [19, 20]. In addition, the yeast might have utilized much of the nutrient for growth rather than for ethanol production [21]. On the other hand, GPC with moderate glucose yield had the highest ethanol yield and since the starch content depends largely on the processing method, more starch component with amylose moiety which can easily be hydrolysed to glucose, might have been deposited in the chaff, [3, 18]. The results obtained in this study, followed closely the observations made by Akpan *et al.*, [16] that reported maximum ethanol content of 8.2% in a mixture of groundnut shell and corncobs using acid hydrolysates, and

Akin-osanaiye *et al.* [8], reported 3.83 to 4.18% in peels of pawpaw.

Table 2. Production of Single Cell Protein by different hydrolysates

Sample	Hydrolysates (%)		
	Acid	<i>A. niger</i>	<i>R. oryzae</i>
RPP	7.30±0.82 ^a	12.60±1.31 ^b	9.20±0.61 ^c
UPP	6.90±0.31 ^a	10.60±0.77 ^b	8.30±0.52 ^{ab}
RCP	6.40±0.41 ^a	9.60±0.51 ^b	7.80±0.36 ^c
WCP	6.00±0.64 ^a	9.10±0.48 ^b	7.50±0.37 ^c
YP	9.10±1.51 ^a	12.10±1.04 ^b	11.00±1.16 ^{ab}
CP	9.70±0.83 ^a	15.50±0.97 ^b	12.90±0.73 ^c
GPC	10.20±0.45 ^{ab}	15.80±1.13 ^b	13.11±0.51 ^c

Values are means ± SD of triplicate determinations. Means on the same row not followed by same superscript differ significantly ($p < 0.05$)

The result for the single cell protein contents of the agricultural wastes using different hydrolyzed methods, are presented in Table 2. Single cell protein, content ranged from 6.00-10.20% 9.10-15.80%, and 7.50-13.11% for acid, *A. niger* and *R. oryzae* catalyzed hydrolysis respectively. For these fermented samples, garri processing chaff had the highest SCP yield and WCP had the least judging from the various hydrolysates. Also, *A. niger* hydrolysates produced the highest amount of SCP in all the samples compared to other hydrolysates. Single cell protein is the most readily measured biomass component [22]. Ajao *et al.*, [2] reported low levels of SCP for cocoyam peel inoculated with *Penicillium expansum*. Also, Akinyele and Agbro, [23] reported SCP level of 8.5 and 11.4% for ripe and unripe plantain peels respectively when subjected to solid state fermentation using *A. niger*. This result obtained, shows the possibility and effectiveness of the enrichment of agrowastes through the fermentation by *S. cerevisiae* and is thus suggestive of the applicability and usefulness in the poultry feed manufacturing industries among others.

Table 3. Production of vinegar from different hydrolysates

Sample	Hydrolysates (%)		
	Acid	<i>A. niger</i>	<i>R. oryzae</i>
RPP	0.11±0.03 ^a	0.14±0.01 ^a	0.13±0.03 ^a
UPP	0.28±0.02 ^a	0.33±0.02 ^a	0.32±0.03 ^a
RCP	0.14±0.01 ^a	0.21±0.03 ^b	0.16±0.02 ^{ab}
WCP	0.20±0.03 ^a	0.25±0.03 ^a	0.23±0.05 ^a
YP	0.90±0.04 ^a	1.07±0.05 ^b	0.93±0.02 ^a
CP	0.36±0.01 ^a	0.55±0.03 ^b	0.45±0.02 ^c
GPC	0.95±0.09 ^a	1.10±0.11 ^a	1.00±0.08 ^a

Values are means ± SD of triplicate determinations. Means on the same row not followed by same superscript differ significantly ($p < 0.05$).

As shown in Table 3, the amount of vinegar produced ranged from 0.11-0.95%, 0.14-1.10%, and 0.13-1.00% for *A. niger*, *R. oryzae* and Acid hydrolysates respectively. The production of acetic acid from these wastes was studied

using *Acetobacter aceti*. Following the above trend, *A. niger* hydrolysate had significantly higher yield over *R. oryzae* and acid hydrolysates for YP and CP samples studied. GPC had the highest yield (1.10%), while RPP had the least (0.11%). The study by Parrondo *et al.*, [11] revealed that fermentation of whey supplemented with lactose, using *Acetobacter aceti* for a longer period produced vinegar having concentration of acetic acid between 5-6% (v/v) with efficiency of biotransformation of 84%. Other researchers reported acetic acid yield of 0.80 to 0.90g acetic acid/glucose using *Clostridium sp.* [8] [24]. The fermentation process used in this study and the substrate employed satisfied FAO requirements in order to serve for food [11]. In this study, agricultural based domestically generated wastes were successfully converted into useful products of economic value using chemical and microbial approach.

6. Conclusions

From the result of this study, it was deducible that *Aspergillus niger* produced the best glucose yield and fermentation by products in majority of the agricultural wastes evaluated, and in general, the hydrolytic potentials of the chemical method was less effective than the applied microbiological sources.

REFERENCES

- [1] Sunil, J.K.(2016) Bioconversion OF waste feedstock into useful products: an insight into recent studies and Investigations, EPRA International Journal of Research and Development (IJRD) 1 (10), 98-103.
- [2] Ajao, A.T., Abdullahi, H.J., Atere, T.G. and Kolawole O.M (2009). Studies on the biodegradation and utilization of selected tuber wastes by *Penicillium expansum*. Biosciences Research Communication 31 (5): 221-227.
- [3] Okolo, B.W., Ezeogu, L.I., Mba, C.N. (1995). Production of raw starch digesting amylase by *A.niger* and *Bacillus alvei* from nature starch sources. J. Sci Food Agric. 69: 109-115.
- [4] Ogunka-Nnoka, C.U., Mepba, H.D., Abbey, B.W and Akaninwor, J.O. (2011). Evaluation of Citric Acid production potentials of food processing wastes. Nig. J. of Nutri. Sci. 32(2): 37-41.
- [5] Amadi, P.U., Ifeanchi, M.O. and Agomuo, E.N. (2017). The effects of different heating periods and exclusion of some fermentation conditions on bioethanol production from plantain pseudo-stem waste using the digestive juice of *Archachatina marginata*, garlic and *Saccharomyces cerevisiae*. *Biofuels*, Taylor and Francis Online Publishing firm DOI: 10.1080/17597269.2017.1292018.
- [6] Busche, R.M. (1991). Extractive Fermentation of Acetic acid. Economic tradeoff between yield of *Clostridium* and concentration of *Acetobacter*. Appl. Biochem. Biotech. 28 (29): 605-621

- [7] Harumi, V., Masitah., H., Ramachandran, K.B., (2003). "Acetic Acid Production by single Step anaerobic fermentation on glucose. In; Proceedings of technical postgraduate symposium (TECHNOLS 2003).
- [8] Akin-Osanaiye, B.C. Nzelibe, H.C and Agbaji, A.S (2005). Production of Ethanol from Carica payaya (paw paw) agro waste: Effect of saccharification and different treatments on Ethanol yield Afri. J. of Biotechnol. 4 (7): 657-659.
- [9] Dharumadurai, D., Lawanya, S, Saha, S., Thajuddin, N; Pannerslvam, A. (2011). Production of Single Cell Protein from Pineapple waste using yeast. Innovative Romanian Food Biotechnol. 8:26-31.
- [10] Nwabueze, T.U., Nwabueze, J.C. (2001). Growth, Nutrient utilization and Biomass production of *S. cerevisiae* from citrus wastes. J. Agric Biotech. Environ. 3:32-37.
- [11] Parrondo, J., Herrero, M., Carcia, L.A and Diaz, M (2003). A Note – production of Vinegar from Whey. J. Inst. Brew. 109(4): 356-358.
- [12] Wahua, T.A. T. (1999). Applied Statistics for scientific studies. Africa Link Press. Aba, Nigeria 68-76.
- [13] Omemu, A.M., Akpan, I., Bankole M.O. and Teniola, O.D (2005). Hydrolysis of raw tuber starches by amylase of *A. niger* AMO7 isolated from the soil. African Journal of Biotechnol. 4(1). 19-25.
- [14] Abaseed, A.E., Lee, Y.Y., Wastson, J.R., (1991). Effect of Transient Heat Transfer and Particle Size on Acid Hydrolysis of Hardwood Cellulose. Bioresour. Technol. 35: 15-21.
- [15] Prado, F.C. Veindernbaghe L.P.S, Wokiechowski, R., Rodrigues-Leon C. and Soccoh, R. (2005). Citric acid production by solid state fermentation on a semi pilot scale using different percentages of treated cassava bagasse. Bras. J. Chem. Eng. 22(4) 115-118.
- [16] Akpan, U.G., Kovo, A.S., Abdullahi, M. and Ijah, J.J. (2005). The Production of Ethanol from Miaze Cobs and Groundnut Shells. AU.J.T. 9 (2): 106-110.
- [17] Adesanya, O; Oluyemi, K; Josiah, S; Adesanya, R; Shittu, L; Ofuson, D., Bankole, M. and Babalola G (2008). Ethanol productions by *S. cerevisiae* from cassava peel Hydrolysates. The Internet Journal of Microbiology 5(1):22-27.
- [18] Ogunka-Nnoka, C.U., Mepba, H.D., Abbey, B.W and Akaninwor, J.O. (2011). Determination of Optimal condition for maximum yield of glucose from food processing wastes. Acid hydrolysis. Nig. J. of Nutri. Sciences. 32(1): 63-67.
- [19] Detroy, R.W., Cunningham, R.I., Bothast, R.J., Hagh, M.O. and Herman, A.J (1982). Bioconversion of wheat Straw cellulose /Hemicellulose to ethanol by *S. uwayen* and *Pachysolen tannophilus*. Biotechnology and Bioeng. 24:1105-1113.
- [20] Amadi, P.U. and Ifeancha, M.O. (2016). Impact of changes in fermentation time, volume of yeast, and mass of plantain pseudo-stem substrate on the simultaneous saccharification and fermentation potentials of African Land Snail slime and yeast, *Journal of Genetic Engineering and Biotechnology* Elsevier B.V, 14 (2), 289-297
- [21] Agu, R. C., Amadife A.E., Ude, C.M., Onyia, A., Ogu, E.O., Okafor, M.C. and Ezejiofor, E (1997). Combined Heat Treatment and Acid Hydrolysis of cassava grate waste (GW) biomass for ethanol production. Waste Management 17 (1):91-96
- [22] Rainbault, M. (1997). General and Microbiological aspects of solid substrate fermentation. Curitiba – Parana, Brazil
- [23] Akinyele, B.J and Agbro O (2007). Increasing the Nutritional value of Plantain wastes by the activities of fungi using the solid state fermentation Techniques. Research Journal of Microbiology 2(2); 117-124.
- [24] Parekh, S.R Cheryyan, M (1990) Acetate Production from glucose by *Clostridium thermoaceticum*. Process Biochemistry International. 117-121.