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Original Research Article

Bioethanol Production and Compositional Changes during Fermentation of Cassava Processing Wastes from a Local Cassava Mill

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A b s t r a c t

Compositional changes during fermentation of cassava processing wastes to produce bioethanol were determined. The naturally–occurring microbial species responsible for fermentation included *Lactobacillus plantarum, L. delbrueckii, L. coryniformis, Bacillus subtilis, Candida tropicalis* and *Saccharomyces cerevisiae* with *L. plantarum* being the most prevalent microorganism. Bacterial counts increased from $3.92 \times 10^5 \pm 0.04$ to $5.95 \times 10^5 \pm 0.07$ cfu/g while fungal species decreased from $2.62 \times 10^5 \pm 0.03$ to $2.10 \times 10^5 \pm 0.01$ cfu/g at the end of fermentation for 72 h. Moisture, protein and fat contents increased from 6.53 ± 0.03, 4.11 ± 0.22 and 4.28 ± 0.05% to 7.75 ± 0.78, 13.92 ± 1.52 and 6.64 ± 0.02% respectively after fermentation. There was a decrease in crude fibre, ash and carbohydrate contents. There was a decrease in magnesium, sodium, potassium, phosphorus, iron, zinc, manganese, aluminium, lead and copper contents with an increase in calcium content. The antinutrients (alkaloid, oxalate, saponin, tannin, phytate, cyanide and lignin) decreased after fermentation. Fermentation brought about 81.46% reduction in the cyanide content. There was significant increase ($p<0.05$) in the amino acid contents. At the end of fermentation for 72 h, actual yield of bioethanol produced was 32%.

K e y w o r d s

Bioethanol
Cassava processing wastes
Compositional changes
Cyanide content
Fermentation

Introduction

Nigeria is the largest producer of cassava, *Manihot esculenta* Crantz, a perennial woody shrub with an edible starchy tuberous root, which grows in tropical and subtropical areas of the world (Burrell, 2003). In Nigeria, both bitter and sweet varieties are cultivated. Varieties with cyanide level less than 50mg HCN/kg of pulp are non–toxic and sweet. Those with cyanide level between 50 – 100mg HCN/kg of pulp are moderately toxic varieties. And those with cyanide level more than 100mgHCN/Kg

of pulp are very toxic and bitter varieties (National Root Crops Research Institute NRCRI, 2004; Kobawila et al., 2005).

Despite their higher cyanide content, the bitter varieties are more predominantly utilized. This is due to the development of the techniques for processing them to safe products. The tubers are detoxified by the hydrolysis of linamarine and lotaustraline by the enzyme linamarase located in the root peels into hydrogen cyanide. The roots are primarily produced for food especially in the forms of garri, fufu and lafun (NRCRI, 2004). This is because cassava roots are quite high in carbohydrates about 60 to 70% for Nigeria cultivars. In 1999, Nigeria produced 33 million tonnes of cassava making it the world's largest producer in that year (FAO 2004). This increased to about 42 million tonnes in 2006.

As a rough estimate, about 10 million tonnes of cassava are processed into garri annually in Nigeria alone (Oboh et al., 2002). During the production of garri, two important biological wastes, that may cause damage to the environment, are generated. They are the cassava peels and the liquid effluent. In Ekiadolor, Ovia North East LGA, the case study for this research, these wastes are discharged into soil and water bodies causing great damage to the environment and also causing air pollution. Since garri is the staple food of the people in the area, the waste can become a source of energy for them. The women trek long distances to fetch firewood to fry the processed cassava into garri, since many cannot afford the high price of kerosene.

Ethanol has been promoted as a solution for a variety of complex problems relating to energy and environment. Compared to fossil fuels, ethanol has the advantage of being renewable, providing cleaner burning, having high octane rating and producing no greenhouse gases. The very little work done on development of bioethanol production technology in Nigeria, the rising and fluctuating petroleum prices, increasing threat to the environment from exhaust emissions and global warming have generated an intense interest in developing an alternative non – petroleum resource (Omale et al., 2010). There is therefore the urgent need for capacity building through the establishment of pilot plants for the adoption and local development of biofuels process technology. As the world largest producer of cassava, Nigeria can harness the potential of cassava as an energy source reducing our dependence on fossil fuel (Malgwi et al., 2002). Small scale batch production of ethanol was carried out from the cassava peels and effluent wastes derived from the local cassava processing mill. This study to determine the production of ethanol from the cassava wastes from a local cassava mill in Ekiadolor, is therefore expected to reawaken awareness about the importance of cassava in Nigeria not only as food but possible feedstock for biofuel production.

Materials and methods

Collection and preparation of samples

The peels collected from the cassava mill were washed, dried and ground. The liquid effluent from the pressed fermenting cassava was collected into a ten liter gallon.

Cassava fermentation

The cassava wastes were weighed and one hundred and fifty 150 ml of cassava effluent was mixed with 200 g of washed, dried, ground cassava peels. Sterile distilled water was added to make up the 500ml mark of the flask and the flasks were stopped with sterile cotton wool covered with aluminum foil. The mixtures were sterilized in an autoclave at 121°C for 15 min to gelatinize the starch. They were allowed to cool and sterile distilled water was aseptically added to make the 500 ml mark of the flask again. Prepared 20% w/v *Saccharomyces cerevisiae* isolates were aseptically inoculated into the flask. The mixture was transferred into a clean fermenter and fermentation of the cassava wastes took place for 72 h.

The distillate collected was measured using a measuring cylinder and the ethanol concentration was determined by measuring its specific gravity after distillation (Maiorella et al., 1981). The specific gravity value was used to determine ethanol concentration from a standard curve prepared using known concentrations of ethanol (Ado et al., 2009). Alcohol yield was read with an alcohol meter (Distillique, South Africa). The energy content of the ethanol produced was determined using an adiabatic bomb calorimeter (Ledikwe et al., 2005). The octane rating of the ethanol was determined by

the use of Zeltex ZX – 101C Portable Octane Analyzer (ASTM, 1985).

Microbiological analyses

The different indigenous microorganisms responsible for fermentation of the wastes were determined and isolated. The bacterial and fungal counts of the substrates were determined using pour plate techniques (Ezema, 2007). Ten ml of wastes collected at the beginning and end of fermentation were aseptically transferred into 90ml of sterile distilled water to give a 10^{-1} dilution and serial dilutions prepared from the suspension to give a range of 10^{-6}. From these dilutions 1ml was asceptically plated out using pour plate method for total viable counts on Nutrient Agar (Lab M Ltd UK), lactic acid bacteria on de Man – Rogosa - Sharpe (MRS) Agar (Lab M Ltd UK), total aerobic mesophiles on Plate Count Agar (Lab M Ltd UK) and total fungal counts on Potato Dextrose Agar (PDA) (Lab M Ltd., UK) supplemented with 10% lactic acid and 0.5% chloramphenicol (AOAC, 2001). The colonies were observed and counted in a Techmel and Techmel USA Counter Model TT 201. The results were expressed as colony forming units per gram (cfu/g). Representatives of the different purified colonies were subjected to various cultural, morphological and biochemical analyses (Sharpe, 1979). Identification was based on Bergey's Manual of Determinative Bacteriology 9th Edition (Holt et al., 1994) and Bergey's Manual of Systematic Bacteriology (Bonne et al., 2009). For fungi identification, wet mount method was used as described by Yarrow (1998).

Proximate analyses

Proximate Analysis of the wastes was carried out to determine their chemical compositions. pH was determined using the method of AOAC (1995), ash content was determined using the method of Pearson (1973), crude fibre content was determined by the tricyclic acid TCA method (IITA, 1990), moisture content was determined according to the oven method of AOAC (1995), fat content was determined using the Soxhlet extraction method as described by Oyeleke (1984), protein content was determined by the Micro-Kjeldahl method as described by Pearson (1973) and modified by Laukevics et al. (1984) and carbohydrate content was given as total carbohydrate by difference.

Mineral and physicochemical analyses

The mineral and physicochemical parameters of the cassava wastes were analyzed. The cyanide content was determined by the alkaline titration method (AOAC, 1990) and titratable acidity was determined using the standard method of AOAC (2000). The mineral contents like calcium, magnesium, boron, aluminium, iron, zinc, manganese, lead, phosphorus were determined using AAS by the procedure of AOAC (1990). Potassium and sodium contents were determined by the use of flame photometer (AOAC, 1990). Amino acids content were determined using the method of Spackman et al. (1958) as described by Uysal et al. (2002). The phytochemical contents (total oxalate, phytate, tannin, alkaloids, lignin and saponin) were determined. Total oxalate by the method of Day and Underwood (1986), phytate by the spectrophotometric method as described by Agoreyo et al. (2012), tannin was by the spectrophotometric method of Trease and Evans (1989), alkaloids by the method of Harborne (1973), lignin by the thioglycolic acid method of Bruce and West (1989) and saponin was determined as described by Abhishek et al. (2011). Specific gravity was measured using the specific gravity bottle or hydrometer (Luther et al., 2004).

Enzyme activity

The amylase activity was determined using the amylase reagent kit (Alli et al., 1998) and linamarase activity was determined by the method of O' Brien et al. (1991).

Statistical analysis of data

The results were presented as mean standard values of triplicates of results. A One – Way Analysis of Variance ANOVA and student's t – test was carried out (Ogbeibu, 2005). Significant difference was accepted at $p \leq 0.05$.

Results and discussion

Results obtained after fermentation of the cassava peel and effluent wastes is presented in Table 1. It showed that there was increase in the bacterial counts and a decrease in the fungal counts after fermentation for 72 h. The high microbial counts of the cassava wastes showed that the fermentation medium was suitable for microbial growth. The initial counts of the microorganisms at the

beginning of fermentation though not significant (p>0.05) became significant at the end of fermentation. The slight reduction in the fungal counts could be attributed to the inhibitory effect of the acid produced by the lactic acid bacteria (Tetchi et al., 2010).

Table 1. Microbial counts (cfu/g) before and after fermentation of cassava waste.

Microbial count	Before fermentation	After fermentation
Bacterial count (cfu/g)	$3.49 \times 10^5 \pm 0.04^c$	$5.95 \times 10^5 \pm 0.07^c$
Fungal count (cfu/g)	$2.62 \times 10^5 \pm 0.03^c$	$2.10 \times 10^5 \pm 0.01^b$
Values are means ± standard deviation (n =3). Means in the same column with the same superscript are not significantly different (p>0.05).		

The microorganisms isolated during fermentation included lactic acid bacteria (*Lactobacillus plantarum, L. delbrueckii* and *L. coryniformis*), *Bacillus subtilis, Candida tropicalis and Saccharomyces cerevisiae*. *L. plantarum* was the most prevalent microorganism with the highest counts during fermentation of the cassava wastes (Table 2). *L. plantarum* converts low molecular weight sugars almost quantitatively into lactic acid thus contributing to the organoleptic qualities and preservation potential of the fermentable product. The ability to produce sufficient amount of reducing sugar from the cassava wastes determine the amount of ethanol produced (Agbogbo and Wenger, 2007). The combination of indigenous organisms like lactobacillus species with high linamarase activity and *B. subtilis* responsible for root softening isolated in this study is favourable. The ability of these organisms to break down cellular structure facilitated the contact between linamarin and endogenous linamarase in cassava (Westby et al., 1997).

Table 2. Total viable count (cfu/g) and percentage prevalence of microbial isolates.

Microbial isolates	Total viable count (cfu/g)		Prevalence (%)
	Before fermentation	After fermentation	
Lactobacillus plantarum	1.23×10^5	2.09×10^5	35.17
Lactobacillus delbrueckii	4.02×10^4	6.85×10^4	11.52
Lactobacillus coryniformis	6.43×10^4	1.09×10^5	18.41
Bacillus subtilis	5.88×10^4	1.00×10^5	16.84
Candida tropicalis	2.54×10^4	2.03×10^4	9.69
Saccharomyces cerevisiae	2.19×10^4	1.76×10^4	8.37

Table 3. Proximate composition of cassava wastes.

Proximate composition (%)	Before fermentation	After fermentation
Moisture	6.53 ± 0.03^b	7.75 ± 0.78^b
Crude Protein	4.11 ± 0.22^a	13.92 ± 1.52^b
Fat	4.28 ± 0.05^b	6.64 ± 0.02^b
Crude Fiber	6.12 ± 0.04^a	5.90 ± 0.06^a
Ash	1.29 ± 0.04^a	0.28 ± 0.04^a
Carbohydrate	77.67 ± 6.10^a	39.41 ± 6.20^a
Values are means ± standard deviation (n =3). Means in the same row with the same superscript are not significantly different (p>0.05).		

The proximate composition of the cassava wastes is represented in Table 3. There was increase moisture, protein and fat contents and decrease ash, crude fiber and carbohydrate contents. The increase in protein during the fermentation of the cassava peels with waste water could be attributed to possible secretion of extracellular enzymes such as amylases, linamarase and cellulose (Oboh et al., 2003) into the cassava mash by the fermenting organisms. The increase in fat content could be attributed to the possibility that fungi could secrete microbial oil (Oboh and Akindahunsi, 2003). The significant increase in protein, fat and moisture of the residue and decrease in crude fiber is of nutritional importance for livestock like swine (Akinfala and Tewe, 2001).

Table 4. Physicochemical composition of the cassava wastes.

Physicochemical composition	Before fermentation	After fermentation
pH	6.68 ± 0.02[a]	3.89 ± 0.06[b]
Specific gravity	1.52 ± 0.01[a]	1.03 ± 0.03[b]
Reducing sugar (%)	61.53 ± 0.21[b]	39.41 ± 0.78[b]
Hydrogen cyanide (mg/kg)	43.75 ± 0.11[b]	8.11 ± 2.08[b]
Titratable acidity (%)	0.02 ± 0.20[b]	3.88 ± 0.65[a]
Values are means ± standard deviation (n =3). Means in the same row with the same superscript are not significantly different (p>0.05).		

The physicochemical composition of the cassava wastes before and after fermentation is represented in Table 4. The pH of the fermenting medium decreased due to the loss of cyanide from the cassava roots. Also, the activities of the lactic acid bacteria caused a decrease in pH which resulted to an increase in titratable acidity (Okoh et al., 2010). The drop in pH and the increase in titratable acidity in the cassava wastes could be attributed to the accumulation of some organic acids such as lactic and acetic acids.

The high microbial counts accounted for higher consumption of soluble sugars and faster drop of pH (Coulin et al., 2006). Though statistically there was no significant difference in the reduction of reducing sugar content, the decrease in content of reducing sugars contributed to the production of lactic acid during the fermentation process. According to Panda et al. (2008), *L. plantarum* can produce α-amylase and reducing sugars from hydrolysed starch. The reduction in the amount of cyanide was due to the grounding of the cassava peels which disrupted the structural integrity of the plant cells, thus allowing cyanogenic glucosides to come in contact with the enzyme linamarase. Also, the fermentation of the peels with the effluent waste water reduced the cyanide content considerably below the deleterious level of 30mg/kg after fermentation (Ubalua, 2007).

Table 5. Enzyme activity during fermentation of the cassava wastes.

Enzyme Activity (1U/ml)	Before fermentation	After fermentation
Linamarase Activity	0.02 ± 0.00[a]	2.63 ± 0.05[b]
Amylase Activity	5.12 ± 0.02[a]	6.28 ± 0.42[b]
Values are means ± standard deviation (n =3). Means in the same row with the same superscript are not significantly different (p>0.05).		

Table 6. Mineral composition of the cassava wastes.

Mineral composition (mg/l)	Before fermentation	After fermentation
Calcium	236.58 ± 1.14[a]	250.31 ± 9.16[a]
Magnesium	93.98 ± 0.32[a]	71.68 ± 3.12[a]
Sodium	410.67 ± 1.39[a]	368.38 ± 2.30[a]
Potassium	533.82 ± 1.81[a]	505.58 ± 4.09[a]
Phosphorus	398.51 ± 1.35[a]	320.68 ± 3.07[b]
Iron	198.97 ± 0.67[a]	170.83 ± 5.38[a]
Zinc	51.88 ± 0.18[a]	37.59 ± 2.35[a]
Manganese	7.54 ± 0.03[a]	5.21 ± 0.51[a]
Aluminium	4.85 ± 0.02[b]	3.03 ± 0.37[a]
Lead	2.53 ± 0.01[a]	0.99 ± 0.05[a]
Copper	5.42 ± 0.02[b]	3.51 ± 0.48[b]
Values are means ± standard deviation (n =3). Means in the same row with the same superscript are not significantly different (p>0.05).		

The enzyme activity of the cassava wastes is presented in Table 5. There was increase in enzyme activity after fermentation. The increase in linamarase activity was due to increased loss in structural integrity which enhanced contact with the endogenous enzyme linamarase. The grinding of the peels improved the contact between microbial enzymes and linamarin

(Okafor and Nwabuko, 2003). Amylase activity increased due to conversion of starch to reducing sugar (Etoa et al., 2005).

The mineral composition of the cassava wastes before and after fermentation is presented in Table 6. The fermentation process caused an increase in calcium level with reduction in magnesium, sodium, potassium, phosphorus, iron, zinc, manganese, aluminium, lead and copper. The reduction in all the minerals except calcium is as a result of their utilization by the fermenting microorganisms as reported by Aderiye and Ogunjobi (1998). Potassium was the highest quantity of mineral present with the micronutrients recording the lowest quantity.

The phytochemical composition of the cassava wastes as presented in Table 7 showed a reduction in all the parameters tested. The cassava waste was susceptible to microbial nutrient enrichment and detoxification causing a significant reduction in all the antinutrients present. After fermentation, the levels of the antinutrients were far below the detrimental dose in food.

The amino acid profile of the cassava waste is presented in Table 8. There was increase in the content of the amino acids tested. The significant increase in protein after fermentation in this study must have been due to the increase in the amino acid composition. These amino acids play significant role in protein synthesis, tissue repairs, hormone synthesis and precursors of heme as well as synthesis of enzymes that catalase biochemical reactions in cells (Maduka et al., 2004).

Table 7. Phytochemical composition of the cassava wastes.

Phytochemical composition	Before fermentation	After fermentation
Oxalate (mg/100g)	14.32 ± 2.00^{a}	5.91 ± 0.08^{a}
Phytate (mg/100g)	107.35 ± 13.54^{b}	61.30 ± 1.54^{a}
Tannin (%)	0.36 ± 0.20^{a}	0.20 ± 0.01^{a}
Alkaloid (%)	4.09 ± 0.30^{b}	0.23 ± 0.02^{a}
Lignin (mg/100g)	2.52 ± 0.03^{b}	0.21 ± 0.37^{a}
Saponin (%)	0.18 ± 0.02^{a}	0.13 ± 0.52^{a}
Values are means ± standard deviation (n =3). Means in the same row with the same superscript are not significantly different (p>0.05).		

Table 8. Amino acid profile of the cassava wastes.

Amino acids (g/100g)	Before fermentation	After fermentation
Arginine	7.83 ± 0.26^{a}	8.34 ± 0.02^{b}
Histidine	0.97 ± 0.05^{b}	1.23 ± 0.16^{b}
Lysine	2.07 ± 0.02^{b}	2.75 ± 0.01^{b}
Tryptophan	0.53 ± 0.01^{a}	0.98 ± 0.03^{a}
Phenylalanine	0.85 ± 0.10^{b}	1.04 ± 0.01^{b}
Methionine	1.01 ± 0.62^{a}	1.38 ± 0.02^{b}
Threonine	3.21 ± 1.52^{a}	3.77 ± 0.02^{b}
Leucine	5.89 ± 1.40^{b}	6.12 ± 0.01^{b}
Valine	1.97 ± 0.04^{b}	2.72 ± 0.04^{b}
Values are means ± standard deviation (n =3). Means in the same row with the same superscript are not significantly different (p>0.05).		

The properties of the ethanol produced from the fermentation of the cassava wastes is presented in Table 9. The volume of the bioethanol produced from the cassava wastes was 51cm^3 and the specific gravity (SG) was 0.9504. The volume multiplied by the SG gives the mass 48.47. The rate of enzymatic hydrolysis of the cassava wastes was expressed by the accumulation of reducing sugar in the hydrolysate. The ability to produce sufficient amount of reducing sugar determines the importance of a particular feedstock for ethanol production (Agbogbo and Wenger, 2007). The low amount of ethanol (32%) from the cassava wastes was due to the low reducing sugar obtained after

hydrolysis and as well as the amounts of the total carbohydrates coupled with a significant portion of proteinous matter in the peels (Ballesteros et al., 2000). The ethanol yield though low was higher than that reported in other studies in which it was reported that cassava peel hydrolysate prepared enzymatically yielded 1.05% and 2.3% ethanol (Adesanya et al., 2008) and 26% from cassava peel (Oyeleke et al., 2012).

Table 9. Properties of ethanol produced from fermentation of cassava wastes.

Parameter	Cassava wastes
Volume (cm^3)	51.00
Specific gravity	0.95
Mass	48.47
Reducing sugar, converted (%)	22.12
Actual yield (%)	32.00
Theoretical yield (%)	35.30
Fermentation efficiency (%)	91.00
Octane rating	36.00
Energy content (KJ/g)	8.32

The percentage reducing sugar converted was used to determine the theoretical yield of the ethanol. The theoretical yield was determined by multiplying the reducing sugar by 0.511 g and dividing by the actual ethanol yield in percent (Maiorella et al., 1981). Both values were comparable. The alcohol fermentation efficiency (FE) or yield in percent depends on the ability of the yeast to utilize particular feedstock based on their characteristics and compositional differences (Nuwamanya et al., 2011). The FE is actual yield divided by the theoretical yield × 100 (Ocloo and Ayernor, 2010).

The energy content of the ethanol was determined in order to ascertain the amount of energy released as fuel. The value of the energy content got (8.32 KJ/g) was lower than that of normal ethanol due to the low yield of ethanol produced. The octane rating is the measure of the resistance of petrol to engine knocking (Kemp et al., 2003). The octane rating of the ethanol from this study is 36. Octane rating does not relate to the energy content of the fuel but it is only a measure of the fuels tendency to burn in a controlled manner.

Conclusion

This research study has determined that ethanol can be produced from cassava wastes, which are the peels and effluent. The production of ethanol from cassava wastes is a means of controlling environmental pollution due to cassava processing. Also, it mitigates the human effects on climate change by producing efficient, clean and renewable energy that can reduce the dependence of Nigeria on fossil fuels.

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