International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (12), pp. 001-006, December, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals



*Full Length Research Paper*

# Fermentation in cassava *(Manihot esculenta* Crantz) pulp juice improves nutritive value of cassava peel

**Naa Ayikailey Adamafio*, Maxwell Sakyiamah and Josephyne Tettey**

Department of Biochemistry, University of Ghana, P. O. Box LG 54, Legon, Ghana.

Accepted 14 October, 2018

**A major challenge in using cassava peel as feed for animals is the presence of cyanogenic glycosides and the low concentration of protein. The present study investigated the possibility of upgrading cassava peels using fermented cassava pulp juice. Cassava pulp juice was squeezed out of grated cassava pulp and fermented for 3 days at ambient temperature. The microorganisms in the fermented pulp juice were identified as *Aspergillus niger*, *Aspergillus flavus* and *Lactobacillus spp.* Non-sterile cassava peels were sun-dried, milled and inoculated with fermented cassava pulp juice over a 7-day period. Controls were treated with either sterile distilled water, autoclaved inoculum or phosphate buffer (pH 5) over the same period. After 7 days, the cyanogenic glycoside content of the peels, determined by the silver nitrate titration method, had decreased to 12.3% ($p < 0.05$) of the value for untreated peels while the cyanogenic glycoside content of the controls was 38.8 - 42.9%. Proximate analysis of 7-day inoculum-treated and untreated cassava peels showed that the protein content of the treated peels had increased 10-fold and significant decreases in starch and fat content were recorded. The fibre content remained unchanged. The present findings show that microorganisms present in fermented cassava pulp juice are capable of enhancing the nutritional value of cassava peels by increasing the protein content and reducing the cyanogenic glycoside content to levels safe for consumption by livestock.**

**Key words:** Cassava peel, pulp juice, cyanogenic glycosides, microorganisms.

## INTRODUCTION

Substantial quantities of cassava (Manihot esculenta Crantz) peel, that could provide carbohydrates for livestock (Ezekiel et al., 2010; Ukanwoko et al., 2009), are generated in Ghana annually from the processing of cassava into starch, chips and gari. Maximum utilization of this bioresource in an integrated agricultural system would alleviate the major challenge of inadequate dry-season feed for livestock in Ghana and generate additional income for cassava processors and farmers. Amino acid-derived β-glycosides of α-hydroxynitriles, termed cyanogenic glycosides, are produced by a variety of plants as defense biomolecules (Gleadow et al., 2008; Bak et al., 2006). The cassava plant produces two toxic

cyanogenic glycosides, linamarin (2-β-D-glucopyranosyloxyl isobutyronitrile) and lotaustralin (methylbutyronitrile), a large proportion of which is present in the peel (Cardoso et al., 2005). The enzymes linamarase and hydroxynitrile lyase which catalyze the degradation cyanogenic glycosides to release hydrogen cyanide (HCN), are sequestered in different tissues of the cassava plant and released when the tissue is disrupted (Kimaryo et al., 2000). Chronic ingestion of fresh or processed cassava peel-based diets containing sub-lethal dietary cyanide has reportedly caused impaired thyroid function and growth, neonatal deaths and lower birth rates in animals (Fatufe et al., 2007; Ernesto et al., 2000). Another limitation to the use of cassava peels as animal feed is its low protein content (Ezekiel et al., 2010).

Sun drying, the commonest method used in the treatment of cassava peels for livestock feeding by subsistence farmers in Ghana, is only partially effective in reducing cyanogenic glycoside content (Tewe, 1989). Various methods of processing, some more effective than others, have been described (Perera, 2010; Kuti and Konoru, 2006). However, it is important to identify methods that are highly

*Corresponding author. E-mail: adamafio@gmail.com.

**Abbreviation: HCN,** hydrogen cyanide.

effective, require no sophisticated equipment and can readily be adopted by subsistence farmers.

Although the use of pure cultures of microorganisms such as *Aspergillus spp, Saccharomyces spp* and *Lactobacillus spp*, or combinations of these is reported to cause a substantial decrease in cyanogenic glycoside content (Oboh, 2006; Oboh and Akindahunsi, 2003), its application by subsistence farmers in Ghana may be undermined by the cost and techniques involved. An alternative approach may be the use of fermented cassava pulp juice, which contains cyanohydrophilic microorganisms and is readily available as a waste product of starch and gari producing industries. The present study was, therefore, aimed at investigating the effectiveness of naturally fermented cassava pulp juice in the remediation of cassava peels.

## MATERIALS AND METHODS

Cassava roots were purchased from Madina market in the Ga East District of Accra, Ghana. The samples were washed with water and peeled to obtain both parenchyma and peels.

### Treatment of peel and cyanogen determination

Peeled cassava parenchyma was grated and pressed in a fine cloth to obtain pulp juice. The juice was allowed to ferment for 3 days at ambient temperature (26 - 32°C). This served as the inoculum. The peels were cut into small segments, sun-dried for 3 days and milled into fine powder. A 5 ml aliquot of the inoculum was added to 3.0 g of milled cassava peels and incubated, without aeration or shaking in capped plastic containers, at 32°C for 1 to 7 days. Control samples were treated with equal volumes of either 0.2 M phosphate buffer (pH 5.3) or autoclaved inoculum. After termination of the fermentation process, samples were dried at 40°C to constant weight and the cyanogenic glycoside content was determined using the silver nitrate method (AOAC, 1965).

### Identification of microorganisms

Nutrient agar plates were prepared from 1.3% nutrient broth and 1.5% agar-agar, inoculated with 0.5 ml of the inoculum and incubated at 37°C for 24 h. The isolates were subcultured to obtain distinct colonies. These were subsequently used to inoculate 6.5% Sabouraud Dextrose Agar plates which were then incubated at 37°C for 3 days. Light microscopy was performed to identify fungi on the basis of their colony colours and hyphal characteristics (Thom and Raper, 1945; Burnett, 1972). In order to identify bacteria, peptone agar plates (2.5% peptone water broth and 1.5% agar-agar) were inoculated aseptically with the distinct colonies from the nutrient agar plates and incubated at 37°C for 24 h. The bacteria were identified on the basis of colony and cell morphology, gram stain reaction and standard biochemical tests (Harley and Prescott, 1990; Buchnan and Gibbons, 1974).

### Nutrient composition of cassava peels

Protein, non-structural carbohydrate composition and content were determined using the macro-Kjedahl method (AOAC, 1970), the Luff-Schoorl method (Kirk and Sawyer, 1991) and a gravimetric method (AOAC, 1970), respectively. Moisture content was also measured (AOAC, 1970) and values of nutrients were expressed on dry weight basis.

### Data analysis

Statistical analysis was performed using Microsoft Excel and SPSS. Analysis of variance was carried out using Statgraphics-plus Software Programme, Version 3.0. Differences were considered significant if p was less than 0.05.

## RESULTS

As shown in Figure 1, the cyanogenic glycoside content of all categories of cassava peels diminished during the 7 day incubation period. However, cassava peels treated with fermented cassava pulp fluid showed the most significant decrease in cyanogenic glycosides ($p < 0.05$).The level dropped to 53.8 mg/kg after the fermented pulp fluid treatment from 351 mg/kg in the dried untreated peels. This represents 12.3% HCN relative to the untreated peels which was set as 100%. The buffer and sterile inoculum treatment gave values of 43.1% and 34.3% respectively after the 7th day of fermentation.

The inoculum-treated peels displayed the highest reduction in HCN content. The buffer-treated control samples showed the least reduction in HCN.

A general increase in protein content was observed for all samples during the treatment period (Figure 2) The most dramatic increase (10.8 fold) in protein occurred in the inoculum-treated peels after the 7th day ($p < 0.05$). No significant alteration in fibre content was recorded (Figure 3). However, the amount of non-structural carbohydrates, comprising starch and sugars, reduced after treatment with both the inoculum and autoclaved inoculum (Figure 4).

The protein content of the inoculum-treated peels increased considerably ($p < 0.05$). There was no significant change in the fibre content of the inoculum-treated peels. The non-structural carbohydrate con-tent of both autoclaved inoculum-treated and inoculum-treated peels reduced significantly($p < 0.05$).

Both fungi and bacteria were present in the inoculum. The fungi were identified as *A. niger* and *A. flavus*. Gram positive motile cocci and rod-shaped (bacilli) bacteria were present in the fermented cassava pulp juice. Biochemical tests showed that the bacteria included lactose fermenters and non gas producers. They neither metabolized indole nor sulphur.

## DISCUSSION

The objective of the present study was to determine whether or not fermented cassava pulp juice would be an effective agent in the bioremediation of cassava peels. A reduction in the pH of the cassava pulp juice from 7.5 to

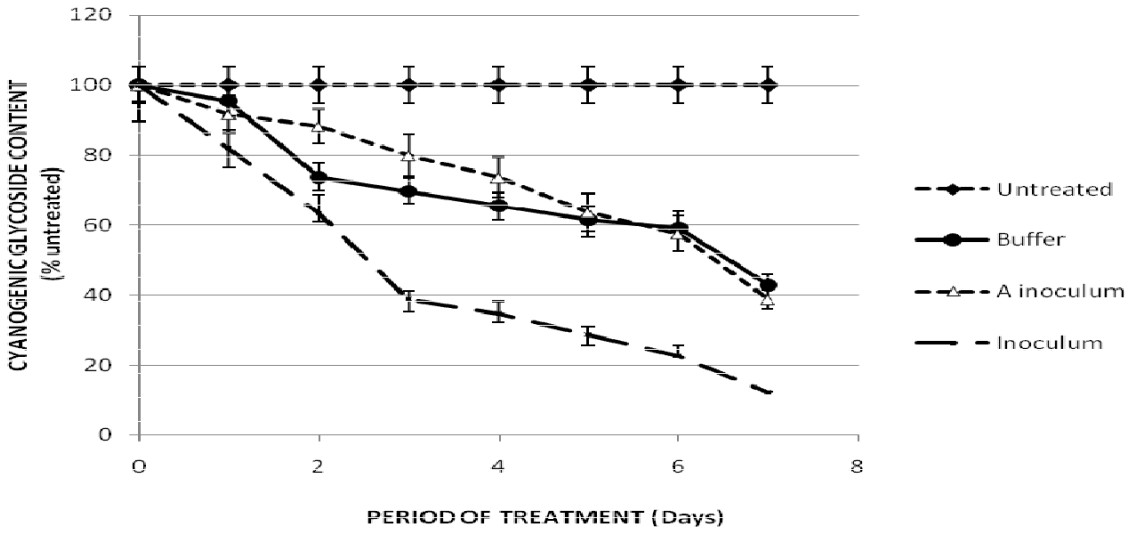


**Figure 1.** Effect of microbial treatment on cyanogenic glycoside content of cassava peel. Dried milled cassava peels were treated with fermented cassava pulp juice, autoclaved inoculum (A-inoculum) or phosphate buffer for 7 days and the amount of cyanogenic glycosides was determined by the silver nitrate method. The values are presented as mean ± SEM.

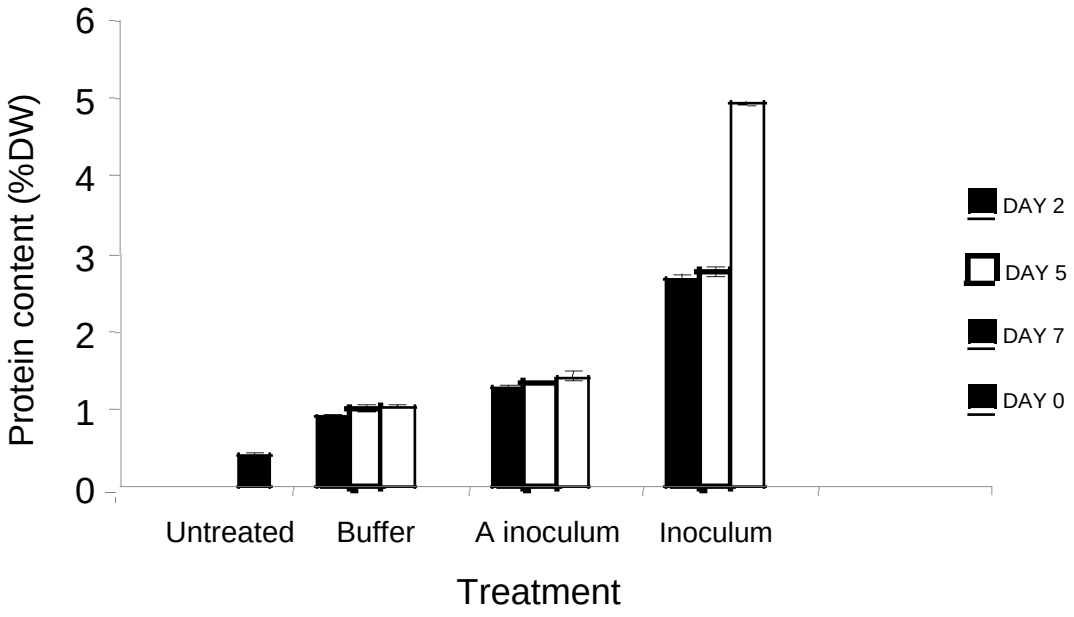


**Figure 2.** Effect of microbial treatment on the protein content of cassava peels. Dried milled cassava peels were treated with fermented cassava pulp juice, autoclaved inoculum (A-inoculum) or phosphate buffer for 7 days and protein content of the peels was determined using the Kjedahl method.

5.3 after 3 days of fermentation prior to the treatment of cassava peels was observed. This necessitated the inclusion of buffer (pH 5.3) treatment to discount the possibility that any alterations in cyanogen content were caused by acidification.

Treatment of the cassava peels with fermented cassava pulp juice resulted in a progressive decrease in cyanogen content during the 7 days period by 86.2% compared with 58.8 and 64.4% for buffer-treated and autoclaved inoculum -treated peels respectively (Figure 2). The observed reduction in cyanogen content of the buffer-treated and autoclaved inoculum-treated peels is attributable to degradation by indigenous cyanophilic microorganisms associated with the non-sterile cassava

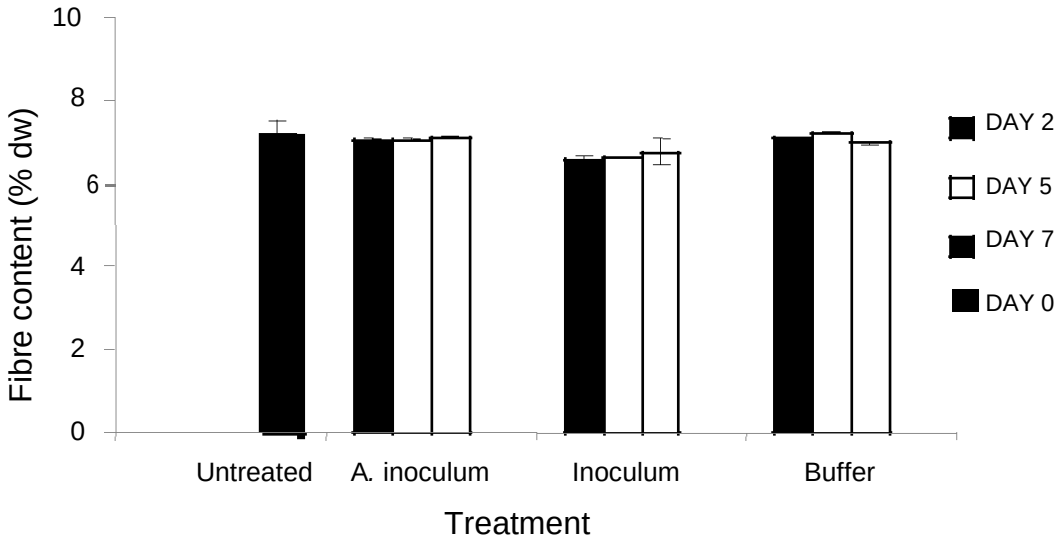


**Figure 3.** The effect of microbial treatment on fibre content of cassava peels. Dried milled cassava peels were treated with fermented cassava pulp juice, autoclaved inoculum (A-inoculum) or phosphate buffer for 7 days and crude fibre was measured using a gravimetirc method.

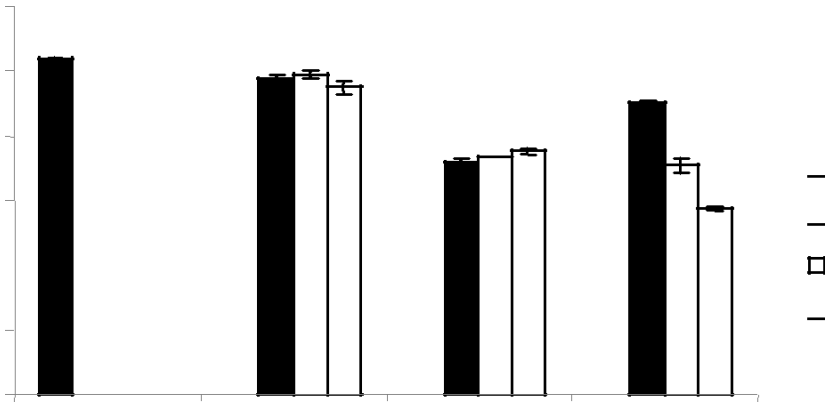

**Figure 4.** Non-structural carbohydrate content of cassava peels after microbial treatment. Dried milled cassava peels were treated with fermented cassava pulp juice, autoclaved inoculum (A-inoculum) or phosphate buffer for 7 days and starch and sugar content of the peels was determined using the Luff-Schoorl method.

peels. This would account, in part, for the decrease in the cyanogen content of the inoculum-treated peels. However, a comparison of the magnitude of reduction in treated and control peels indicates that cyanogenic glycoside degrading microorganisms were present in the fermented cassava pulp juice and contributed significantly to the level of reduction observed. This is consistent with the observation by Amoa-Awua et al. (1997) that all yeasts and moulds identified in traditional cassava dough inocula exhibited linamarase activity and were therefore capable of degrading cyanogenic glycosides.

The extent of reduction of cyanogens in the treated cassava peel compares favourably with the findings of studies conducted using pure strains of *Saccharomyces cerevisae* and *Lactobacillus spp.* (Oboh, 2006; Amoa-Awua and Jakobsen, 1995). Furthermore, the present findings confirm the observation by other researchers (Tweyongyere and Katongole, 2002; Wickham, 2001) that microbial treatment achieves a far greater degree of

cyanide detoxification than methods such as drying, steaming and boiling, which reduce cyanogen content by approximately 27, 16 and 47%, respectively. Cyanophilic microorganisms have been shown to possess the enzymes linamarase, hydroxynitrile lyase and cyanide hydratase that catalyze the sequential degradation of cyanogenic glycosides into HCN which is subsequently converted into formamide which they use as both a nitrogen and carbon source.

Analysis of the nutrient composition of inoculum-treated and control cassava peels showed a considerable increase in protein content of the latter by day 7 (Figure 3), probably reflecting a rise in microbial mass as well as an increase in the concentration of extracellular microbial enzymes. In contrast to the effect on protein content, there was no significant alteration in fibre content. This is advantageous since it indicates that the cellulose and hemicellulose components of fibre, vital sources of energy for ruminants, were conserved. In contrast, non-structural carbohydrate content decreased somewhat (Figure 4). Presumably, the microorganisms oxidized carbohydrates and lipids to obtain energy. The enhanced protein content of the inoculum-treated peels should be beneficial from the standpoint of animal nutrition. The observation that the quantity of non-structural carbohydrates, comprising starch and sugars, reduced after treatment with both the inoculum and autoclaved inoculum suggests that amylolytic microorganisms associated with the non-sterile peels were largely responsible for this reduction.

Since a natural process of fermentation was employed in the present study, the spectrum of indigenous microorganisms present should be fairly similar to that present in naturally-fermented cassava dough, *Agbelima.* A mixed population of microorganisms, mainly lactic acid bacteria, *Bacillus* species, moulds and yeasts are involved in the fermentation of cassava dough (Amoa-Awua et al., 1996). Since this product has been consumed for centuries, in Ghana, Togo and Benin, without any deleterious effects, it is highly improbable that the consumption of inoculum-treated cassava peels by livestock would lead to adverse consequences. Nonetheless, the identification of *A. flavus* in the inoculum used in the present study necessitates future studies on mycotoxin content, to ascertain the wholesomeness of fermented cassava pulp juice-treated cassava peels as an animal feed resource. Future studies would also investigate whether the inclusion of GRAS (Generally Recognized As Safe) microorganisms in the fermentation process would increase the velocity of the fermentation process appreciably.

## Conclusion

In conclusion, the findings of the present study show that liquid waste discarded by cassava processing industries, can be fermented and effectively utilized to achieve remediation of cassava peels, another waste product. The fermented pulp juice contains cyanophilic microorganisms which are capable of reducing the levels of cyanogenic glycosides in cassava peels to non-toxic levels and also improving the nutritional value of the peels by increasing the protein content of the peels appreciably. The cassava pulp juice can be readily obtained from starch and gari industries and distributed to subsistence farmers for the purpose of improving the nutritional value of cassava peels after sun-drying. This would be an effective and simple means of detoxifying and enriching cassava peels as a feed resource for livestock production.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance provided by Mr. K. Bako of the Department of Botany and Ms. R. Nyarko of the Department of Biochemistry in the identification of microorganisms.

## REFERENCES

AOAC (1965). Official Methods of Analysis (10th edn). Horwitz W. (ed.) Association of Official Analytical Chemists, Washington, USA p. 341.

AOAC (1970). Official Methods of Analysis (11th edn). Association of Official Analytical Chemists, Washington, USA p. 438.

Amoa-Awua WKA, Jakobsen M (1995). The role of *Bacillus* sp in the fermentation of cassava. J. Appl. Bacteriol., 79: 250-256.

Amoa-Awua WKA, Appoh FE, Jakobsen M (1996). Lactic acid fermentation of cassava dough into agbelima. Int. J. Food Microbiol. 31: 87-98.

Bak S, Paquette S, Morant M, Morant A, Saito S, Bjarnholt N, Zagrobelny M, Jørgensen K, Osmani S, Simonsen H, Pérez R, Heeswijck T, Jørgensen B, Møller B. (2006). Cyanogenic glycosides: A case study for evolution and application of cytochromes p450. Phytochem. Rev. 5: 309-329.

Buchnan RE, Gibbons NE (1974). Bergey's Manual of Determinative Bacteriology (8th edn). Waverly Press Inc. Baltimore, USA pp. 270-575.

Burnet HL (1972). Illustrated Genera of Imperfect Fungi, Burgess Publishing Company, Minneapolis, Minnesota, USA pp. 34-45.

Cardoso AP, Mirione E, Ernesto M, Massaza F, Cliff J, Haque MR, Bradbury HJ (2005). Processing of cassava roots to remove cyanogens. J. Food Composition and Analysis 18: 451-460.

Ernesto M, Cardoso AP, Nicala D, Mirione E, Massaza F, Cliff J, Haque MR, Bradbury JH (2002). Persistent konzo and cyanogens toxicity from cassava in northern Mozambique. Acta.Tropica. 82: 357-362.

Ezekiel OO, Aworh OC, Blaschek HP, Ezeji TC. (2010). Protein enrichment of cassava peel by submerged fermentation with*Trichoderma viride* (ATCC 36316). Afr. J. Biotechnol. 9: 187-194.

Fatufe AA, Akanbi IO, Saba GA, Olowofeso O, Tewe OO (2007). Growth performance and nutrient digestibility of growing pigs fed a mixture of palm kernel meal and cassava peel meal. Livestock Research for Rural Development. Volume 19, Article #180. Retrieved September 26, 2008, from http://www.cipav.org.co/lrrd/lrrd19/12/fatu19180.htm

Gleadow RM, Haburjak J, Dunn JE, Conn ME, Conn EE (2008). Frequency and distribution of cyanogenic glycosides in *Eucalyptus* L'Hérit. Phytochem. 69: 1870-1874.

Harley PJ, Prescott ML (1990). Laboratory Exercises in Microbiology, W.C. Brown Publishers, Toronto, Canada.

Kimaryo VM, Massawe GA, Olasupo NA, Holzapfal WN (2000). The use

of a starter culture in the fermentation of cassava for the production of ''kivunde'', a traditional Tanzanian food product. Int. J. Food Microbiol. 56: 179-190.

Kirk RS, Sawyer R (1991). Sugars and preserves. In: Pearson's Composition and Analysis of Foods, Longman Scientific and Technical, Essex pp. 183-235.

Kuti JO, Konoru HB (2006). Cyanogenic glycosides content in two edible leaves of tree spinach (*Cnidoscolus* sp) J. Food Composition and Analysis 19: 556-561.

Oboh G (2006). Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisae* and *Lactobacillus* sp solid media fermentation techniques. Elect. J. Biotechnol. 9: 203-206.

Oboh G, Akindahunsi AA (2003). Biochemical changes in cassava products(flour and gari) subjected to Saccharomyces cerevisae solid media fermentation. Elect. J. Biotechnol. 82: 559-602.

Perera CO (2010). Removal of Cyanogenic Glycoside from Cassava during Controlled Drying. Drying Technol. 28: 68-72.

Tewe OO (1989). Indices of cassava safety for livestock feeding. In: M. Bokanga,. Essers AJA, Poulter N, Rosling H, Tewe O. (eds). Int. Soc. Hort. Sci. pp. 241-249.

Thom C, Raper KB (1945). A Manual of the *Aspergilli,* The Williams and Wilking Company, Baltimore, USA pp. 25-68.

Tweyongyere R, Katongole I (2002). Cyanogenic potential of cassava peels and their detoxification for utilization as livestock feed. Vet. Human Toxicol. 44: 366-369.

Ukanwoko AI, Ahamefule FO, Ukachukwu SN (2009). Nutrient Intake and Digestibility of West African Dwarf Bucks Fed Cassava Peel-Cassava Leaf Meal Based Diets in South Eastern Nigeria. Pak. J. Nutr. 8: 983-987.

Wickham LD (2001). FAO report Small-scale processing of starchy staplescaricomcountries.http//journals2.iranscience.net800/www.fao.org/ag/ags/Agsi/starchfinal/hs_257Ewickhamrev3proof.htmCASSA retrieved on 1104-2006.