Nigerian Journal of Biochemistry and Molecular Biology 0189-4757/96 $3.0 + 0.00
Volume 26, No. 1, 2011. pp. 1-9 (Printed in Nigeria)

NJBMB/004/11

Nutritional and toxicological aspects of *Aspergillus niger* fermented cassava (*Manihot esculenta,* Crantz) products-based diets

Oboh, G.
Biochemistry Department, Federal University of Technology, P.M.B. 704 Akure, Nigeria

ABSTRACT: This study sought to evaluate the nutritional and toxicological potential of *Aspergillus niger* fermented cassava products (flour and gari). Cassava mash was fermented with pure strain of *A. niger* for 72 days and subsequently processed to flour and gari (the forms in which it is popularly consumed in Nigeria). Three weeks old Wistar rats were assigned into 3 groups: 1-3 such that Group 1 was fed with the basal diet (control) while Groups 2 and 3 were fed with the *A. niger* fermented gari- and flour-based diets (40 % inclusion) and water *ad libitum* for 21days. Feed intake, weight gain and feaces were monitored while haematological indices, serum enzymes and metabolites were determined at the end of the feeding experiment. The results revealed that there was no significant change ($P>0.05$) in the average daily feed intake, daily weight gain, feed conversion ratio, apparent and dry matter digestibility, and nitrogen intake of the rats fed *A. niger* fermented cassava products diet. However, there was significant increase ($P<0.05$) in the serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in rats fed *A. niger* fermented cassava products; while there was no significant ($P>0.05$) change in the total bilirubin and albumin in rats fed cassava flour. Furthermore there were no significant ($P>0.05$) changes in the Packed Cell Volume (PCV) and White Blood Cell (WBC), while there was significant increase ($P<0.05$) in the Red Blood Cell (RBC). However, the *A. niger* fermented cassava products (flour and gari) could not be considered safe due to the elevated serum GPT and GOT which is an indication of possible damage to the liver and heart, despite the good nutritional and haematological attributes of the *A. niger* fermented cassava products.

Keywords: Cassava, *Aspergillus niger*, Flour, Gari, Fermentation, Toxicity

INTRODUCTION

Cassava (*Manihot esculenta,* Crantz) is a perennial vegetatively propagated shrub cultivated throughout the lowland tropics for its starchy roots. It is of great economic importance to several countries of Africa where its consumption (in terms of carbohydrate content) exceeds those of other crops. The advantage it has over other root crops include its easy propagation, high yield, pest and drought resistance (Cock, 1985, O'Brien *et al.,* 1991). However, certain varieties contain a large amount of cyanogenic glucosides (linamarin and lotaustralin) which can be hydrolysed to hydrocyanic acid (HCN) by the endogenous enzyme (linamarase) when the plant tissue is damaged during harvesting, processing or other mechanical processes (Conn, 1973). Another drawback for the utilization of cassava as food is that the protein content is very low, and cases of malnutrition have been reported in places where cassava is the staple constituent of the diet (Bailey, 1961).

Many efforts have been made to remove these constraints on the utilization of cassava. Two principal methods are available for increasing the protein content of fermented cassava products. First, through controlled fermentation during which microflora are produced in large numbers in the mash (Reinbault and Alazard, 1980). This method of upgrading the protein content of cassava has been developed in some countries. Protein enrichment through solid media fermentation of cassava products had been experimented with *Aspergillus fumigatus* (Reade and Gregory, 1975), *Rhizopus oryzae* (Vlavonou, 1988; Akindahunsi *et al*., 1999; Oboh and Oladunmoye, 2007), *Aspergillus niger* (Oboh *et al.,* 2002) and *Saccharomyces cerevisae* (Oboh and

***Corresponding Author**
E-mail: goboh2001@yahoo.com

Akindahunsi, 2003; Oboh, 2006). The second method involves adding protein to the deficient food from external sources in such a way as not to alter significantly the organoleptic qualities of the original food (Collins and Falasinnu, 1977; Oshodi, 1988; Odetokun *et al.*, 1998). However, the most viable option is via the biotechnological route by way of the "generally regarded as safe" (GRAS) organisms (Ejukonemu and Nwafor, 2004). These GRAS organisms have the potential of not only beefing up the protein content of cassava and it's by products, but also reducing their fibre level and anti- nutritional load through enzymatic hydrolytic fermentation (Dhilon and Skirvaran, 1999; Belewu and Jimoh, 2005).

However, some microbial activities are usually accompanied by secretion of some harmful metabolites (mycotoxins) (Oboh *et al*., 2000) that may be very dangerous to the eventual consumer; therefore a lot of questions had emanated on the safety of fungi fermented cassava products for human consumption. This study therefore sought to investigate the nutritional quality and safety of *Aspergillus niger* fermented cassava products (flour and gari) using rats as model.

MATERIALS AND METHODS

Materials

Sweet variety of Cassava tubers (less than 50 mg/kg cyanide content) freshly harvested from the Research Farm of the Federal University of Technology, Akure, Nigeria was used in the study. The chemicals used were of analytical grade, and distilled water was used as solvent. Pure culture of *Aspergillus niger* were collected from Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria.

Methods

Sample preparation

The cassava tubers were peeled, crushed, pressed using hydraulic press, and the pressed pulp was later subjected to fermentation (Oboh, 2006). A known amount (1 kg) of the processed pulp was spread in a tray (about 50 cm diameter) to an average layer thickness of 2 cm. A 10 g freshly sub-cultured pure strains of *Aspergillus niger* in 73 ml nutrient solution [containing urea (8.0 g), $MgSO_4.2H_2O$ (7.0 g), KH_2PO_4 (1.3 g) and citric acid (2.0 g)] was carefully added to the solid matrix in order to obtain a well homogenized mixture. The mash was allowed to ferment for three days; the incubation temperature and the relative humidity of the air were 30ºC and 90-93% respectively, the fermented cassava mash was subsequently processed to gari and flour (Oboh *et al*., 2002).

Animal grouping and feeding parameters

The nutritional quality was determined using Wistar rats grouped into three. Animals in each group were housed in individual wire cages in a room maintained at room temperature. The diet was formulated (Table 1) using a modified method of Aletor (1993), the fermented cassava flour and gari were included at 40% replacement of the corn flour in the basal diet (Table 1). Group 1 (control) were fed with the basal diet (Table 1), while groups 2-3 were fed with the formulated diet. Feed intake, daily weight gain, feed efficiency and apparent and dry matter digestibility were computed. At the end of the 21-day feeding period, the rats were sacrificed by asphyxiation and blood was withdrawn by heart puncture. The serum obtained was subsequently stored at -18^{o}C until analysis.

Determination of haematological and biochemical parameter

The haematological parameters (PCV, RBC and WBC) were determined according to the standard procedures (Aning *et al*., 1998). The biochemical parameters (albumin, bilirubin, GOT, GPT) were also determined following the Instructions contained in the assay kits.

Data Analysis

The results of the four replicates were pooled and expressed as mean ± SEM. One-way analysis of variance (ANOVA) and the least significance difference (LSD) of the data was carried out using the procedure described by Zar (1984). Significance was accepted at $p \leq 0.05$.

RESULTS

The production chart for micro-fungi fermented cassava products is depicted in Figure 1. The feed utilization and performance as presented in Table 2, revealed that there was no significance difference (P>0.05) in the average daily feed intake of those fed basal (9.8g/day) and *A. niger* fermented cassava products based diet [Flour (8.6 g/day), Gari (9.9 g/day)]. While the average daily weight gain of the rats was 2.2 g/day for those fed the fermented cassava flour and 3.0 g/day for those fed *A. niger* fermented cassava-gari, there was no significant difference in the average daily weight gain of the animals fed the control diet and those fed *A. niger* fermented cassava products. The Feed:Gain ratio (feed conversion ratio) for the *A. niger* fermented cassava-gari was not different from the control. There was no significant difference (P>0.05) in the average nitrogen intake of the rats maintained on *A. niger* fermented products and those placed on the basal diet (control).

Furthermore, there was no significant difference (P>0.05) in the apparent and dry matter digestibility of both fermented cassava products and the control (Table 3). There was significant increase (P<0.05) in the activities of serum GOT and GPT of rats fed *A. niger* fermented cassava products compared to the basal diet fed animals. In contrast, there was no significant difference (P>0.05) in the serum total bilirubin contents of rats fed *A. niger* fermented cassava flour and the basal diet, whereas the serum total bilirubin content of animals fed *A. niger* fermented cassava-gari was significantly reduced (P<0.05). In addition, there was no significant difference (P>0.05) in the serum albumin content of rats fed *A. niger* fermented cassava flour whereas the serum albumin content of rats fed *A. niger* fermented cassava-gari significantly increased (Table 4).

Rats maintained on *A. niger* fermented cassava product resulted in PCV that compared well with the control whereas the RBC increased significantly in the animals maintained on the fermented cassava products (Table 5). Furthermore, there was no significant difference (P>0.05) in the WBC of rats fed *A. niger* fermented cassava flour and the basal diet whereas animals fed *A. niger* fermented cassava-gari produced significantly reduced (P<0.05) WBC.

DISCUSSION

Successful use of biotechnology for plant propagation and breeding could dramatically raise crop productivity and overall food production. Tissue culture techniques are already being used to produce more drought and disease resistant varieties of different crops including cassava. However, fermentation techniques in solid media such as protein-enriched cassava products can improve the nutritional value of the crop (Oboh *et al.*, 2002; Oboh and Akindahunsi, 2003; Oboh, 2006). The nutritive value and safety of *A. niger* fermented cassava products was the focus of the present investigation.

The absence of significant alteration in the average daily weight gain, daily feed intake, feed: gain ratio and nitrogen intake of rats fed *A. niger* fermented cassava products compared to those fed the basal diet suggest that the fermented cassava products will support growth. If rats gain weight and show good feed-conversion efficiency and overall performance, it is an indicator that the diets are of high enough quality (Van Weerden, 1999). However, the Feed:Gain ratio of the *A. niger* fermented cassava products are within the same range with what was reported for albino rats fed sorghum rootless and Brewer's dried grain (Aning *et al*., 1998). This reveals that the *A. niger* fermented cassava products diet would support growth.

The apparent and dry matter digestibility of the *A. niger* fermented cassava flour and

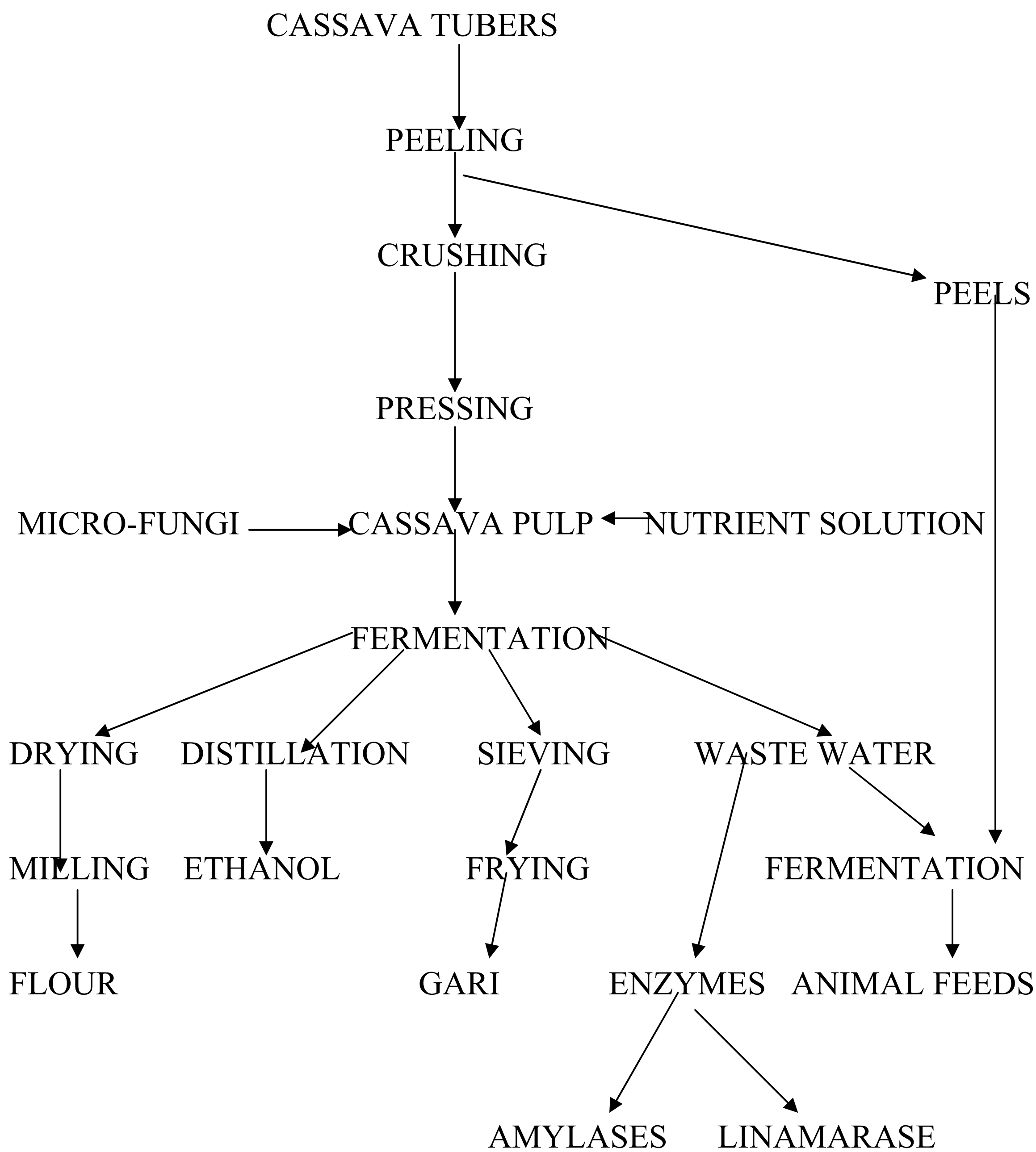

Fig.1: Production chart for micro-fungi fermented cassava products

Table 1: Composition of the basal diet

Sample	Quantity
Maize	66.6
Groundnut cake	14.0
Fish meal	5.0
Palm oil	10.0
[a]Vitamin premix	1.0
[b]Mineral premix	3.4

[a]vitamin premix was obtained from the nutritional Biochemical Corporation, Ohio, U.S.A.

[b] The mineral premix, contained per kilogram mixture: $CaHPO_4$, 735.0g; K_2HPO_4, 81.0g; K_2SO_4, 68.0g; NaCl, 30.6g; $CaCO_3$, 21.0g; Na_2HPO_4, 21.4g; MgO, 25.0g and trace metal mixture, 18.0g. The constituents of the trace metal mixture were: Ferric citrate, 31.0g; $ZnCO_3$, 4.5g; $MnCO_3$, 23.4g; $CuCO_3$, 1.85g; KI, 0.04g and citric acid to make 100g.

*The protein content of the *Aspergillus niger* fermeted cassava flour and gari was 16.23 % and they were included at 40% equi-weight replacement of the basal diet

Table 2: Nutrient utilization and performance of albino rats fed *Aspergillus niger* fermented cassava products

Sample	Control	Flour	Gari
Daily weight gain (g/rat/day)	3.0 ± 0.4^a	2.2 ± 0.6^a	3.0 ± 0.7^a
Daily feed intake (g/rat/day)	9.8 ± 1.2^a	8.6 ± 0.8^a	9.9 ± 1.0^a
Feed: Gain ratio	3.3 ± 0.5^a	4.0 ± 0.8^a	3.3 ± 0.5^a
Nitrogen intake (g/rat/day)	0.3 ± 0.0^a	0.2 ± 0.0^a	0.3 ± 0.0^a

Values represent means of four replicates

Means with the same superscripts along the same row for each product (flour and gari) are not significantly different (P>0.05)

Table 3: Apparent and dry matter digestibility of *A. niger* fermented cassava products

Sample	Control	Flour	Gari
Apparent Digestibility (%)	86.5 ± 3.1^{a}	88.9 ± 1.3^{a}	86.6 ± 6.4^{a}
Dry Matter Digestibility (%)	87.2 ± 4.0^{a}	89.8 ± 1.6^{a}	87.1 ± 6.4^{a}

Values represent means of four replicates.
Means with the same superscript along the same row for each product (flour and gari) are not significantly different (P>0.05)

Table 4: Changes in selected serum biomolecules of rats fed *A. niger* fermented cassava products

Parameter	Control	Flour	Gari
GOT (IU/L)	267.0 ± 7.0^{a}	338.0 ± 8.0^{b}	491.0 ± 11.0^{c}
GPT (IU/L)	39.0 ± 4.0^{a}	72.0 ± 2.0^{b}	86.0 ± 2.0^{b}
Total Bilirubin (mg/dl)	3.4 ± 0.2^{b}	2.9 ± 0.3^{ab}	2.5 ± 0.3^{a}
Albumin (mg/dl)	0.2 ± 0.0^{b}	0.2 ± 0.0^{a}	0.5 ± 0.1^{b}

Values represent means of four replicates.
Means with the same superscript letter(s) along the same row are not significantly different (P>0.05)
GPT- Glutamate pyruvate transaminase, GOT - Glutamate oxaloacetate transaminase.

Table 5: Haematological parameters of rats fed *A. niger* fermented cassava products

Sample	Control	Flour	Gari
PCV (%)	25.7 ± 0.6^{a}	25.3 ± 0.6^{a}	25.0 ± 1.0^{a}
RBC($x10^{6}/\mu l$)	3.9 ± 0.1^{a}	4.5 ± 0.2^{b}	5.1 ± 0.0^{c}
WBC ($x10^{6}/\mu l$)	8.4 ± 0.2^{b}	8.5 ± 0.2^{b}	4.5 ± 0.1^{a}

Values represent means of four replicates.
Means with the same superscript letter(s) along the same row for each product (flour and gari) are not significantly different (P>0.05)
PCV - packed cell volume; RBC - red blood cell; WBC - white blood cell.

gari was not altered since the values compared well with the animals maintained on basal diet. Furthermore, the apparent digestibility of the *A. niger* fermented cassava products was higher than that of white beans-81.3% (Bressani, 1999), and naturally fermented cassava product-81.71% (Aletor, 1993), but lower than that of casein (95.2%) reported by Bressani (1999). This indicates that the protein in the diet was highly digestible, and could possibly be attributed to the reduction in tannin during fermentation (Oboh *et al.*, 2002). This is further corroborated by lack of significant difference (P>0.05) between the dry matter digestibility of the basal and *A. niger* fermented cassava products (flour and gari). Although, these values are lower than that of raw sweet potato (90.4%) root diet fed to pigs, but compared favourably with that of the cooked sweet potato root (85.5%) diets fed to pigs (Canope and Tamiya, 1977). This suggest that *A. niger* fermented cassava products have high digestibility.

In view of the fact that some microbial activities are usually accompanied by secretion of some harmful metabolites (mycotoxins) (Oboh *et al.*, 2000), the safety of *A. niger* fermented cassava products was evaluated in the present study. The rise in the serum GOT activity in the rats fed fermented cassava products could be the consequence of some metabolites such as Aflatoxin that might have been produced by the organism during the fermentation process (Oboh *et al.,* 2000). Aflatoxin B_1 has been implicated in liver damage (Oboh and Akindahunsi, 2005). Therefore, increase in serum GOT activity may be attributed to damage to either the liver or the heart of the animals (American Liver Foundation, 1995; David and Johnston, 1999). Furthermore, this rise in serum GPT activity confirms a possible damage to the liver of rats fed *A. niger* fermented cassava products. The elevated serum GOT and GPT in the rats fed *A. niger* fermented cassava products agrees with the findings of Oboh and Akindahunsi (2005), where rats fed *S. cerevisae* fermented cassava flour had elevated serum GOT and GPT. Normally, the blood contains low levels of these transaminases, but after damage to the cell membrane, these enzymes are liberated into the serum. Elevated GPT and GOT levels can also be attributed to liver tissue damage and cardiac necrosis. Since GOT increases when the cell membrane of the heart and liver are disrupted, elevated activity of GPT is a more specific and reliable indicator of liver injury (American Liver Foundation, 1995; David and Johnston, 1999).

The absence of an effect on the serum bilirubin content of the albino rats fed *A. niger* fermented cassava products suggest that the *A. niger* fermented cassava products did not interfere with the production of the rats bilirubin. It is also an indication of absence of hyperbilirubineamia that is usually found in haemolytic anemia as a result of the inability of the liver to adequately sequester the bile pigment from the blood (Oboh and Akindahunsi, 2005). Furthermore, the increase in the serum albumin content of the rats fed *A. niger* fermented gari diet cannot be caterigocally stated, however it could be speculated that the amino acids in the diet may have been mobilized for albumin synthesis. The *A. niger* fermented cassava products will not obstruct biosynthesis of albumin in the liver (American Liver Foundation, 1995; David and Johnston, 1999).

The results of the PCV, RBC and WBC counts compared favourably with the results reported on albino rats fed sorghum and brewer's grains (Aning *et al*., 1998), cassava products (Aletor, 1993) and *S. cerevisae* fermented cassava flour (Oboh and Akindahunsi, 2005) to the extent that the *A. niger* fermented cassava products would not cause haemolysis. The low WBC count by rats fed *A. niger* fermented gari indicates absence of infection in the animals.

In conclusion, the *A. niger* fermented cassava products (flour and gari) could not be considered safe due to the elevated serum GPT and GOT which is an indication of possible damage to the liver and heart, despite the good nutritional and haematological attributes of the *A. niger* fermented cassava products.

REFERENCES

Akindahunsi, A. A., Oboh, G. and Oshodi, A. A. (1999). Effect of fermenting cassava with *Rhizopus oryzae* on the chemical composition of its flour and gari, Riv Ital Sostanze Grasse. 76: 437-440.

Aletor, V. A. (1993). Cyanide in garri. 2: Assesssment of some aspects of the nutrition, biochemistry and haematology of the rats fed garri containing varying residual cyanide levels. Int J Food Sci Nut. 44: 289 - 292.

American Liver Foundation (1995): Liver function tests, http://www.gastro.com/liverpg/ifts.htm

Aning, K. G., Ologun, A. G., Onifade, A., Alokan, J. A., Adekola, A. I. and Aletor, V. A. (1998). Effects of replacing dried brewer's grains with sorghum rootlets on growth, nutrient utilization and some blood constituents in the rat. Animal Feed Sci. Tech., 71: 185-190.

Bailey, K. V. (1961). Rural Nutrition Studies in Indonesia III, Epidemiology of hunger Edema in the cassava area, Trop. Geo. Med.,13: 289-295.

Belewu, M. A. and Jimoh, N. O. (2005). Blood, carcass and organ measurements as influenced by Aspergillus' treated cassava waste in the diet of West African Dwarf (WAD) goat. Global J. Agric. Sci., 4: 125-128.

Bressani, R. (1999). Nutritional evaluation in humans. Bioconversion of organic residues for rural communities. http://www.unu.edu/hp/unupbooks/80434e/80434E11.htm

Canope, J. and Tamiya, B. (1977). Preliminary experiments in the use of chlorella as human food. Food Technol. J., 8: 179-182.

Cock, H. J. (1985). Cassava new potential for a neglected crop. Colorado, Boulder: Westview press.

Collins, J. L. and Falasinnu, G. A. (1977). Yam (*Dioscorea spp)* flour fortification with soy flour. J. Food Sci., 42, 821-833.

Conn, E. E. (1973). Cyanogenic glucosides: their occurrence biosynthesis and function. In chronic cassava toxicity. Nestle, B. and Mclntyer, B. (eds). Canada, Ottawa: International Development Research Centre. p. 55-63.

David, E. and Johnston, M. D. (1999). Special considerations in interpreting liver function tests. American Family Physician. Published by the American Academy of Family Physicians. http://www.aafp.org/afp/990415ap/2223.html

Dhilon, J. K. and Shirvaram, N. (1999). Biodegradation of cyanide compounds by a Pseudomonas species. Can. J. Microb., 45: 201-208

Ejukonemu, F. E. and Nwafor, O. E. (2004). Bioconversion of cassava wastes for protein enrichment using amylolytic fungi. A preliminary report. Global J. Pure & App. Sci., 10:505-507.

Oboh, G. (2006). Nutrient enrichment of cassava peels using *Saccharomyces cerevisae* & *Lactobacillus spp* solid media fermentation techniques. Elect. J. Biotech., 9: 46-49.

Oboh, G. and Akindahunsi, A. A. (2003). Biochemical changes in cassava products (flour & gari) subjected to S*accharomyces cerevisae* solid media fermentation. Food Chem., 82: 599-602.

Oboh, G. and Akindahunsi, A. A. (2005). Nutritional and toxicological evaluation of *Saccharomyces* cerevisae fermented cassava flour. J. Food Comp. Anal., 18: 731 – 738.

Oboh, G. and Akindahunsi, A. A. and Oshodi A. A. (2000). Aflatoxin and moisture content of micro-fungi fermented cassava products (flour and gari). App. Trop. Agric., 5: 154-157**.**

Oboh, G., Akindahunsi, A. A. and Oshodi, A. A. (2002). Nutrient and anti-nutrient content of *Aspergillus niger* fermented cassava products (flour and gari). J Food Comp. Anal., 15: 617-622.

Oboh, G., Akindahunsi, A. A. and Oshodi, A. A. (2003). Dynamics of Phytate-Zn balance of fungi fermented cassava products (Flour & Gari). Plant Food Hum. Nut., 58: 1-7.

Oboh, G. and Oladunmoye, M. K. (2007). Biochemical changes in Micro-Fungi fermented Cassava Flour produced from Low- And Medium-Cyanide variety of Cassava Tubers. Nut. Health., 18: 355 - 367

O'Brien, G. M., Taylor, A. J. and Poulter, N. H. (1991). Improved enzymatic assay for cyanogens in fresh and processed cassava J. Sci. Food Agric., 56: 277-289.

Odetokun, S. M., Aiyesanmi, F. A. and Esuoso, K. O. (1998). Enhancement of nutritive value of pupuru, a fermented cassava products. Riv Ital Sostanze Grasse, 75: 155-158.

Oshodi, A. A. (1988). Protein enrichment of foods that are protein deficient. II. Fortification of processed cassava (Gari) with bovine blood plasma protein concentrate. Nig. J. App. Sci., 6: 61- 64.

Reade, A. E. and Gregory, K. F. (1975). High temperature protein Enriched feed from cassava fungi. App. Microb., 30: 897-907.

Reinbault, M. and Alazard, D. (1980). Culture method to study fungal growth in solid fermentation. Eur. J. App. Microb. Biotech., 9: 199-209.

Van Weerden, E. J. (1999). Nutritional evaluation of bioconversion products for farm animal. http://www.unu.edu/hq/unupbooks/80434e/804340z.htm

Zar, J. H. (1984). Biostatistical Analysis. USA: Prentice-Hall, Inc.. p. 620.