# **Original Research Article**

# Fermentation reduces cyanide content during the Production of Cassava Flours from Sweet and Bitter Cassava Tuber Varieties

# ABSTRACT

Sweet and Bitter Cassava tubers were fermented for production of cassava flours. The samples were peeled, washed with potable water and cut into slices of 5-6cm length. They were steeped separately in water to ferment spontaneously for 4 days. The samples were serially diluted and 0.1 ml aliquote inoculated on appropriate media and incubated for isolation of bacteria and fungi. Physico-chemical, cyanide and proximate status of the fermented samples were determined. Staphylococcus aureus, Lactobacillus spp., Bacillus spp., Klebsiella spp., Corynebacterium spp., Aspergillus niger, Penicillium, Mucor, Rhizopus and Candidia species were isolated. Lactobacillus, Bacillus and Candida species remained till the end of fermentation. Sweet cassava had total viable bacterial counts range:  $3.6 \times 10^2 - 4.1 \times 10^5$  cfu/ml; coliform counts:  $2.7 \times 10^2$  cfu/ml - $3.5 \times 10^5$  cfu/ml and fungal counts  $1.6 \times 10^2$  cfu/ml -  $2.3 \times 10^5$  cfu/ml while bitter cassava had total viable bacterial counts:  $3.3 \times 10^2$  -  $3.7 \times 10^5$  cfu/ml; coliform counts:  $2.3 \times 10^2$  -  $3.2 \times 10^5$  cfu/ml and fungal counts of  $1.3 \times 10^2$  to  $2.1 \times 10^5$  cfu/ml. TA of both cassava samples increased after fermentation while the hydrogen cynanide and pH decreased after fermentation in both cassava tubers varieties. Fermentation reduced the cyanide content in both cassava varieties flours to acceptable limits and eliminated food borne pathogens from the floors thus making the cassava flours safe for human consumption.

# Key words: Cassava tubers, cyanide content, fermentation, food safety, microorganisms.

# **1.0 INTRODUCTION**

Cassava (*Manihot esculenta* Crantz) is a tropical root crop that serves as staple food for millions of people in the developing world (Akoroda, 1995). Cassava is classified based on the level of cyanide content into sweet and bitter varieties (Oboh *et al.*, 2002). Varieties with low cyanide less than 50 mg HCN/kg of pulp are non-toxic and sweet. Those with cyanide level above are bitter and toxic (National Root Crops Research Institute NRCRI, 2004). The sweet varieties are mainly used as thirst quenchers and snacks while the bitter varieties are for processing into flour and flour products (Nweke, 1994).

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. Cassava roots are used as human food either fresh when low in cyanogens; it's the case of sweet variety; or in many processed forms and products, mostly starch, flour, and for animal feed; it's the case of bitter variety. Over 90% of the cassava processed in Africa is used for human nutrition as fermented products (Mensah, 1997). Several varieties of cassava exist but it is possible to gather them in two groups: the sweet ones and the bitter ones (Assanvo *et al.*, 2002).

Cassava is processed therefore to remove or reduce the toxic cyanogenic glucoside (which is broken down to release toxic hydrogen cyanide), improve palatability as well as serve as a means of preservation (Yeoh and Sun, 2001). Nigeria is the largest producer of cassava in the world (FAO, 2008) with about 45 million metric tonnes and its cassava transformation is the most advanced in Africa (Egesi *et al.*, 2006). Cassava is grown throughout the tropic and could be regarded as the most important root crop, in terms of area cultivated and total production (Ano, 2003). It is a major food crop in Nigeria (Ogbe *et al.*, 2007). It is essentially a carbohydrate food with low protein and fat. The edible part of fresh cassava root contains 32–35% carbohydrate, 2-3% protein, 75–80% moisture, 0.1% fat, 1.0% fibre, and 0.70–2.50% ash (Oluwole, *et al.*, 2004). The production of cassava for human consumption has been estimated to be 65% of cassava products, while 25% is for industrial use, mostly starch 6% or animal feed 19% and 10% lost as waste (Fish and Trim, 1993).

Cassava fermentation either naturally or with selected microbial inoculums extensively enhances the nutritional potentials of cassava and its byproducts both for human and livestock consumption (Aro, 2008). Natural fermentation of plant material is widely used in underdeveloped countries to transform and preserve vegetables because of its low technology and energy requirements and the unique organoleptic properties of the final product.

In spite of the desirability of cassava for consumption as food and animal feed, it contains some toxic compounds such as cyanogenic glycosides, linamarin, and lotaustralin which are highly toxic. Thus, the consumption of an inadequately processed cassava product for prolonged periods may result in chronic toxicity. However, the toxicity of the cyanogens is as a result of inadequate processing (Bradbury *et al.*, 2006). Cassava roots are highly perishable and a lot of postharvest losses occur to this commodity during storage due to high physiological activities and activities of

microorganisms that enter bruises received during harvesting as well as the inherent high moisture content of fresh roots, which promote both microbial deterioration and unfavourable biochemical changes in the commodity (Wenham, 1995).

This study is aimed at investigating the effect of fermentation in reducing the cyanide contents of cassava during the production of cassava flours.

# 2.0 MATERIALS AND METHODS

# 2.1 SAMPLE COLLECTION AND PROCESSING

Freshly harvested sweet and bitter cassava varieties were collected from National Root Crops Research Institute, Umudike, Abia state. The Sweet Cassava (TMS 419) and Bitter Cassava (TMS 419) varieties were sorted, peeled, washed with potable water and drained. A portion of the sweet and bitter cassava tubers (5kg each) was cut into slices of 5-6cm length and soaked separately in a big bowel? (bowl) containing water . The pieces were completely submerged in water and the bowel? uncovered to allow for exposure to air (Oyewole and Afolami, 2001). Both were left for 4 days to ferment at ambient condition (27-32°C). During the fermentation, the steep water of both cassava types was taken aseptically for different microbiological analysis in the laboratory. After fermentation, the pulp was removed, water squeezed out and spread on a tray and sundried for 3 days.

### 2.2 MICROBIOLOGICAL ANALYSIS OF SAMPLES.

**2.2.1** Ten ml of the fermenting steep water was collected with sterile pipette after vigorous shaking and serially diluted (Dhawale and Lamaster, 2003) after which 0.1 ml aliquot of 10<sup>-4</sup> dilution was aseptically inoculated by Spread Plate on Nutrient agar (also used for total viable count); MacConkey agar (also used to total coliform counts); De Man Rogosa Sharpe (MRS) Agar (also used for total lactic acid bacteria counts) and Sabouraud Dextrose Agar (SDA) for fungal isolation and count. The bacterial plates were incubated at 37 °C for 48 hrs while the fungal plates were incubated at 22 °C for 5 days. The total viable counts for bacteria and fungi were then determined. The various bacterial colonies isolated were sub-cultured by streaking to obtained pure cultures. They were later identified based on their colonial morphology, biochemical and sugar fermentation tests (Rachael and Oluwamodupe, 2012).

The Fungal isolates were identified macroscopically and microscopically using lactophenol cotton blue in which a portion of the fungi were collected using sterile needle and placed on the microscope slide. Few drops of lactophenol cotton blue were added on it followed by teasing using needle. It was covered with cover slips and viewed under the microscope using x10 and x40 objective lenses.

# 2.3 DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS OF SAMPLES

The pH, total titratable acidity and temperature of the fermenting samples were monitored during the fermentation time (Sedden and Murphy, 2003) for every 12 hours.

#### 2.4 PROXIMATE ANALYSES OF FERMENTED CASSAVA TUBERS

The proximate analyses: fat, crude protein, crude fiber, free fatty acid, moisture content and Minerals of the fermented cassava tubers were determined according to AOAC (2005), described in NAFDAC, SOP (2011).

# 2.5 STATISTICAL ANALYSIS

The results were presented as Mean  $\pm$  Standard deviation of triplicate values. A one way analysis of variance (ANOVA) and Duncan multiple range test were carried out. Significant difference was accepted at P $\leq$  0.05.

# 3.0 RESULTS

Table 1 shows the bacterial isolates from the fermentation of sweet and bitter cassava tuber varieties. They include: *Klebsiella* spp, *Bacillus* spp, *Escherichia coli, Staohylococcus aureus, Lactobacillus* spp, and *Corynebacterium* spp.

Table 2 shows the fungal isolates from the fermentation of sweet and bitter cassava varieties. They include, *Aspergillus niger, Mucor* spp, *Penicillium* spp, *Rhizopus* spp, and *Candida* spp.

Table 3 shows the occurrence of bacterial isolates. *Lactobacillus* spp, had the highest occurrence in both the sweet and bitter cassava while *E. coli* and *Staphylococcus aureus* had the least occurrence in both varieties.

Table 4 shows the occurrence of fungal isolates. *Candida* spp, had the highest occurrence in both sweet and bitter cassava while *Penicillium* spp, had the lowest occurrence in both varieties.

Table 5 shows the total bacterial viable counts of fermenting Sweet and Bitter Cassava samples. The highest count for Sweet Cassava was  $5.6 \ge 10^4$  cfu/ml after 72 hrs while the highest count for Bitter Cassava was  $5.0 \ge 10^4$  cfu/ml after 72 hrs.

Table 6 shows the total coliform counts from fermenting Sweet and Bitter Cassava samples. The highest count for fermenting sweet cassava was  $3.5 \times 10^5$  cfu/ml after 96 hrs while that for fermenting bitter cassava was  $3.1 \times 10^5$  cfu/ml after 96 hrs

Table 7 shows the total fungal counts for fermenting Sweet and bitter cassava samples. The highest count for sweet cassava was  $2.3 \times 10^5$  cfu/ml after 96 hrs while that for the bitter cassava was  $2.1 \times 10^5$  cfu/ml after 96 hrs

Table 8 shows the pH and temperature of fermenting Sweet and Bitter cassava samples. The lowest pH for Sweet Cassava was 4.5 after 96 hrs while that for Bitter Cassava was 3.9 after 96 hrs. The highest temperature recorded for Sweet Cassava was 27.8 °C after 12 hrs while that for Bitter cassava was 27.7 °C.

Table 9 shows the TTA and hydrogen cyanide content of fermenting sweet and bitter cassava tuber varieties. The percentage TTA increased from 0.65 to 0.71 in the sweet cassava and from 0.73 to 0.75 in the bitter cassava.

Table 10 shows the proximate composition of fermenting sweet and bitter cassava samples before and after fermentation. There was increase in the moisture content, crude protein, crude fiber and ether extract in both sweet and bitter cassava tubers after 72 hours of fermentation while there was a decrease in the ash content and carbohydrate of both cassava tubers after fermentation.

Colony shape	Microscopy	Motility	Gram reaction	Catalase	Coagulase	Mannitol	Citrate	Indole	Fructose	Galactose	Mannose	Xylose	Glucose	lactose	Sucrose	Maltose	Carbohydrate utilization	Probable isolates
whitish, circular convex on NA	Rod occurring single	non motile	+	+	-	A-	+	-	A-	A-	A-	-	AG	AG	A-	NA	A-	Klebsiella spp
Cream coloured, large, irregular and translucent on NA	Large with terminally tipped ends.	motile	+	+	+	A-	+	-	AG	AG	AG	AG	+	+	A-	+	A-	<i>Bacillus</i> spp
Pinkish, circular on MA	Rods in singles	Motile	-	+	-	A-	+	+		A-	A-	-	AG	AG	A-	A-	AG	Escherichia coli
Milky, raised on NA	cocci in clusters	ND	+	+	+	A-		-	A-	A-	A-	-	A-	A-	A-	A-	A-	Staphylococcus aureus
Small and greyish white colour on MRS	large rods occurring singly and in pairs, non- sporing	non motile	+	-	5		+	-	А-	A-	A-	-	+	+	-	+	AG	Lactobacillus spp
Small, smooth, cream to yellow on NA	short rods, clusters at jointed ends	non motile	+	+	K		Ŧ	-	-	-	A-	-	+	-	+	-	AG	Corynebacterium spp

# Table 1: Bacterial isolates from fermenting sweet and bitter cassava samples

Keys: + = present; - = Absent; A-= Acid; AG= Acid and Gas; ND = Not done; NA: Not applicable

# Table 2: Morphological, identification and characterization of fungi from fermenting sweet and bitter cassava tubers

۵.

Macroscopy	Microscopy	Probable isolate
Very common colours (black or white)	Black with sulfurs area on the surface celled spores (conidia in chains) developing at the end of the sterigma	Aspergillus niger
White surface with brown reverse	Filamentous and non mucor septate without cilia. Thick wall is formed called zygospore.	<i>Mucor</i> spp
Large fluffy white colonies almost covering the whole surface	Non-septate branch and hyphae enlarge at the apex to form conidiophore, thus produce brownish black conidia	Penicillium spp
Large fluffy milky colonies which later turns black on SDA	Non-septate hyphae with right sporangiospore connected by stolon and rhizoids, dark pear shaped sporangium on the hemispherical columella	<i>Rhizopus</i> spp
Dry, rough and whitish on SDA	Single balls on direct microscopy, circular shapes and buds	Candida spp

Sweet cassava								
Isolates	0 hr	24 hrs	<b>48 hrs</b>	72 hrs	96hrs			
<i>Klebsiella</i> spp	-	+	+	+	-			
Bacillus spp	+	+	+	+	+			
Escherichia coli	+	+	+	-	-			
Staphylococcus aureus	+	+	+	-				
Lactobacillus spp	+	+	+	+	+			
Corynebacterium	+	+	+	+	-			
spp								
		Bitter ca	ssava					
Bacillus spp	+	+	+	+	-			
Escherichia coli	+	+	+	-	-			
Staphylococcus	+	+	+		-			
aureus								
Lactobacillus spp	+	+	+	+	+			
Key: + means presen	t. – mean	s absent						

 Table 3: Occurrence of bacterial isolates from fermenting sweet and bitter cassava tuber varieties

# Table 4: Occurrence of fungi in fermenting Sweet and bitter cassava varieties (%)

		Sweet cassa	ava			
Fungal isolates	0 hr	24 hrs	48 hrs	72 hrs	96hrs	
Aspergillus spp	- <	+	+	+	-	
Mucor spp			+	+	+	
Penicillium spp			+	+	-	
Rhizopus spp	- \ \	-	+	+	-	
Candida spp	+	+	+	+	+	
		Bitter cass	ava			
Aspergillus spp	-	+	+	+	-	
Mucor spp	-	-	+	+	+	
Penicillium spp	-	-	+	+	-	
Rhizopus spp	-	-	+	-	-	
Candida spp	+	+	+	+	+	

**Keys**: + = Present: - = Absent

Sample	Hours	Counts (Cfu/ml)
FSC	0	$3.6 \times 10^2$
	24	$4.8 \times 10^2$
	48	$5.3 \times 10^2$
	72	$5.6 \times 10^4$
	96	$4.1 \ge 10^5$
FBC	0	$3.3 \times 10^2$
	24	$4.2 \ge 10^2$
	48	$4.8 \ge 10^2$
	72	$5.0 \ge 10^4$
	96	$3.7 \times 10^5$

 Table 5: Total Bacterial viable counts of fermenting Sweet and Bitter Cassava samples

Keys: FSC- Fermenting sweet cassava; FBC- Fermenting bitter cassava

Sample	Hours	Counts(Cfu/ml)
FSC	0	$2.7 \times 10^2$
	24	$3.8 \times 10^2$
	48	$4.C \ge 10^2$
	72	$4.9 \ge 10^4$
	96	$3.5 \times 10^5$
FBC	0	$2.3 \times 10^2$
	24	$3.2 \times 10^2$
	48	$4.0 \ge 10^2$
	72	$4.4 \times 10^4$
	96	$3.1 \times 10^5$

Keys: FSC- Fermenting sweet cassava; FBC- Fermenting bitter cassava

Sample	Hours	Counts (Cfu/ml)
FSC	0	$1.6 \times 10^2$
	24	$2.4 \times 10^2$
	48	$2.9 \times 10^2$
	72	$3.2 \times 10^4$
	96	$2.3 \times 10^5$
FBC	0	$1.3 \times 10^2$
	24	$2.2 \ge 10^2$
	48	$2.5 \times 10^2$
	72	$3.0 \ge 10^4$
	96	$2.1 \ge 10^5$

 Table 7: Total Fungal count from fermenting sweet and bitter cassava tubers.

Keys: FSC- Fermenting sweet cassava; FBC- Fermenting bitter cassava

Table 8: pH and temperature of ferment	ting	Swee	t and	Bitter	cassava s	amples

Sample/hours	0	12	24	36	48	60	72	84	96		
FSC	6.6	6.3	6.0	5.7	5.5	5.3	5.0	4.8	4.5		
FBC	5.9	5.7	5.5	5.3	5.0	4.8	4.5	4.2	3.9		
Temperature											
Sample/hours	0	12	24	36	48	60	72	84	96		
FSC	27.7	27.8	27.8	27.9	26.8	26.7	26.6	25.9	25.8		
FBC	27.7	27.7	27.8	27.8	26.8	26.6	26.5	27.8	25.7		

Keys: FSC- Fermenting sweet cassava; FBC- Fermenting bitter cassava

Samples	TTA (%)	HCN (%)
SCB	$0.65 \pm 0.01^{\circ}$	$10.03 \pm 0.01^{b}$
SCA	$0.71{\pm}0.01^{b}$	$5.22 \pm 0.03^{d}$
BCB	$0.73 \pm 0.01 a^{b}$	$10.12 \pm 0.01^{a}$
BCA	$0.75{\pm}0.01^{a}$	5.41±0.01°

Table 9: Percentage TTA and HCN changes in fermenting sweet and bitter cassava tubers

Values are mean  $\pm$ SD; values with different superscript along the column are significantly different (P $\leq$ 0.05).

**Keys:** SCB: Sweet cassava before fermentation; BCB: Bitter cassava before fermentation; SCA: Sweet cassava after fermentation; BCA: Bitter cassava after fermentation

# Table 10: Proximate composition of sweet and bitter cassava samples before and after fermentation.

Sample	Dry matter	Moisture content	Crude protein	Crude fibre	Ether extract	Ash content	Carbohydrate
SCB	$33.75 \pm 0.02^{b}$	$66.25 \pm 0.02^{\circ}$	0.75±0.01 <sup>c</sup>	0.62±0.03 <sup>c</sup>	1.71±0.01d	1.27±0.01 <sup>a</sup>	29.41±0.01 <sup>b</sup>
SCA	$30.15 \pm 0.01^{\circ}$	69.85±0.01 <sup>b</sup>	$1.87{\pm}0.01^{a}$	$1.82 \pm 0.01^{b}$	$1.92 \pm 0.01^{b}$	$0.55 \pm 0.01^{\circ}$	$23.94 \pm 0.02^{\circ}$
BCB	$34.04{\pm}0.00^{a}$	$65.95 {\pm} 0.01^{d}$	$0.62{\pm}0.03^{d}$	$0.66 \pm 0.01^{\circ}$	$1.81 \pm 0.02^{\circ}$	$1.02 \pm 0.01^{b}$	$29.94{\pm}0.04^{a}$
BCA	$20.75 \pm 0.03^{d}$	79.25±0.03 <sup>a</sup>	1.79±0.01 <sup>b</sup>	$1.95{\pm}0.01^{a}$	$1.98{\pm}0.01^{a}$	$0.37{\pm}0.01^{d}$	$14.65 \pm 0.06^{d}$

Values are mean  $\pm$ SD; values with different superscript along the column are significantly different (P $\leq$ 0.05).

**Keys:** SCB: Sweet cassava before fermentation; BCB: Bitter cassava before fermentation; SCA: Sweet cassava after fermentation; BCA: Bitter cassava after fermentation

# DISCUSSION

Microbial fermentation for the production of cassava flour from Sweet Cassava (TMS 419) and Bitter cassava (TMS 419) tuber varieties were carried out. Whatever the variety of cassava, the changes in the content of microorganisms throughout the cassava fermentation was slightly similar but it was observed that microflora was slightly more abundant in the fermentation of sweet cassava than the bitter cassava This was believed to be due to the low moisture content of the latter and also its higher cyanogenic glucoside content which had inhibitory effect on microbial growth (Rainbault, 1995). This result agrees with Assanvo *et al.* (2006).

Lactic acid bacteria, *Bacillus* spp. yeast, and filamentous fungi have been shown to be present in traditional fermented cassava (*lafun*) (Oyewole and Odunfa, 1989). This corresponds to the work done by Obadina *et al.*, (2007) in which he isolated similar fungi from 'fufu' flour stored at different relative humidity in ambient condition.

*Lactobacillus* spp., showed the highest acid producing ability and it does not only aid in fermentation, it also contributes positively to the shelf-life of the food product. This type of food will be suitable for rural people where refrigeration and modern storage facilities are not available. The presence of *Bacillus* spp and *Klebsiella* spp during fermentation contributed to the retting of the tubers. *Bacillus* spp also contributed to the production of amylase enzyme necessary for the breakdown of starch to sugars which are needed for the growth of other fermenting microorganism and forms resistance spores which tolerate adverse conditions for many days. The low percentage of *S. aureus* in the food proves its safety. This same principle is applied to the enteric pathogen; *E. coli* (Broke *et al.*, 1998).

The high bacterial load recorded in this study may be attributed to the poor microbial quality of the materials used in precedent cassava fermentation. This high bacterial content included the normal LAB involved in the lactic acid fermentation as well as others undesirable bacterial such as coliforms. The high coliform counts were probably induced by the contamination of exposure of bowl to environmental atmosphere in the course of fermentation processing as well as by the faecal contamination. Coliforms are generally very thermal sensitive so their high number during the preparation of starter culture could be favoured by the absence of thermal step in the course of fermentation processing.

However, it has been reported that the presence of coliforms in fermented cassava could contribute a health hazard to the consumer and could also act as potential spoilage agent of fermented cassava dishes and foods (Okpokiri *et al.*, 1985; EEC, 2005).

The rapid increase in microbial population at the beginning must have been due to the abundance of nutrients needed for their growth (Tetchi *et al.*, 2010). After this period, the growth in the fermenting medium of the cassava fractions decreased especially for the fungal population at the end of fermentation due to competition for nutrients and high acidity (Abodjo – Kakou *et al.*, 2010). Moorthy and Mathew (1998) stated that cassava fermentation is associated with the fermentative activities of bacteria and yeasts. But the decrease of their population is probably due to the depletion of substrates and the bacterial competition although their counts remained relatively constant at the end of the fermentation as observed by Coulin *et al.*, (2006) during "attiéké" fermentation.

The increase of acidity during fermentation could contribute probably to inhibition acts of LAB which developed abundantly against other undesirable Microorganisms resulted in a decrease growth of coliforms as shown by their low number at the end of the fermentation. The lowering of their counts can be also explained by the fact that they do not play an important role for the acidification during fermentation. They probably produce relatively high levels of cell-wall degrading enzymes which contributed to textural changes in the cassava pulp (Mante *et al.*, 2003; Obilie *et al.*, 2004) or also antibiotic agent as Nisine (Valat *et al.*, 2003).

At time (0 hour), sweet cassava variety had pH 6.6 and bitter cassava variety had pH (5.9). During the fermentation process, there was significant decrease in the pH and the pH of the bitter variety remained lower than that of the sweet variety throughout fermentation. At the end of the fermentation period, i.e after 96 hours, the bitter variety had lower pH of 3.9 than the sweet variety which had pH of 4.5. Lower pH in the steeping water of bitter cassava could be explained by the presence of high amount of cyanhydric acid accumulated through the degradation of the cyanogenic glycosides such as amygdalin, linamarin, and linseed cyanogensm by lactic acid bacteria (Lei *et al.*, 1999). At the pH about 5, the cyanohydrins formed disintegrate to form HCN (Fomunyam *et al.*, 1985) which would be evaporated latter from the fermented cassava pulp. Indeed, fermentation allowed the elimination of more than 90% of endogenous cyanide compounds in the cassava roots after 48 hrs.

The changes in percentage TTA and hydrocyanide content of fermenting sweet and bitter cassava tuber varities were also determined. The percentage TTA increased from 0.65 to 0.71 in the sweet cassava and from 0.73 to 0.75 in the bitter cassava. There was a reduction in the hydrocyanide content of both the sweet and bitter cassava tuber varities from 10.03 to 5.22 and from 10.12 to 5.41 respectively. The greater TTA observed in the cassava samples may be due to the higher water content in the fermenting medium which encourages the activities of the fermenting microflora. This has been reported to be attributed to the activities of lactic acid bacteria on carbohydrates in the fermenting cassava (Oyewole and Afolami, 2001). Acid production had been reported to be dependent on microflora and processing conditions (Oyewole, 1990). The low HCN appears to be caused by conditions which encourage a greater breakdown of cyanogenic compounds of HCN.

Higher moisture content observed in cassava tuber in this study might have been as a result of higher water absorption by the fermenting cassava. The reduction in the ash and carbohydrates

had been attributed to the leaching of these nutrients into the soaking water and microbial utilization (Oyewole and Odunfa, 1989).

### Conclusion

The microbial fermentation for the production of cassava flour from sweet and bitter cassava tuber varieties reduced the cyanide contents of the flour of the two cassava varieties to acceptable level safe for human consumption. Pathogenic microorganisms were eliminated from the cassava flours due to fermentation.

# REFERENCES

- Abodjo Kakou, C., Tago Guehi, S., Olo, K., Akissi Kouame, F., Koffi Nevmy, R and Marina Koussemon, C. Biochemical and microbial changes during traditional spontaneous lactic acid fermentation process using two varieties of cassava for production of a "Alladjah" starter. *International Food Research Journal*, 2010, 17: 563-573.
- Akoroda, M. O (1995). Alleviating hunger in Africa with root and tuber crops. *African Journal* of Tropical Root Crops, 1:41-43.
- Ano, A.O (2003. )Studies on the effect of Liming on the Yield of two cassava cultivars," NRCRI Annual Report, 2003.
- AOAC. (2005) Association of Official Analytical Chemists. Official Methods of Analysis 18th edition, Washington D C, USA.
- Aro, S. O. (2008). Improvement of the nutritive quality of cassava and it's by products through microbial fermentation. *African Journal of Biotechnology*, 7(25): 4789-4797.
- Assanvo, J. B., Agbo, G. N., Behi, Y. E. N. and Coulin, P. (2006). La microflore du ferment de manioc pour la production de l'attiéké Adjoukro à Dabou (Côte d'Ivoire). Bioterre, *Review International. Science*. De la vie et la terre N° spécial, 286-299.
- Assanvo, J. B., Agbo, G. N., Behi, Y. E. N. and Coulin, P. (2002). La microflore du ferment de manioc pour la production de l'attiéké Adjoukro à Dabou (Côte d'Ivoire). Bioterre, *Review International. Science*. De la vie et la terre N° spécial, 286-299.
- Bradbury, J. H., Cumbana, A., Mirione, E and Cliff, J (2006) "Reduction of cyanide content of cassava flour in Mozambique by the wetting method," *Food Chemistry*, vol. 101, no. 3, pp. 894–897.
- Brooke, G. F., Butel, J. S and Mores, S. A (1998) Spore-forming gram positive bacilli: Bacillus and Clostridium species In: Medical Microbiology, Jawez *et al.* (ed) 21st Ed. Large Medical book, McGraw Hill, New York, 1998, 182.

- Coulin P., Farah Z., Assanvo J., Spillmann H., and Puhan, Z. (2006). Characterisation of the microflora of attie'ke', a fermented cassava product, during traditional smallscale preparation. *International Journal of Food Microbiology*, 106: 131 136.
- Dhawale, S. and LaMaster, A. (2003). Microbiology Laboratory manual. The mcHill Company.Inc. USA
- EEC, (2005). Commission regulation (EC) N° 2073/2005 relating to microbiological criteria for foodstuffs. *Official Journal of the European Communities*, L338 of 15 November 2005.
- Egesi, C., Mbanaso, E., Ogbe, F., Okogbenin, E and Fregene, M (2006). "Development of cassava varieties with high value root quality through induced mutations and marker-aided breeding," NRCRI, Umudike Annual Report.
- FAO, (2008) "Corporate Document Repository," The impact of HIV/ AIDS on the agricultural sector, http://www.fao.org/ docrep/005/Y4636E/y4636e05.htm.
- Fish, D. M. and Trim, D. S. (1993). A review of research in the drying of cassava. *Tropical Science*, 33, (2):191–203.
- Fomunyam, R.T., Adegbola, A.A. and Oke, O.L. (1985). The stability of cyanohydrins. *Food Chemistry*, 17: 221–225.
- Mante, E.S., Sakyi-Dawson, E. and Amoa Awua, W.K.A., (2003). Antimicrobial interactions of microbial species involved in the fermentation of cassava dough into agbelima with particular reference to the inhibitory effect of lactic acid bacteria on enteric pathogens, *Journal of Food Safety*. 31;34-45.
- Mensah, P. (1997). Fermentation the key to food safety assurance in Africa. *Food Control* 8: 271-278.
- Moorthy, S.N. and Mathew, G. (1998). Cassava fermentation and associated changes in physicochemical and functional properties. *Critical Review in Food Science and Nutrition* 38: 73-121.
- NAFDAC (2016) Good practices for pharmaceutical quality control laboratories
- NRCRI (2004) Guide to cassava cultivation in Nigeria, National Root Crops Research Institute Umudike, 121.
- Nweke, F. I. (1994). Cassava processing in sub-Saharan African: the implications for expanding cassava production. *Outlook on Agriculture*, 23: 197-205.
- Obadina, A.O., Oyewole, O.B and Odubayo, M.O (2007). Effect of storage on the safety and quality of "fufu" flour. *Journal of Food Safety*, 27: 148-156.
- Obilie, E. M., Tano-Debra, K and Amoa-Awua, W.K (2004) Souring and breakdown of cyanogenic glucosides during the processing of cassava into akyeke. International *Journal of Food Microbiology*, 93(1): 115-121.
- Oboh, G., Akindahunsi, A.A. and Oshodi , A. A. (2002) Nutrient and antinutrient contents of *Aspergillus* fermented cassava products (flour and garri). *Journal of Food Composition* and Analysis 15(5): 617 622.
- Ogbe, F.O., Emehute, J. K. U and Legg, J (2007) "Screening of cassava varieties forwhitefly populations," NRCRI Annual Report.
- Okpokiri, A.O, Ijeoma, B.C., Alozie, S.O and Ejiofor, M. A. N (1985). Production of improved Cassava, *Nig. Food Journal* 2, 145 148.

- Oluwole, O. B., Olatunji, O.O. and Odunfa, S. A. (2004). "A process technology for conversion of dried cassava chips into gari," *Journal of Food Science and Technology*, vol. 22, pp. 65–77.
- Oyewole, O. B and and Afolami, A. O (2001) Quality and preference of different cassava varieties for "lafun" production. *The Journal of Food Technology in Africa* (6)1: 27-29
- Oyewole, O. B., and S. A. Odunfa. 1989. Microbiological studies on cassava fermentation for "lafun" production. *Food Microbiology*. 5:125–133.
- Oyewole, O. B. 1990. Optimization of cassava fermentation for fufu production: effects of single starter cultures. *Journal of Applied Bacteriology*, 68:49–54
- Tetchi, F.A., Solomen, O.W., Celah, K.A and Geoges, A.N (2010) effects of cassava variety and fermentation on biochemical and microbiological of raw artinasal startar for attieke production. Innovation rom. *Food biotechnology*, 10 40-47
- Valat C., Champiat D.,, Loiseau G., Raimbault M. and Montet D. (2003). Use of ATP bioluminescence to determine the bacterial sensitivity threshold to a bacteriocin. *Luminescence* 18: 254-258.
- Wenham, J.E. (1995) "Post-harvest deterioration of cassava.ABiotechnology perspective," F.A.O. Plant Production and Protection Paper 130, Food and Agriculture Organization of the United Nations, Rome, Italy,.
- Yeoh, H. H and Sun, F (2001) (Assessing cyanogen content in cassava-based food using the enzyme-dipstick method. *Food Chem Toxicol.* 39(7):649-53.