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: $3.6x10^2$ - $4.1x10^5$cfu/ml; coliform counts: $2.7x10^2$cfu/ml - $3.5x10^5$cfu/ml and fungal counts $1.6x10^2$cfu/ml - $2.3x10^5$cfu/ml while bitter cassava had total viable bacterial counts: $3.3x10^2$ - $3.7x10^5$cfu/ml; coliform counts: $2.3x10^2$ - $3.2x10^5$cfu/ml and fungal counts of $1.3x10^2$ to $2.1x10^5$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^{-4} 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 ^{o}C for 48 hrs while the fungal plates were incubated at 22 ^{o}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 ± 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≤ 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 x 10^4 cfu/ml after 72 hrs while the highest count for Bitter Cassava was 5.0 x 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 x 10^5 cfu/ml after 96 hrs while that for fermenting bitter cassava was 3.1 x 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 2.3×10^5 cfu/ml after 96 hrs while that for the bitter cassava was 2.1×10^5 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 $^\circ$C after 12 hrs while that for Bitter cassava was 27.7 $^\circ$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.

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

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-	*Bacillus* 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	+	-	-	-	+	-	A-	A-	A-	-	+	+	-	+	AG	*Lactobacillus* spp
Small, smooth, cream to yellow on NA	short rods, clusters at jointed ends	non motile	+	+	-	-	+	-	-	-	A-	-	+	-	+	-	AG	*Corynebacterium* spp

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

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.	*Mucor* 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	*Rhizopus* spp
Dry, rough and whitish on SDA	Single balls on direct microscopy, circular shapes and buds	*Candida* spp

10

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

	Sweet cassava				
Isolates	0 hr	24 hrs	48 hrs	72 hrs	96hrs
Klebsiella spp	-	+	+	+	-
Bacillus spp	+	+	+	+	+
Escherichia coli	+	+	+	-	-
Staphylococcus aureus	+	+	+	-	-
Lactobacillus spp	+	+	+	+	+
Corynebacterium spp	+	+	+	+	-
	Bitter cassava				
Bacillus spp	+	+	+	+	-
Escherichia coli	+	+	+	-	-
Staphylococcus aureus	+	+	+	-	-
Lactobacillus spp	+	+	+	+	+

Key: + means present. – means absent

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

	Sweet cassava				
Fungal isolates	0 hr	24 hrs	48 hrs	72 hrs	96hrs
Aspergillus spp	-	+	+	+	-
Mucor spp	-	-	+	+	+
Penicillium spp	-	-	+	+	-
Rhizopus spp	-	-	+	+	-
Candida spp	+	+	+	+	+
	Bitter cassava				
Aspergillus spp	-	+	+	+	-
Mucor spp	-	-	+	+	+
Penicillium spp	-	-	+	+	-
Rhizopus spp	-	-	+	-	-
Candida spp	+	+	+	+	+

Keys: + = Present: - = Absent

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

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

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

Table 6: Total coliform count from fermenting sweet and bitter cassava tubers

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

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

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

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

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

Table 8: pH and temperature of fermenting Sweet and Bitter cassava samples

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

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

Samples	TTA (%)	HCN (%)
SCB	0.65 ± 0.01^c	10.03 ± 0.01^b
SCA	0.71 ± 0.01^b	5.22 ± 0.03^d
BCB	$0.73\pm0.01a^b$	10.12 ± 0.01^a
BCA	0.75 ± 0.01^a	5.41 ± 0.01^c

Values are mean ±SD; values with different superscript along the column are significantly different (P≤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 ± 0.02^b	66.25 ± 0.02^c	0.75 ± 0.01^c	0.62 ± 0.03^c	$1.71\pm0.01d$	1.27 ± 0.01^a	29.41 ± 0.01^b
SCA	30.15 ± 0.01^c	69.85 ± 0.01^b	1.87 ± 0.01^a	1.82 ± 0.01^b	1.92 ± 0.01^b	0.55 ± 0.01^c	23.94 ± 0.02^c
BCB	34.04 ± 0.00^a	65.95 ± 0.01^d	0.62 ± 0.03^d	0.66 ± 0.01^c	1.81 ± 0.02^c	1.02 ± 0.01^b	29.94 ± 0.04^a
BCA	20.75 ± 0.03^d	79.25 ± 0.03^a	1.79 ± 0.01^b	1.95 ± 0.01^a	1.98 ± 0.01^a	0.37 ± 0.01^d	14.65 ± 0.06^d

Values are mean ±SD; values with different superscript along the column are significantly different (P≤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.

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