Effects of Microbial Fermentation on Cyanide Contents and Proximate Composition of Cassava Tubers

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Abstract: Yellow and white varieties of cassava (*Manihot esculenta*) tubers were fermented for the reduction of cyanide content and nutritional improvement. The samples were steeped separately in rain water and allowed to ferment spontaneously for 4 days. The fermenting steep water was inoculated by Spread Plate method on Nutrient, De Man Rogosa Sharpe and McConkey Agar for bacterial isolation and on Sabouraud Dextrose Agar for isolation of fungi in triplicates and incubated. The isolates include: *Staphylococcus aureus, Escherichia coli, Bacillus* spp*, Lactobacillus* spp*, Enterobacter* spp*, Aspergillus* spp*, Candida* spp*,* and *Saccharomyces* spp. Fermentation caused a reduction in the cyanide content from 9.24±0.01 to 2.93±0.02 mg/100g and 9.85±0.03 to 3.15±0.04 mg/100g in the yellow and white cassava varieties respectively. The pH decreased in value while the titratable acidity increased in value for both cassava varieties. Proximate analyses showed significant increase in protein, crude fibre and moisture values in both cassava varieties but a decrease in ash and carbohydrate contents. Fermentation significantly reduced the cyanide contents ($p \leq 0.05$) and improved nutritional status of the fermented cassava tubers. The fermented food was found pathogen-free thus safe for consumption.
Key words: Cassava tubers, cyanide, fermentation, food safety, microorganisms, nutritional status

INTRODUCTION

Cassava (*Manihot esculenta*) is an extensively cultivated crop and a staple food for millions of people in the tropical regions of Africa, Latin America and Asia. Globally, in terms of annual production, it is the fifth most important food crop after maize, rice, wheat and potato (FAOSTAT, 2011). The tuber consists of 20 to 25% starch but very limited quantities of proteins, fats, vitamins and Minerals. Traditionally Cassava roots are processed in a number of ways that vary from one region to another resulting in different end products like *gari, fufu, lafun, farinha, pande, yucca*. Despite all the usefulness of cassava, its uses as food source is limited by its perishability, its low protein content and its potential toxicity (Andrew, 2002).

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Cassava roots are potentially toxic due to the presence of cyanogenic glycosides, linamarin and a small amount of lotaustralin which are catalytically hydrolyzed to release toxic hydrogen cyanide (HCN) when the plant tissue is crushed (Seri *et al*., 2013). Hydrolysis and subsequent removal of liberated HCN takes place during various processing stage (Cumbara *et al*., 2007). Most processing techniques have been developed in different parts of the world to reduce the HCN content to an acceptable level (Etsuyankpa *et al*., 2015). The processing methods could lead to reduction in the cyanide content in cassava products to improve its palatability and convert it into a storable form (Owuamanam *et al*., 2010).

In Nigeria as in most African countries, cassava roots are processed into different products as a means of preservation due to their perishability (Andrew, 2002). Physiological deterioration occurs in cassava roots, 2-5 days after harvesting followed by microbial deterioration 3-5 days later (Victor and Chidi, 2010).

The consumption of cassava and its derived products which contains large amounts of HCN may be responsible for such visible manifestations as goiter and cretinism, tropical ataxic neuropathy and konzo (Cardoso *et al*., 2005). Unhydrolysed cyanide remaining in cassava roots after fermentation can constitute a health problem for the consumers (Nartley, 2010).

This research work was aimed at determining the effect of fermentation on cyanide content and the proximate composition of cassava tubers.

MATERIALS AND METHODS

Sample collection and processing

The Cassava roots tubers (yellow and white varieties) used in this work were purchased at National Roots Crop Research Umudike, Abia State while the study was conducted at the Microbiology Laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. One kilogram (1Kg) each of yellow and white cassava varieties was peeled and cut into pieces of 20 g each and then washed with rain water. The samples were steeped separately in tap water in a clean, transparent 10 litre plastic buckets and labeled accordingly. The buckets were placed on a clean table and left uncovered to ferment spontaneously for 4 days.

Determination of Cyanide content of fermented and unfermented cassava tubers

The cyanide content of the fermented cassava tubers was determined using the Alkaline picrate method (Okoko, 2011).

Microbiological Analysis of cassava tuber samples.

Ten ml each of the steep water of the fermenting cassava tubers was collected every 24 hours with the aid of a sterile 10 ml pipette and serially diluted. 0.1ml aliquot from 10^{-4} dilution was inoculated by spread plate method in triplicate onto previously prepared Nutrient Agar, De Man Rogosa Sharpe (MRS) Agar for isolation of Lactic acid bacteria, McConkey Agar for bacterial isolation and on Sabouraud Dextrose Agar (SDA) for isolation of fungi. The media were also used for determination of total microbial counts respectively (Cheesbrough, 2005). The Nutrient and MRS agar plates were incubated at 37^{o}C for 48 hours while the SDA culture plates were incubated at 22^{o}C for 5 days. The isolates were sub-cultured to obtained pure cultures and identified based on colonial morphology, Gram reaction, biochemical characteristics and sugar fermentation tests and then identified (Bergey and Holt, 2000).

Proximate Analysis of Fermented Cassava

The proximate analyses for moisture, ash, fats, carbohydrates (Tsai *et al.,* 2015) and protein (AOAC, 2005). The physico-chemical profiles (titratable total acidity and pH) were determined according to Nwafor *et al*. (2015).

Statistical analyses

The data were analyzed using the SPSS Statistical software. Comparison of means was done using the one-way analysis of variance (ANOVA).

RESULTS

Microbial isolates

Table 1 shows that five bacteria namely: *Staphylococcus aureus, Escherichia coli, Bacillus* spp, *Lactobacillus* spp and *Enterobacter* spp were isolated from the work. Table 2 shows the *Aspergillus* spp, *Saccharomyces* spp and *Candida* spp were isolated from the fermentation of the cassava tubers.

Total microbial Counts

Table 3 shows that the total bacterial count of Yellow and White Cassava varieties were in the range of 3.0×10^{2} to 8.9×10^{4}cfu/ml and 2.8×10^{2} to 8.8×10^{4}cfu/ml respectively while the total coliform count was in the range of $2.2\text{x}10^{3}$ to 7.6×10^{3}cfu/ml and $2.5\text{x}10^{3}$ to 7.4×10^{3}cfu/ml for yellow and white Cassava varieties respectively. The total fungal counts for the Yellow and White Cassava varieties were in the range of 0 to $8.0\text{x}10^{4}$ and 0 to $8.1\text{x}10^{4}$ respectively.

Microbial Succession of cassava tubers Fermentation

In Table 4, result of microbial succession analyses show that among the bacterial isolates, only *Lactobacillus* spp was isolated at the end of the fermentation while among the fungi, only *Saccharomyces* spp was isolated at the end of the fermentation for both yellow and white cassava varieties. The pathogens (*E. coli and S. aureus*) that were part of the starter cultures were not isolated at the end of the fermentation.

Physico-chemical Composition of fermented cassava tubers

In Table 6, the pH of the fermenting medium decreased during fermentation from 6.20 (on day 0) to 3.38 and from 6.40 to 3.14 for the yellow and white cassava varieties respectively while the TTA increased from 0.02 to 0.06 and from 0.03 to 0.07 for yellow and white cassava varieties respectively.

The hydrogen cyanide content decreased from 9.24±0.01 to 2.93±0.02 and from 9.85±0.03 to 3.15±0.04 mg/100g for yellow and white Cassava varieties respectively.

Proximate Composition of cassava

The proximate composition of the cassava is represented in Table 7. There was a significant increase ($p \leq 0.05$) in the moisture content (69.42±0.02 to 72.42±0.01 and 67.65±0.01 to 70.21±0.01 in yellow and white cassava respectively); crude protein (0.72±0.01 to 1.86±0.01 and 0.56±0.01 to 1.83±0.01 in yellow and white cassava respectively) and fats (1.76±0.01 to 1.98±0.00 and 1.82±0.02 to 1.95±0.01 in yellow and white cassava varieties). There was a decrease in the crude fiber content (1.78±0.01 to 0.54±0.01 and 1.84±0.01 to 0.63±0.01 in yellow and white Cassava varieties respectively); Ash (1.26±0.01 to 0.52±0.01 and 1.15±0.01 to 0.56±0.01 in yellow and white Cassava varieties respectively) and carbohydrate (27.90±0.00 to 21.43±0.02 and 28.40±0.0 to 23.61±0.01 in yellow and white Cassava varieties respectively).

Table 1: Morphological and Biochemical characteristics of bacterial isolates from fermented cassava tubers

S/N	Colonial morphology	Microscopy	Gram reaction	Catalase	Citrate	Oxidase	Coagulase	Methyl Red	Voges Proskauer	Indole	Spore stain	Glucose	Lactose	Sucrose	Fructose	Maltose	Mannitol	Probable Isolates
1	Opaque, golden yellow, spherical	Irregular cocci in clusters	**+**	+	+	-	+	+	-	-	-	A	A	A	-	-	+	*Staphylococcus aureus*
2	Pink, Irregular, Smooth, Entire	Short rods	**-**	-	-	-	-	+	-	+	-	A	A	+	A/G	A	A	*Escherichia coli*
3	Opaque, flat, irregular pinhead,	Long/short rods	**+**	+	+	-	-	-	+	-	+	A/G	-	-	A	-	-	*Bacillus* spp
4	Raised, Convex, smooth, opague	Long slender rods	**+**	+	-	-	-	-	-	-	-	A	A	-	A/G	A/G	-	*Lactobacillus* spp
5	Brown, large colonies	Short rods	**+**	-	-	-	-	-	+	-	-	A	A	-	A	A	A/G	*Enterobacter* spp

Key: + mean positive, - means negative, A/G = Acid/Gas positive

Table 2: Morphology of Fungal isolates from fermented cassava tubers

Colony characteristics	Cell morphology	*Organism*
Whitish at first then black with yellow reverse and a cottony texture.	Branch septate hyphae, conidophores.	*Aspergillus* spp
Flat, smooth, glistening and cream in color.	Unicellular, globose and budding, hyphae are absent.	*Saccharomyces* spp
White to cream colored, smooth colonies.	Pseudohyphae	*Candida* spp

Table 3: Total microbial Counts (CFU/g)

Time (hour)	**Bacterial count**		**Coliform count**	
	Yellow Cassava	White Cassava	Yellow Cassava	White Cassava
0	$3.0x10^2$	$2.8x10^2$	$2.2x10^3$	$2.5x10^3$
24	$6.5x10^4$	$6.3x10^4$	$6.5x10^4$	$6.4x10^4$
48	$7.9x10^3$	$7.7x10^3$	$7.6x10^3$	$7.4x10^3$
72	$8.6x10^3$	$8.5x10^3$	$5.9x10^3$	$5.7x10^3$
96	$8.9x10^4$	$8.8x10^4$	$3.0x10^4$	$3.9x10^4$
		Fungal counts		
Time (Hour)	Yellow Cassava	White Cassava		
0	0	0		
24	$2.3x10^2$	$2.5x10^2$		
48	$6.4x10^4$	$6.6x10^4$		
72	$7.2x10^3$	$7.5x10^3$		
96	$8.0x10^4$	$8.1x10^4$		

Table 4: Microbial Succession of cassava tubers fermentation (hrs)

Isolates	**Yellow cassava**					**White cassava**				
	0	24	48	72	96	0	24	48	72	96
Escherichia coli	+	+	+	-	-	+	+	+	-	-
Lactobacillus spp	-	+	+	+	+	-	+	+	+	+
Bacillus spp	-	+	+	+	-	-	+	+	+	-
Enterobacter spp	-	+	+	-	-	-	+	+	-	-
Staphylococcus aureus	+	+	-	-	-	+	+	-	-	-
Aspergillus spp	-	+	+	+	-	-	+	+	+	-
Saccharomyces spp	-	+	+	+	+	-	+	+	+	+
Candida spp	-	+	+	+	-	-	+	+	+	-

Table 5: Physico-chemical profile of fermented cassava tubers

Physio-chemical composition	Yellow cassava		White cassava	
	Before fermentation	After fermentation	Before fermentation	After fermentation
pH	6.20	3.38	6.40	3.14
Hydrogen Cyanide(mg/100g)	9.24 ± 0.01^{b}	2.93 ± 0.02^{d}	9.85 ± 0.03^{a}	3.15 ± 0.04^{c}
Titratable acidity (%)	0.02	0.06	0.03	0.07

Values are mean standard deviation. Means in the same row between with the same superscripts are not significantly different (P≤0.05)

Table 7: Proximate Compositions of unfermented and fermented Cassava varieties

Cassava variety	Proximate Composition %	Moisture Content	Crude protein	Crude fiber	Fats	Ash	Carbohydrate
Yellow Cassava	Unf	69.42 ± 0.01^{c}	0.72 ± 0.01^{b}	0.54 ± 0.01^{d}	1.76 ± 0.01^{c}	1.26 ± 0.01^{a}	27.90 ± 0.01^{a}
	Fer	72.42 ± 0.01^{a}	1.86 ± 0.01^{a}	1.78 ± 0.01^{b}	1.98 ± 0.00^{a}	0.52 ± 0.01^{d}	21.43 ± 0.02^{d}
White Cassava	Unf	67.65 ± 0.01^{d}	0.56 ± 0.01^{c}	0.63 ± 0.01^{c}	1.82 ± 0.02^{b}	1.15 ± 0.01^{b}	28.40 ± 0.00^{a}
	Fer	70.21 ± 0.01^{b}	1.83 ± 0.01^{a}	1.84 ± 0.01^{a}	1.95 ± 0.01^{a}	0.56 ± 0.01^{b}	23.61 ± 0.01^{c}

Key: Unf = unfermented, Fer = fermented

Values are mean standard deviation. Mean in the same column with the same superscripts are not significantly different (P≤0.05)

DISCUSSION

The microorganisms isolated from the fermentation of the yellow and white cassava tubers varieties are in agreement with the findings of Umeh and Odibo (2014) and Ihenetu *et al*., (2017). Some of these organisms as suggested by Fagbemi and Ijah, (2005) may have originated from the water used for fermentation, surrounding air or the bowls used for the steeping and retting of the tubers. The methods of preparation, water used for steeping, surface micro flora of raw materials , atmosphere where processing takes place and the handlers all affect the types of microorganisms isolated during fermentation processes (Abegaz, 2007). A wide spectrum of microorganisms is involved during fermentation processes but a few types usually determine the quality of the end product (Abegaz, 2007).

The fungi associated with the fermentation in this work are similar to those isolated by Guira *et al*., 2016. Although Umeh and Odibo (2014) stated that *Lactobacillus* spp, *Candida* spp and *Saccharomyces* spp cause complete retting (Fagbemi and Ijah, 2005). This is possibly due to the similarity in the varieties of cassava used in the work.

The increase in microbial counts may be due to favorable conditions such as nutrients, oxygen which allowed the multiplication of the microorganisms. The rapid increase in microorganisms within the first 72 hr of fermentation was possibly due to abundance of nutrient useful for their growth. The proliferation of coliform, especially in the early and intermediate stages of the fermentation is characteristic of mixed acid fermentation (Fagbemi and Ijah, 2005). The lowering of the coliform counts later in the fermentation time can be due to acidification of the medium by lactic acid bacteria (Schwan *et al.*, 2016). The increase in the counts of the yeasts and lactic acid bacteria in the water in the latter stages of fermentation probably may be as a result of increased acidity of the retting water which favoured their growth (Umeh and Odibo, 2014).

The pathogens: *E. coli, S. aureus* and *Aspergillus* spp did not survive till the end of the fermentation due to the antagonistic metabolites from lactic acid bacteria which are known to include bacteriocins, hydrogen peroxides, diacetyl and organic acids (Olaoluwa *et al*., 2013) and yeasts which produce alcohols. The elimination of *S. aureus and E. coli* from the fermented food is an important finding in this work. The yeast survived at the low pH till the end of the fermentation. Yeasts are presumably involved in breaking down starch to simple sugars utilized by the bacteria. The coexistence and positive interactions involving yeasts and lactic acid bacteria in fermented cassava have also been documented (Mante *et al.,* 2003).

The significant decrease in cyanide level after fermentation ($p \leq 0.05$) agrees with Obueh and Ikenebomeh, (2014). The decrease in the cyanide level after fermentation indicates that fermentation is a detoxification process leading to a reduction in the cyanide content. This is another important finding from this research. Several health problems such as tropical ataxic neuropathy, endemic goitre, spastic paraparesis and konzo have been associated with consumption of inadequately processed cassava roots and cyanide is found to be the most toxic factor restricting the consumption of cassava roots and leaves (Montagnac *et al*., 2009).

It is widely known that consumption of cassava varieties in their raw or even boiled state cause serious poisoning (International cyanide Management Institute (ICMI), 2012). The yellow variety of cassava was found to have lower post-fermentation cyanide content than the white variety possibly because the unfermented tuber has higher cyanide content than the white variety.

Ihenetu *et al*. (2017) reported that retting of cassava roots allows softening of the roots from further processing and the reduction of the potentially toxic cyanogenic glycosides present in the roots. The reduction in cyanide is attributed to changes in the texture in the plant tissues which makes it possible for vacuole bound cyanogenic glycoside to diffuse and come in contact with membrane bound linamarase (Danladi *et al*., 2016).

The decrease in the cyanide content may be due to various activities taking place during cassava mash fermentation through the assistance of endogenous microbial enzymes (Cumbara *et al*., 2007). Yeast and fungi as reported by Aworrh, (2008) contribute to cassava tissue breakdown by cellulase production leading to a more intimate interaction between linamarase and cyanogenic compounds of cassava, linamarin and lotaustralin resulting in the formation of glucose and acetone and resultant detoxification of cassava (Aworrh, 2008).

Fermentation reduces the cyanide level and reduction to safe cyanide level is achieved through further processing of the fermented cassava into different products like *fufu*, *abacha*. Akinrele (2010) stated that 0.3mg/100g is the acceptable safe level of cyanide content of cassava and cassava products for consumption.

The fermentation of cassava caused an increase in the protein and moisture content and a decrease in crude fiber, ash and carbohydrate for both cassava varieties. Similar result was reported by Obueh *et al*., (2017). The increase in protein content could be due to accumulation of single cell proteins of microbes associated with the fermentation process (Obueh *et al*, 2017). The slight increase in moisture could be attributed to the steeping water (Ojokoh *et al*., 2014). The hydrolysis of the carbohydrate of the cassava during fermentation to reducing sugar was due to the production of hydrolytic enzymes by the microbial flora present which they used as carbon sources for their energy generation and build up of biomass and possible transformation to other molecules such as protein and fat (Ahaotu *et al*., 2011). There was a significant decrease in the ash and crude fiber contents after fermentation in the microbial flora. Since ash is a measure of the total minerals present within a food, the reduction in its level could be as a result of mineralization during microbial metabolism (Aloys and Ming, 2006). The decrease in crude fiber content of the cassava could enhance digestibility in animals' gastrointestinal tract if the fermented wastes are eventually included in livestock ratio formulation (Guira *et al*., 2016).

The increase in protein and moisture and a decrease in crude fiber are of nutritional importance especially as cassava is a staple food for the rural people where alternative animal proteins are expensive to come by (Obueh *et al*., 2017).

CONCLUSION

Microbial fermentation was found to have significant effect in the reduction of the cyanide contents. It also increased the protein contents of both cassava varieties and eliminated pathogens from the fermented cassava tubers.

RECOMMENDATION

Based on our findings, cassava must be fermented to reduce the cyanide content before consumption.

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