MICROBIOLOGICAL AND PROXIMATE COMPOSITIONS OF FIVE CASSAVA FRACTIONS SUBJECTED TO SUBMERGED FERMENTATION PROCESS

[1*]Obueh, Henrietta Omokhele,[2]Ikenebomeh, Marcel James, [3]Kolawole,Sunday Ekanah
[1*]Biology Department, College of Education, P. M. B 1144, Ekiadolor, Benin City, Edo State, Nigeria
Email: hettyobueh72@yahoo.com Phone number: 2348056112904
[2]Microbiology Department, University of Benin, P. M. B 1154, Benin City, Edo State, Nigeria.
[3] National Open University of Nigeria, Benin Study Centre
*E-mail: kolasunde@gmail.com

Abstract
The microbiological and proximate compositions of five cassava fractions subjected to submerged fermentation process for 72 h were determined. The five cassava fractions from sweet and bitter varieties included diced (cassava fraction A), grated (cassava fraction B), Peel + effluent (cassava fraction C), effluent (cassava fraction D) and peel + water (cassava fraction E). The isolated microorganisms included Lactobacillus plantarum, L. coryniformis, L. delbrueckii, Lactococcus lactis, Bacillus subtilis, Klebsiella aerogenes, Candida tropicalis, Saccharomyces cerevisiae and Fusarium solani. F. solani was absent in cassava fractions A, B and E of the sweet cassava variety and cassava fractions A and B of the bitter cassava variety. L. plantarum was the most prominent microorganism with mean prevalence in the order B> C> D> A> E and B> C> A> D> E for the sweet and bitter cassava varieties respectively. There was an increase in the bacterial counts and a decrease in the fungal counts of the cassava fractions. Microbial population was highest in cassava fraction B. There was a significant increase ($p \leq 0.05$) in protein, moisture and fat and a decrease in crude fibre, ash and carbohydrate contents of all the cassava fractions, with the sweet variety higher than the bitter variety. Cassava fraction B had the highest moisture, protein and fat contents and cassava fraction E had the lowest contents for both cassava varieties. The nutrient enhancement of the cassava fractions is of nutritional importance to increase productivity, efficiency and quality output of cassava.

Key words: Microbiological, Proximate, Cassava fractions, Submerged fermentation process

Submitted: 11.12.2016 Reviewed: 22.03.2017 Accepted: 24.03.2017

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a tropical root crop that serves as a food security for millions of people in the developing world (Akoroda, 1995). The different types of cassava are described by the term cultivars or varieties. 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 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).

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). Submerged fermentation or liquid state fermentation technique is the one in which water is always in a free state while food nutrients in the form of carbon, nitrogen, phosphorus and others are in a suspended or dissolved state (Balagopalan *et al.*, 2002). The cassava roots, whole or cut into pieces are soaked in water for the duration of fermentation (Oyenole and Odunfa, 1989). Submerged fermentation affects carbohydrate, protein and mineral contents of cassava roots. A wide spectrum of microorganisms has been

implicated in submerged fermentation. Obile *et al.* (2004) identified *Bacillus spp, Leuconostoc spp, Klebsiella spp, Corynibacterium spp, Lactobacillus spp, Aspergillus spp, Candida spp and Geotrichum spp* during the submerged fermentation of cassava. These microorganisms utilize the sugars produced from the carbohydrate or starch and convert them into organic acids. During fermentation, only few microorganisms were able to withstand the increasing acidity of the medium (Oyewole, 1995). The objectives of this study was to determine the microbiological and proximate compositions of five cassava fractions during submerged fermentation process and also ascertain the nutritional potential of the cassava fractions.

2. MATERIAL AND METHODS

Collection and preparation of cassava

Freshly harvested sweet and bitter cassava varieties were collected from cassava farms at Ekiadolor (Lat 6° 29' N, Long 5° 35' E) in Edo State, Nigeria. The sweet and bitter cassava varieties were washed with distilled water and peeled. The peeled cassava were washed thoroughly with distilled water and processed to obtain five cassava fractions A, B, C, D and E of the two cassava varieties as follows:

A = Two hundred (200) g peeled cassava roots, diced with a sterile knife into uniform sizes (5 – 7 cm), washed and soaked in 150 ml of sterile distilled water.

B = Two hundred (200) g peeled cassava roots, grated with a grater and put in 150 ml of sterile distilled water.

C = Two hundred (200) g of dried, ground, cassava peels mixed in 150 ml of cassava fermentation waste water.

D = Two hundred (200) g of cassava fermentation waste water mixed in 150 ml sterile distilled water.

E = Two hundred (200) g of dried, ground cassava peels mixed in 150 ml of sterile distilled water.

The different cassava fractions were each weighed into separate 500 ml conical flasks. Sterile distilled water was added to make up the mark of the flask and the flasks were stopped with sterile cotton wool covered with aluminium foil. The mixtures were sterilized in an autoclave at 121^{o}C for 15 min to gelatinize the starch. They were allowed to cool and sterile distilled water was aseptically added to make the 500 ml mark of the flask again. The mixtures were transferred into a sterile fermenter and fermentation of the cassava fractions took place at room temperature 30^{o}C for 72 h. Samples were taken aseptically for different analyses in the laboratory.

Microbiological analyses

The different naturally occurring microorganisms responsible for fermentation were determined using appropriate serial dilutions and pour plate method on nutrient agar (Lab M Ltd), plate count agar (Lab M Ltd), de-Man-Rogosa Sharpe agar (Lab M Ltd) for total viable counts of bacteria at 30^{o} - 35^{o}C for 24 – 48 h and potato dextrose agar (Lab M Ltd) supplemented with 10% lactic acid and 0.5% chloramphenicol (AOAC, 2001) at 25^{o}C for 2 – 5 days. The colonies were observed and counted and results expressed as colony forming units per gram (cfu/g). Representatives of the different purified colonies were subjected to various cultural, morphological and biochemical analyses. Bacterial identification was based on Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994) and fungal identification was by wet mount method as described by Yarrow (1998).

Proximate analyses

The moisture, protein, crude fiber, fat, ash determined using the methods of AOAC (2005) and the reducing sugar was determined by the method of Kimaryo *et al.* (2000). The moisture was determined by weighing each sample in a dish and then drying the samples in a hot air oven at 105^{o}C for 3 h until a constant weight was determined. The difference between the

initial weight of the dish containing the sample and the final dry weight was the moisture content. Protein content was by Kjeldahl method. A known weight, 0.5 g each of the samples was weighed and digested by using 98% concentrated hydrochloric acid (HCl) with the formation of ammonium sulphate $(NH_4)_2 SO_4$. Through the alkalinization with sodium hydroxide (NaOH), ammonia was displaced from the ammonium sulphate via steam distillation. The distillate was titrated with 0.01 M HCl until a grey colour, the end point was reached. Percentage crude protein was total percentage nitrogen × 6.25. Ash content was determined by placing 5 g of each sample in a crucible and incinerated in a muffle furnace at 550°C for 4 h, cooled in a dessicator and then weighed. The difference in the weight of the crucible without sample before and after ashing was the ash content. Fat content was determined by Soxhlet extraction method in ethyl ether. A known weight, 5 g of each sample was ground and placed in a porous cellulose thimble. The thimble was then placed in the extraction chamber, suspended above the extraction flask containing 200 ml ethyl ether and extraction was carried out for about 5 – 6 h. The flasks containing the fat was oven dried at 105 – 110°C for 1 h, cooled and weighed. Crude fiber was determined by the enzymatic-gravimetric method. One gram of each sample was weighed into a 500 ml conical flask and 100ml of TCA (tricyclic acid) digestion reagent was added. The content was boiled for 40 min, allowed to cool and filtered through a 15 cm diameter No 4 Whatman filter paper. The residue was then washed six times with hot water and four times with industrial spirit and then transferred into a weighed porcelain dish, dried over night at 105°C, transferred to a dessicator and weighed when cool. Then the residue was ashed in a muffle - furnace at 600°C for 6 h, cooled and weighed. To determine the reducing sugar, the samples were first treated with excess α – amylase for 2 h at pH 5.5 and heated to 100°C, cooled to 60°C and excess glucoamylase added. The samples were centrifuged and then one ml of diluted supernatant of the sample was mixed with 1 ml of DNS (3,5 dinitrosalicylic acid), heated to 100°C for 5 min in a water bath and cooled to room temperature at 30°C. The absorbance of the colour developed was read at 540 nm. The concentration of residual sugar was determined against a glucose standard graph. All the proximate analyses were carried out in triplicates and calculated in percentage.

Statistical analyses

The results were presented as mean ± standard deviation of triplicate values. A one way ANOVA (Analysis of Variance) and Duncan Multiple Range test were carried out. Significant difference was accepted at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The bacterial and fungal counts for the sweet and bitter cassava fractions are presented in Table 1 and 2. The microbial counts were higher in the sweet cassava variety fractions than in the bitter cassava variety fractions before and after fermentation. The bacterial counts were between $8.60 \times 10^4 \pm 0.02$ cfu/g to $6.88 \times 10^5 \pm 0.02$ cfu/g and $4.10 \times 10^4 \pm 0.03$ cfu/g to $6.29 \times 10^5 \pm 0.03$ cfu/g for the sweet and better cassava fractions after 72 h. The fungal counts ranged from $1.60 \times 10^4 \pm 0.01$ cfu/g to $2.84 \times 10^5 \pm 0.03$ cfu/g and $3.00 \times 10^3 \pm 0.01$ cfu/g to $2.55 \times 10^5 \pm 0.02$ cfu/g for the sweet and bitter cassava fractions after fermentation. The grated fraction B had the highest microbial count. There was a decrease in fungal counts after fermentation for all the fractions.

Table 3 showed the mean percentage occurrence of microbial isolates for the cassava fractions. The isolated microorganisms included *Lactobacillus plantarum, L. coryniformis, L. delbrueckii, Lactococcus lactis, Bacillus subtilis, Klebsiella aerogenes, Candida tropicalis, S. cerevisiae* and *Fusarium solani*. *Lactobacillus plantarum* was the most prevalence of 30.24% and 28.69% for the sweet and bitter cassava variety respectively. *Fusarium solani* was absent in fractions A, B and E of the sweet cassava and fractions A and B of the bitter cassava.

Table 1: Bacterial Counts (cfu/g) for Sweet and Bitter Cassava Varieties and Their Fractions

Cassava Variety	Cassava Fractions	Before Fermentation	After Fermentation
Sweet	**A**	$3.31 \times 10^5 \pm 0.02^d$	$5.34 \times 10^5 \pm 0.24^b$
	B	$3.76 \times 10^5 \pm 0.03^b$	$6.88 \times 10^5 \pm 0.02^a$
	C	$3.49 \times 10^5 \pm 0.04^c$	$5.95 \times 10^5 \pm 0.07^c$
	D	$3.63 \times 10^5 \pm 0.04^a$	$6.71 \times 10^5 \pm 0.24^b$
	E	$3.20 \times 10^4 \pm 0.02^e$	$8.60 \times 10^4 \pm 0.02^c$
Bitter	**A**	$3.09 \times 10^5 \pm 0.33^b$	$5.05 \times 10^5 \pm 0.07^c$
	B	$3.53 \times 10^5 \pm 0.02^a$	$6.29 \times 10^5 \pm 0.03^a$
	C	$3.28 \times 10^5 \pm 003^c$	$5.45 \times 10^5 \pm 0.16^d$
	D	$3.42 \times 10^5 \pm 0.07^a$	$6.13 \times 10^5 \pm 0.02^d$
	E	$1.40 \times 10^4 \pm 0.03^d$	$4.10 \times 10^4 \pm 0.03^e$

Values are means ± standard deviation (n=3). Means in the same column between the cassava fractions with same superscripts are not significantly different (P> 0.05). A = Diced B = Grated C = Peel + Effluent D = Effluent E = Peel + Water

Table 2: Fungal Counts (cfu/g) for Sweet and Bitter Cassava Varieties and Their Fractions

Cassava Variety	Cassava Fractions	Before Fermentation	After Fermentation
Sweet	**A**	$1.74 \times 10^5 \pm 0.02^d$	$1.41 \times 10^5 \pm 0.01^b$
	B	$3.37 \times 10^5 \pm 0.03^a$	$2.84 \times 10^5 \pm 0.03^a$
	C	$2.62 \times 10^5 \pm 0.03^c$	$2.10 \times 10^5 \pm 0.01^b$
	D	$3.11 \times 10^5 \pm 0.07^b$	$2.62 \times 10^5 \pm 0.02^a$
	E	$2.00 \times 10^4 \pm 0.01^e$	$1.60 \times 10^4 \pm 0.01^c$
Bitter	**A**	$1.22 \times 10^5 \pm 0.04^b$	$0.78 \times 10^5 \pm 0.07^c$
	B	$2.93 \times 10^5 \pm 0.02^c$	$2.55 \times 10^5 \pm 0.02^b$
	C	$2.33 \times 10^5 \pm 0.02^c$	$1.93 \times 10^5 \pm 0.05^c$
	D	$2.51 \times 10^5 \pm 0.03^a$	$2.10 \times 10^5 \pm 0.04^a$
	E	$1.10 \times 10^4 \pm 0.06^d$	$3.00 \times 10^3 \pm 0.01^d$

Values are means ± standard deviation (n=3). Means in the same column between the cassava fractions with same superscripts are not significantly different (P> 0.05). A = Diced B = Grated C = Peel + Effluent D = Effluent E = Peel + Water

Table 3: Mean Percentage Occurrence of Microbial Isolates in the Sweet and Bitter Cassava Variety Fractions

		Microbial Isolates (%)								
Cassava Variety	**Cassava Fractions**	*Lactobacillus plantarum*	*Lactobacillus coryniformis*	*Lactococcus lactis*	*Lactobacillus delbrueckii*	*Bacillus subtilis*	*Klebsiella aerogenes*	*Candida tropicalis*	*Saccharomyces cerevisiae*	*Fusarium solani*
Sweet	**A**	32.34	12.42	1.17	10.75	14.75	5.58	10.67	12.32	0.00
	B	34.34	15.41	1.25	11.42	15.84	3.75	9.67	8.32	0.00
	C	33.33	16.50	1.83	10.83	13.92	4.58	10.09	7.99	0.93
	D	32.67	12.08	0.92	10.75	15.75	8.75	10.58	7.50	1.00
	E	18.50	14.75	0.67	5.17	27.47	8.50	24.94	0.00	0.00
	Mean	30.24	14.23	1.16	9.78	17.55	6.23	13.19	7.23	0.39
Bitter	**A**	30.75	10.75	2.09	11.33	15.08	6.33	11.67	12.00	0.00
	B	33.84	10.00	2.25	11.58	14.33	7.34	12.75	7.91	0.00
	C	32.50	16.08	2.67	9.83	12.83	4.75	9.49	9.98	1.87
	D	30.09	11.17	0.58	10.83	14.92	11.42	10.83	9.36	0.80
	E	16.25	14.58	0.92	4.92	22.42	8.41	31.75	0.00	0.75
	Mean	28.69	12.52	1.70	9.70	15.92	7.65	15.29	7.85	0.68

There was significant increase in moisture (6.11 ± 0.21% to 7.32 ± 0.90%), protein (3.98 ± 0.14% to 15.75 ± 0.84%), and fat (4.01 ± 0.02 to 8.18 ± 0.04%) for the sweet cassava variety and moisture (5.94 ± 0.25% to 6.22 ± 0.22%), protein (3.48 ± 0.15% to 14.03 ± 0.38%) and fat (3.43 ± 0.04% to 7.26 ± 0.01%) for the bitter cassava variety after fermentation. There was decrease in crude fibre (6.52 ± 0.04% to 4.96 ± 0.06% and 8.95 ± 0.06% to 4.03 ± 0.83%), ash 1.76 ± 0.30 to 0.54 ± 0.06 and 3.14 ± 0.14% to 0.36 ± 0.03%) and carbohydrate (77.62 ± 2.10% to 44.47 ± 0.31% and 75.06 ± 3.11% to 42.24 ± 0.11%) for sweet and bitter cassava varieties respectively. The cassava fraction B had the highest moisture, protein and fat contents and cassava E had the lowest contents for both varieties (Table 4 and 5).

Table 4: Proximate Compositions of Sweet and Bitter Cassava Variety Fractions Before Fermentation

Cassava Variety	Cassava Fractions	Proximate Composition %					
		Moisture	Crude Protein	Fat	Crude Fiber	Ash	Carbohydrate
Sweet	**A**	6.24 ± 0.30^{a}	4.29 ± 0.62^{b}	4.19 ± 0.01^{a}	6.22 ± 0.01^{a}	1.25 ± 0.03^{a}	77.81 ± 2.10^{a}
	B	6.01 ± 0.04^{a}	4.38 ± 0.10^{b}	3.82 ± 0.03^{b}	6.00 ± 0.02^{c}	0.76 ± 0.10^{d}	79.03 ± 3.01^{b}
	C	6.53 ± 0.03^{b}	4.11 ± 0.22^{c}	4.28 ± 0.05^{c}	6.12 ± 0.04^{a}	1.29 ± 0.04^{a}	77.67 ± 6.10^{a}
	D	6.81 ± 0.05^{c}	3.77 ± 0.11^{e}	4.01 ± 0.02^{d}	6.43 ± 0.04^{a}	1.07 ± 0.07^{b}	77.91 ± 2.70^{b}
	E	4.97 ± 0.11^{d}	3.33 ± 0.10^{c}	3.76 ± 0.06^{a}	7.82 ± 0.05^{b}	4.39 ± 0.02^{e}	75.70 ± 1.20^{c}
Bitter	**A**	5.98 ± 0.25^{a}	3.63 ± 0.10^{a}	3.81 ± 0.04^{b}	8.45 ± 2.09^{a}	2.94 ± 0.11^{a}	74.85 ± 2.70^{c}
	B	6.34 ± 0.30^{b}	3.97 ± 0.10^{b}	3.29 ± 0.02^{b}	8.13 ± 1.40^{c}	2.02 ± 0.20^{b}	76.09 ± 3.10^{a}
	C	6.42 ± 0.10^{b}	3.56 ± 0.20^{b}	3.22 ± 0.04^{b}	9.34 ± 2.00^{a}	2.37± 0.10^{b}	75.09 ± 2.10^{b}
	D	6.59 ± 0.30^{a}	3.44 ± 0.30^{b}	3.63 ± 0.04^{b}	8.67 ± 1.08^{a}	2.59 ± 0.20^{a}	75.55 ± 4.51^{b}
	E	4.35 ± 0.10^{d}	2.79 ± 0.10^{c}	3.19 ± 0.01^{a}	10.16 ± 3.90^{b}	5.79 ± 0.30^{e}	73.72 ± 2.00^{c}

Values are means ± standard deviation (n=3). Means in the same column between the cassava fractions with same superscripts are not significantly different (P> 0.05). A = Diced B = Grated C = Peel + Effluent D = Effluent E = Peel + Water

Table 5: Proximate Compositions of Sweet and Bitter Cassava Variety Fractions After Fermentation

Cassava Variety	Cassava Fractions	Proximate Composition %					
		Moisture	Crude Protein	Fat	Crude Fiber	Ash	Reducing sugar
Sweet	**A**	6.97 ± 2.39^{a}	21.08 ± 1.62^{a}	9.40 ± 0.06^{a}	5.80 ± 0.08^{b}	0.50 ± 0.02^{b}	44.21 ± 1.41^{c}
	B	8.04 ± 0.74^{b}	23.51 ± 0.50^{b}	10.01 ± 0.02^{b}	5.66 ± 0.02^{b}	0.44 ± 0.1^{c}	33.79 ± 0.09^{b}
	C	7.75 ± 0.78^{c}	13.92 ± 1.52^{c}	6.64 ± 0.02^{c}	5.90 ± 0.06^{a}	0.28 ± 0.04^{a}	39.41 ± 6 .20^{a}
	D	7.94 ± 0.47^{d}	11.67 ±0.16^{d}	7.25 ± 0.05^{d}	4.92 ± 0.08^{c}	0.21 ± 0.07^{d}	37.82 ± 6 52^{d}
	E	5.89 ± 0.11^{e}	8.62 ± 0.40^{d}	5.61 ± 0.04^{a}	2.53 ± 0.05^{d}	1.28 ± 0.02^{e}	67.14 ± 1.20^{e}
Bitter	**A**	6.02 ± 0.25^{c}	18.52 ± 0.17^{c}	8.55 ± 0.01^{a}	4.24 ± 0.03^{b}	0.39 ± 0.01^{a}	39.11 ± 1.07^{a}
	B	7.23 ± 0.39^{a}	22.74 ± 0.16^{b}	9.54 ± 0.01^{a}	4.13 ± 0.02^{c}	0.35 ± 0.02^{c}	37.04 ± 0.09^{b}
	C	6.49 ± 0.20^{b}	11.33 ± 0.36^{a}	5.22 ± 0.01^{b}	4.30 ± 0.05^{a}	0.19 ± 0.08^{b}	41.11 ± 2.12^{c}
	D	6.72 ± 0.03^{d}	10.04 ± 0.83^{d}	6.09 ± 0.01^{c}	4.25 ± 0.03^{d}	0.11 ± 0.01^{d}	28.12± 4. 20^{d}
	E	4.64 ± 0.01^{e}	7.52 ± 0.06^{a}	4.90 ± 0.02^{b}	3.22 ± 0.02^{c}	0.75 ± 0.03^{d}	65. 05 ± 1.60^{e}

Values are means ± standard deviation (n=3). Means in the same column between the cassava fractions with same superscripts are not significantly different (P> 0.05). A = Diced B = Grated C = Peel + Effluent D = Effluent E = Peel + Water

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). The microbial isolates were more abundant in the sweet cassava fractions than in the bitter cassava fractions due not only to the low moisture content of the latter (Assanvo *et al.*, 2006) but also to their higher cyanogenic glucoside content, which had inhibitory effect on microbial growth (Rainbault, 1995). *Lactobacillus plantarum* was the most prevalent organism due to its high prevalence in all cassava fractions except the control E (Henshaw *et al*., 2012). The mean prevalence was higher in the sweet cassava variety than the bitter cassava variety and the organism dominated the microbial population (Nybom *et al.*, 2008) due to its ability to maintain a pH gradient between the inside and the outside of the cell in the presence of a large amount of acetate or lactate (McDonald *et al.*, 1990). It therefore has ability to convert low molecular weight sugars almost quantitatively into lactic acid. The high prevalence of *Bacillus subtilis* during fermentation contributed to the rotting of the root tubers and production of amylase enzymes necessary for the breakdown of starch to sugar. *B. subtilis* forms resistant spores which can tolerate adverse conditions and can survive in adverse environment for many days (Brooke *et al*., 1998). *Candida tropicalis* was the only fungus present in the cassava fraction E of the sweet cassava variety. The high occurrence of *C. tropicalis* in all the cassava fractions could be attributed to the fact that *C. tropicalis* grows on soluble starch, corn and cassava powder without requiring that these substrates be previously hydrolyzed. *C. tropicalis* possesses the enzyme α – amylase needed to hydrolyze starch (Assanvo *et al*., 2006). The presence of the least prevalent organism *Fusarium solani* could have been due to contamination as *F. solani* is a natural flora of the soil. Although yeasts and moulds are known to have an important role in food processing particularly in fermentation, the growth of moulds is undesirable as they contaminate the food (Moorthy and Mathew, 1998).

The fermentation of the cassava fractions caused an increased in protein, fat and moisture contents and a decrease in fibre, ash and carbohydrate contents for both cassava varieties. This was in agreement with the works of Adesanya *et al.* (2008) and Oboh and Elusiyan (2007). The increase in protein content may be because of the microbial flora which degraded the cassava fractions readily and could have secreted some extracellular enzymes (proteins) in the cassava fractions (Akindahunsi *et al.*, 1999). The increase in fat content could be attributed to fact that microorganisms could produce microbial oil (Oboh and Akindahunsi, 2003). The higher increase in protein and fat contents of the cassava fractions of the sweet variety than the bitter variety could be due to the higher cyanide content in the bitter variety which inhibited the growth of the fungi present, thereby limited the amount of protein added as single cell protein (Oboh and Akindahunsi, 2003) and microbial oil secreted by the fungi (Nuwamanya *et al.*, 2010). The highest increase in the grated cassava fraction B could be due to the increased surface area making it easier for microbial action during fermentation. The slight increase in moisture could be attributed to the addition of water to the cassava fractions prior to fermentation (Ojokoh *et al*., 2012). The reduction of the carbohydrate content of the cassava fractions after fermentation to reducing sugar was due to the production of hydrolytic enzymes by the microbial flora present which they used as carbon source and transformed them to other macro molecules or metabolites such as protein and fat (Oboh, 2006). More sugar was used as the percentage mean reducing sugar converted in the sweet cassava variety fractions than the bitter variety fractions because of the higher

carbohydrate content. Conversely, the breakdown of carbohydrate content is as a result of the ability of the fungal/bacterial complex to hydrolyze starch to glucose and ultimately the glucose will be used by the same organisms as a carbon source to synthesize fungal/bacterial biomass rich in protein (*Oboh et al*., 2002).

There was a significant decrease in the ash and crude fibre contents after fermentation by 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 the minerals present being used up by the fermenting organisms as a mineral source during their metabolism (Aderiye and Ogunjobi, 1998). The decrease in crude fibre content of the cassava fractions by could enhance digestibility in animals' gastro-intestinal tracts if the fermented wastes are eventually included in livestock ration formulation (Oboh and Oladundoye, 2007). Therefore, the increase in protein, fat and moisture and a decrease in crude fibre are of nutritional importance.

4. CONCLUSION

The submerged fermentation process of the cassava fractions enhanced the productivity, efficiency and quality output of the cassava. The fungal/bacterial complex increased the nutritional potentials of the cassava fractions.

5. REFERENCES

[1] 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.

[2] Aderiye, B. J and Ogunjobi, A. A. Fermentation of yam: microbiology and sensory evaluation of cooked fermented yam tissues. *Plant foods for Human Nutrition*, 1998, 52: 49-54.

[3] Akindahunsi, A. A, Oboh, G., Oshodi A. A. Effect of fermenting cassava with *Rhizopus oryzae* on the chemical composition of its flour and gari. *La Rivista Italiana Delle Sostanze Grasse*, 1999, 76: 437-440.

[4] Akoroda, M. O. Alleviating hunger in Africa with root and tuber crops. *African Journal of Tropical Root crops*, 1995, 1:41-43.

[5] AOAC. Association of Official Analytical Chemists. Bacteriological Analytical Manual online US Food and Drug Administration, Washington DC, 2001, 946.

[6] AOAC. Association of Official Analytical Chemists. Official Methods of Analysis 18th edition, Washington DC, USA, 2005.

[7] Aro, S. O. Improvement of the nutritive quality of cassava and it's by products through microbial fermentation. *African Journal of Biotechnology*, 2008, 7(25): 4789-4797.

[8] Assanvo, J. B., Agbo, G. N., Behi, Y. E. N., Coulin., P and Farah, Z. Microflora of traditional starter made from cassava for Attieke production in Dabou (Cote d'Ivoire). *Food Control*, 2006, 17: 37-41.

[9] Balagopalan, C., Padmaja, G and George, M. Improving the nutritional value of cassava products using microbial techniques. FAO – corporate document repository. Animal Production Health Paper 95, 2002.

[10] Brooke, G. F., Butel, J. S and Mores, S. A. 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.

[11] Holt, J.G., Krieg, N.R., Sneath, P. H. A., Stanley J. T and S. T. Williams. Bergey's Manual of Determinative Bacteriology, Williams and Wilkins, Baltimore Maryland, 1994, 1568.

[12] Kimaryo, V. W., Massawi, G. A., Olasupo, W. A and Holzapfel, W. H. The use of a starter culture in the fermentation of cassava for the production of 'kivunde' a traditional Tanzanian food product. *International Journal of Food Microbiology*, 2000, 56: 176-190.

[13] McDonald, L.C., Fleming, H. P and Hassan, H.M. Acid tolerance of *Leuconostoc mesenteriodes* and *Lactobacillus plantarum*. *Applied Environmental Microbiology*, 1990, 56: 2120-2124.

[14] Moorthy, S. N. and Matthew, G. Cassava fermentation and associated changes in physiological and functional properties. *Critical Review in Food Science Nutrition*, 1998, 38: 73-121.

[15] NRCRI. Guide to cassava cultivation in Nigeria, National Root Crops Research Institute Umudike, 2004, 121.

[16] Nuwumanya E., Baguma, Y., Emmabux, N., Taylor J and Rubaihayo, P. Physicochemical and functional characteristics of cassava starch in Ugandan varieties and their progeny. *Journal of Plant Breeding and Crop Science*, 2010, 2: 1-11

[17] Nweke, F. I. Cassava processing in sub-Saharan African: the implications for expanding cassava production. *Outlook on Agriculture*, 1994, 23: 197-205.
[18] Nybom, S. M., Collado, M. C., Surono, I. S., Salminen, S. J and Meriluoto, J. A. Effect of glucose removal of microcystin – LR by viable commercial probiotic strains and strains isolated from dadih fermented milk. *Journal of Agricultural and Food Chemistry*, 2008, 56 (10): 3714-20.
[19] Obile, E. M., Tano-Debra, K and Amoa-Awua, W.K. Souring and breakdown of cyanogenic glucosides during the processing of cassava into akyeke. International *Journal of Food Microbiology*, 2004, 93(1): 115-121.
[20] Oboh, G. Nutrient enhancement of cassava peels using a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus spp* solid media fermentation techniques. *Electronic Journal of Biotechnology*, 2006, 9(1): 71-78
[21] Oboh, G and Elusiyan, C. A. Changes in the nutrient and anti-nutrient content of micro-fungi fermented cassava flour produced from low and medium cyanide variety of cassava tubers. *African Journal of Biotechnology*, 2007, 6 (18): 2150-2157.
[22] Oboh, G. and Akindahunsi, A. A. Biochemical changes in cassava products (flour and garri) subjected to *Saccharomyces cerevisiae* solid media fermentation. *Food Chemistry*, 2003, 82 (4): 599-602.
[23] Oboh, G., Akindahunsi, A.A. and Oshodi , A. A. Nutrient and antinutrient contents of *Aspergillus* fermented cassava products (flour and garri). *Journal of Food Composition and Analysis*, 2002, 15(5): 617 – 622.
[24] Ojokoh, A. O., Abiola, A.B. and Law, R. T. Changes in nutrient and antinutrient composition of popcorn and groundnut composite flour subjected to solid substrate fermentation. *African Journal of Agricultural Research*, 2012, 7(23):3439-3445.
[25] Oyewole, O. B and Odunfa, S.A. Microbiological studies on cassava fermentation for lafun production. *Food Microbiology*, 1999, 5: 125-133.
[26] Oyewole, O. B, Application of biotechnology to cassava processing in Africa in Transformation Alinentaire du Manioc: In T. Agbor Egbe, A Brauman, D. Griffon, S. Treche (eds) 1995 editions, ORSTOM, 1995, 277-286.
[27] Raimbault, M. Importance des bacteries lactiques dans les fermentations du manioc. In Agbor Egbe, Brauman, Griffon, Treche. Transformation Alimentaire du Manioc ed crestom, Paris, 1995, 747 pp.
[28] Tetchi, F. A., Solomon, O. W., Celah, K. A and Georges, A. N. Effect of cassava variety and fermentation time on biochemical and microbiological characteristics of raw artisan starter for Attieke production. *Innovative Romanian Food Biotechnology*, 2012, 10 (3): 40-47.
[29] Yarrow, D. Methods for isolation, maintenance and identification of yeasts :In Kurtzman, C. P., J. W. Fell eds. The yeast: A Taxonomic study, Elsevier Science B.V Amsterdam, 1998, 77-100.
[30] Yeoh, H. H and Sun, F. Assessing cyanogens content in cassava – based food using the enzymes in cassava based food using the enzyme – dipstick method. *Food and Chemical Toxicology*, 2001, 39(7): 649 - 653.