INFLUENCE OF *LACTOBACILLI* STARTER CULTURES ON THE NUTRITIONAL CONTENT AND ANTI-NUTRITIONAL FACTORS OF FERMENTED CASSAVA FOR *USI* (EDIBLE STARCH) PRODUCTION IN NIGERIA

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Abstract
Usi, an indigenous food in Delta State, Nigeria, produced from fermented cassava has no documented information about the nutritional and anti-nutritional content when fermented with starter cultures even though fermentation is known to engender nutritional improvement on food products. This study therefore aimed at investigating the effect of starter culture fermentation on the nutritional quality of cassava for usi production.
Cassava tubers (TME 30572), fermented both spontaneously (control experiment) and with Lactobacilli starter cultures (singly and in-combination) for 72 hours were analyzed for proximate and anti-nutritional factors using standard methods. Data were statistically analyzed at 5% level of probability.
Spontaneously-fermented sample had lower moisture content (5.68%), crude fat (0.23%), crude fibre (1.91%) and higher carbohydrate content (90.30%) than the starter-fermented samples which had (6.79-7.35%), (0.25-0.39%), (1.93-2.24%) and (88.31-89.63%) respectively. However, higher protein content ranging from 1.02% to 1.24% was observed in the starter-fermented mashes. The least tannin content (33.4 mg/g) was evident in the spontaneously-fermented sample while cyanide was not detected in both the spontaneously- fermented sample and when the starters were combined.
Although, spontaneously-fermented sample exhibited better moisture content, crude fibre content, tannin and cyanide content, utilization of starters brought about improved protein content. Furthermore, the consortium of the three starters showed better nutritional results than when utilized singly.

Key words*: Cassava fermentation; Lactic Acid Bacteria; Proximate; Starter culture; Usi*

Received: 23.06.2018 Reviewed: 10.09.2018 Accepted: 28.09.2018

1. INTRODUCTION

Cassava, rated as the 4th most important root crop in developing countries, is one of the most useful tropical crops with a world output increase of 4.6% between 2013 and 2014, having Nigeria as one of the major producers (FAO, 2014). It is widely exploited as a cheap energy source despite major limitations like the presence of toxic cyanogenic glucosides, low protein content and short postharvest shelf life.
Cassava roots were reported to be rich in carbohydrates with most of it being present as starch hence, its utilization by most people in the Tropics as sources of carbohydrate (Morgan and Choct, 2016). They are deficient in protein with about 0.7% to 1.3% fresh weight (Ngiki *et al*., 2014). Even though, cassava tuber has been criticized for its low and poor protein content, it produces more weight of carbohydrate per unit area than other staple food crops under comparable agro-climatic conditions thus, being an energy-dense food and therefore ranked high for its energy value of 250 x 10^3 cal/ha/day as compared to rice, wheat, maize and sorghum (Jisha *et al*., 2010). The edible starchy flesh comprises some 60% to 70% total weight of the root as water (Morgan and Choct, 2016). This forms the major component and it is between the range 60.3% and 87.1% according to (Zvinavashe *et al.*, 2011). The tubers are usually processed, mostly through fermentation to overcome the aforementioned problems into various products such as *usi* (edible starch), which is a major staple diet of the Itsekiri and Urhobo in Delta, Nigeria.
Usi is an energy-rich food containing about 84% starch. Since the food is a product of fermentation, it is expected that fermentation

process will confer some beneficial effect on both the improvement of its proximate composition and anti-nutritional factors. However, there is paucity of information to confirm this. Hence, this study was conducted to investigate the effect of both spontaneous fermentation and the use of different starter cultures on the nutritional qualities of *usi.*

2. MATERIALS AND METHODS

Cassava samples

Healthy cassava variety TME 30572 was obtained from the International Institute of Tropical Agriculture, Ibadan. Oyo State, Nigeria.

Starter culture

Lactobacillus pentosus F2A (Accession number KJ778115), *L. plantarum subsp. argentolarensis* F2B (KJ778116) *and L. plantarum* F2C (KJ77117) previously isolated from a spontaneously-fermented cassava and identified (Oyinlola *et al*., 2016) using the NCBI Basic Local Alignment Search Tools (BLAST) were used as starter cultures.

Preparation of cassava for *usi* fermentation

Cassava tubers were visually assessed, peeled, washed with clean tap water, cut into pieces and sterilized using 0.1% HgCl in 70% ethanol followed by rinse with sterile distilled water (Adetunde and Onilude, 2010). One hundred gram was grated and the pulp were fermented both spontaneously (control experiment) and with *Lactobacilli* starter cultures, under sterile conditions in 2-litre sterile distilled water in a 5-litre capacity fermenter for 72 hours.

Inoculum size determination

The starters were inoculated into sterile de Man Rogosa and Sharpe (MRS) broth and incubated at 30^{o}C for 48 hours. Aliquot (1 ml) of broth cultures were introduced into another batch of sterile broth and incubated at 30^{o}C for 24 hours. At the end of incubation period, the broth cultures were centrifuged (Himac CR21GII, Japan) at 5000×g for 10 minutes. The supernatant was decanted while the pellet was washed with sterile distilled water and re-centrifuged before being suspended in sterile normal saline.

The inoculum size was determined using McFarland standard. Dilutions were made with sterile normal saline to McFarland standard (No 4) using a spectrophotometer (Cecil CE 1011, Cambridge, England) to give 0.669 optical density at 600 nm, resulting in an approximate cell density of 1.2×10^{9} CFU/ml. Aliquot (5 ml) of resultant diluent was used as inoculum singly and in-combination to inoculate 100 g of the steeped cassava and fermentation was allowed for 72 hours at room temperature. Spontaneously-fermented cassava served as the control experiment. Samples for analysis were taken at the end of the fermentation period.

Nutritional analysis

Moisture content, crude protein, fat, fibre, ash and total carbohydrate were estimated according to Association of Official Analytical Chemists methods (AOAC, 2005).

Anti-nutritional factor analysis

Determination of cyanide content

The cyanide content of the fermented cassava mash was determined using the method of (Ojimelukwe, 1997). Twenty gram (20 g) of sample was homogenized in 200 ml distilled water for 10 minutes. The homogenate was incubated for 18 hours at room temperature after which 100 ml of 5% $NaHCO_3$ was added to it before distillation. After distillation, the filtrate was collected and titrated against 0.2% iodine solution using 1% starch as indicator. Percentage cyanide content was calculated using the titre value.

Estimation of phytic acid

The phytic acid was determined using the procedure described by (Markkar *et al.*, 1993). Two gram (2 g) of sample was weighed into 250 ml conical flask. A hundred millilitre concentrated HCl acid (2% v/v) was used to soak the sample in the conical flask for 3 hours and then filtered through a double layer of Whatman filter papers. The filtrate (50 ml) was placed in 250 ml beaker and 100 ml of distilled water was added. Ten millilitres (10 ml) of 0.3% (w/v) ammonium thiocyanate solution was added to the solution as indicator. The solution was titrated with standard iron chloride solution, which contained 1.95 mg

iron per millilitre. The end point colour was slightly brownish-yellow which persisted for 5 minutes. The percentage phytic acid was calculated using the titre value.

Estimation of tannin content

The method described by (Markkar *et al*., 1993) was adopted. Four hundred milligram (400 mg) of sample was placed in 500 ml conical flask and 40 ml diethyl ether containing 1% acetic acid (v/v) was added, this was properly mixed to remove the pigment materials. The supernatant was carefully discarded after 5 minutes and 20 ml of 70% aqueous acetone was added. The flask was sealed with cotton plug covered with aluminum foil, then kept in shaker for 2 hours for extraction. The content of the flask was filtered through Whatman filter paper (No. 1) and the filtrate was used for analysis. Aliquot (50 ml) of tannin extract from the sample was introduced into test tube and the volume was made up to 100 ml with distilled water. Folin-Ciocalteua reagent (0.5 ml) was added, mixed properly after which 2.5 ml of 20% (w/v) sodium carbonate solution was added and further mixed. The mixture was kept for 40 minutes at room temperature, and absorbance was read at 725 nm using spectrophotometer (Cecil CE 1011, Cambridge, England). Tannin concentration was estimated from tannic acid standard curve.

Statistical analysis

Data obtained were subjected to Analysis of variance (ANOVA) using SPSS software at 5% level of significance to determine differences and were presented as mean ± standard deviation of three replicates.

3. RESULTS AND DISCUSSION

The proximate composition and anti-nutritional factor of the fresh and spontaneously fermented cassava presented in **Table 1** showed that the fresh cassava variety used for this study had 87.90% total carbohydrate and protein content as low as 1.02% which makes it a predominantly starchy food. Moisture content of 7.28% observed was slightly lower than the range (7.31% - 8.40%) reported during the analysis of the nutritional quality of *fufu* from different cassava cultivars (Etudaiye *et al*., 2012). These authors also put the range of ash content and crude fibre to be between 0.15% – 1.5% and 0.12% – 0.65% respectively, among the different cultivars. These values were lower than what was observed in this study with 1.57% ash and 1.75% crude fibre. However, Manano *et al*. (2018) reported lower moisture content of 5.43%, ash content 1.05% and higher crude fibre 1.06% in fresh cassava while much higher moisture content (8.98%), 1.87% ash and 3.23% crude fibre was recorded in fresh cassava during the study on effect of duration of fermentation on *gari* quality by Irtwange and Achimba (2009). Phytate, tannin and cyanide content of the fresh cassava root were 0.3 mg/g, 35.4 mg/g and 0.1 mg/g respectively. All these findings were indications that different cassava cultivars have varied nutritional content.

After 72-hour fermentation with starter cultures, the moisture content of the cassava mash ranged between 6.79% and 7.35% (**Table 2**). This indicated that the fermentation process brought about both an increased and reduced moisture content in the samples. Decrease in moisture content after fermentation is an indicator for stable shelf life and this could be due to uptake of water by the fermentation substrate which resulted in their soft and porous texture after fermentation. However, increase in moisture after fermentation has been linked to hydrolytic activity of the fermenting organisms whereby moisture could be released as part of their metabolic products (Adegbehingbe, 2014). As shown in the results obtained, fermentation with starter cultures increased moisture content than the spontaneous fermentation (5.68%) and this could be as a result of faster utilization of the substrates by the starters which released moisture as one of their end products since spontaneous fermentation has been characterized with a longer lag phase.

Table 1: Proximate composition and anti-nutritional factor of fresh and spontaneously-fermented cassava

		Fresh cassava	Spontaneously-fermented cassava
Proximate composition (%)	Moisture content	7.28 ± 0.03	5.68 ± 0.42
	Crude protein	1.02 ± 0.12	0.95 ± 0.12
	Crude fat	0.48 ± 0.01	0.23 ± 0.02
	Crude fibre	1.75 ± 0.03	1.91 ± 0.03
	Ash	1.57 ± 0.04	0.93 ± 0.06
	Total carbohydrate	87.9 ± 0.11	90.3 ± 0.52
Anti-nutritional factor (mg/g)	Phytate	0.30 ± 0.004	0.29 ± 0.001
	Tannin	35.4 ± 0.03	33.4 ± 0.01
	Cyanide	0.10 ± 0.01	0.00

Values are means ±Standard Deviation of three replicates

Table 2: Proximate composition and anti-nutritional factor of starter-fermented cassava

		Lactobacillus pentosus F2A	*Lactobacillus plantarum subsp. argentolarensis F2B*	*Lactobacillus plantarum F2C*	*Combination of the three starters*
Proximate composition (%)	Moisture content	6.88 ± 2.25^{c}*	7.32 ± 0.06^{b}	7.35 ± 0.02^{a}	6.79 ± 0.03^{d}
	Crude protein	1.02 ± 0.12^{b}	1.02 ± 0.12^{b}	1.24 ± 0.13^{a}	1.24 ± 0.13^{a}
	Crude fat	0.31 ± 0.02^{b}	0.25 ± 0.01^{d}	0 29 ± 0.02^{c}	0.39 ± 0.02^{a}
	Crude fibre	1.93 ± 0.05^{c}	1.94 ± 0.05^{c}	1.96 ± 0.02^{b}	2.24 ± 0.02^{a}
	Ash	0.93 ± 0.02^{b}	0.89 ± 0.02^{c}	0.78 ± 0.03^{d}	1.03 ± 0.01^{a}
	Total carbohydrate	89.63 ± 0.18^{a}	88.63 ± 0.16^{b}	88.37 ± 0.13^{c}	88.31 ± 1.28^{d}
Antinutritional factor (mg/g)	Phytate	0.32 ± 0.003^{a}	0.32 ± 0.003^{a}	0.28 ± 0.001^{b}	0.27 ± 0.004^{b}
	Tannin	46.4 ± 0.4^{a}	35.5 ± 0.03^{c}	40.6 ± 0.02^{b}	34.6 ± 0.02^{d}
	Cyanide	0.20 ± 0.01^{b}	0.10 ± 0.01^{c}	0.80 ± 0.02^{a}	0.0^{d}

Values are means ±Standard deviation of three replicates
* Means reported with the same superscript in each row indicated no significant difference ($p \leq 0.05$).

Samples fermented with consortium of the starters had the most reduced (6.79%) moisture content. This was closely followed by those fermented with *L. pentosus* F2A starter, having 6.88% moisture content. Even though these values were lower than the moisture content (7.28%) of the fresh unfermented cassava, fermentation with starter cultures brought about overall increased moisture content when compared with the value obtained (5.68%) in the spontaneously fermented sample.

The spontaneously-fermented cassava mash had lower protein content (0.95%) when compared to the fresh cassava, while there was no significant difference (1.02%) in the protein content when starters *L. pentosus F2A* and *L. plantarum subsp. argentolarensis F2B* were utilized. However, *L. plantarum F2C* and combination of the three starters had the highest protein content (1.24%) after the 72-hour fermentation. Earlier studies on protein improvement in foods involved fortification and supplementing with protein-rich legume based substrates (Adetunde *et al*., 2011), but as observed in this study, where it was indicated that starter fermented samples had increased protein content than the spontaneously fermented samples, Tilahun *et al*. (2013) confirmed that fermentation with starter cultures have been proven to be an effective method of protein improvement especially in cassava fermentation. Structural proteins are

part of the microbial cell and this could have contributed to the protein content increase observed even though, such increase has been attributed to the ability of microbial biomass to secrete cellulolytic enzymes that break down non-starch polysaccharides to monomer sugars. These are easily metabolized to protein and further extensive hydrolysis to amino acid and other simple peptides (Ezekiel and Aworh, 2013).

Crude fat was generally reduced after both fermentations even though, starter fermented samples had higher fat content (between 0.25% and 0.39%) when compared with the spontaneously fermented samples (0.23%).

Ash content was lower in fermented samples (0.78% -1.03%) than the fresh cassava (1.57%) however, samples fermented with the combined starter had the highest ash content (1.03%) while those fermented with single starters ranged between 0.78% and 0.93%. Reduction in ash content after both fermentations from 1.57% in the fresh cassava to as low as 0.78% could be ascribed to leaching of soluble mineral elements into the fermenting medium or as a result of enzymatic hydrolysis of food components into their absorbable forms. Similar decrease after fermentation was also consistent with the report of Oduah *et al*. (2015) during the analysis of effect of fermentation on quality and composition of cassava mash as well as Atti (2000) when fermenting millet. However, observable increase in ash content was reported by Sanusi *et al.* (2013) and Adeyemi *et al*. (2012) during the fermentation of *Jatropha curcas* and *Candida albidum* seeds with fungi.

Crude fibre of the starter fermented samples ranged between 1.93% and 2.24%. This was higher than the fibre content (1.91%) of the spontaneously fermented sample. A general increase in crude fibre observed after fermentation was similar to the findings of Oyewole and Ogundele (2001) who also reported an increase in crude fibre of *fufu* as fermentation progressed even though, it was suggested that increasing crude fibre interferes with other nutrients thereby, making such nutrients unavailable for use (Adeyemi *et al.*, 2012). However, both fermentations showed increased fibre content when compared with the fresh cassava which had 1.75% and this finding indicated that the use of starter cultures and fermentation generally, could lead to increased crude fibre of cassava. On the contrary, Igbabul *et al.* (2014), during the fermentation of cocoyam, observed a decrease in crude fibre which was reported as an indication of softening of the fibrous tissues during fermentation and the decrease was attributed to microbial bio-conversion of carbohydrates and lignocelluloses into protein.

Total carbohydrate of all samples increased after the 72-hour fermentation in both starter- and spontaneously-fermented mashes with ranges from an initial 87.9% in the fresh cassava to 90.6%. The samples fermented with single starters had higher carbohydrate content (88.4% - 89.6%) than that of the combined starter (88.3%) However, the spontaneously-fermented sample had the highest total carbohydrate (90.3%) after fermentation. This general increase in total carbohydrate as fermentation time progressed could be a factor of decrease in moisture content as suggested by Igbabul *et al*. (2014) during the fermentation of cocoyam flour or the proportionate increase in protein content (Oboh *et al*., 2002).

Since fermentation has been known to denature anti-nutritional factors and increase nutritional values of food products (Nambisan,2011), decrease in anti-nutrients was expected and such could be attributed to leaching and microbial activities (Nwosu, 2010). The fresh cassava sample had 0.30% phytate. Samples fermented with starters *L. pentosus* F2A and *L. plantarum subsp. argentolarensis* F2B had increased phytate content (0.32%) while others showed significant reduction. Phytate reduction could be as a result of the effect of phytase activity, an endogenous enzyme from the substrate or inherent in the organisms. The enzyme hydrolyzes the phytic acid in the fermented food preparations into inositol and orthophosphate (Ragon *et al*., 2008). However, contrary to expectation, the increased phytate (0.32%) observed when starters *L. pentosus*

F2A and *L. plantarum subsp. argentolarensis* F2B were utilized could not be established, but might be as a result of some plant metabolites being converted to phytate-like products as suggested by Akindahunsi *et al*. (1999) who observed similar phytate increase during the fermentation of cassava flour and *gari.*

L. plantarum F2C had 0.28% phytate while the combined starter had the least phytate content (0.27%) and this could be due to the effect of combined microbial action.

The spontaneously-fermented sample had the most reduced tannin content (33.4%). Samples fermented with single starters exhibited increased tannin content (35.5% - 46.4%) while the combination of the three starters showed reduced tannin (34.6%) when compared to the initial 35.4% tannin content of the fresh cassava.

Cyanide content of samples fermented with *L. pentosus F2A*, *L. plantarum subsp. argentolarensis F2B* and *L. plantarum F2C* had 0.2%, 0.1% and 0.8% respectively whereas there was no detectable cyanide when the combined starter was utilized. Non detection of cyanide in mashes fermented with the mixed culture could be said to be due to combined effect of the mixed starter or from the effect of other cyanide-degrading organisms in the spontaneous fermentation as no cyanide was also detected in the spontaneously-fermented cassava. This reduction in cyanide had mostly been linked to the fermentation involving water since it has been established to be the simplest method to reduce cyanide content as the water will swell the cells and allow linamarase to come into contact with linamarin, thus, initiating cyanide hydrolysis (Flibert *et al*., 2016). Samples fermented with the single starters did not satisfy the Standards Organization of Nigeria regulation which puts the minimum tolerant cyanide level at 0.05 mg/g (SON, 1985) even though, the deleterious level was put at 0.03 mg/g (Tweyongyere and Katongole, 2002). In spite of both, observed values deviated from the findings of (Oboh and Akindahunsi, 2003) who put the usual cyanide content of major cassava products in Nigeria as 0.019 mg/g.

Conclusively, despite the fact that fermentation process had significantly improved effect on the nutritional content of the cassava as observed in the spontaneous fermentation, utilization of starter cultures, most especially the combined starter elicited a more desired effect. Furthermore, the starters, even though belonged to the same genus, exhibited significantly different nutritional qualities.

5. REFERENCES

[1]. FAO 2014. FAO (Food and Agriculture Organization of the United Nations). Food Outlook Biannual report on global food market.

[2]. Morgan, N. K. and Choct M. Cassava: Nutrient composition and nutritive value in poultry feed. *Animal Nutrition.* **2(4)** 2016:253-261

[3]. Ngiki, Y. U., Igwebuike, J.U. and Moruppa, S.M. Utilization of cassava products for poultry feeding: a review. *International Journal Science Technology*. **2(6)** 2014:48-59

[4]. Jisha, S. Padmaja G. and Sajeev, M. S. Nutritional and textural studies on dietary fiber-enriched muffins and biscuits from cassava-based composite flours. *Journal of Food Quality*. **33** 2010:79–99.

[5]. Zvinavashe, E., Elbersen, H. W., Slingerland, M., Kolijn, S. and Sanders, J. P. M. Cassava for food and energy: exploring potential benefits of processing of cassava into cassava flour and bioenergy at farmstead and community levels in rural Mozambique. *Biofuels, Bioproducts and Biorefining* **5(2)** 2011:151–164.

[6]. Oyinlola, K.A., Onilude, A.A. and Garuba, O. E. Towards the development of a common starter culture for *fufu* and *usi* (edible startch): Screening for potential starters. *International Journal of Food Studies.* **5** 2016: 61-72

[7]. Adetunde, L. A. and Onilude, A. A. Comparison of the effect of particulate materials and some osmoregulators on lactic fermentation of new local white cassava variety "Bianbasse" using both spontaneous and starter cultures. *African Journal of Microbiology Research*. **4(14)** 2010:1480 – 1485.

[8]. AOAC. Official Method of Analysis of Chemists. AOAC International, 18th ed.Maryland USA. (2005).

[9]. Ojimelukwe, P. C. Effects of different processing treatments on soy/cassava weaning diets. In Proceedings of the 10th Annual Conference of the Biotechnology Society of Nigeria, eds. Garba S.A., Ali U.D., Lamai S.L., Sadiku S.O.E. & Chukwedo A.A.pp. 81–91, Minna, Nigeria: Federal University of Technology. 1997.

[10]. Markkar, H. P. S., Blummer, M., Browy, N. K. and Becker, K. Gravimetric of Tannins and their correlation with chemical and protein precipitation

methods. *Journal of Science Food and Agriculture*. **61** 1993:161-165.
[11]. Etudaiye, A., Nwabueze, T. U. and Sanni L.O. Nutritional Quality and Preference of *Fufu* processed from selected Cassava Mosaic Disease (CMD) Resistant Cultivars. *Advances in Applied Science Research*. **3(5)** 2012: 2687-2692.
[12]. Manano, J., Ogwok, P. and Byarugaba-Bazirake, G. W. Chemical Composition of Major Cassava Varieties in Uganda, Targeted for Industrialization. *Journal of Food Research*. **7(1)** 2018:1-9
[13]. Irtwange, S. V. and Achimba, O. Effect of the Duration of Fermentation on the Quality of *Gari*. *Current Research Journal of Biological Sciences*. **1(3)** 2009: 150-154.
[14]. Adegbehingbe, K. T. Effect of fermentation on nutrient composition and anti-nutrient contents of ground Lima bean seeds fermented with *Aspergillus fumigatus, Rhizopus stolonifer* and *Saccharomyces cerevisiae. International Journal of Advanced Research.* **2(7)** 2014: 1208-1215.
[15]. Adetunde, L. A., Onilude, A. A. and Adetunde, I. A. Effect Of particulate materials on lactic fermentation of new local white variety cassava ("Bianbasse") using both spontaneous and starter culture. *Nature and Science*. **9(1)** 2011: 50-56.
[16]. Tilahun, A. T., Shimelis, A. E., Gulelat, D. H. and Tilahun, B. G. Effect of processing on physicochemical composition and anti -nutritional factors of cassava (Manihot esculenta crantz) grown in Ethiopia. *International Journal of Science Innovations and Discoveries*. **3(2)** 2013: 212-222
[17]. Ezekiel, O. O. and Aworh, O. C. Solid State Fermentation of Cassava Peel with *Trichoderma viride* (ATCC 36316) for Protein Enrichment. *International Journal of Biology, Food Veterinary Agriculture and Engineering.* **7** 2013:667–674.
[18]. Oduah, N.O., Adepoju, P.A., Longe, O., Elemo, G.N. and Oke, O.V. Effects of Fermentation On The Quality And Composition Of Cassava Mash (Gari). *International Journal of Food Nutrition and Safety.* **6(1)** 2015: 30-41
[19]. Atti, J. V. Development, nutritional evaluation and acceptability of processed millet (*Eleusine coracana*), soybean (*Glycine max*) and sesame (*Sesanum indicum*) flours and blends. Ph.D. Dissertation. Department of Home Science and Nutrition, University of Nigeria Nsukka. pp. 23-26. 2000.
[20]. Sanusi, G. O., Belewu, M. A. and Oduguwa, B. O. Changes in chemical composition of *Jatropha curcas* kernel cake after solid-state fermentation using some selected fungi. *Global Journal of Biological, Agriculture and Health Science.* **2(2)** 2013: 62-66.
[21]. Adeyemi, O. T., Muhammad, N. O. and Oladiji, A. T. Biochemical assessment of the mineral and some antinutritional constituents of *Aspergillus niger* fermented *Chrysophyllum albidum* seed meal. *African Journal of Food Science.* **6(1)** 2012: 20-28.
[22]. Oyewole, O. B. and Ogundele, S.L. Effect of length of fermentation on the functional characteristics of fermented cassava *fufu*. *Journal of Food Technology.* **6(2)** 2001: 38-40.
[23]. Igbabul, B. D., Amove, J. and Twadue, I. Effect of fermentation on the proximate composition, antinutritional factors and functional properties of cocoyam (*Colocasia esculenta*) flour. *African Journal of Food Science and Technology*. **5(3)** 2014: 67-74.
[24]. Oboh, G., Akindahunsi, A. A. and Oshodi, A. A. Nutrient and anti nutrient content of Aspergillus niger fermented cassava products (flour and gari). *Journal of Food Composition and Analysis.* **15(5)** 2002: 617-622
[25]. Nambisan, B. Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. *Food Chemistry and Toxicology*. **49** 2011:690-693.
[26]. Nwosu, J. N. Effect of soaking, blanching and cooking on the anti-nutritional properties of asparagus bean (*Vigna sesquipedis*) flour. *Science Nature*. **8(8)** 2010:163-167.
[27]. Ragon, M., Aumelas, A., Chemardin, P., Santiago, S., Moulin, G. and Boze, H. Complete hydrolysis of myo-inositol hexakisphosphate by a novel phytase from Debaryomyces castellii CBS 2923. *Applied Microbiology and Biotechnology*. **78** 2008:47–53
[28]. Akindahunsi, A. A., Oboh, G. and Oshodi, A. A. Effect of fermenting cassava with *Rhizopus oryzea* on the chemical composition of its flour and gari. *La Rivista Italiana Delle Sostanze Grasse.* **76** 1999: 437-440.
[29]. Flibert, G., Tankoano, A and Savadogo, A. African cassava Traditional Fermented Food: The Microorganism's Contribution to their Nutritional and Safety Values-A Review. *International Journal of Current Microbiology and Applied Sciences*. **5(10)** 2016: 664-687
[30]. Standards Organization of Nigeria (SON). 1985. Nigerian Food Standards.
[31]. Tweyongyere, R. and Katongole, I. Cyanogenic potential of cassava peels and their detoxification for utilization as livestock feed. *Veterinary and Human Toxicology*. **44** 2002: 366-369.
[32]. Oboh, G. and Akindahunsi, A. A. Biochemical changes in Cassava products (flour & gari) subjected *to Saccharomyces cerevisae* solid media fermentation. *Food Chemistry*. **82(4)** 2003: 599-602.