International Journal of Food Nutrition and Safety, 2021, 12(1): 10-27

Modern Scientific Press

*International Journal of Food Nutrition and Safety*

Journal homepage:www.ModernScientificPress.com/Journals/IJFNS.aspx

**ISSN: 2165-896X**
*Florida, USA*

*Article*

# Effect of *in- situ* Storage and Fermentation on the Microbial Population, Mineral Composition, and Anti-nutritional Properties of American Yam Bean (*Pachyrizus erosus*) Tubers and Their Flours

**Josephine Ohuche [a], Chidi Ezeama[b] and Victor Ntukidem[c, *]**

[a]*Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University,Awka, Anambra State. Nigeria*

[b]*Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria*

[c]*Department of Food Science and Technology, University of Uyo, Akwa Ibom State, Nigeria*

*Author to whom correspondence should be addressed: vicksonybless@gmail.com

*Article history*: Received 14 May 2021, Revised 1 July 2021, Accepted 1 July 2021, Published 12 July 2021.

**Abstract:** This study elucidated the efficacy of *in-situ* (underground) storage and fermentation of American yam bean (*Pachyrhizus erosus*) tubers on the microbial population, mineral composition and anti-nutritional properties of the flour. Seeds of American yam bean were planted in a farm at Effium, Ebonyi state, Nigeria for seven months. The fresh tubers after harvest were stored underground for the period of 3 months. Fresh and stored tubers were collected every month to produce flour for analyses. Both freshly harvested and stored tubers were washed, hand peeled, rewashed, sliced and fermented for 72 h, dried in a hot air oven (55 $^{o}$C, 6 hr), milled and sieved to obtain fermented flour. A similar procedure was repeated on the tubers, but not fermented to obtain unfermented flour. The flour samples were analyzed for microbial population, mineral composition, and anti-nutritional properties using standard methods. Fermentation of the tuber was appropriate as the anti-nutrient (HCN, Tannin, Oxalate and saponin) content of the flour samples significantly ($P<0.05$) decreased except for alkaloids. Fermentation also significantly ($P<0.05$) decreased calcium, sodium and phosphorus contents (mg/100g) of the flour from 9.52 to 8.43, 7.34 to 6.87 and 12.44 to 11.72 respectively for the tuber stored for 3 months. The microbial load of all the flour samples was significantly ($P<0.05$) low as

shown in the total viable count (<$10^1$ CFU/g to 5.40x$10^1$ CFU/g, <$10^1$ CFU/g to 9.60x$10^1$ CFU/g) and total fungal counts (<$10^1$ CFU/g to 1.80x$10^1$ CFU/g, <$10^1$ CFU/g to 3.40x$10^1$ CFU/g) of the unfermented and fermented flours respectively. The total coliform count for all the tubers during fermentation were <$10^1$ CFU/g. It is evident that the American yam bean (*Pachyrhizus erosus*) tuber could be stored underground for at least 3 months to avoid post-harvest losses. The tubers when fermented or not fermented, could produce flours of low anti-nutrient contents and of acceptable mineral and microbial quality.

**Keywords**: *Pachyrhizus erosus,* fermentation, anti-nutrient, mineral composition, microbial load, *in-situ* storage

## 1. Introduction

*Pachyrhizus erosus* otherwise known as American yam bean or Mexican yam bean is one of the legumes that have been underutilized especially in the tropics and subtropics (Jacobsen *et al*., 2015). American yam bean taxonomically contains about three species that are closely related with each other. They include Amazonian yam (*Pachyrhizus tuberosus*), Mexican yam bean (*Pachyrhizus erosus)* and the Andean yam bean (*Pachyrhizus ahipa*). American Yam beans are closely related to soybeans (Ndirigwe *et al*., 2017) and produce huge quantities of seed that are rich in protein and good fatty acids (Gruneberg *et al*., 1999). Despite its wide utilization, they are not edible by humans as it contains a toxic iso-flavonoid called rotenone in toxic quantity (Ndirigwe *et al*., 2017). American yam beans despite its various usage and utilization are not regarded as African crop.

In Nigeria, American yam bean crop is non-indigenous, but has high food value and high yield in areas of similar climatic conditions. Unlike other leguminous crops where the seeds are the edible part, the tuber of American yam bean is the edible part. However, this edible part (the tuber) of American yam bean is underutilized globally due to its poor post-harvest handling which in turn affect the shelf stability. American yam bean tubers deteriorate shortly after harvest under ambient storage. This drastically impedes the full utilization of the tuber. There is scarcity of report regarding the effect of storage and fermentation on the anti-nutritional composition, microbial safety and mineral composition of the tuber and its flour.

The present level of malnutrition and food insecurity in most African Countries including Nigeria calls for effort on research and development of readily available food crops that are underutilized. Appropriate storage conditions of these root crops will reduce scarcity thereby improving food security in Nigeria. Some fermented foods on the other hand are carriers of probiotics (health-promoting bacteria), which are useful to the body. So, this study will reveal how storage and fermentation affect

the food values and microbial load of the flour. Proper handling and processing methods for the tuber after harvest could be established from the study. The present study aimed to investigate the effects of *in-situ* storage and fermentation on the microbial population, mineral composition and anti-nutritional properties of the tuber and its flour. It is hoped that the information from this study will give an insight on storage, fermentation, processing, and preservation of the tubers, to enhance food security in Nigerians also for farmers and researcher on the utilization of this root crop for new food product development purposes.

## 2. Material and Methods

### *2.1. Collections of Seeds*

American yam bean (*Pachyrhizus erosus*) seeds (Fig. 1a) were collected, identified and authenticated by Dr. Amadi of National Root Crop Research Institute, Umudike, Abia State, Nigeria, and planted in an experimental farm at Effium, Ebonyi State, Nigeria according to the method described by Doporto *et al*., (2011) and as guided by Dr. Amadi of the same Institute.

### *2.2. Planting Site Description*

Effium is a larger city found in Ebonyi State, Nigeria. It is located in Ohaukwu Local Government Area, which made up of Uffium, Ezza and Amuda. Its geographical coordinates are 6º 38’ 0” North, 8º 4’ 0” East. Effium has 6.63 latitude and 8.06 longitude, and it is positioned at altitude of 115 meters above sea level. Effium has an estimated population of 86,945. The popular markets in Effium include Nwafia Effium, Inikiri Benard, Nweke Ndegu and Eke Amuda It operates on the WAT time zone as Abakaliki City.

### *2.3. Seed Planting Procedure and Cultivation*

The seeds (Fig. 1a) were soaked overnight in lukewarm water before planting. About 2 to 4 seeds were planted in each hole of the ridges. The holes were 20 to 25 cm apart and the soil kept wet. The tubers were harvested at the end of the seventh months (1st February, 2019 to 31st September, 2019) of planting after maturity.

### *2.4. In-Situ Storage of the Harvested Tubers*

After harvesting of the crop, part of it was stored under the ground (*in-situ* storage) for a period three months (See Fig. 1 c-d). A ground of about 0.5 meters deep and 0.6 meters wide was dug under a shade, the tubers void of bruises were buried and covered with excavated earth to prevent direct sun-

light on them. Each month, samples were taken from the stored tubers for subsequent analyses for a period of 3 months, starting from zero (0) month.

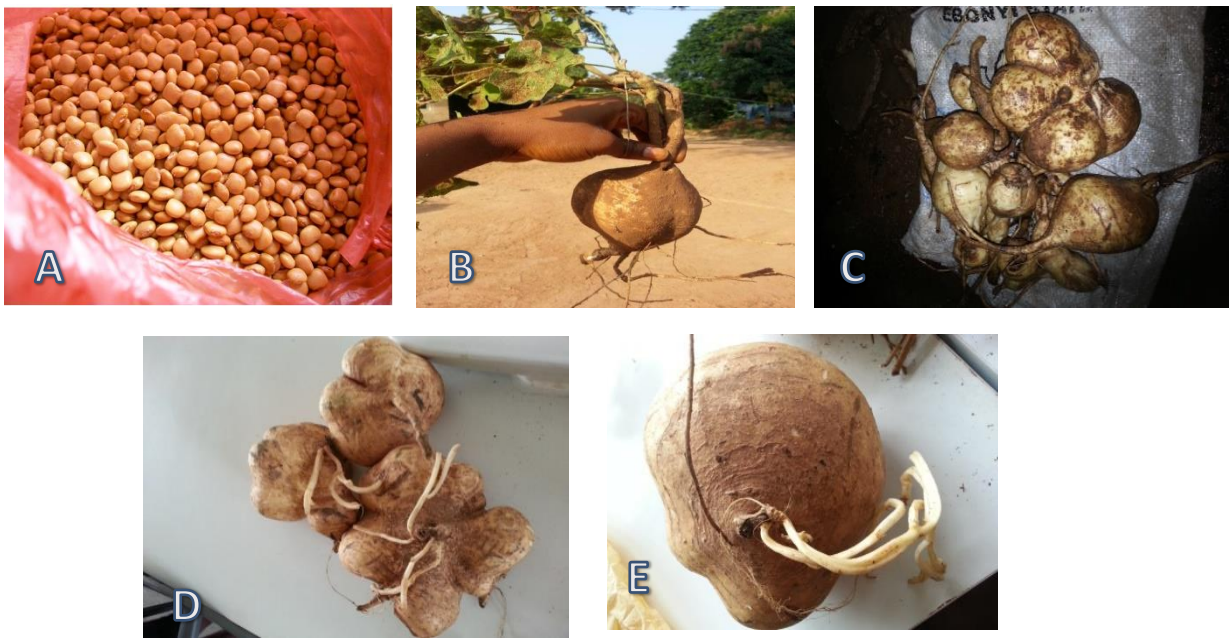


**Figure 1**. Plate A= American Yam Beans (*Pachyrhizus erosus*) seed, Plate B = Picture of freshly harvested (0 month) American Yam Beans, Plate C =Picture of American Yam Beans stored for 1 month, Plate D= Picture of American Yam Bean stored for 2 months, Plate E = Picture of American Yam Beans stored for 3 months.

*2.5. Processing of Pachyrhizus erosus Flour*

This was performed following the method described by Doporto *et al*., (2011) with some modifications. Freshly harvested tubers (Fig. 1b) were sorted to remove bruised tubers and divided into four portions, which were then assigned to various treatments of 0- month storage (freshly harvested), 1-month storage, 2 months storage, and 3 months storage. The storage system used was *in-situ* (underground). The fresh tubers at the point of harvest (0 month) were washed in water, hand-peeled with knife, and re-washed. Peeled tubers were then sliced to about 3.0mm thickness with the use of a vegetable slicer and shared into two halves of 1 kilogram each. One half (1 kg) of the sliced American yam bean tubers were then oven-dried (model: OVH-102, Nig.) at 55°C for 6 hr. The sample was then milled and the flour obtained and stored in an airtight container for analyses.

The other portion (1 kg) was fermented for 72 hr (3 days). The fermentation type used was solid-substrate-submerged whereby the peeled and sliced tubers were soaked in water as in the case of fermentation of cassava for fufu production. At the end of the fermentation, the fermented tubers was dried at 55ºC in the oven (model: OVH-102, Nig.) for 6 hr and milled. The flour obtained was sieved to fine particle and stored in a ziplock container for analyses. These processes and analysis were repeated

every month for the other two treatments (unfermented and fermented portions) for a period of three (3) months.

### *2.6. Analysis Conducted*

#### *2.6.1. Microbial load determination*

*Culture media preparation*

Preparation and sterilization of all microbial culture media used were carried out according to their manufacturer's instruction in conical flasks. The total viable count, total fungal, Lactic acid bacteria (LAB) and coliform bacteria of the samples were determined using Tryptone Soy Agar, Potato Dextrose Agar (Lab. Tech. India), DeMan Rogosa Sharp Agar (Oxiod, Uk) and Eiosine Methylene Blue Agar respectively.

*Enumeration of microbial loads*

The pour plate technique as described by Ezeama (2007) was used. Sterile distilled water (9 mL) was dispensed into four labeled sterile test tubes. One milliliter (1 mL) of the desired sample was added to 9 mL of distilled water (sterile) in a test-tube to get $10^{-1}$ dilution. The sequence was continued in similar way until $10^{-4}$ dilution strength was obtained. An aliquots of 1.0 mL from test-tube $10^{-3}$, was introduced into each sterile Petri dish and 10 mL of sterile molten medium allowed to cool to about 44ºC to 46ºC was poured into each of the plates and swirled gently. Then the agar was allowed to solidify on a level surface. On setting, the culture plate were incubated at room temperature (28 ± 2ºC) for 120 h for 3-5 days for fungi and at 35-37 ºC for 48 h for bacteria. Plates with 25 colonies and above were selected and the count was recorded as colony forming unit per grams (CFU/g).

#### *2.6.2. Anti-nutritional composition determination*

Hydrogen cyanide and Oxalate were determined following the methods described by Onwuka (2005). Tannin, Alkaloid and Saponin were conducted using the procedure of AOAC (2012).

#### *2.6.3. Determination of mineral content*

Elemental (Sodium, Potassium, Calcium, Magnesium Iron, and Zinc) analysis was determined using standard methods of AOAC (2012).

### *2.7. Statistical Analysis*

Completely randomized Experimental design was adopted. All determinations were conducted in triplicates. Data generated from laboratory analysis were exposed to two-way analysis of variance (ANOVA), using the Statistical Package for the Social Science (SPSS) (version 20). The mean scores

were separated using New Duncan's Multiple Range Test. Significant difference was determined at $P<0.05$ level of probability

## 3. Results and Discussion

### *3.1. Effects of Storage and Fermentation on the Microbial Load of Pachyrhizus erosus Tuber*

The microbial load of fermented American yam bean tuber during storage and fermentation is as shown in Table 1. The results showed that unfermented freshly harvested American yam bean tuber (0 hr) had a total viable count of $<10^1$ CFU/g while at 72 hr had the highest viable counts of $1.08 \times 10^2$ CFU/g. The rate of bacterial proliferation also increased with an increase in the period of fermentation. A similar trend was recorded for American yam bean tubers stored for periods of one, two and three months. These unfermented samples (0 hr) recorded total viable counts of $<10^1$ CFU/g but after 72 hr of fermentation, had the viable counts of $1.24 \times 10^2$, $1.34 \times 10^2$ and $1.40 \times 10^2$ CFU/g respectively. The rate of bacterial proliferation in the samples increased with increased fermentation time as well as storage time of the tubers. This increase could be due to prolonged fermentation, which resulted to increased concentration of lactic acid bacteria (LAB) as similarly reported by Sunny-Roberts *et al*., (2012) during fermentation of cassava-tempeh flour mixes.

The population of fungi increased from $<10^1$ to $4.40 \times 10^1$ CFU/g (Table 1) and this was quite lower than the total viable counts. The fresh unfermented American yam bean tuber (0 hr) had the least viable count ($<10^1$ CFU/g) while tubers fermented for 72 hr had the highest count ($4.40 \times 10^1$ CFU/g). The same trend was observed for American yam bean tubers stored for a period of one to three months while the unfermented samples (0 hr) had the least viable count of $<10^1$CFU/g. Tubers stored for 1, 2 and 3 months had the highest counts of $4.60 \times 10^1$, $5.00 \times 10^1$ and $5.40 \times 10^1$ CFU/g respectively after fermentation for 72 hr. It was also observed that increased fermentation period and storage periods resulted to increased total fungal counts. This increase could be attributed to the low pH of the fermenting medium, which resulted from increased production of organic acid by fermenting organisms, which favoured the growth of yeasts (Sunny Robert *et al*., 2012).

The population of lactic acid bacteria in the samples also ranged from $<10^1$ to $6.40 \times 10^1$ CFU/g for the control (unfermented freshly harvested American yam bean tuber (0 hr)). The lowest viable count ($<10^1$ CFU/g) was recorded for unfermented freshly harvested (0 hr) while when fermented for 72 hr recorded the highest count of $6.40 \times 10^1$ CFU/g. The same trend was observed for tubers stored for 1 month. The unfermented (0 hr) recorded the least count ($<10^1$CFU/g) while when fermented for 72 hr had the highest count ($7.80 \times 10^1$CFU/g). Similarly, samples stored for 2 and 3 months recorded counts $<10^1$ CFU/g while after fermentation for 72 hr had the counts of $8.4 \times 10^1$ and $8.60 \times 10^1$ CFU/g

respectively (Table 1). The results also indicated that the population of lactic acid bacteria in the samples increased as fermentation and storage progresses. Olanipekun and Adelakun (2015) reported similar findings of increased LAB load during fermentation of soybean. Lactic acid acts as a flavouring agent and also as antimicrobial substances, which inhibits the growth of pathogenic organisms (Olanipekun and Adelakun, 2015).

The population of coliform detected in all the samples fermented within a period of 0 to 72 hr was $<10^1$ CFU/g. This insignificant count is probably due to the low pH caused by increased population of lactic acid bacteria, which did not support the survival, and growth of coliform within the period of fermentation. This supports the results of Abdjo *et al*., (2010) who reported that antimicrobial property of lactic acid bacteria leads to production of bacteriocins while some other antimicrobial agents have been shown to have inhibitory effect on coliforms.

**Table 1:** Effect of storage (0-3 months) and fermentation (0-72 hr) on the Microbial load (CFU/g) of American yam bean tubers

| **POF (Hours)** | **0 hr** | | **12 hr** | | **24 hr** | | **36 hr** | | **48 hr** | | **60 hr** | | **72 hr** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **POS (Months)** | **TV (x10$^1$)** | **TFC (x10$^1$)** | **TVC (x10$^1$)** | **TFC (x10$^1$)** | **TVC (x10$^1$)** | **TFC (x10$^1$)** | **TVC (x10$^1$)** | **TFC (x10$^1$)** | **TVC (x10$^2$)** | **TFC (x10$^1$)** | **TVC (x10$^2$)** | **TFC (x10$^1$)** | **TVC (x10$^2$)** | **TFC (x10$^1$)** |
| 0 | $<10^1$ | $<10^1$ | $2.60^d$ | $1.00^d$ | $5.00^d$ | $2.00^d$ | $7.40^d$ | $2.80^c$ | $0.98^d$ | $3.60^c$ | $0.87^c$ | $4.00^c$ | $1.08^d$ | $4.40^d$ |
| 1 | $<10^1$ | $<10^1$ | $5.80^c$ | $1.80^c$ | $6.90^c$ | $2.10^c$ | $8.80^c$ | $3.00^b$ | $1.00^c$ | $3.60^c$ | $1.16^b$ | $4.40^c$ | $1.24^c$ | $4.60^c$ |
| 2 | $<10^1$ | $<10^1$ | $6.80^b$ | $2.00^b$ | $7.40^b$ | $2.40^b$ | $9.00^b$ | $3.00^b$ | $1.02^b$ | $3.80^b$ | $1.18^b$ | $4.60^b$ | $1.34^b$ | $5.00^b$ |
| 3 | $<10^1$ | $<10^1$ | $7.00^a$ | $2.20^a$ | $7.80^a$ | $2.60^a$ | $9.40^a$ | $3.20^a$ | $1.16^a$ | $4.40^a$ | $1.28^a$ | $5.00^a$ | $1.40^a$ | $5.40^a$ |
| **POS (Months)** | **TLC (x10$^1$)** | **TCC (x10$^1$)** | **TLC (x10$^1$)** | **TCC (x10$^1$)** | **TLC (x10$^1$)** | **TCC (x10$^1$)** | **TLC (x10$^1$)** | **TCC (x10$^1$)** | **TLC (x10$^1$)** | **TCC (x10$^1$)** | **TLC (x10$^1$)** | **TCC (x10$^1$)** | **TLC (x10$^1$)** | **TCC (x10$^1$)** |
| 0 | $<10^1$ | $<10^1$ | $1.60^c$ | $<10^1$ | $3.00^d$ | $<10^1$ | $4.60^d$ | $<10^1$ | $5.00^d$ | $<10^1$ | $5.80^c$ | $<10^1$ | $6.40^d$ | $<10^1$ |
| 1 | $<10^1$ | $<10^1$ | $4.00^b$ | $<10^1$ | $4.80^c$ | $<10^1$ | $5.80^c$ | $<10^1$ | $6.20^c$ | $<10^1$ | $7.20^b$ | $<10^1$ | $7.80^c$ | $<10^1$ |
| 2 | $<10^1$ | $<10^1$ | $4.80^a$ | $<10^1$ | $5.00^b$ | $<10^1$ | $6.00^b$ | $<10^1$ | $6.70^b$ | $<10^1$ | $7.20^b$ | $<10^1$ | $8.40^b$ | $<10^1$ |

Means ± standard deviations of triplicate determinations expressed as CFU/g. Two means along the same column with different superscripts are significantly ($P<0.05$) different.

TVC = Total viable count, TFC = Total fungal count, TLC = Total lactic acid bacteria count, TCC = Total coliform count, POS= Period of storage in months, POF = Period of fermentation in hours

*3.2. Mineral Content of Raw and Fermented American Yam Bean (Pachyrhizus erosus) Tuber*

Table 2 shows the mineral content of raw and fermented (72 hr) American yam bean tubers. Calcium content of raw American yam bean tuber was 8.77 mg/100g and it was significantly ($P<0.05$) higher than that of the fermented tuber which was 7.93 mg/100g.These values were much lower than the average value of calcium content (50 mg/100g) reported by Laurie *et al*., (2012) for fresh tubers of 12 sweet potato cultivars suggesting that the American yam bean tubers would not meet the body's daily need for calcium. Calcium element is of utmost significance especially in blood clotting, contraction of muscle, and neurological role, repairs of bone and teeth and function in the regulation of metabolic processes (Senga *et al*., 2013) and in the safeguarding of the veracity of the intracellular strengthen materials (Karau *et al*., 2012; Adjatin *et al*., 2013).

Magnesium was present in little quantity though the raw tuber (5.95 mg/100g) was significantly ($P<0.05$) higher than the fermented tuber (5.45 mg/100g). These values were much lower than 32 mg/100g reported for yam by Adepoju (2012) suggesting that the American yam bean tubers would not meet the body's daily need for magnesium which is 40 mg/100g a day. According to Alinnor and Oze (2011), magnesium is also essential as calcium. In that, it helps in the metabolism in bones as well as prevention of cardiovascular diseases. Magnesium also is significant in regulation of blood pressure and in the release of insulin. Research has it that, magnesium mediates in avoidance of cardiomyopathy, prevention of growth delay, muscle degeneration etc. (Andzouana and Mombouli, 2012; Adjantin *et al*., 2013).

Sodium content of the raw tuber was recorded as 6.57 mg/100g while that of the fermented tuber was found to be 6.34 mg/100g. These values were lower than the range (29.00 to 34.00 mg/100g) reported by Sanoussi *et al*., (2016) for sweet potato. According to Gropper *et al*., (2005); and Ojimelukwe *et al*., (2005) sodium is very necessary for body fluid and electrolyte balance, glucose absorption and osmosis.

Potassium was among the least abundant macro-mineral and it was found to be significantly ($P<0.05$) higher in the raw tuber (4.91 mg/100g) than in the fermented tuber (4.72 mg/100g). Also, the potassium content of the raw and fermented samples were much lower than the range (338.00 to 407.04 mg/100g) reported by Ellong *et al*., (2014) for sweet potato. Potassium is essential for enzyme, protein, and carbohydrate metabolism (Umesh, 2009). It is also vital for maintenance of body fluid, water, and electrolyte balance as well as regulation of heart rhythm and nerve action (Gropper *et al*., (2005); and Ojimelukwe *et al*., (2005).

Phosphorous was one of the most abundant minerals in the tubers. The phosphorous content of the raw tuber was 12.02 mg/100g while that of the fermented tuber was 11.45 mg/100g.These values were much below 163 mg/100g reported for raw yam (Adepoju, 2012). Phosphorous is necessary for

development of bones and teeth, cell activity and maintenance of body. It is also a component of sugar phosphate involved in carbohydrate metabolism (Gropper *et al*., (2005); and Ojimelukwe *et al*., (2005).

Iron content of the raw tuber was 0.81 mg/100g while the fermented tuber had 0.74 mg/100g of iron. The values obtained in study were in agreement with the range of (0.73 to 1.26 mg/100g) reported on a fresh weight basis by Laurie *et al*., (2012) for sweet potato. Deficiency of iron causes illness like anemia, which is common among children, just like vitamin A deficiency (Sanoussi *et al*., 2016).

Zinc element happens to be one of the most targeted micronutrient in food fortification (La Frano *et al*., 2014). The results showed that the raw tuber contained significantly ($P<0.05$) higher amounts of zinc (0.60 mg/100g) than the fermented tuber (0.48 mg/100g). However, the values obtained here is far lower than the 1.70 % reported as RDA per day for zinc for both children and adults (Sanoussi *et al*., 2016) hence indicating that American yam bean is not a good source of zinc.

**Table 2:** Mineral content (mg/100g) of raw and fermented (72 hr) American yam bean American yam bean tubers

| Samples | Ca | Mg | Na | K | P | Fe | Zn |
|---|---|---|---|---|---|---|---|
| Raw | $8.77^{a}$±0.03 | $5.95^{a}$±0.01 | $6.57^{a}$±0.04 | $4.91^{a}$±0.01 | $12.02^{a}$±0.01 | $0.81^{a}$±0.01 | $0.60^{a}$±0.00 |
| Fermented | $7.93^{b}$±0.04 | $5.45^{b}$±0.00 | $6.34^{b}$±0.00 | $4.72^{b}$±0.01 | $11.45^{b}$±0.00 | $0.74^{b}$±0.00 | $0.48^{b}$±0.01 |

Means ± standard deviations of triplicate determinations. Two means along the same column with different superscripts are significantly different ($P<0.05$).

*3.3. Anti-nutrient Properties of Raw and Fermented American Yam Bean Tubers*

Table 3 shows the anti-nutrient compositions of raw and fermented American yam bean tubers. Saponin was found to be higher in the raw tuber (0.65 mg/100g) than in the fermented tuber (0.49 mg/100g). Hydrogen cyanide (HCN) content of the raw tuber (1.29 mg/100g) was higher than that of the fermented tuber (0.83 mg/100g). However, these values were within the permissible level (10 mg) for humans set by regulatory bodies (Igbabul *et al*., 2014). The tannin content of the raw tuber (0.48 mg/100g) was not significantly different from that of the fermented tuber (0.46 mg/100g) suggesting that fermentation had no effect on tannin content of American yam bean tuber. The oxalate content followed a similar trend as tannin and the raw tuber (0.33 mg/100g) had similar concentration of oxalate as the fermented tuber (0.46 mg/100g) suggesting that fermentation did not reduce the level of oxalate in American yam bean tuber. The alkaloid content of the raw tuber was naturally low (0.24) and decreased significantly ($P<0.05$) after 72 hr fermentation. Generally, the decrease in anti-nutrients of the fermented tubers was in line with the findings of other researchers (Igbabul *et al*., 2014) because

fermentation is known to reduce concentration of anti-nutrients in food by action of the fermenting organisms.

**Table 3:** Anti-nutrient composition (mg/100g) of raw and fermented (72 hr) American yam bean (American yam bean) tuber

| Samples | Saponin | HCN | Tannin | Oxalate | Alkaloid |
|---|---|---|---|---|---|
| Raw | $0.65^{a}$±0.00 | $1.29^{a}$±0.01 | $0.48^{a}$±0.02 | $0.55^{a}$±0.04 | $0.24^{a}$±0.00 |
| Fermented | $0.49^{b}$±0.02 | $0.83^{b}$±0.00 | $0.46^{a}$±0.00 | $0.46^{a}$±0.00 | $0.19^{a}$±0.02 |

Means ± standard deviations of triplicate determinations. Two means along the same column with different superscripts are significantly different (P<0.05).

*3.4. Effect of Storage and Fermentation on the Microbial Load of the Flour Samples from Fresh and Stored Unfermented and Fermented American Yam Bean Tubers*

The microbial load of flour samples produced from American yam bean tubers after *in-situ* storage and fermentation are shown in Table 4. The total viable counts of the unfermented ($UT_0$, $UT_1$, $UT_2$ and $UT_3$) and fermented American yam bean flours ($FT_0$, $FT_1$, $FT_2$ and $FT_3$) produced ranged from 1.20 to 3.60 x $10^1$ CFU/g and 3.20 to 6.20 x $10^1$ CFU/g respectively. The TVCs were low but increased gradually with increased period of *in-situ* storage of the tubers for the unfermented and fermented flours. This could be attributed to proliferation of microorganisms during *in-situ* storage and packaging of the products. It has also been reported that the number of organisms will increase if materials is not adequately cleansed and sanitized (Ezeama and Amajor, 2015). It was also evident that significant (P<0.05) differences existed between the unfermented flours from zero (0) month of storage to three months and the fermented flours followed a similar trend but had higher bacterial load than the unfermented flours which might be attributed to proliferation of these organisms during *in-situ* storage and fermentation of the tubers used for flour production.

The total fungal count ($TFC_S$) of unfermented and fermented flours from American yam bean tubers stored underground for three months ranged from <$10^1$ to 1.80 x $10^1$ CFU/g in the unfermented flours and from 1.40 to 3.40 x $10^1$ CFU/g in the fermented flours. The results showed that the TFCs of American yam bean flours increased with increased storage time in both the unfermented and fermented flours. This could be due to the proliferation of these organisms during *in-situ* storage as a result of increased acidity condition brought about by lactic acid bacteria. The result also showed significant (P<0.05) difference in the total fungal count during the storage time of the tubers for the unfermented and fermented flours. The fungal load of the flours from unfermented American yam bean tubers increased with storage time and they had lower fungal loads than the flours from fermented American yam bean tubers. It was also observed that the $TFC_S$ of the fermented flour was within a close range during the second and third month of storage suggesting that there was no increase in the fungal load of

$FT_3$ probably because of increased Lactic acid bacteria activities, which resulted in increased acidity in $FT_3$.

**Table 4:** Effect of storage and fermentation on microbial load of flours of American yam bean tubers

| **Samples** | **Total viable count (x $10^1$ CFU/g)** | **Total fungal count (x $10^1$ CFU/g)** |
|---|---|---|
| $UT_0$ | $1.80^d$ x $10^1$ | $<10^1$ |
| $UT_1$ | $3.60^c$ x $10^1$ | $1.20^c$ x $10^1$ |
| $UT_2$ | $4.80^b$ x $10^1$ | $1.60^b$ x $10^1$ |
| $UT_3$ | $5.40^a$ x $10^1$ | $1.80^a$x $10^1$ |
| | Total viable count (x $10^1$ CFU/g) | Total fungal count (x $10^1$ CFU/g) |
| $FT_0$ | $4.60^d$ x $10^1$ | $1.40^c$ x $10^1$ |
| $FT_1$ | $5.00^c$ x $10^1$ | $1.60^b$ x $10^1$ |
| $FT_2$ | $8.60^b$ x $10^1$ | $3.40^a$ x $10^1$ |
| $FT_3$ | $9.60^a$ x $10^1$ | $3.40^a$ x $10^1$ |

**Key**

**$UT_0$ =** flour of unfermented tubers stored for 0 month, **$FT_0$** = flour of fermented tubers stored for 0 month

**$UT_1$** = flour of unfermented tubers stored for 1 month, **$FT_1$** = flour of fermented tubers stored for 1 month

**$UT_2$** = flour of unfermented tubers stored for 2 months, **$FT_2$** = flour of fermented tubers stored for 2 months

**$UT_3$** = flour of unfermented tubers stored for 3 months, **$FT_3$**= flour of fermented tubers stored for 3 months

### *3.5. Effects of in-situ Storage and Fermentation on the Mineral Content of Flours from American Yam Bean* Tubers

Minerals element are important constituents of human diet and also seen as cofactors for many biological and metabolic activities. The mineral content of flours from *American yam bean* tubers after *in-situ* storage and fermentation are presented in Table 5. In terms of calcium content of the flour samples, storage improved the calcium content of the all the flour samples (from 8.52 - 9.52 mg/100g) except for $FT_3$ (8.43 mg/100g) where calcium content decreased. This observed increase in calcium content of most of the samples was due to moisture losses that further helped to inhibit the activity of the fermenting enzymes. The decrease in calcium content of the fermented samples was ssignificantly ($P<0.05$) difference and may be due to increased activity of the fermenting enzymes during fermentation (Chikwendu *et al*., 2014). This suggests that fermentation had no beneficial effect on calcium availability. Calcium is noted for growth repairs and bone formation.

The concentration of magnesium was found to increase significantly ($P<0.05$) from 5.83 – 6.70 mg/100g for all the flour samples as a result of storage of the *American yam bean* tubers. However, it was also found to decrease significantly as a result of fermentation. This therefore implies that fermentation did not improve magnesium content of the flours. Magnesium is essential in maintaining

normal muscle and nerve function as well as promotes a healthy immune system thereby making the bones strong.

The sodium content of the unfermented flours (samples $UT_1$, $UT_2$ and $UT_3$) increased significantly recording values of 6.84, 7.17 and 7.34 mg/100g during storage for 1, 2 and 3 months respectively. For the fermented flour samples ($FT_1$, $FT_2$ and $FT_3$), sodium was observed to decrease during 1 month of storage, which recorded 5.73 mg/100g while a significant ($P<0.05$) increase was recorded during storage for 2 and 3 months (6.27 and 6.87mg/100g respectively), implying that storage of the tuber had variable effects particularly on the fermented flours. Fermentation on the other hand, resulted in decreased sodium content of the flours implying that fermentation did not improve sodium content of the flours. This may be due to fermentation of soluble salts and leaching into solution.

In terms of potassium content of the flour samples, storage however, increased the potassium content of the flours significantly from 4.78 - 5.52 mg/100g. The increase was also the same for samples $FT_1$, $FT_2$ and $FT_3$ (4.25, 4.78 and 4.86 mg/100g) as significant difference ($P<0.05$) was observed between them in terms of their potassium content. Meanwhile, fermentation caused a significant decrease in potassium content of all the flour samples.

Phosphorus content of the flour samples obtained in this study reveals that**,** storage was noted to have increased the phosphorus content of the unfermented flour samples $UT_2$ (12.36 mg/100g) and $UT_3$ (12.44 mg/100g) while the same was noted for the fermented flours with exception of $FT_1$ (10.27 mg/100g) which recorded a decrease in phosphorus content. The significantly higher phosphorus content of the unfermented flour samples compared with fermented ones suggests that fermentation decreased the phosphorus content of the flours.

Iron content of the flour samples revealed that**,** storage for 1, 2 and 3 months increased the iron content of all the flours from 0.68 – 0.87 mg/100g except $FT_1$ (0.46 mg/100g) which was not significantly ($P>0.05$) different from $FT_o$ (0.44 mg/100g) in terms of their iron content. These variations could be due to slight differences in the tuber composition. There was also a significant decrease in the level of iron of the fermented samples (0.62 – 0.73 mg/100g) compared with the unfermented ones. Thus fermentation did not enhance the iron content of the flour.

Zinc content of the flour sample revealed that, storage resulted to a corresponding increase in zinc content from 0.50 – 0.56 mg/100g with increased duration of storage, fermentation decreased the zinc content of all the flour samples This therefore implies that tuber storage between 1 to 3 months improved the zinc content of *American yam bean* flour.

Generally, the observations in this study regarding a decrease in mineral content of the fermented flours were contrary to the reports of other researchers who showed that fermentation improved the bioavailability of minerals such as iron, magnesium, and zinc as a result of phytic acid hydrolysis (Roday, 2007). The variations may be attributed to the fact that the mineral content of the flour samples

in this study may have leached into the fermenting medium. Furthermore, little or no information is available on the effect of storage on mineral content of *American yam bean* flours. However, the observed increase in mineral content of the flours in this study due to tuber storage could be attributed to moisture losses, which tend to increase the concentration of minerals in the flour.

**Table 5:** Effect of storage and fermentation on mineral content (mg/100g) of flours from American yam bean tubers

| Samples | Calcium | Magnesium | Sodium | Potassium | Phosphorous | Iron | Zinc |
|---|---|---|---|---|---|---|---|
| $UT_0$ | $8.52^{e}$±0.10 | $5.83^{c}$±0.02 | $6.30^{d}$±0.01 | $4.78^{d}$±0.00 | $11.82^{b}$±0.10 | $0.68^{cd}$±0.00 | $0.50^{bcd}$±0.02 |
| $FT_0$ | $7.83^{g}$±0.00 | $5.16^{d}$±0.00 | $6.13^{e}$±0.02 | $4.25^{e}$±0.02 | $10.66^{d}$±0.05 | $0.62^{d}$±0.02 | $0.44^{e}$±0.01 |
| $UT_1$ | $8.72^{d}$±0.02 | $6.25^{d}$±0.02 | $6.84^{c}$±0.08 | $5.17^{c}$±0.02 | $11.87^{b}$±0.04 | $0.74^{bc}$±0.05 | $0.52^{abc}$±0.00 |
| $FT_1$ | $8.25^{f}$±0.02 | $6.00^{c}$±0.02 | $5.73^{f}$±0.01 | $4.78^{d}$±0.02 | $10.27^{e}$±0.04 | $0.65^{d}$±0.00 | $0.46^{de}$±0.02 |
| $UT_2$ | $9.19^{b}$±0.01 | $6.40^{c}$±0.14 | $7.17^{b}$±0.02 | $5.29^{b}$±0.01 | $12.36^{a}$±0.08 | $0.83^{a}$±0.02 | $0.53^{ab}$±0.00 |
| $FT_2$ | $8.87^{c}$±0.04 | $5.87^{c}$±0.03 | $6.27^{d}$±0.04 | $4.77^{d}$±0.04 | $11.26^{c}$±0.02 | $0.76^{b}$±0.02 | $0.49^{cde}$±0.01 |
| $UT_3$ | $9.52^{a}$±0.00 | $6.70^{a}$±0.14 | $7.34^{a}$±0.08 | $5.52^{a}$±0.10 | $12.44^{a}$±0.22 | $0.77^{ab}$±0.02 | $0.56^{a}$±0.04 |
| $FT_3$ | $8.43^{e}$±0.02 | $6.26^{b}$±0.04 | $6.87^{c}$±0.04 | $4.86^{d}$±0.05 | $11.72^{e}$±0.02 | $0.73^{bc}$±0.02 | $0.55^{ab}$±0.01 |

Means ± standard deviation of triplicate determinations. Two means along the same column with different superscripts are significantly ($P<0.05$) different.

**Key**

**$UT_0$ =** flour of unfermented tubers stored for 0 month, **$FT_0$** = flour of fermented tubers stored for 0 month

**$UT_1$** = flour of unfermented tubers stored for 1 month, **$FT_1$** = flour of fermented tubers stored for 1 month

**$UT_2$** = flour of unfermented tubers stored for 2 months, **$FT_2$** = flour of fermented tubers stored for 2 months

**$UT_3$**= flour of unfermented tubers stored for 3 months, **$FT_3$** = flour of fermented tubers stored for 3 months

### *3.6 Effects of in-situ storage and fermentation on the anti-nutritional compositions of flours from American yam bean tubers.*

Anti-nutritional compositions of flour sample from *American yam bean* tubers after *in-situ* storage and fermentation of the tubers are shown in Table 6. The hydrogen cyanide (HCN) content of the flours was relatively low, ranging from 0.65 to 0.80 mg/100g and 0.36 to 0.48 mg/100g for unfermented and fermented flour samples respectively. Igbabul *et al*., (2014) reported 10 mg/1kg as recommended safe level for HCN, which the values from this study, was far below the recommended value. Little or no information is available on the effect of tuber storage on HCN content of the flour samples. In this study, storage increased the HCN content of samples $UT_2$ and $UT_3$ (0.73 and 0.80 mg/100g respectively) as well as samples $FT_1$ (0.45 mg/100g) and $FT_2$ (0.48 mg/100g). However, the HCN content of were within safe limits as stated above. Fermentation was observed to decrease the HCN content of all the flour samples. This was in agreement with the report of Kobawilla *et al*., (2005) which

reported a 70 to 75 % reduction in cyanogenic glycosides content of fermented roots and leaves of cassava. The decrease in HCN content of the fermented flour may be attributed to its hydrolysis by fermenting organisms (Igbabul *et al*., 2014).

In terms of tannin content of the flour samples, *in-situ* storage increased the tannin content of samples $UT_1$ (0.45 mg/100g) and $UT_2$ (0.46 mg/100g) while those of other samples were not affected significantly ($P>0.05$). Fermentation of the tubers caused a significant ($P<0.05$) decrease in the tannin content of all the flours. This reduction in tannin content of the fermented flour samples may have been due to the processing method adopted and activities of the microbial enzymes involved during fermentation. Tannins are bioactive ingredients and protective covers, that hindering the activities of enzymes. Tannins helps in encouraging the disruption of the plasma membrane, by withdrawal of substrates needed for the microbial growth (Rodrighes *et al*., 2005). Ekpo *et al*. (2004) reported that, processing methods such as fermentation could reduce most popular toxicant in food.

The alkaloid content of the flour samples ranged from 0.165 to 0.215 mg/100g and 0.076 to 0.140 mg/100g for unfermented and fermented flours respectively. With regards to storage, the results showed that alkaloid content of samples $UT_2$ and $UT_3$ (unfermented samples) increased significantly ($P<0.05$) but was highest for $UT_3$ (0.215 mg/100g) while that of $UT_1$ was not significantly ($P<0.05$) affected. As for the fermented flour samples, only $FT_3$ recorded a significant ($P<0.05$) increase from 0.085 to 0.140 mg/100g. In addition, all the flours were safe for consumption as the alkaloids were within tolerable limits. Fermentation decreased the alkaloid content of all the flours. This finding was in line with the reports of Igbabul *et al*., (2014) who stated that fermentation reduced alkaloid content of cocoyam flour.

The highest oxalate content was recorded in $UT_3$ (0.54 mg/100g) while the lowest value was 0.29 mg/100g in $FT_3$. The results showed that storage increased the oxalate content of samples $UT_1$ (0.42 mg/100g), $UT_2$ (0.43 mg/100g) and $UT_3$ (0.54 mg/100g). Similar trends were also observed for the fermented flours except for $FT_3$ (0.29 mg/100g) where storage decreased the content. The effect of fermentation on all the flour samples significantly ($P<0.05$) decreased oxalate content. This was in line with the reports of Ojokoh *et al*., (2014) and it could be attributed to the combined effect of various microorganisms involved during the natural fermentation. This reduction is of great importance because oxalate can form complexes with divalent metals, which in turn makes them unavailable for metabolic activities [30]. The values recorded for oxalate content of the flours were far below the lethal level (105 mg/100g) as reported by World Health Organization.

In terms of saponin content of the flour samples, little or no information is available on the level of storage on saponin content in American yam bean flour. The saponin content was relatively higher in the unfermented flour samples (0.35 to 0.45 mg/100g) than in the fermented ones (0.26 to 0.38 mg/100g). In this work, *in-situ* storage decreased the saponin content of the flour samples $UT_1$ and $UT_3$ but increased that of $UT_2$ indicating significantly effects of storage on the unfermented flours. For the

fermented flours, a significant decrease ($P<0.05$) in saponin content was recorded in all the flour samples. It was observed that, fermentation decreased the saponin content of all the flour samples in this study. This reduction is in line with the reports of Ekpo *et al*., (2004) who reported that most popular toxicant could be reduced by processing methods such as fermentation.

**Table 6:** Effect of storage and fermentation on anti-nutritional (mg/100g) compositions of flours from American yam bean tubers

| Samples | HCN | Tannin | Alkaloid | Oxalate | Saponin |
|---|---|---|---|---|---|
| $UT_0$ | $0.65^{c}\pm0.02$ | $0.42^{a}\pm0.02$ | $0.165^{bc}\pm0.021$ | $0.39^{bc}\pm0.00$ | $0.42^{ab}\pm0.02$ |
| $FT_0$ | $0.43^{e}\pm0.02$ | $0.29^{c}\pm0.01$ | $0.562^{dc}\pm0.000$ | $0.32^{de}\pm0.05$ | $0.35^{c}\pm0.00$ |
| $UT_1$ | $0.68^{c}\pm0.01$ | $0.45^{a}\pm0.01$ | $0.085^{d}\pm0.001$ | $0.42^{b}\pm0.01$ | $0.38^{bc}\pm0.02$ |
| $FT_1$ | $0.45^{d}\pm0.00$ | $0.32^{bc}\pm0.02$ | $0.170^{bc}\pm0.000$ | $0.35^{cde}\pm0.00$ | $0.26^{e}\pm0.00$ |
| $UT_2$ | $0.73^{d}\pm0.01$ | $0.46^{a}\pm0.02$ | $0.180^{b}\pm0.000$ | $0.43^{b}\pm0.04$ | $0.45^{a}\pm0.00$ |
| $FT_2$ | $0.48^{de}\pm0.00$ | $0.36^{b}\pm0.00$ | $0.076^{d}\pm0.002$ | $0.37^{bcd}\pm0.02$ | $0.32^{cd}\pm0.02$ |
| $UT_3$ | $0.80^{a}\pm0.00$ | $0.42^{a}\pm0.01$ | $0.215^{a}\pm0.021$ | $0.54^{a}\pm0.02$ | $0.35^{c}\pm0.00$ |
| $FT_3$ | $0.36^{d}\pm0.02$ | $0.28^{c}\pm0.00$ | $0.140^{c}\pm0.028$ | $0.29^{e}\pm0.00$ | $0.26^{e}\pm0.05$ |

Means ± standard deviation of triplicate determinations. Two means along the same column with different superscripts are significantly ($P<0.05$) different

**Key**

**$UT_0$ =** flour of unfermented tubers stored for 0 month, **$FT_0$** = flour of fermented tubers stored for 0 month

**$UT_1$** = flour of unfermented tubers stored for 1 month, **$FT_1$** = flour of fermented tubers stored for 1 month

**$UT_2$** = flour of unfermented tubers stored for 2 months, **$FT_2$** = flour of fermented tubers stored for 2 months

**$UT_3$** = flour of unfermented tubers stored for 3 months, **$FT_3$** = flour of fermented tubers stored for 3 months

## 4. Conclusion

This study showed that American yam bean that happens to be a foreign crop to tropical Africa, could be grown in Nigeria. It also evidences that shelf life of the American yam bean tubers after harvest could be extended using *in-situ* (underground) storage for a period of at least three (3) months. Fermentation of the American yam bean tubers significantly ($P<0.05$) decreased the anti-nutritional constituents, particularly oxalate, hydrogen cyanide, tannin and saponin, contained in the tubers and its flours although, they were originally below the recommended levels in this crop. During the tuber fermentation, it was evident that the microbial load increased with increase in fermentation time but within the tolerable limit.

## Declaration of Competing Interest

The authors declare they have no conflicting interests.

## Acknowledgements

The authors are grateful to Dr. Amadi of National Root Crop Research Institute, Umudike, Abia State, Nigeria, who identified and authenticated the seeds of *Pachyrhizus erosus* and for technical assistance on the planting procedures. The Authors also would like to express their gratitude to the technical staff of Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria for their assistance.

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