Original Research Article

EFFECTS OF FERMENTATION AND EXTRUSION ON THE ANTINUTRIENT COMPOSITION OF UNRIPE PLANTAIN AND PIGEON PEA BLENDS

ABSTRACT

This study was designed to investigate effects of fermentation and extrusion on the antinutrient composition of unripe plantain and pigeon pea blends. The blended samples were prepared in three combinations (A=100g unripe plantain; B= 70g unripe plantain: 30g pigeon pea; C= 50g unripe plantain: 50g pigeon pea) and separated into four batches (i.e. first batch = preconditioned and fermented; second batch = extruded; third batch = fermented and extruded; and fourth batch = unfermented/unextruded). The blended samples were fermented for 96 hours using semi-solid state fermentation. The pH, temperature and total titratable acidity (TTA) of the fermented samples were determined. A total number of fifteen microorganisms comprising 9 bacteria, 2 yeasts and 4 molds were isolated and identified as; *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Lactobacillus mali*, *Streptococcus lactis*, *Saccharomyces cerevisiae*, *Candida utilis*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus candidus*, and *Mucor hiemalis*. The pH and total titratable acidity (TTA) significantly varied during fermentation. The anti-nutrient content of fermented and extruded blends decreased significantly ($P<0.05$) when compared to the raw blends. Hence, it can be concluded based from the available information from this study that fermentation and extrusion decreased the antinutrient composition of unripe plantain and pigeon pea blends.

Keywords: Unripe plantain, pigeon pea, fermentation, extrusion

1. Introduction

Comment [Y1]: The introduction lack information on anti-nutrient properties and their effects, types advantages.

Fermentation and extrusion improve the nutritional value of foods by reducing the water-binding capacity of cereal flour. This allows the fortified to have a free-flowing consistency even with high proportion of flour. Extrusion has been reported as an effective processing treatment to improve the nutritional quality of cereals (Amadou *et al*., 2011). In the developing world, fermentation is one of the oldest technologies used for food processing and preservation. Fermentation reduces antinutrient properties of foods. It can be described as a desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes (Muchoki *et al.*, 2010.) Extrusion cooking technology has been described as a process in which raw materials are heated and worked upon mechanically while passing through compression screws (Iwe, 2003).

Plantain (*Musa paradisiaca)* is a giant perennial crop, cultivated in many tropics and subtropical countries of the world (Akomolafe and Aborisade 2007). Plantains are staple food that provides 60 million people with 25% calories (FAO, 2005). Plantain is used as a source of starchy staple food for millions of people in Nigeria. Mature plantain pulp is rich in iron, potassium and vitamin A but low in protein and fat (Adeniji *et al.*, 2006). Unripe plantain meal is usually consumed by diabetics to reduce postprandial glucose level. This is because the propensity of individual to develop diabetes and obesity is due to the increased consumption of carbohydrate-rich foods with a high glycemic index (Oboh and Erema, 2010).

Pigeon peas are leguminous shrubby herb, with trifoliate leaves, yellow flowers and flattened pods that is much cultivated especially in the tropics (Damaris, 2007). Pigeon pea is well adapted to the tropical regimes. One of the best solutions to protein energy malnutrition in developing countries is supplementing cereals with protein rich legumes. Pigeon pea flour has been tested and found to be suitable as a protein source for supplementing cereal food products due to its high level of protein, iron and phosphorus (Harinder *et al*., 1999).

The main objective of this study is to evaluate the fermentation and extrusion effects on the antinutrient compositions of unripe plantain and pigeon pea flour blends for human consumption.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Green matured unripe plantain and pigeon pea seeds used for this study were obtained from Oja Oba, Akure metropolis in Ondo state, Nigeria.

2.2 Processing of Unripe Plantain Flour

The unripe plantain was sorted for maturity and cleaned by washing with water. The clean unripe plantains were peeled and sliced thinly into 2 mm diameter and sun dried for 72 hours. The dried unripe plantain was then fed into a Bentall attrition mill (Model 200L090). The milled flour was sieved with 0.25 mm mesh sieve into fine flour and kept in an air tight container.

2.3 Processing of Pigeon pea Flour

Pigeon pea seeds were cleaned by sorting out dirt and stones. The cleaned pigeon pea seeds were coarsely milled to separate the coat from the cotyledon. The husk was separated from the seed by blowing air into it. The dehulled pigeon pea seeds were milled into fine flour using an attrition mill after which it was sieved through 0.25 mm mesh. The pigeon pea flour was kept in an airtight container.

Comment [Y2]: winnowing

2.4 Formation of pigeon pea-plantain Blends

Comment [Y3]: formulation

The unripe plantain and pigeon pea flours were formulated in the ratio of (unripe plantain: pigeon pea) 100:0; 70:30; and 50:50.

Comment [Y4]: justification

Sample A (100:0) = 100% unripe plantain flour

Sample B (70:30) = 70% unripe plantain flour and 30% pigeon pea flour

Sample C (50:50) = 50% unripe plantain flour and 50% pigeon pea flour

Comment [Y5]: proportions (%) or amount (g)

2.5 Fermentation and Extrusion of Flour Blends

A batch of the flour blend was fermented using semi- solid state fermentation for 96 hours. 70 ml of sterilized water was added to 100 g of each sample in cleaned containers and properly sealed. The fermentation process was terminated by oven drying at 60°C for 24 hours. Two batches of samples were subjected to extrusion cooking. The first batch consists of the unfermented blends. The blends were hydrated and preconditioned by adding 50 ml of water to 1000 g of the sample and manually mixed in a sterile bowl to ensure even distribution of water. The samples were extruded using a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg,

Comment [Y6]: sepate the value from the unit

Germany). The second batch of the samples consists of the fermented samples. The fermented samples were also extruded using a Brabender 20DN single screw laboratory extruder (BrabenderOHG, Duisburg, Germany). The samples were extruded at 100°C, 20 revolution per minute and feeding rate of 30 kg/h. All the extrudates were air dried for 12 hours after which they were stored at 32°C in sterile polyethylene bags and kept in properly labeled air tight containers. The control which consists of the raw blends which were neither fermented nor extruded was kept in air tight containers.

2.6 Microbiological Analysis of the Samples

Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosa sharpe agar was used to isolate lactic acid bacteria. Techniques were enumerated by using appropriate serial dilution and pour plate techniques. The bacterial culture was incubated at 37°C for 18 to 24 hours, fungal plates were inverted and incubated at 24°C for 48 to 72 hours. De Man Rogosa sharpe agar plates were incubated at 32°C for 18 to 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of Fawole and Oso (2007) and (Cheesbrough, 2006).

2.7 Determination of pH and TTA

The pH of all fermenting samples was determined at 24 hours interval using a pocket size pH meter. A 1 g of the sample was dissolved in 10 ml of distilled water and filtered. The pH meter was calibrated with buffer solutions of pH 4, 7 and 9, this was followed by dipping the electrode of the pH meter into the sample solution and the observed pH was read and recorded in triplicates. The total titratable acidity of the fermenting samples was determined at 24 hours interval. A 2 g of macerated sample was weighed into a beaker. 20 ml of distilled water was added to it, it was mixed and filtered. 10 ml of the filtrate was measured into a beaker and 2 drops of phenolphthalein indicator was added into it. This was titrated with 0.1 M sodium hydroxide (NaOH) solution and the titre value was read. Total titratable acidity was expressed as percent (%) lactic acid. The acidity was calculated as: TTA= Titre value × 9 mg/100. The pH and TTA of the samples were carried out according to the method described by AOAC (2012).

2.8 Determination of Antinutrients

2.8.1 Determination of phytate

The phytate content in each sample was determined using the modified method of Chen (2004). Phytate was extracted by adding 0.1g of the sample into 100 ml 0.2M HCI and shaken for 1 hour before centrifuging at 5000 rpm for 15 minutes. A 0.5 ml of the supernatant was pipetted into a test tube fitted with ground glass stopper before adding 1ml acidic ammonium iron (3) sulphate dodecahydrate (0.2g $NH_4Fe(SO_4)_2$)

2.8.2 Determination of alkaloid content

The sample (100g) was grounded and then extracted with methanol for 24 hours in a continuous extraction (soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45^{o}C to dryness. A part of this residue was dissolved in 2 N HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10ml volumetric flask and diluted to volume with chloroform. Presence of alkaloid was confirmed by Dragen droff's method. A part of extract was dissolved in dilute HCL and 2 drops of Dragon drop's was added, a crystalline precipitate indicates presence of alkaloid (Ali *et al.*, 2012).

Comment [Y7]: molarity or normality

2.8.3 Determination of saponin

The sample was grounded and 20g of each were put into a conical flask and 100 cm^3 of 20 % aqueous ethanol was added. The samples were place over a hot water bath for 4 hours with continuous stirring at 55^{o}C. The mixture was filtered and the residue re extracted with another

200 ml of 20 % ethanol. The combined extracts were reduced to 40ml over water bath at 90^{o}C. The concentrate was transferred into 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether was later discarded. The purification process was repeated and 60 ml of n- butanol was added. The combined n-butanol extract was washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight; the saponin content was calculated as percentage (Amir *et al.*, 2011).

$$\% \text{ Saponin} = \frac{\text{Initial weight} - \text{final weight of the sample}}{\text{Initial weight}} \times 100$$

2.8.4 Determination of phenol

The concentration of phenolics in the sample was determined using spectrophotometric method (Singleton *et al*., 1999). Methanolic solution of the sample in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of the sample, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5 % $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.5 ml methanol, 2.5 ml 10 % Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5 % of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45^{o}C for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read in (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed

in terms of gallic acid equivalent (mg of GA/g of extract) (Singleton *et al.,* 1999; Milan, 2011).

2.8.5 Determination of tannin content

A 200mg of finely ground sample was weighed into a 50 ml sample bottle. Ten (10) ml of 70 % aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker for 2 hours at 30^{o}C. Each solution was then centrifuged and the supernatant stored in ice. A 0.2 ml of each solution was pipetted into test tubes and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml stock and the solution made up to 1 ml with distilled water. A 0.5 ml of folin reagent was added to both sample and standard followed by 2.5 ml of 20% Na_2CO_3. The solutions were then vortexed and allowed to incubate for 40 minutes at room temperature after which absorbance was read at 725 nm against a reagent blank concentration of the samples from a standard tannic acid curve (Banso and Adeyemo, 2010).

3. Statistical Analysis

Statistical analyses of the Data were obtained using SPSS statistical software (SPSS for window version 20). Data obtained as mean standard deviations were analyzed by Analysis of Variance (ANOVA), followed by Duncan's New Multiple Range Test($P<0.05$) to determine the significant differences between the mean values.

4. RESULTS

4.1 Microorganisms Isolated During

Fermentation of Samples

A total number of fifteen (15) microorganisms comprising nine (9) bacteria, two (2) yeasts and four (4) molds were isolated and identified as; *Bacillus subtilis*, *Bacillus cereus, Micrococcus luteus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc*

mesenteroides, Lactobacillus mali, Streptococcus lactis, Saccharomyces cerevisiae, Candida utilis, Aspergillus niger, Aspergillus fumigatus, Aspergillus candidus, and *Mucor hiemalis.*

4.2 pH of Unripe Plantain and Pigeon Pea Flour Blends during Fermentation

The pH variations during the fermentation ofunripe plantain and pigeon pea blends are shown in Fig. 1. Sample A gradually decreased from 5.80±0.00 to 5.10±0.03, Sample B decreased from 6.0±0.00 to 5.30±0.00, and sample C, decreased from 5.90±0.00 to 5.20±0.00.

4.3 Total Titratable Acidity of Unripe Plantain and Pigeon Pea Flour Blends during Fermentation

Variations in titratable acidity (TTA) during fermentation of unripe plantain and pigeon pea blends are represented in Fig. 2. Sample A had TTA of 1.20±0.00 at 0 hour; this increased to 2.20±0.00 and 4.40±0.00 at 24 hours and 48 hours and increased slightly to 4.5±0.00 at 72 hours and finally decreased to 3.70±0.00 at 96 hours. Sample B increased from 1.00±0.00 at 0 hour and increased to 2.20±0.00 at 24 hours, decreased slightly to 2.00±0.00 at 48 hours and increased to 6.60±0.00 at 72 hours and finally decreased to 3.6±0.00 at 96 hours. Sample C at 0 hour increased from 1.10±0.00 to 2.30±0.00 at 24 hours and increased to 3.60±0.00 at 48 hours, decreased to 2.60±0.00 at 72 hours and finally decreased to 3.6±0.00 at 96 hours.

4.4 Anti-Nutritional Composition of Unripe Plantain and Pigeon Pea Flour Blends.

4.4.1 Changes in alkaloid content

The alkaloid content of unripe plantain and pigeon pea blends are shown in Table 1. The result obtained from the anti-nutrient composition of the samples indicated that the raw samples contained the highest alkaloid content ranged from 1.47±0.03 to 3.83±0.02. There was no significant difference ($P \leq 0.05$) in the fermented-unextruded blends (0.75±0.02 to 1.13±0.04) and unfermented-extruded blends (0.88±0.03 to 1.46±0.01). The fermented-extruded blends recorded significantly low alkaloid content ranging from 0.55±0.01 to 1.03±0.05.

4.4.2 Changes in phenol content

The phenol content of unripe plantain and pigeon pea blends are shown in Table 1. There was significant difference between the phenol content of all the blends. Raw blends had the highest phenol content with values ranged from 11.17±0.01 to 17.03±0.00. Fermented blends had phenol content ranging from 2.35±0.03 to 15.29±0.01. Extruded unfermented blends had phenol content with values ranging from 1.13±0.0 to 9.15±0.03. Extruded fermented blends had the lowest phenol content ranging from 1.03±0.01 to 7.52±0.01.

4.4.3 Changes in tannin content

Tannin content of the blends is shown in Table 1. There was significant difference ($P \leq 0.05$) between sample A, B and C of the raw blends (0.85±0.01, 1.63±0.00 and 1.28±0.00 respectively). There was no significant difference ($P \leq 0.05$) for samples A and B for the fermented blends (0.47±0.00 and 0.39±0.00 respectively), but sample C recorded significant difference ($P \leq 0.05$). Extruded unfermented blends had no significant difference for sample B and C (1.07±0.01 and 1.03±0.01 respectively) but sample A recorded significant difference ($P \leq 0.05$). The extruded fermented blends had values ranging from 0.25±0.01 to 1.13±0.01.

4.4.4 Changes in saponin content

Saponin content of the blends is shown in Table 1 The raw blends had the highest saponin content (1.32±0.08 to 1.99±0.07). Saponin content reduced significantly in the fermented unextruded blends having values that ranged from (0.31±0.04 to 0.78±0.06). There was slight decrease in the saponin content of unfermented extruded (1.25±0.24 to 1.52±0.13) and fermented extruded blends (0.57±0.13 to 1.07±0.23).

4.4.5 Changes in phytate content

Phytate content of the blends are shown in Table 1. There was significant difference ($P \leq 0.05$) in the phytate content of the raw blends, fermented blends and fermented-extruded blends. The extruded-unfermented blends of sample B and C (18.15±0.02 and 18.03±0.01 respectively) showed no significant difference but extruded-fermented sample A showed significant difference ($P \leq 0.05$). The raw blends had the highest phytate content with values that ranged from 21.35±0.04 to 23.17±0.01 for samples A to C. Fermented samples had the least phytate content with values that ranged from 11.53±0.23 to 15.25±0.00 for sample A to C.

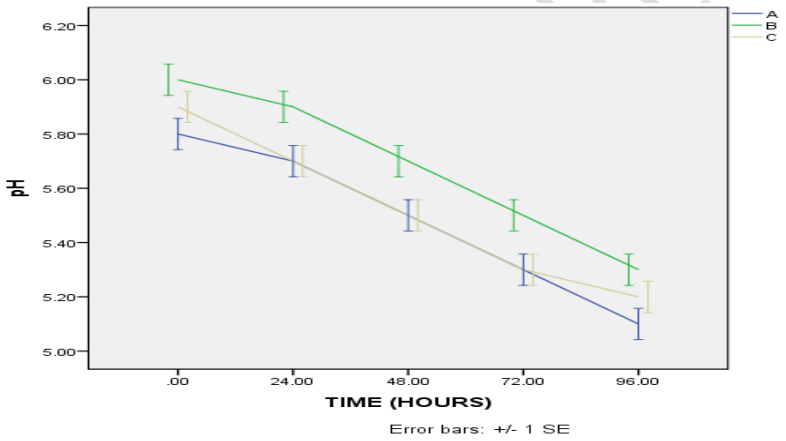

Figure 1: pH variation during fermentation of unripe plantain and pigeon pea blends
Keys
A= 100g Unripe Plantain flour;

B= 70g unripe plantain flour and 30g pigeon pea flour;

C= 50g unripe plantain flour and 50g pigeon pea flour

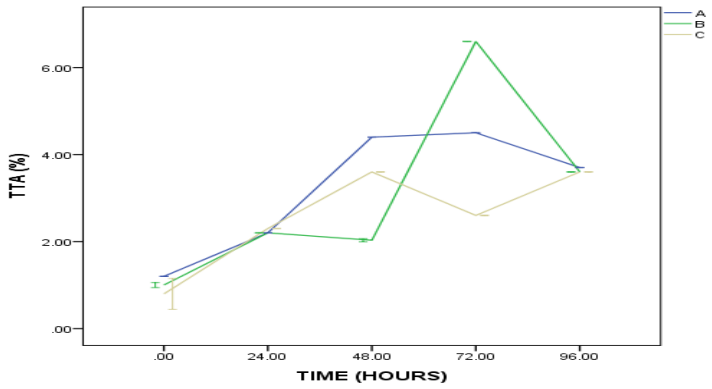

Figure 2: Variations in Total titratable acidity during the fermentation of unripe plantain and pigeon pea blends

Keys
A= 100g Unripe Plantain flour;
B= 70g unripe plantain flour and 30g pigeon pea flour;
C= 50g unripe plantain flour and 50g pigeon pea flour

Table 1: Anti-nutrient composition of unripe plantain and pigeon pea blends (mg/g)

Sample codes	Alkaloid **(mg/g)**	Phenol **(mg/g)**	Tannin **(mg/g)**	Saponin **(mg/g)**	Phytate **(mg/g)**
ARF	1.47±0.01^{d}	11.17±0.00^{g}	0.85±0.01^{ab}	1.32±0.00^{c}	23.17±0.01^{g}
BRF	2.25±0.01^{e}	17.03±0.01^{j}	1.63±0.02^{d}	1.79±0.01^{e}	21.35±0.02^{f}
CRF	3.83±0.00^{f}	16.25±0.00^{j}	1.28±0.00^{c}	1.99±0.00^{f}	22.69±0.01^{f}
AFU	1.13±0.01^{c}	2.35±0.00^{c}	0.47±0.00^{a}	0.78±0.00^{ab}	13.37±0.00^{b}
BFU	1.07±0.00^{c}	15.29±0.00^{i}	0.39±0.02^{a}	0.69±0.01^{a}	11.53±0.00^{a}
CFU	0.75±0.01^{b}	13.48±0.01^{h}	1.15±0.00^{b}	0.31±0.01^{a}	15.25±0.00^{d}
AUE	1.46±0.00^{d}	1.13±0.01^{a}	0.75±0.00^{ab}	1.25±0.00^{c}	17.43±0.01^{d}
BUE	1.37±0.00^{d}	4.27±0.00^{b}	1.07±0.00^{b}	1.30±0.00^{c}	18.15±0.00^{e}
CUE	0.88±0.00^{b}	9.15±0.01^{f}	1.03±0.00^{b}	1.52±0.00^{d}	18.03±0.01^{e}
AFE	1.03±0.01^{c}	1.03±0.01^{a}	0.25±0.00^{a}	0.57±0.00^{a}	15.31±0.00^{c}
BFE	0.71±0.02^{b}	7.52±0.00^{e}	1.13±0.01^{b}	0.63±0.00^{a}	12.03±0.01^{a}
CFE	0.55±0.00^{a}	5.97±0.01^{d}	1.01±0.00^{b}	1.07±0.02^{b}	13.77±0.00^{b}

Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different ($P \leq 0.05$)
Keys: ARF= Raw unripe plantain flour 100g; BRF= Raw unripe plantain flour 70g and raw pigeon pea flour 30g; CRF= Raw unripe plantain flour 50g and raw pigeon pea flour 50g; AFU= Fermented unextruded unripe plantain flour 100g; BFU= Fermented unextruded unripe plantain flour 70g and pigeon pea 30g; CFU= Fermented unextruded unripe plantain flour 50g and pigeon pea 50g; AUE= Unfermented extruded unripe plantain flour 100g; BUE= Unfermented extruded unripe plantain flour 70g and pigeon pea 30g; CUE= Unfermented extruded unripe plantain flour 50g and pigeon pea 50g; AFE= Fermented extruded unripe plantain flour 100g; BFE= Fermented extruded unripe plantain flour 70g and pigeon pea 30g; CFE= Fermented extruded unripe plantain flour 50g and pigeon pea 50g

5. DISCUSSION

There were varied microbial populations during the fermentation of unripe plantain and pigeon pea flour blends. The increase in microbial population between 0 and 48 hours could be attributed to various microorganisms adapting to the fermentation environment. Jeff- Agboola, (2007) also recorded increase in microbial counts during natural fermentation of African yam beans (*Sphenostylis sternocarpa* Harms). Also the decrease in microbial population between 48 and 96 hours may be due to reduction in pH which could have inhibited the growth of some microorganisms like *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus luteus* in the fermenting media. Similar results were reported by Odion-Owase *et al.* (2018) who stated that the results of the microbial counts in fermented pigeon pea showed decrease in the microbial counts as fermentation progressed. As fermentation of unripe plantain and pigeon pea flour blends progressed, the pH of the samples decreased. Odion-Owase *et al.* (2018) also recorded decrease in pH during the fermentation of pigeon pea. The lowering of pH could be due to the high carbohydrate composition in unripe plantain and pigeon pea blends which might have been degraded to organic acids. This finding is similar to that of Hassan *et al.* (2015) who stated that the decrease in pH may be as a result of the activities of microorganisms on the fermentable substrate which led to the hydrolysis of complex organic compounds of the substrate thereby producing acid and ethanol. The acids produced led to a decrease in pH and increase in total

titratable acidity which consequently resulted in decrease in microbial loads (Hassan *et al.*, 2015). However, the fermentation results of this research suggest that it is acidic where pH of fermenting media decreases with increase in total titratable acidity (TTA).

Comment [Y8]: Acidic medium

The microorganisms that were present in the fermenting media varied, comprising of a total of fifteen microorganisms which include, nine (9) bacteria, two (2) yeasts and four (4) molds. These were isolated and identified as; *Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Lactobacillus plantarum, Lactobacillus fermentum, Leuconostoc mesenteroides, Lactobacillus mali, Streptococcus lactis, Saccharomyces cerevisiae, Candida utilis, Aspergillus niger, Aspergillus fumigatus, Aspergillus candidus,* and *Mucor hiemalis.* This is similar to the findings of Ojokoh and Udeh (2014) that legume supplemented products had a greater microbial diversity and higher microbial populations. *Staphylococcus aureus, Bacillus subtilis*, *Bacillus cereus*, and *Micrococcus luteus* were isolated between 0 and 24 hours of fermentation. However, as fermentation progressed, these less desirable bacteria disappeared. This could be as a result of their inability to tolerate the low pH of the fermenting media. The inability to detect these less desirable microorganisms in the fermentation media after 24 hours agrees with the observation of Wakil and Ajayi (2013). The involvement of microorganisms like *Staphylococcus aureus* may be attributed to handling because they are normal flora of the skin. Okigbo and Omodamiro (2011) isolated *Staphylococcus aureus* from human skin which is a normal flora of the skin. Between 24 and 48 hours *Bacillus* species constituted the major bacteria in samples B and C while *Aspergillus* species were the dominant fungi. These could be the microbial flora associated with pigeon pea as these samples have been supplemented with pigeon pea. Similar findings were observed by Olunlade *et al.* (2013) who isolated *Bacillus* species and *Aspergillus* species from maize-pigeon pea biscuit. Since the major components of the samples

are carbohydrate, protein and fat; the microbes that are responsible for the fermentation must have the ability to utilize these three components. *Bacillus* species were dominant in samples B and C during the fermentation process. Enujiugha (2009) confirmed that *Bacillus* species are known to have proteolytic and lipolytic ability. At 72 and 96 hours, lactic acid bacteria were dominant in all the fermenting media. This corresponds with the findings of Ezeama and Ihezie (2006) that fermentation of most carbohydrate foods are lactic acid fermentations.

The results of anti-nutrient components revealed that extrusion cooking reduced phytate levels. It could be expected that lowering this compound would enhance the bioavailability of minerals such as Zinc and Iron in the extrudates since phytic acid has been implicated in making these minerals unavailable as reported by Anuonye *et al*. (2009). Decrease in phytate content may be partly due to the formation of insoluble complexes between phytate and microbial degradation. It could also be due to the heat labile nature of phytic acid. The effect of fermentation on phytic acid may be due to the activity of enzyme like phytase produced by fermenting micro flora (Amit *et al*., 2017). The reduction in phytate level of fermented blends could be interpreted as the main reason behind the observed increase in the concentrations of minerals in the fermented samples. Reduction in the phytate content was also reported by Hassan *et al.* (2015) when he evaluated the effect of blanching and fermentation on cocoyam.

The level of tannin was significantly reduced in the unfermented extruded blends, fermented unextruded blends as well as fermented extruded blends. Decrease in tannin after fermentation may be as a result of microbial activity during fermentation like tannase and metabolites for microbial fermentation (Sorour *et al.,* 2017). Tannins form insoluble complexes with proteins thereby decreasing protein digestibility (Uzoechina, 2007). Tannins also decrease palatability, cause damage to intestinal tract and enhance carcinogenesis

(Anuonye *et al.,* 2012). Reduction of tannin in the fermented blends may be as a result of the fact that tannins are polyphenols and all polyphenolic compounds are water soluble in nature (Udenzi *et al.,* 2007). Therefore, reduction in tannin content of the fermented blends may be attributed to leaching out of phenols into the fermenting media (Udenzi *et al.,* 2007). Similar result was also reported by Hassan *et al.* (2015). Korus *et al*. (2007) attributed the decrease in the phenolic content of beans to high temperature when he evaluated the effect of extrusion on the phenolic composition and antioxidant activity of dry beans. Suazo *et al*. (2014) reported that the concentration of phenol reduced with fermentation.

Saponin content was reduced in all the flour blends but significantly reduced in fermented extruded blends. Saponins have anti-hyper cholesterol, anti- inflammatory, cardiac depressant property and also appear to kill or inhibit cancer cells without killing the normal cells in the process (Ojinnaka *et al.,* 2016). The saponin content of the pigeon pea flour samples were reduced by each of the processing methods (fermentation and extrusion). The raw samples had the highest saponin values increasing from 1.32±0.00mg/g to 1.99±0.00mg/g while the fermented extruded blends had the lowest saponin values ranging from 0.57±0.00mg/g to 1.07±0.02 mg/g. It has been reported that processing techniques such as boiling, sprouting and fermentation reduce anti-nutritional content of legume flours (Nwanekezi *et al*., 2017).

Alkaloids are important poisons produced by plants as active defense against aggressors (Kasprowicz-Potocka *et al.,* 2017). There was significant difference ($P<0.05$) in the alkaloid content of the unripe plantain and pigeon pea flour samples. The raw samples had the highest alkaloid content (1.47±0.01mg/g to 3.83±0.00mg/g) in comparison with the fermented extruded samples with a significant reduction ranging from 1.03±0.01mg/g to 0.55±0.00mg/g in the total

alkaloid content. Alkaloids are nitrogen containing naturally occurring compounds found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms (Kasolo *et al.,* 2010). The reduction of alkaloids due to boiling, sprouting and fermentation had been reported respectively by Nwanekezi *et al.* (2017). The presence of alkaloids in this study may lead to healing of wounds, varicose ulcers, hemorrhoids, frostbite and burn in herbal medicines as reported by Ndulaka and Obasi (2018).

The rate at which these anti-nutrients affect the availability of nutrients by chelating the nutrients and making them available for utilization in the system will be relatively reduced. Since the higher the level of anti-nutrient, the lower the bioavailability of the nutrient and minerals contained therein (Fagbemi and Ojokoh, 2016).

5.1 CONCLUSION

This investigation shows that fermentation and extrusion of the blending of unripe plantain and pigeon pea has the potential of reducing the antinutrient compositions of the samples thereby producing enriched complementary food for improving the health of malnourished children of developing countries.

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