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# COMPARATIVE STUDY OF NUTRITIONAL AND MICROBIAL EVALUATIONS OF PACKAGED COMPOSITE FLOURS OF *DISCOREA SPP, MUSA SPP., GLYCINE MAX., ZINGIBER OFFICINALE* AND A LOCAL EXPOSED BLEND "ELUBO"

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## ABSTRACT

A blend from several plant materials' roots, tubers, grains, and legumes is known as swallow composite flour. In this study, the dried composite flours (Composite A) and a regional blend called "elubo" (Composite B) were compared for their nutritional value and microbiological health. The maceration method was used to prepare the aqueous extract of Composite A and B, and standard protocols were observed in all adopted analyses. The results showed that there were significant differences ($P<0.05$) among the samples. Proximate analysis revealed higher values in Composite A than in B, with high Protein (8.60±0.02%) and Crude fiber (13.73%), while CHO (42.77±0.59 %) and moisture (4.32±0.05%) of Composite B are lesser. Phytochemical screening showed high Flavonoids (41.97±0.39), Alkaloids (35.40±0.37), Tannin (30.10±0.32), phenol (40.38±0.45) and cardiac glycoside (30.35±0.86), than Composite B, Saponin (26.15±0.75) and Terpenoid (22.08±0.35) were low. DPPH, Nitric oxide and reducing power scavenging activity (%Inhibition), in 25, 50, 75 and 100µg/ml increased in Composite A. Some minerals were present except for the absence of Lead and Cadmium from Composite A. The total plate count of microbial load activities for Composite B (147250) was high and Composite A (102000) low, amongst *Escherichia coli* (110 and 200cfu/g), *Staphylococcus aureus* (180 and 350cfu/g) in 25 and 50 µg/ml concentration, yeast and mold were not detected in both samples. This packed flour formula is naturally preserved, nutritious, has medicinal effects, and has lower microbiological activities as compared to market Composite B and ICMSF (International Commission for Microbiological Specification for Foods) criteria.

**Keywords:** Swallow, Flour, Composite, Proximate, Antioxidants.

## 1.0 INTRODUCTION

In Nigeria, there have been several attempts at overcoming the nutritional of yam or cassava-based diets by fortifying with soya bean, which has high protein content of good quality (Kolapo & Sanni, 2005). The use of soybean flours and other legume flours to increase the nutritional contents of yam have been explored (Ashaye & Lawal, 2001). Composite flours are considered firstly as blends of many flours for the production of leavened breads, baked products, porridges, snacks, swallow meals and so on (Shittu *et al.*, 2007). A flour made by blending varying proportions of more than one non-wheat flours with or without wheat flour and utilized for production of unleavened or leavened baked products that are traditionally produced from wheat flour and increase essential nutrients in human diet is referred to as composite flour (Shittu, Raji & Sanni 2007). The reason to mix different flours can be economically, nutritionally and medicinally justified. Healthy swallow composite flour, is a mixture of dried powder product based on plant materials, obtained

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from roots, tubers, cereals, and legumes, while the "Elubo" is a locally blend recipe from dried yam tuber (*Discorea spp*). In addition, Abulude & Ojediran (2006) reported that, to fortify yam flour with plantain, soybean flour and other legumes, improves its viscosity and texture of yam flour paste. Various species of yam tubers among them are *Dioscorea rotundata, Dioscorea alata and Dioscorea cayenensis* have been processed into yam flour (Akinwande *et al.,* 2008) and results showed that they are good raw materials for yam flour production. Results of previous studies on fortification of yam, cassava and plantain flours using soybean has shown that fortification improves nutritional quality of resulting meals, including Amala (Abulude & Ojediran, 2006). Yam (*Dioscorea spp*) is the major staple and main calorie source in some tropical regions. During the harvest season, yam is very cheap and affordable source of food for low-income families. Yam is unpopular in some other parts of the world where it is considered as inferior to cereal due to its low protein content. In spite of its low protein, yam consists of a good proportion of all the essential amino acids and a fairly good source of minerals and dietary fiber (Ukpabi & Omodamiro, 2008). Plantain is a staple food commonly consumed in the tropical regions of the world, is extremely low in fat and protein but high in fibre and starch. Unripe plantain is a good source of vitamin A, B6 and C which helps maintain vision, good skin and builds immunity against diseases. It is also rich in potassium, magnesium and phosphate when cooked green (Ogazi, 1996). FAO (2009) reported that more than 2.5 million metric tons of plantains are produced in Nigeria. Traditionally unripe plantain can be processed into flour. The flour is mixed with boiling water to make a stretchable paste (known as Amala) which is eaten with various soups. However, plantain is increasingly finding a gradual application in weaning food formulation and composite flour preparations. Soybean has been recognized to be an ideal grain for meeting protein and energy requirement of both man and animal. Soybean is probably the world's most valuable crop, used as feed by billions of livestock, as a source of dietary protein and oil by millions of people, and in the industrial manufacture of thousands of products. Soybean is such an extremely rich source of protein, energy, vitamins and minerals (Nwokolo & smart, 1996) Soybeans have great potential in overcoming protein-calorie malnutrition. Although soybean is not indigenous to Africa, it has received tremendous popularity as a cheap protein source in Nigeria (Nwabueze, 2007). Ginger (*Zingiber officinale*) is a root or an underground stem (rhizome) known to contain gingerols (oleoresin) with several health benefits. It reduces the risk of colon cancer, obesity, diabetes, cardiovascular diseases, cold related-diseases and arthritis (Barley-Shaw, *et al.,* 2008). Ginger is one of the most common species that is used to add flavour to meals. Studies have also shown its hypoglycemic properties (Iroaganachi *et al.*, 2015). Considering the health benefits of yam, unripe plantain, soybean and ginger, their incorporation in the preparation of composite flour will enhance nutritional, medicinal and health status of the consumers. Several studies have reported the use of plantain flour and ginger to manage Diabetes mellitus in Nigeria (Ndife *et al.*, 2011), but the possibility of combining them in a typical diet that included soybean is unknown. Hence, this study is aimed at comparing the nutritional and microbial evaluations of composite flours produce from *discorea spp, musa spp., glycine max., zingiber officinale* with a local blend "Elubo"

## 2. METHODOLOGY

### 2.1. Materials

The yam tuber (*Dioscorea rotundata*), unripe plantain (*musa spp*), soybean seeds (*Glycine max)* and ginger roots (*zingiber officinale*) and other reagents, equipments used, were purchased from an open market source located in ketu Lagos state, Nigeria

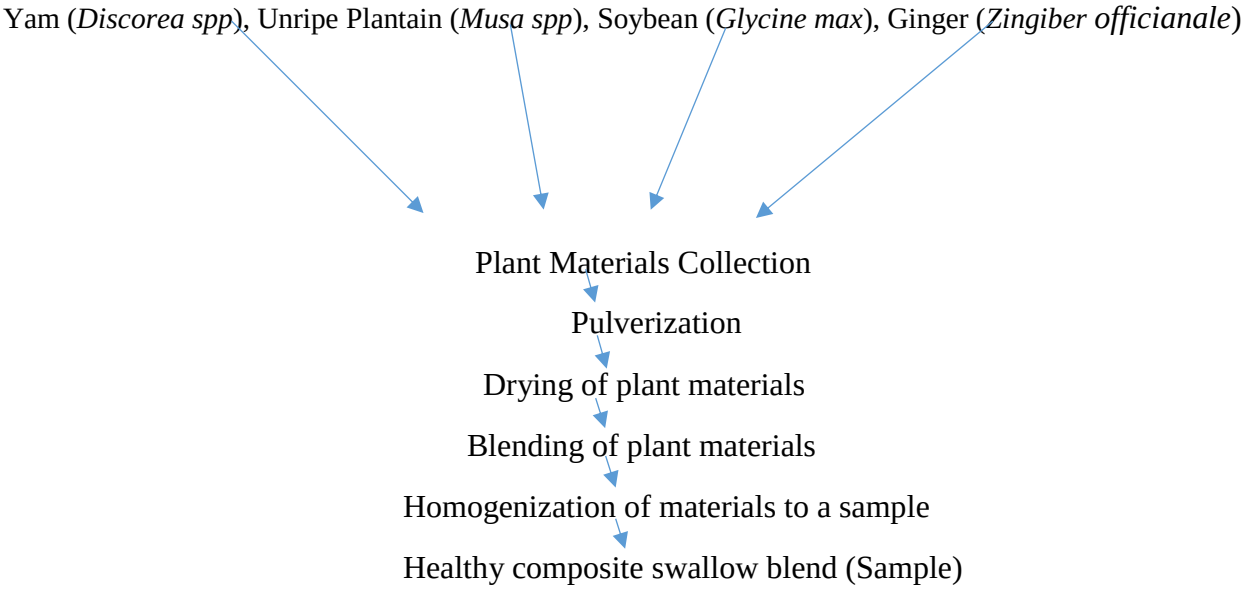


*Figure 1: Flow chart of composite flour production*

**2.1.1 Preparation of Yam Flour**

Yam flour was produced following the method described by (FIIRO, 2005). The yam tubers were washed to remove sand, dirt and other adhering materials. The yam tubers were peeled sliced to 0.02mm thickness, were sun dried for two days and oven dried at 60℃ for 24hrs which was followed by milling using a hammer mill and the yam flour was sieved, packaged in a Ziploc bag and stored in a dry place S prior to analysis.

**2.1.2 Processing of plantain flour**

Fresh unripe plantain was peeled, sliced using slicer and sun dried for two days and then, in an oven at 60℃ for 24 hours. Dried sample was ground into powder.

**2.1.3 Processing of soybeans**

Soybean flour was prepared by the method described by (Kulkarni *et al.*, 1991). Soybeans were sorted to remove particles, defective seeds and stones before cleaning thoroughly washed in clean tap water, followed by dehulling using manual method i.e. hand rubbing within two palms, after dehulling, the soybean seeds were sun dried and then in an hot air oven at 70°c for 10hours. After drying the soybean hulls were removed by winnowing, the dried samples were milled to fine powder and sieved through a standard sieve of 400µm particle size. The flour was packaged in a Ziploc bag and stored in a dry place prior to analysis.

**2.1.4 Processing of Ginger Powder**

Fresh ginger roots were sorted and washed to remove soil and other foreign materials then sliced to thin layers and dried in an oven at 60oC for 24 hours before milling to powder.

**2.2 Formulation of Yam, Unripe Plantain, Soybeans and Ginger Flour Blends**

A 100g of the blended swallow composite (Composite A) was prepared with 35g, 30g, 27g and 8g from the combinations of Yam, unripe plantain, soybean and ginger respectively, as blends mixed in different proportions using Nutri-survey linear programming software version 2007 to obtain the formulations; Composite A=100% with Yam = 35%, unripe plantain =30%, Soybean = 27%, Ginger = 8%, while the market place "Elubo" 100% Yam (Composite B), was purchased dried and ready for analysis

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**2.3 Methods of Analysis**

The Atomic Absorption Spectrophotometer (AAS Model SP9) was used to analyze iron and calcium content of the flours, while the sodium and potassium contents of the flours were determined using flame photometer.

**2.4 Proximate analysis**

The moisture content was determined in a hot-air at 105ºC 3 hours circulating oven (Galenkamp). Ash was determined by incineration (550ºC) of known weight of sample in a muffle furnace (Hot oven, Gallencamy, UK). Crude fat was determined by the exhaustively extracting a known weight of sample in petroleum ether (boiling point 40-60ºC) using Tecatorsoxtec (Model 2043). Protein content (Nx 6.25) was determined by the micro-kjeldahl method (No 978.04). Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide. Carbohydrate content was determined by difference that is addition of all the percentages of moisture, fat, crude protein, ash and crude fiber and subtracting from 100%. This gave the amount of nitrogen free extract otherwise known as carbohydrate by the Association of official Analytical Chemist [18] Bulk density, water and oil absorption capacities were determined using the methods reported by Arisa *et al*., (2013). Foaming capacity, solubility, swelling capacity and phytochemical screening of the samples were carried out.

**2.5 Phytochemical Determination**

2.5.1 Flavonoid was determined by the method of Boham & Kocipal-Abyazan, (1994). The whole solution was filtered through Whatman filter paper No 42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.5.2 Alkaloid was determined by the alkaline precipitation gravimetric method described by Harbone, (1973). 5 g of the sample was weighed into 50 mL of 10% acetic acid solution in ethanol in a 250 mL beaker. The mixture was shaken and allowed to stand for 4 hour then filtered with Whatman No. 42 filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation using a steam bath. Alkaloid in the extract was precipitated by drop-wise addition of ammonium hydroxide ($_4$paper and washed with 1% ammonia solution ($NH_4OH$), dried in the oven at 80oC for 1 hour. It was later cooled in a desiccator and reweighed. By weight difference, the weight of alkaloid was determined and expressed as percentage of the sample analyzed, using the formula. % Alkaloid = W2-W1 X 100 W 1 Where: W= weight of sample W1= weight of empty filter paper W2= weight of paper + alkaloid precipitate.

2.5.3 Saponin was determined by the method of Nahapetian & Bassiri, (1974). 20 g of sample was dispersed in 200 mL of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90ºC. The concentration was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ethyl layer was discarded. The purification process was repeated. 60 mL of n-butane extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage. Tannin was determined using Follins Dennis spectrophotometric method according to Pearson (1976). 5g of the sample was dispersed in 50 mL of distilled water and shaken. The mixture was allowed to stand for 30 min at room temperature and shaken every 10 min. at the end of the 30 min,

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the mixture was filtered through Whatman filter paper and the filtrate was used for the experiment. Two milliliters (2 mL) of the extract was measured into 50 mL volumetric flask. Similarly, 5 mL of standard tannic acid solution and 5 mL of distilled water were measured into separate flask to serve as standard (Pearson, 1976) and blank respectively. They were further diluted with 35 mL distilled water separately and 1 mL of Follin-Dennis reagent was added to each of the flask, followed by 2.5 mL of saturated sodium carbonate solution ($NA_2CO_3$). The content of each flask was then made up to 50 mL at room temperature. The absorbance of the developed colour was measured at 620 nm wavelength in spectrophotometer. Readings were taken with the reagent blank at zero

**2.6 Antioxidant determination:**

Solution of the sample extract (1ml) was mixed with 3ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4m ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 950°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm. The total antioxidant capacity was expressed as equivalent of ascorbic.

**2.6.1 DPPH radical scavenging activity assay:**

An aliquot of 0.5ml of extract in ethanol (95%) at different concentration (25, 50, 75, 100ug/ml) was mixed with 2.0ml of reagent solution (0.004g of DPPH in 100ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the black. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517nm. The scavenging effects was calculated using the expression: % inhibition = [A0 - A1] × 100/A0

Where AO is the absorbance of the blank sample and A1 is the absorption of the extract.

In vitro lipid peroxidation assay: The reaction mixture containing liver homogenate (0.2ml). Tris- HCL buffer (20Mm Ph 7.0.0.1ml), $Fecl_2$ (2mM, 0.1ml), ascorbic acid (10Mm, 0.1 ml), and 0.5 ml plant extract (25 - 100ug/ml) in a final volume of 1ml. The reaction mixture was incubated at 37°C for 1hour. Lipids peroxidation was measured as malondialdehyde (MDA) using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HCL (TBA- TCA reagent: 0.375% w/v TBA; 15% w/v TCA and 0.25N HCL). The incubated reaction mixture was mixed with 2ml of TBA- TCA reagent and heated in a boiling water bath for 15 minutes. After cooling, the flocculent precipitate was removed by centrifugation at 10,000g for 5 minutes. Finally, malondialdehyde concentration in the supernatant fraction was determined spectrophotometrically at 535nm. Ascorbic acid was used as standard.

**2.6.2 Nitric oxide scavenging assay:**

4ml sample of plant extract or standard solution of different concentrations (25, 50, 75, 100ug/ml) were taken in different test tubes and 1ml of sodium nitroprusside (5mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30°C to complete the reaction. A 2ml sample was withdraw from the mixture and mixed with 1.2ml of Griess reagent (1% sulphanilamide), 0.1% naphthylethylene diamine dihydrochloride in 2% $H_3PO_4$). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylene diamine was measured at 550nm (Alisi *et al.*, 2008) Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation: [(A0-A1)/A0] × 100.

Where, A0 is the absorbance of the control and A1 is the absorbance of the extract or standard.

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**2.6.3 Reducing power assay:**
Various concentrations of the extracts (20 to 100ug/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml), the mixture was incubated at 50°C for 20 min. aliquots of trichloroacetic acid (2.5ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 mins. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml), the absorbance was measured at 700nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16ug/ ml) was used as standard.

**2.7 Microbial Evaluation**
Microbiological analysis of the flour Composites A and B, were carried out using the adopted, pour plate method and described by ICMSF, (1996).

**2.8 Design and Data Analysis**
Data generated were subjected to one-way analysis of Variance (ANOVA) in randomized block to test significant variations ($P<0.05$) among mean values obtained. The values used for each treatment were in triplicate. Where significant differences existed Duncan's multiple range test was applied to indicate where the differences occurred using Genstat statistical package 2005, 8TH edition (Genstat Procedure Library Release PL16). Where Composite A=100% with Yam = 35%, unripe plantain =30%, Soybean = 27%, Ginger = 8%, while the market place "Elubo" 100% Yam (Composite B) were analyzed.

**3. RESULTS AND DISCUSSION**
**RESULTS**
Table 1: Proximate composition of the crude sample of the composite blend A and B

| **Parameters (%)** | **Composite A** | **Composite B** | **t (p)** |
|---|---|---|---|
| Carbohydrate | $42.77 \pm 0.59^{b}$ | $68.14 \pm 0.29^{a}$ | 38.59 (<0.001) |
| Crude Protein | $8.60 \pm 0.02^{a}$ | $4.33 \pm 0.08^{b}$ | 51.78 (<0.001) |
| Crude Fat | $0.66 \pm 0.02^{a}$ | $0.50 \pm 0.05^{b}$ | 2.971 (0.041) |
| Crude Fibre | $13.78 \pm 0.56^{a}$ | $12.76 \pm 0.55^{a}$ | 1.299 (0.264) |
| Moisture Content | $4.68 \pm 0.05^{b}$ | $5.32 \pm 0.06^{a}$ | 8.194 (0.001) |
| Crude Ash | $5.90 \pm 0.04^{a}$ | $4.59 \pm 0.18^{b}$ | 7.104 (0.002) |

Table 1 showed the proximate composition of the formulated flour blend. There were significant difference ($P<0.05$) in the moisture content of Composite A and B flour blends. The moisture content of the composite flour (Composite A) was 4.68% while the other flour blend (Composite B) showed slight increase of 5.32%. These results were within the moisture content (<10%) of flour reported by Iroaganachi *et al*., (2015). Moisture content of foods or processed products determines the shelf stability of products. Hence, the composite flour (Composite A), is more stable compared to Composite B due to its lowest moisture content. The protein content of composite A (8.60 %), was twice higher than composite B blend (4.33%). The recommended daily allowance for protein in diabetic patients is 15-20% of the total calories (Chatterjir *et al*., 2012). The highest protein value recorded in composite A could be attributed to the inclusion of soybean. The fat content was slightly higher

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in composite A (0.66 %) than in c B (0.50 %), which is least as showed in Table 1. Composite B (68.14%) had a high carbohydrate content than Composite A (42.77 %). The recommended daily allowance for carbohydrate in diabetics is 50-70% of the total calories (Vansunder, 2006) Crude fiber was significantly different ($P<0.05$) among the samples. Composite A (13.78%) had higher fiber content compare to Composite B (12.76%). However, these values were not in agreement with Agoyero *et al*., (2011), who reported 10.43% and 10.11% for sundried and oven-dried flours respectively. Dietary fiber is important for human digestive health and regular bowel movement. It also helps to full the stomach for longer time; it can improve cholesterol and blood sugar levels and assist in preventing diseases such as diabetes, heart disease and bowel cancer. Ash is the inorganic residue after water and organic matter have been removed by burning food sample. Ash content was significantly different ($p < 0.05$) among the samples. The ash content of Composite A (5.90%) was higher than Composite B (4.59%).

Table 2: Qualitative and Quantitative Phytochemical Screening of the crude sample of the composite blend A and B

| **Parameters mg/100g** | **Flavonoid** | **Phlobatanin** | **Alkaloid** | **Tannin** | **Phenol** | **Saponin** | **Steroid** | **Terpenoid** | **Cardiac Glycoside** |
|---|---|---|---|---|---|---|---|---|---|
| | Present | Absent | Present | Present | Present | Present | Absent | Present | Present |
| Composite A | 41.97 ± 0.39[a] | - | 35.40 ± 0.37[a] | 30.10 ± 0.32[a] | 40.38 ± 0.45[a] | 30.10 ± 0.75[a] | - | 22.08 ± 0.35[a] | 30.35 ± 0.86[a] |
| Composite B | 36.65 ± 1.22[b] | - | 33.72 ± 0.88[a] | 28.77 ± 0.36[a] | 39.54 ± 0.49[a] | 26.15 ± 0.56[b] | - | 24.02 ± 0.29[a] | 26.96 ± 0.69[b] |
| t (p) | 4.154 (0.014) | - | 1.760 (0.153) | 2.761 (0.051) | 1.263 (0.275) | 4.220 (0.013) | - | 4.268 (0.130) | 3.390 (0.036) |

Value represent Mean ± standard error of mean

**3.1 Phytochemical analysis**

Table 2 shows the phytochemical constituents of the 2 (two) flour blends. The lowest terpanoid (22.08 mg/100g) was observed in Composite A and was followed by Composite B (24.02 mg/100g). The highest Flavonoid content (41.97mg/100g) was observed in Composite A, while Composite B had 36.65 mg/100g. Saponin was lower in Composite B (26.15mg/100g) than in Composite A (30.10mg/100g) and saponins are known to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic permeabilization of the intestine and paralysis of the sensory system) properties (Price *et al*., 1987) Flavonoids, alkaloids and tannins are polyphenolic compounds with antioxidant properties. Phenolic compounds in plants, are responsible for the contribution to colour, sensory and antioxidant properties of food (Robbins, 2003) The low phytochemical values of this study are significantly lower than ($p<0.05$) the results of (Eleazu, *et al.,* 2011) who reported that the levels of saponin in the flour are quite too low to cause any deleterious effects. Phlabotannins and steroids were absent in this study

Table 3: Antioxidant properties of Composite A and Composite B

| DPPH Scavenging Activity (%Inhibition) | | | | |
|---|---|---|---|---|
| **Concentration** | **25µg/ml** | **50µg/ml** | **75µg/ml** | **100µg/ml** |
| Composite A | 42.95 ± 0.63[b] | 54.92 ± 1.05[b] | 66.83 ± 0.63[b] | 74.11 ± 0.42 |
| Composite B | 38.76 ± 0.83[c] | 41.42 ± 0.63[c] | 49.46 ± 0.42[c] | 54.59 ± 0.42 |
| Ascorbic acid | 46.46 ± 0.35[a] | 61.38 ± 0.13[a] | 74.40 ± 0.17[a] | 83.25 ± 0.73 |
| **F (p)** | **36.900 (<0.001)** | **205.200 (<0.001)** | **814.500 (<0.001)** | **726.000 (0.001)** |
| Nitric oxide Scavenging Activity (%Inhibition) | | | | |
| Composite A | 23.07 ± 0.33[b] | 37.17 ± 0.67[b] | 57.72 ± 0.33[b] | 70.66 ± 0.45[b] |
| Composite B | 21.50 ± 0.33[c] | 35.20 ± 0.11[b] | 55.51 ± 0.56[b] | 67.00 ± 0.11[b] |
| Ascorbic acid | 54.38 ± 1.30[a] | 67.74 ± 0.65[a] | 71.43 ± 0.65[a] | 84.56 ± 1.63[a] |
| **F (p)** | **540.900 (<0.001)** | **1130.000 (<0.001)** | **264.100 (<0.001)** | **89.670 (<0.001)** |
| Reducing power Scavenging Activity (%Inhibition) | | | | |
| Composite A | 0.189 ± 0.001 | 0.224 ± 0.001[b] | 0.198 ± 0.004[c] | 0.351 ± 0.001[c] |
| Composite B | 0.200 ± 0.005 | 0.298 ± 0.004[b] | 0.291 ± 0.003[b] | 0.425 ± 0.003[b] |
| Ascorbic acid | 0.230 ± 0.002 | 0.369 ± 0.005[a] | 0.549 ± 0.005[a] | 0.744 ± 0.006[a] |
| **F (p)** | **45.030 (<0.001)** | **375.500 (<0.001)** | **1984.000 (<0.001)** | **2844.00 (<0.001)** |

Value represent Mean ± standard error of mean

The enhanced Composite A with soybean, ginger, and unripe plantains is full of antioxidants that have the power to shield the body from oxidative stress. These bioactive substances include phenolics, carotenoids, dopamine, norepinephrine, and ascorbic acid (vitamin C), (Someya *et al.,* 2002). DPPH scavenging activity increased with the increasing other of the concentration in Composite A than in Composite B, but was compared with the standard ascorbic acid. The highest DPPH was obtained in the composite flour A (74.11 µg/ml), which stipulates the essence of the flour blend enrichment as reported by (Mahloko *et al., (*2019). The nitric oxide Scavenging capacity was displayed increasing, in the other of increasing concentration in Composite A, but higher than Composite B, when compared with the standard (Ascorbic acid). The incorporation of unripe plantain and ginger flour into the yam flour significantly improved the antioxidant capacity of composite flour compared to the 100% yam flour, according to Pacheco-delahaye *et al.*, (2008). The reducing power scavenging activity also shows an increasing effect with the trend of the concentration in Composite B than in Composite A and when compared to the standard. This is in tandem with the report of Farooqui and Farooqui, (2018) that the main classes of flavonoids detected in plantains are quercetin, myricetin, and kaempferol. Flavonoids are also active against many infectious diseases (bacterial and viral diseases), cardiovascular diseases, cancers, and other age-related diseases (Farooqui & Farooqui, 2018).

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Table 4: Mineral Composition of Composite A and B

| ±Parameters | Composite A (PPM) | Composite B (PPM) |
|---|---|---|
| Calcium | 162.34 ± 0.04[a] | 152.26 ± 0.22[a] |
| Sodium | 117.80 ± 0.09[a] | 109.48 ± 0.41[a] |
| Magnesium | 59.06 ± O.12 [b] | 54.25 ± 0.14 [b] |
| Potassium | 140.58 ± O.33[a] | 128.73 ± 0.01 [b] |
| Lead | Absent | Absent |
| Iron | 7.33 ± 0.07 [a] | 5.62 ± 0.23 [b] |
| Zinc | 3.38 ± 0.12 [a] | 2.51 ± 0.14 [b] |
| Copper | 2.24 ± 0.04 [a] | 1.16 ± 0.32 [b] |
| Cadmium | Absent | Absent |
| Manganese | 2.45 ± 0.09[a] | 0.38 ± 0.22 [b] |

Table 4 showed the mineral composition of the Composites A and B, with Lead (Pb) and Cadmium (cd) absent from the samples. The mineral content tends to be high, owing to various degree of formulation of samples. Flour blend A contained significant higher ($P<0.05$) quantities of mineral content compared to Composite B, where calcium (Ca) was higher (162.34 ppm)) in Composite A than in Composite B (152.26 ppm). Calcium plays an important role in muscle contraction, transmitting messages through the nerves and the release of hormones and is also an important mineral in the formation of teeth and bones. Calcium is an important component of intracellular processes that occur within insulin responsive tissues like muscle and adipose tissues. From the table, potassium content in Composite A, is relatively higher (140.58ppm), when compared with Composite B and potassium is an important mineral in the body that regulates fluid balance, muscle contraction and nerve signals. High potassium may reduce blood pressure and water retention, protect against stroke and prevent osteoporosis and kidney stones while low dietary potassium (K) is associated with increased risk of incident diabetes (Chatterjir, *et al*., 2012).The highest iron content was seen in Composite A (7.33ppm) and the least was observed in Composite B (5.62ppm). Iron influences glucose metabolism, insulin action and it also interferes with insulin inhibition of glucose production by the liver (Chatterjir, *et al.*, 2012). The highest sodium content was observed in Composite A (117.80ppm), higher than Composite B (109.48ppm), Sodium is essential for life. It helps to control the body's fluid balance. It send nerve impulses and affect muscle function.

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**Table 5: Microbial Activities in Composite A and B**

| Parameter cfu/g | Concentration | Composite A Parametric count | Composite B Parametric count | Levofloxacin | Official (ICMSF) Limits | | F (p) |
|---|---|---|---|---|---|---|---|
| | | | | | GMP | MAX | |
| 25µg/ml | Escherichia coli | 38 | 110 | $25.17 \pm 0.29^{b}$ | $10^{2}$ | $10^{3}$ | 7.705 (0.002) |
| | Staphylococcus aureus | 80 | 180 | $28.33 \pm 0.29^{a}$ | | | |
| | Yeast | ND | ND | - | $10^{3}$ | $10^{6}$ | |
| | Mold | ND | ND | - | $10^{3}$ | $10^{5}$ | |
| 50µg/ml | Escherichia coli | 90 | 200 | $29.83 \pm 0.29^{b}$ | $10^{2}$ | $10^{4}$ | 8.144 (<0.001) |
| | Staphylococcus aureus | 100 | 350 | $33.17 \pm 0.29^{a}$ | | | |
| | Yeast | ND | ND | - | $10^{3}$ | $10^{6}$ | |
| | Mold | ND | ND | - | $10^{3}$ | $10^{5}$ | |
| Total plate count | | 102000 | 147250 | - | $10^{4}$ | $10^{5}$ | |

Note : ND means Not detected

Table 5, showed the analysis of microbial qualities of 5 parameters that were evaluated according to the ICMSF (International Commission for Microbiological Specification for foods), are: *Escherichia coli, Staphylococcus aureus,* Yeast, mold and Total plate count of Composite A and B. In the average plate count of 5 assay medium, Composite B has a higher value in TSA (147250cfu/g) as compared to Composite A (102000). This microbial limit report for Composite A and B displayed *Escherichia coli* detected and high in Composite B parametric count (110 and 200cfu/g respectively) of 25 and 50µg/ml, than in Composite A (38 and 90cfu/g respectively). *Staphylococcus aureus* was detected and high in Composite B (180 and 350cfu/g respectively) with concentrations of 25 and 50 µg/ml. Yeast and Mold of both samples, were not detected. Pelletier and Lawrence, (1996) reported that bacteria are lysed by immune cells which cause toxic reaction that may lead to blood pressure, respiratory failure, reduced oxygen delivery and lactic acidosis (manifestation of septic shock), which disagrees with the present study due to the reduced parametric count of both gram positive bacteria and gram negative bacteria and non-detection of yeast and mold Composite A (Packaged) than in Composite B (Exposed) (Kim & Chun, 2005). Kim and Chun, (2005), reported that mold is a filamentous or "furry" fungus and has the distinction in the plant world for requiring no light to grow, it reproduces, by forming spores which are very hardy and can survive under extreme conditions in both dry and harsh environments. The spores can travel

through outdoor and indoor air, which may land on a surface where moisture is present and start growing

## CONCLUSION

Yam, unripe plantain, soybean, and ginger flour blended in the right amounts have great nutritional and therapeutic properties. Low fat, low phytochemicals, and dietary fiber that increase transit time through the bowels and facilitate bowel movement. Calcium is crucial for the release of hormones, the transmission of information through the nervous system, and the contraction of muscles. Potassium is a vital mineral that controls fluid equilibrium, muscle contraction, and nerve messages in the body. Low dietary potassium (K) is linked to an increased risk of incident diabetes, while high potassium may lower blood pressure and water retention, protect against stroke, prevent osteoporosis, and avoid kidney stones. Besides, this study revealed that enriching yam flour with unripe plantain, soybean and ginger flour, can reduce the problem of food security in the sub-Sahara region of Africa where malnutrition is prevalent. It was evident that Composite A, had more nutritional value, medicinal properties and less microbial growth to cause any deleterious effects.

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