

# Evaluation of Minerals, Vitamins and Phytochemicals in High Fiber Biscuits Produced from Blends of African Breadfruit, Maize and Coconut Flours

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## ABSTRACT

Composite flours from blends of African Breadfruit flour, Maize flour and Coconut grits were used in the production of High fiber biscuits. The samples obtained from blend ratio of Whole breadfruit flour: Maize flour: Coconut grits of 70:20:10, 60:30:10, 45:45:10, 30: 60:10 and 20: 70: 10 were coded A, B, C, D and E respectively. A sample obtained from blend ratio of Hulled breadfruit flour: Maize flour: Coconut grits of 45:45:10 was coded F, while proprietary flour obtained from 100% Wheat which was coded C served as a control. The samples were evaluated for vitamins, minerals and phytochemicals content. The products obtained from flour blends were significantly ( $p < 0.05$ ) higher in potassium, phosphorus, calcium and magnesium, while the product from wheat flour was significantly ( $p < 0.05$ ) higher in sodium. There was significant ( $p < 0.05$ ) difference in vitamin composition of samples, with the samples containing high proportion of whole breadfruit flour significantly ( $p < 0.05$ ) higher in Vitamins A, B<sub>2</sub> and B<sub>3</sub>, while the wheat flour sample was significantly ( $p < 0.05$ ) higher in Vitamin B<sub>1</sub>. There was no significant ( $p > 0.05$ ) difference in Vitamin C content of Samples A, B and G, which were significantly ( $p < 0.05$ ) higher than Samples C, D, E and F.

The samples obtained from blended flours were significantly ( $p < 0.05$ ) higher in saponins, tannin, alkaloid, flavanoid and phytate, which were the phytochemicals considered. Generally, the blended flour biscuits compared favorably with the wheat flour biscuit, thus suggesting that high fiber biscuits from local agricultural materials can be exploited for industrial purposes.

**Keywords:** Flours, Biscuits, Vitamin, Mineral, Phytochemical.

## 1 INTRODUCTION

The gradual drop in fiber in diets calls for development of recipes, formula and products that would restore the level of dietary fiber (Reddy, 2008). Diets based on whole grains are increasing day by day due to various health benefits associated with them as they are good sources of dietary fiber, antioxidants, vitamins, etc. In fact, there has been a trend to incorporate bran from high protein fiber sources into cereal products (Hegazy *et al.*, 2009).

High fiber diet has been shown time and again to reduce heart disease, regulate blood sugar (reduce type 2 diabetes risk), improve digestive function, increase satiety (aids in achieving healthy weight) and helps to prevent certain cancers (O'Conore *et al.*, 2003).

Rapid urbanization and a change in life style have led to an increase in the demand for ready to eat convenience foods, most of which are wheat based. Wheat is a cereal grain made up of 1.68% minerals, 61.7% carbohydrate, 12.2% moisture, 10.4% protein and 1.56% fat (Pamplona and George, 2004). Wheat has a poor essential amino acid (lysine and tryptophan) pattern (Okoh, 1998), and is also implicated in celiac disease (intolerance to wheat gluten).

The production of wheat in Nigeria is far below the domestic requirement. Many developing countries, including Nigeria, spend substantial foreign exchange on importation of wheat, leading to underutilization of indigenous agricultural materials.

There is need to develop an adequate substitute for wheat from locally grown crops with comparable or better nutritional quality and adequate levels of dietary fiber.

African bread fruit (*Treculia africana* Decne) is a member of the Moraceae family. It is of African Origin but is now grown in most tropical and sub-tropical countries (Aguet *et al.*, 2007). The seeds contain 10% vegetable oil, 17% protein and 40% Carbohydrate as well as several minerals and vitamins (Enibe, 2001). It is rich in calcium, phosphorus, iron, potassium, carotene and vitamin B (Wang *et al.*, 2011). The seeds can be roasted, boiled and fried before consumption or they can also be ground into

flour which can be used as substitute for wheat flour in bakery products (Ijehet *et al.*, 2010). The seeds can also be dried and milled into flour known as breadfruit flour, which can be used to produce a variety of baked foods such as cookies (Akubor and Badifu, 2004), biscuit (Olaoye *et al.*, 2007), extruded ready-to-eat snacks (Samaila and Nwabueze, 2013; Okafor and Ugwu, 2014).

Maize is one of the most important cereal grains in the world, providing nutrients for humans and animals. It is a major source of carbohydrate, protein, vitamin B, vitamin A (Yellow maize) and minerals. It serves as a basic material for production of starch, oil, protein, alcoholic beverage, food sweeteners and more recently fuel (FAO, 1992). The yellow maize cultivar was chosen for this work because it is rich in pro vitamin A (beta-carotene) needed for good eye sight.

Coconut (*Cocos nucifera*) is a member of the *Arecaceae* family (palm). Coconut has one of the highest percentages of fiber among all plant foods. Seventy-five percent of the total carbohydrate content is fiber (Fife, 2005), and good spread of vitamins and minerals. Coconut can be used as an ingredient in weight control foods (Thampman, 1993), curry and chutney formulations (Arumughan *et al.*, 1993), breakfast cereal (Okafor and Usman, 2014) and ready-to-eat snack (Okafor and Ugwu, 2014).

Biscuits are chemically leavened, baked, ready to eat, quick snacks with good eating quality and long shelf life (Singh *et al.*, 1993). Although not eaten as the main course meal, biscuits are known to provide moderate levels of macro and micronutrients, depending on flour source, ingredients and processing methods.

It is not uncommon to observe the presence of phytochemicals in raw and processed agricultural materials, some of which may be beneficial or deleterious to nutrients availability and health in humans. Tannin, flavonoid, alkaloid, phytate and saponin are some of the commonly found phytochemicals in cereals, legumes and nuts. The fact that the base materials for the flour blend cut across this group require that their residual levels in the biscuit product be ascertained.

It is expected that quality biscuit produced from blended flours of African breadfruit, maize and coconut will not only provide another variety of high fiber biscuit, but will reduce the high import bills on importation of wheat in Nigeria and sub-Saharan Africa.

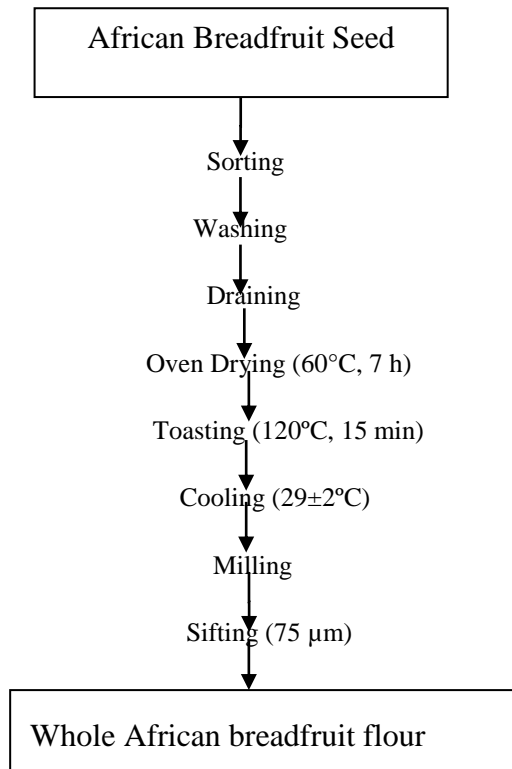
## 2.0 MATERIALS AND METHOD

### 2.1 Collection of materials

Mature seeds of African breadfruit (*Treculia africana*), Maize (*Zea mays*) and Husked coconut (*Cocos nucifera*) were purchased from Umuahia Main Market in Abia State, Nigeria. The samples were authenticated at the Department of Plant science, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Other materials purchased include sugar, margarine, eggs, sodium bicarbonate, vanilla and milk.

### 2.2 Preparation of whole African breadfruit flour

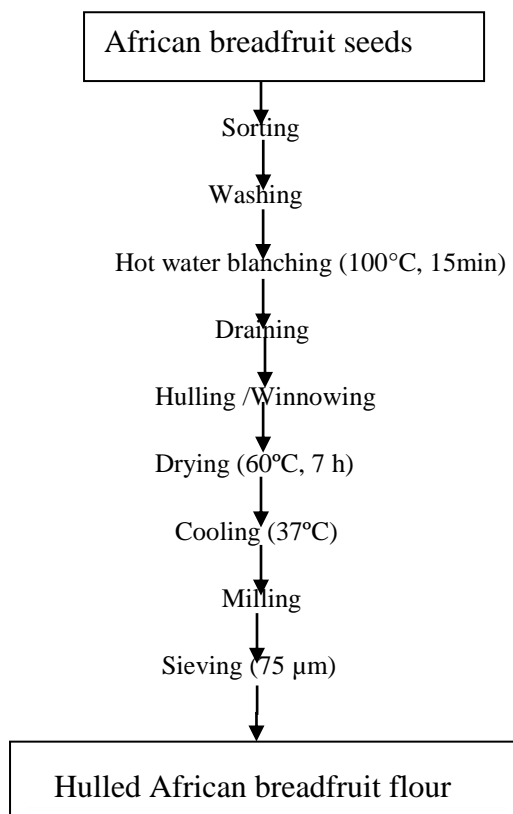
Approximately 5 kg of African breadfruit seeds were sorted to remove spoilt seeds and stones, washed in a basin of tap water till they were freed from slimy materials, dust and other extraneous matters. The clean seeds were left to drain in a plastic basket and subsequently dried in a hot air oven (Gallenkemp 300 Plus, England) at 60°C for 7 h. The seeds were then roasted at 120°C for 15 min in a 60 cm wide shallow pan using gas cooker, cooled to room temperature (29±2°C) before milling in a commercial disc attrition mill (7hp, China). The flour was sieved using a 75 µm screen and stored in an air tight container at room temperature until use (see Figure 1).



**Figure 1: Production of whole African breadfruit flour**

### 2.3 Preparation of hulled African breadfruit flour

The method described by Samaila and Nwabueze(2013) for the production of breadfruit flour was slightly modified. Approximately 6 kg of African breadfruit seeds were sorted and cleaned as before. The clean seeds were blanched in hot water at 100°C for 15 min in an aluminum pot, poured into a plastic basket to drain, and air dried for 15 min. The seeds were cracked in a commercial attrition mill (7hp China) and the hulls winnowed manually. The hulled seeds were dried in a hot air oven (Gallenkemp, 300 Plus, England) at 60°C for 7 h, cooled to 37°C before milling to pass through a 75 µm mesh sieve and stored in an air-tight container at room temperature until use (see Figure 2).



**Figure 2: Production of hulled African breadfruit flour**

#### 2.4 Production of maize flour

Approximately 5 kg of dry maize grains were sorted to remove spoilt seeds, stone and other extraneous matters before milling in a disc attrition mill (7hp China) and stored in an air tight container.

#### 2.5 Production of partially defatted coconut grits

The method of Okafor and Ugwu (2014) was used. Approximately 6 kg of coconut were manually cracked to remove the shell. The meat was manually scraped with a sharp knife to remove the brown skin, reduced to grits with stainless hand grater, homogenized in boiling water, filtered through a clean muslin cloth and pressed to obtain the defatted coconut. This was repeatedly rinsed with hot water (70°C) to minimize residual fat. The defatted coconut was then dried to constant weight in the hot air oven at 60°C, cooled, and stored in an air tight container.

#### 2.6 Preparation of flour blends

Five samples of blended flours were prepared by substituting Whole African breadfruit flour, Maize flour and Coconut grits on a w/w proportions of 70 : 20 : 10, 60 : 30 : 10, 45 : 45 : 10, 30 : 60 : 10, 20 : 70 : 10 respectively; while a sixth sample was obtained by substituting Hulled African breadfruit flour, Maize flour and Coconut grits on a w/w proportion of 45:45:10 respectively (see Table 1).

**Table 1: Formulation of flour blends**

	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Hulled breadfruit flour	-	-	-	-	-	45%
Whole breadfruit flour	70%	60%	45%	30%	20%	---
Maize Flour	20%	30%	45%	60%	70%	45%
Coconut grits	10%	10%	10%	10%	10%	10%

## 2.7 Wheat flour

Approximately 2kg of proprietary wheat flour which was purchased from a Bakery Market in Umuahia was used as a control.

## 2.8 Biscuit production

Production of biscuits followed the method of Kure *et al.* (1998). Approximately 40g of sugar and 30g of margarine were mixed at medium speed using Master Chef Mixer (MC-HM6630) until fluffy. To this was added 150g of flour, 10g of egg, 10g of milk, 1.5g of baking powder, 0.5g of vanilla and 90ml of water. Each sample was mixed until a uniform smooth paste or dough is obtained. This was then rolled to a sheet of about 0.25 cm thickness on a board using a rolling pin. Cookie cutters were used to cut the sheet into desired shapes and sizes which were subsequently baked in an oven at about 150°C for 20 min, allowed to cool, packed and stored at ambient conditions.

## 2.9 Mineral analysis

The mineral content of the test samples was determined by the ash acid extraction method described by James (1995). A measured weight of each sample (5 g) was burnt to ashes in a muffle furnace at 550°C. The resulting ash was dissolved in 10 ml of 2M HCL solution and diluted to 100 ml in a volumetric flask using distilled water, and then filtered. The filtrate was used for the mineral analysis (calcium, magnesium, potassium, sodium and iron).

### 2.9.1 Determination of calcium and magnesium

Calcium and magnesium contents of the samples extract were carried out by versanate EDTA complexometric titration described by James (1995). A measured volume of each 20 ml was dispersed into a conical flask and pinch doses of the masking agents (potassium cyanide, potassium ferrocyanide, hydroxylamine hydrochloride) were added to it. Ammonia buffer (20 ml) was added to adjust the pH to 10.0. A pinch of the indicator Erichrome blank T was added and then the mixture was shook very well. It was titrated against 0.02N EDTA solution, until the color changes from mauve to a permanent deep blue color. This titration gave a reading for combined concentration of Ca and Mg ions. This was as a result of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  forming complexes at pH 10.0 with EDTA. A second titration was conducted to determine Ca alone. This was a repeat of the previous one with a slight change, in that 10% NaOH solution was used to raise the pH of the digest to 12.0 and then was titrated with 0.02N EDTA using selechrome dark blue as indicator in place of Erichrome blank T. At pH 12.0  $\text{Ca}^{2+}$  complexes with EDTA. A reagent blank was titrated to serve as control. The experiment was repeated two more times. The calcium and magnesium contents were calculated separately using the formula;

$$\% \text{ Calcium or Magnesium} = \frac{100}{w} \times \text{EW} \times N \times \frac{V_f}{V_a} \times T - B \dots \dots \dots (1)$$

Where:

W = weight of sample analyzed

EW = Equivalent weight

N = Normality of EDTA

$V_f$  = Total volume of extract

$V_a$  = Volume of extract titrated

T = Titre value of sample

B = Titre value of blank

### 2.9.2 Determination of potassium and sodium

Flame photometry was used to determine the concentrations of potassium and sodium as described by James, (1995). The instrument (photometer) was set up according to the manufactures instructions. The equipment was switched on and allowed to stay for about 10 min. The gas and air inlets were opened and the start knob was turned on, the equipment being self-igniting. After ignition, the flame was adjusted to a non-luminous (blue) flame. Standard potassium and sodium solutions were prepared separately and each was diluted to a concentration of 2, 4, 6, 8 and 10 ppm. The appropriate filter was selected i.e. for potassium and sodium. The highest concentration standard solution (10 ppm) was aspirated and its emission intensity adjusted to 100 units. Thereafter, starting with the least concentrated (2 ppm), each standard solution was aspirated and caused to spray over the non-luminous butane gas flame. The emission intensity was read directly on the instrument and readings were recorded. The sample digest was also aspirated and their readings recorded. The emission intensities of the standards were plotted against their concentrations to obtain a standard curve (calibration graph) for each element. Subsequently, the optical density emissions recorded from each of the samples was matched against those in the curve. Using the curve to extrapolate the quantity of each potassium and sodium ions in the sample, the experiment was repeated two more times to get a mean concentration. The concentration of the test mineral was calculated as follows;

$$\text{Kmg}/100\text{g} \frac{100}{W} \times \frac{1}{1000} \times X_x \times \frac{V_f}{V_a} D \dots \dots \dots (2)$$

Where:

W = Weight of sample used

X = Concentration (in ppm) from curve

V<sub>f</sub> = Total volume of extract

V<sub>a</sub> = Volume of the extract (digest) flame

D = Dilution factor where applicable

**2.9.3 Determination of phosphorus**

Analysis of phosphorus content was conducted using Varian AA240 Atomic Spectrophotometer (AAS) according to the method of APHA (1995). Approximately 2 g of the sample was weighed into a digestion flask and 20 ml of the acid mixture (650 ml conc. HNO<sub>3</sub>, 80 ml perchloric acid, 20 ml Conc. H<sub>2</sub>SO<sub>4</sub>) was added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to 100 ml mark. Appropriate dilutions were then made for each element. A series of standard metal solution in the optimum concentration range were prepared daily by diluting the single stock element solutions with water containing 1.5 ml concentrated nitric acid/liter. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for phosphorus was prepared by plotting the absorbance of standard versus the concentration.

**2.10 Vitamin analysis**

**2.10.1 Determination of carotenoids (Provitamin A)**

Vitamin A was determined by the calorimetric method of Kirk and Sawyer (1991). Approximately 1 g of the sample and standard were mixed with 30 ml of absolute alcohol and 3 ml of 5% KOH solution was added to it and was boiled for 30 min under reflux. After washing with distilled water, vitamin A was extracted with 150ml of diethyl ether. The extract was evaporated to dryness at low temperature and then dissolved in 10 ml of isopropyl alcohol. Exactly 1 ml of standard Vitamin A solution was prepared and that of the dissolved extract were transferred to separate cuvettes and their respective absorbance were read in a spectrophotometer at 325 nm with a reagent blank at zero.

$$\text{Conc. of Vitamin A in Sample} = \frac{\text{Abs of Sample}}{\text{Abs of Std}} \times \text{conc. of STD} \dots \dots \dots (3)$$

**2.10.2 Determination of thiamine (Vitamin B<sub>1</sub>)**

The spectrophotometric method, described by Okwu (2004) was used for determination of the B Vitamins. Exactly 5 g of each sample was homogenized with 50 ml of 1N ethanolic sodium hydroxide and the homogenate was filtered to obtain the filtrate to be used for the analysis. An aliquot (10 ml) of the filtrate was treated with equal volume of 0.1N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution in a flask. Standard thiamine solution was prepared and diluted to a chosen concentration (0.5). An aliquot of the standard thiamine solution was also treated with 10 ml of the dichromate solution (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) in a separate flask while a reagent blank was set up by treating 10 ml of the ethanolic sodium hydroxide with the potassium dichromate solution. The absorbance of the sample and the standard solutions was measured in a spectrophotometer at a wavelength of 360 nm with the reagent blank to be used to calibrate the instrument at zero. The thiamine content was calculated using the formula:

$$\text{Thiamine} \frac{\text{mg}}{100\text{g}} = \frac{100}{W} \times \frac{A_u}{A_s} \times \frac{C}{1} \times \frac{V_f}{V_a} \times D \dots \dots \dots (4)$$

Where:

W - Weight of sample analyzed

A<sub>u</sub> = Absorbance of sample

A<sub>s</sub> = Absorbance of standard solution

C = Concentration (mg/ml) of standard solution

V<sub>f</sub> = Total volume of filtrate

V<sub>a</sub> = Volume of filtrate analyzed

D = Dilution factor where applicable

**2.10.3 Determination of riboflavin (Vitamin B<sub>2</sub>)**

Approximately 1 g of sample was weighed into a conical flask and was dissolved with 100 ml of deionized water. This was shaken thoroughly and heated for 5 min and allowed to cool and then filtered. The filtrate was poured into cuvettes and their respective wavelengths for the vitamins set to read the absorbance using spectrophotometer.

Vitamin B<sub>1</sub> = 261nm

Vitamin B<sub>2</sub> = 242nm

$$\text{Vitamin conc. (mg/\%)} = \frac{\text{AXD.FX Vol. of cuvette}}{\dots} (5)$$

E

Where A = Absorbance

E = Extinction co-efficient = 25 for B<sub>1</sub> and B<sub>2</sub>

DF = Dilution factor

**2.10.4 Determination of niacin (Vitamin B<sub>3</sub>)**

A measured weight (5 g) of each sample was treated with 50 ml of 1N sulphuric acid (H<sub>2</sub>SO<sub>4</sub> solution) and was shaken for 30 min. The mixture was treated further with 3 drops of aqueous ammonia and filtered. The filtrate (extract) was used for the analysis. Standard niacin (nicotinic acid) solution was prepared and diluted as desired. 10 ml portion of the standard solution, sample extract and 10 ml of the acid solution (treated with a drop of ammonia) was dispensed into separate flasks to serve as standard, the sample and reagent blank respectively. Each of them was treated with 5 ml of normal potassium cyanide solution and acidified with 5 ml of 0.02N H<sub>2</sub>SO<sub>4</sub> solution; its absorbance was read in a spectrophotometer at a wavelength of 470 nm. The reagent blank was used to calibrate the instrument at zero. Niacin content was calculated using the formula;

$$\text{niacin mg/100g} = \frac{100}{W} \times \frac{A_u}{A_s} \times \frac{C}{1} \times \frac{V_f}{V_a} \times D \dots\dots\dots (6)$$

Where:

W - Weight of sample analyzed

A<sub>u</sub> = Absorbance of sample

A<sub>s</sub> = Absorbance of standard solution

C = Concentration (mg/ml) of standard solution

V<sub>f</sub> = Total volume of filtrate

V<sub>a</sub> = Volume of filtrate analyzed

D = Dilution factor where applicable

C = Concentration of standard solution

V<sub>f</sub> = Total volume of filtrate

V<sub>a</sub> = Volume of filtrate analyzed

D = Dilution factor where applicable

**2.10.5 Determination of ascorbic acid (Vitamin C)**

The method described by Okwu and Josiah, 2006 was used. Exactly 10g of the sample was extracted with 50ml EDTA/TCA (50g in 50ml of water) extracting Solution for 1 hour and filtered through a Whatman filter paper into a 50ml volumetric flask and made up to the mark with the extracting solution. Twenty (20ml) of the-extract was pipette into a 250ml conical flask and 10ml of 30% K.I was added and also 50ml of distilled water added. This was followed by 2ml of 1% starch indicator. This was titrated against 0.0ml CuSO<sub>4</sub> solution to a dark end point.

$$\text{Vit. C} \left( \frac{\text{mg}}{100\text{g}} \right) = 0.88 \times \frac{100}{5} \times \frac{V_f}{20} \times \frac{T}{1} \dots\dots\dots (7)$$

Where:

V<sub>f</sub> = Volume of extract

T = Sample titre – blank titre.

**2.11 Phytochemical analysis**

**2.11.1 Determination of tannin**

The Folin-Denis spectrophotometric method was used as described by Onwuka, (2005). About 1.0g of the test sample was dispersed in 10ml distilled water and agitated. The mixture was left to stand for 30min at room temperature. It was shaken for 5min after standing for 50min. The extract to be used for the analysis was gotten after centrifugation. 2.5ml of the supernatant was dispersed into a 50ml volumetric flask. In the same way, 2.5ml of the standard tannic acid solution was dispersed into a separate 50 ml flask. Folin-Denis reagent (1 ml) was measured into each flask, followed by 2.5ml of saturated Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was then diluted to mark in the flask (50ml), and it was incubated for 90min at room temperature. The absorbance was measured at 250nm in a Genway model 6000 electron spectrophotometer. Readings was taken with the reagent blank at zero. The tannin content was calculated as:

$$100 \% \text{ tannin} = \frac{A_n}{A_s} \times C \times \frac{100}{W} \times \text{Five} \dots\dots\dots (8)$$

Where:

A<sub>n</sub> = Absorbance of test sample

A<sub>s</sub> = Absorbance of standard solution

C = Concentration of standard solution

W = Weight of sample used

V<sub>f</sub> = Total volume of extract



V<sub>a</sub> = Volume of extract analyzed

### 2.11.2 Determination of alkaloid

The alkaloid content of the test sample was determined by the gravimetric method as described by Onwuka, (2005). About 5.0g of the test samples was dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was properly shaken and allowed to stand for 4h before filtration. The filtrate was allowed to evaporate to one quarter (1/4) of the original volume. A drop of the cone NH<sub>4</sub>OH was added to the remaining filtrate to precipitate the alkaloids. The precipitate was filtered off with a weighed filter paper and then washed with 1% NH<sub>4</sub>OH solution. The precipitate in the filter paper was dried in an oven at 60°C for 50min and reweighed.

**Note:** By weight difference, the weight of alkaloid were determined and expressed as a percentage of the sample weight analyzed. It was calculated as;

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{W} \times 100 \dots \dots \dots (9)$$

Where:

W = Weight of sample

W<sub>1</sub> = Weight of empty filter paper

W<sub>2</sub> = Weight of paper plus precipitate.

### 2.11.3 Determination of phytic acid (phytate)

This was determined using method described by Onwuka (2005). The sample was first extracted with 0.2N HCL, 0.5ml of the extract solutions was pipetted into a test tube fitted with a round glass stopper. Ferric acid solution (1 ml) was added and the tube was covered well. The tube was later heated in a boiling water bath for 30min. After heating, the tube was cooled in ice water for 15min and allowed to adjust to room temperature. The tube was then mixed and centrifuged for 30 min at 3000rpm. The supernatant (1ml) was transferred to another tube and 1.5ml of 2, 2, Bipyridine solution and the absorbance were measured at 519nm against distilled water.

Calculation:

$$\text{Phytic Acid (mg/g)} = \left( \frac{v_f}{v_x} \times \frac{100}{w} \times \frac{1}{100} \right) \dots \dots \dots (10)$$

Where:

V<sub>f</sub> = Total volume of extract

V<sub>x</sub> = Volume of extract used

w = weight of sample used

x = ppm of curve.

### 2.11.4 Determination of saponins

Saponins determination was carried out by the method described by Hamzah *et al.* (2014). Approximately 5g of the test sample was milled and weighed into a conical flask and 100cm<sup>3</sup> of aqueous ethanol was added. The solution was heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with 20% ethanol at 100°C. The combined extract was reduced to 40cm<sup>3</sup> over a water bath at 90°C. The concentrate was transferred into 250cm<sup>3</sup> separating funnel and 20cm<sup>3</sup> diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the other layer was discarded. The purification process was repeated. The N-butanol extract (60cm<sup>3</sup>) was washed twice with 10cm<sup>3</sup> 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;

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W_s} \times 100 \dots \dots \dots (11)$$

Where;

W<sub>2</sub> = weight of beaker + extract

W<sub>1</sub> = weight of beaker

W<sub>s</sub> = weight of sample

### 2.11.5 Determination of flavonoid

The method described by Hamzah *et al.* (2014) was used. Exactly 5 g of the milled sample was weighed into 250cm<sup>3</sup> beaker and extracted with 100cm<sup>3</sup> of 95% ethanol for 30min at room temperature. The whole solution was filtered through Whatman filter paper no 42 (125mm). The filtrate was transferred later into a crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weight of flavonoids was determined by difference and expressed as percentage of the sample weight analyzed as shown;



$$\% \text{ flavonoids} = \frac{W_2 - W_1}{W_s} \times 100 \dots \dots \dots (12)$$

Where;

$W_2$  = weight of crucible + extract

$W_1$  = weight of crucible

$W_s$  = weight of sample

### 2.12 Statistical analysis

Statistical analysis was performed using SPSS version 17.0. Analysis of variance (ANOVA) was conducted on the mean values and significance difference was determined using Duncan Multiple range test.

## 3.0 RESULTS AND DISCUSSION

### Mineral composition of biscuits

The result for mineral composition of biscuit samples as seen in Table 2 show that there was significant ( $p < 0.05$ ) difference in potassium content, with the exception of Samples A and F that both recorded the highest value of 0.57mg/g. The higher values of blended flour biscuits might be attributed to appreciable amount of this mineral in African breadfruit and maize. The values were however lower than the 1086.67 to 5350 mg/kg for potassium content of high protein fiber snacks reported by Opeoluwa *et al.* (2015). The difference might be due to variation in flour and other ingredients used. Potassium is an essential nutrient and has important role in the synthesis of amino acid and protein in man.

There was significant ( $p < 0.05$ ) difference in sodium with the wheat flour biscuit recording the highest value of 5.0mg/g. The wide gap in sodium content of the control and the blended flour samples suggest that wheat is a good source of sodium. The high sodium value of the wheat biscuit is in conformity with the 3346.67 mg/kg for sodium content of wheat fiber snacks reported by Opeoluwa *et al.* (2015). The amount of sodium and potassium in the blended flour samples is an indication that the snacks will be beneficial for people with cardiovascular diseases (Ogbuagu *et al.*, 2011). Evidence has also shown that high intake of potassium against sodium prevent cardiovascular diseases ( Ogbuagu *et al.*, 2011; Opeoluwa *et al.*, 2015).

The samples were significantly ( $p < 0.05$ ) different in phosphorus with the blended flour biscuits recording higher values. The comparatively higher values of phosphorus in Samples C and D may be attributed to the fair share of this mineral in African breadfruit and maize. There was significant ( $p < 0.05$ ) difference in calcium, with the blended flour biscuits showing higher values. However, there was no significant ( $p < 0.05$ ) difference in calcium content of Samples A, B and E with a common value of 0.60mg/g, apparently indicating an even distribution of this mineral in African breadfruit and maize. Calcium intake in diabetics had been shown to be beneficial and likely to reduce osteoporosis in older diabetics (Cryer, *et al* 1994).

There was significant ( $p < 0.05$ ) difference in magnesium content of samples with the wheat flour biscuit lowest with 0.05mg/g. The probable mineral enrichment of the blended flour biscuits contributed by the whole breadfruit fraction is corroborated by the 0.53mg/g of potassium, 0.87mg/g of calcium, 0.17mg/g of magnesium and 0.53mg/g magnesium reported by Ejidike and Ajileye (2007) for African breadfruit seed hull.

The good mineral spread of the blended flour biscuits as evidenced in this report indicates that the products will significantly complement the daily mineral requirements of consumers.

**Table 2: Mineral composition of biscuit samples**

Sample	Potassium (mg/g)	Sodium (mg/g)	Phosphorous (mg/g)	Calcium (mg/g)	Magnesium (mg/g)
A	0.51 <sup>b</sup> ±0.00	0.49 <sup>e</sup> ±0.00	0.35 <sup>f</sup> ±0.00	0.60 <sup>a</sup> ±0.00	0.12 <sup>b</sup> ±0.00
B	0.57 <sup>a</sup> ±0.00	0.70 <sup>b</sup> ±0.00	0.51 <sup>c</sup> ±0.00	0.60 <sup>a</sup> ±0.00	0.18 <sup>a</sup> ±0.00
C	0.46 <sup>d</sup> ±0.00	0.47 <sup>f</sup> ±0.00	0.71 <sup>a</sup> ±0.00	0.40 <sup>e</sup> ±0.00	0.12 <sup>b</sup> ±0.00
D	0.32 <sup>f</sup> ±0.00	0.54 <sup>d</sup> ±0.00	0.68 <sup>b</sup> ±0.00	0.50 <sup>b</sup> ±0.00	0.06 <sup>c</sup> ±0.00
E	0.49 <sup>c</sup> ±0.00	0.53 <sup>d</sup> ±0.00	0.34 <sup>g</sup> ±0.00	0.60 <sup>a</sup> ±0.00	0.18 <sup>a</sup> ±0.00
F	0.57 <sup>a</sup> ±0.00	0.63 <sup>c</sup> ±0.00	0.37 <sup>d</sup> ±0.00	0.40 <sup>e</sup> ±0.00	0.12 <sup>b</sup> ±0.00
G	0.36 <sup>e</sup> ±0.00	5.00 <sup>a</sup> ±0.00	0.36 <sup>e</sup> ±0.00	0.03 <sup>d</sup> ±0.00	0.05 <sup>d</sup> ±0.00

Mean values within a column with different superscript are significantly ( $P < 0.05$ ) different.

A = WBF:MF:DCG (70: 20: 10); B = WBF:MF:DCG (60: 30: 10); C = WBF:MF:DCG (45: 45: 10) D = WBF:MF:DCG (30: 60: 10); E = WBF:MF:DCG (20: 70: 10); F = HBF:MF:DCG (45: 45: 10) G = 100% WF ( Control)

\*WBF = Whole Breadfruit Flour, HBF = Hulled Breadfruit Flour, MF = Maize Flour, WF=Wheat flour.

### Vitamin composition of biscuits

Result in Table 3 show that there was significant ( $p < 0.05$ ) difference in caratenoids (Provitamin A) content of samples with Sample A recording the highest value of 1955  $\mu\text{g}/100\text{g}$ . The significant reduction in this provitamin with reduction in whole

breadfruit flour substitution suggests that this Vitamin A precursor may be concentrated in the seed hull. This is expectedly augmented by the yellow maize purposely used for its carotenoid content. The values obtained correlated with the 2.42 to 6.01 mg/100g for ready to eat snacks as reported by Okafor and Ugwu (2014). Vitamin A is involved in immune function, vision, reproduction and cellular communication (IOM, 2001).

There was significant ( $p<0.05$ ) difference in thiamine (Vitamin B<sub>1</sub>) content of the samples with the wheat flour biscuit recording the highest value of 31.17mg/100g, closely followed by Sample A with 27.99mg/100g. The simultaneous increase in the thiamine profile with breadfruit flour substitution is an indication that African breadfruit seeds are rich in this vitamin. The values obtained are much higher than the 0.130 to 0.423mg/100g reported by Okafor and Ugwu (2014) for ready to eat snacks made from blends of breadfruit, cashew nut and coconut. They are also in excess of the recommended minimum daily intake of 0.2 to 1.0mg (Belitz *et al.*, 2009). There was significant ( $P<0.05$ ) difference in riboflavin (Vitamin B<sub>2</sub>) content of samples with Sample A leading with 4.36mg/100g and closely followed by 4.22mg/100g of Sample G (control). The change in Vitamin B<sub>2</sub> profile followed similar trend as in Vitamin A, thus confirming that legumes are good source of vitamins, and that the B vitamins are more concentrated in the hull region of African breadfruit seeds. Again the values obtained exceeded the recommended daily intake of 0.3 to 1.6mg (Belitz *et al.*, 2009).

There was significant ( $p<0.05$ ) difference in Vitamin B<sub>3</sub> content of the samples with Sample F leading with 4.37mg/100g closely followed by Sample A with 4.23mg/100g, while the wheat flour biscuit (control) recorded a distant least of 1.38mg/100g. This equally took similar pattern as in Vitamins B<sub>1</sub> and B<sub>2</sub>, and as before will be attributed to the rich deposit of the B vitamins in African breadfruit seeds. The values are much higher than the 0.157 to 0.477mg/100g reported by Okafor and Ugwu (2014) for ready to eat snacks produced with African breadfruit, cashew nut and coconut flour blends. The B vitamins are needed for carbohydrate and protein metabolism, and are essential for growth, well structuring and functioning of the cells (Ihekoronye and Ngoddy, 1985).

**Table 3: Vitamin composition of biscuit samples**

Sample	Provitamin A µg/100g	Thiamine B1 (mg/100g)	Riboflavin B2 (mg/100g)	Niacin B3 (mg/100g)	Ascorbic-acid C (mg/100g)
A	1955.95 <sup>a</sup> ±0.01	27.59 <sup>b</sup> ±0.13	4.36 <sup>a</sup> ±0.01	4.23 <sup>b</sup> ±0.00	23.72 <sup>a</sup> ±0.02
B	1945.91 <sup>b</sup> ±0.01	25.53 <sup>c</sup> ±0.01	4.25 <sup>b</sup> ±0.01	4.21 <sup>b</sup> ±0.00	22.00 <sup>a</sup> ±0.00
C	1010.10 <sup>c</sup> ±0.00	20.42 <sup>e</sup> ±0.03	4.18 <sup>c</sup> ±0.01	4.18 <sup>b</sup> ±0.01	14.07 <sup>b</sup> ±0.01
D	1018.50 <sup>d</sup> ±0.27	17.36 <sup>f</sup> ±0.01	4.15 <sup>c</sup> ±0.05	4.06 <sup>c</sup> ±0.01	8.55 <sup>c</sup> ±0.01
E	333.43 <sup>e</sup> ±0.18	17.21 <sup>g</sup> ±0.01	1.17 <sup>d</sup> ±0.00	4.05 <sup>c</sup> ±0.01	4.77 <sup>d</sup> ±0.01
F	976.89 <sup>f</sup> ±0.06	20.67 <sup>d</sup> ±0.07	1.08 <sup>e</sup> ±0.01	4.37 <sup>a</sup> ±0.17	4.95 <sup>d</sup> ±0.01
G	1919.63 <sup>c</sup> ±0.21	31.17 <sup>a</sup> ±0.01	4.22 <sup>b</sup> ±0.01	1.38 <sup>d</sup> ±0.01	21.11 <sup>a</sup> ±0.01

Mean values within a column with different superscript are significantly ( $P<0.05$ ) different.

A = WBF:MF:DCG (70: 20: 10); B = WBF:MF:DCG (60: 30: 10); C = WBF:MF:DCG (45: 45: 10) D = WBF:MF:DCG (30: 60: 10); E = WBF:MF:DCG (20: 70: 10); F = HBF:MF:DCG (45: 45: 10) G = 100% WF ( Control)

There was significant ( $p<0.05$ ) difference in Vitamin C content of some of the samples with Sample A excelling with 23.72mg/100g while sample E was the least with 4.77mg/100g. There was no significant ( $p>0.05$ ) difference in the Vitamin content of Sample A and Sample G (control), suggesting that Vitamin C may be similarly deposited in cereals and legumes. However, the higher values in the samples containing whole breadfruit flours might imply that the vitamin is more concentrated in the hull. The values compared favorably with the value 17.3mg/100g reported by Olapade and Ogunade (2014), except for Samples D, E and F with less hull. Vitamin C is a strong water soluble antioxidant that helps the body develop resistance against infectious agents and scavenges harmful free radicals.

### 3.3 Phytochemical levels in biscuit samples

Results in Table 4 show that there was significant ( $p<0.05$ ) difference in the saponins content of the biscuits with Sample A recording the highest presence of 0.19mg/100g, while Sample G (control) had the lowest value of 0.10mg/100g. The saponins content increased with the level of substitution of the whole breadfruit flour, suggesting that this phytochemical could be more concentrated in the hull. The lower value of saponins in wheat flour biscuit might be due to less presence in the wheat grain, or as

a result of processing method that might have reduced the presence in the proprietary flour used. Samaila and Nwabueze (2013) reported that processing causes reduction of antinutrients in food products. Such processing steps might include hulling, soaking and heating. However, application of heat in breadfruit processing should be controlled to avoid loss of nutrient (Giami *et al.*, 2001). The low levels obtained in the samples are desirable since saponins have been reported to lower plasma cholesterol concentration (Ojinnaka *et al.*, 2013) and inhibit growth of cancer cells (Onimawo and Akubor, 2005).

There was significant ( $p < 0.05$ ) difference in tannin levels of the samples with Sample A showing the highest value of 0.71mg/100g, while Sample G (control) was the lowest with 0.08mg/100g. The levels in other breadfruit containing samples show that this substance is not restricted to the hull of the breadfruit seed, since the value was significantly high in the sample containing hulled breadfruit flour. Chung *et al.* (1998) has observed that tannins are water soluble polyphenols and have been reported to have anticarcinogenic and antimutagenic potentials which protect cellular oxidative damage, including lipid peroxidation in man.

Furthermore, the presence of tannins and saponins in the formulated snacks could have some health benefits as noted by Adeyeye (1998).

**Table 4: Phytochemical levels in biscuit samples**

Sample	Saponins mg/100g	Tannin mg/100g	Alkaloid mg/100g	Flavonoid mg/100g	Phytate mg/100g
A	0.19 <sup>a</sup> ±0.00	0.71 <sup>a</sup> ±0.01	1.01 <sup>a</sup> ±0.01	1.99 <sup>a</sup> ±0.00	0.09 <sup>a</sup> ±0.00
B	0.16 <sup>b</sup> ±0.01	0.53 <sup>b</sup> ±0.00	0.47 <sup>c</sup> ±0.01	1.21 <sup>c</sup> ±0.01	0.02 <sup>b</sup> ±0.00
C	0.16 <sup>b</sup> ±0.01	0.27 <sup>d</sup> ±0.01	0.42 <sup>d</sup> ±0.03	1.18 <sup>c</sup> ±0.01	0.02 <sup>b</sup> ±0.00
D	0.15 <sup>b</sup> ±0.00	0.18 <sup>e</sup> ±0.01	0.52 <sup>b</sup> ±0.03	1.10 <sup>d</sup> ±0.00	0.02 <sup>b</sup> ±0.01
E	0.13 <sup>e</sup> ±0.01	0.14 <sup>f</sup> ±0.01	0.30 <sup>e</sup> ±0.00	1.04 <sup>e</sup> ±0.05	0.01 <sup>c</sup> ±0.00
F	0.13 <sup>e</sup> ±0.00	0.46 <sup>c</sup> ±0.01	0.50 <sup>bc</sup> ±0.00	1.52 <sup>b</sup> ±0.03	0.01 <sup>c</sup> ±0.001
G	0.10 <sup>d</sup> ±0.00	0.08 <sup>g</sup> ±0.00	0.10 <sup>f</sup> ±0.00	0.98 <sup>e</sup> ±0.01	0.01 <sup>c</sup> ±0.00

Mean values within a column with different superscript are significantly ( $P < 0.05$ ) different.

A = WBF:MF:DCG (70: 20: 10); B = WBF:MF:DCG (60: 30: 10); C = WBF:MF:DCG (45: 45: 10) D = WBF:MF:DCG (30: 60: 10); E = WBF:MF:DCG (20: 70: 10); F = HBF:MF:DCG (45: 45: 10) G = 100% WF (Control).

There was significant ( $p < 0.05$ ) difference in alkaloid with the highest levels of 1.01mg/100g in Sample A, and the lowest (0.10mg/100g) in sample G. The higher level in breadfruit containing samples might be due to its lower presence in wheat. There was significance ( $p < 0.05$ ) difference in flavonoid content of samples with Sample A leading with 1.99mg/100g and closely followed by Sample F with 1.52mg/100g; while Sample G recorded the lowest level of 0.98mg/100g. The relatively high levels of flavonoid in Sample F which contain hulled breadfruit flour suggest that it is distributed in the seed rather than concentrated in the hull. The lower level in the control might mean that wheat contain less flavonoid than breadfruit.

There was significant ( $p < 0.05$ ) difference in phytate composition with the highest level of 0.09mg/100g is found in Sample A, while Samples E, F and G had the lowest equal levels of 0.01mg/100g each. The higher presence of this antinutrient in samples containing higher amounts of whole breadfruit flour suggests that this substance is predominant in the seed hull. Abubakar *et al.* (2010) reported phytate level of 0.86mg/100g for sweet potato dishes. Anounye *et al.* (2012) has noted that phytic acid forms insoluble complex with certain trace elements, zinc, iron and copper reducing their bioavailability with resultant effects of reduced turnover of hemoglobin production and impaired metabolic process. The low levels of phytate in the entire sample is desirable since its presence in foods was reported to possess beneficial effects as it contains antioxidants that eliminate free radicals from the body system (Pamplona-Roger, 2006). However, high levels of this insoluble salt are undesirable since this may obstruct kidney glomerulus/tubules leading to kidney stones (Coe, 2005). The tannin and phytate levels of all the snacks considered were lower than the ranges 0.11 to 0.44mg/100g and 0.5 to 1.13mg/100g respectively as reported by Omah and Okafor (2015) for cookies obtained from blend of millet, pigeon pea composite flour and cassava cortex, which make these biscuit samples safer. Meanwhile, the disparity in the anti-nutrient contents of the snacks is expected due to the difference in the raw materials used.

## CONCLUSION

It is evident from this research that high fiber biscuits produced from flour blends of African Breadfruit, maize and coconut possess comparative mineral, vitamin and phytochemical spread. Although superior in mineral and vitamin profile, the higher levels of phytochemicals in samples containing whole breadfruit flour, particularly those with antinutritional properties, require further research on the inclusion of seed hull in flour development for high fiber snacks. Attention should be paid towards exploiting the rich mineral and vitamin endowment in agricultural materials in our domain, while paying due attention to keeping their residual phytochemicals at acceptable limits.

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