The effect of plantain and tigernut flours substitution on the antioxidant, physicochemical and pasting properties of wheat-based composite flours

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The effect of plantain and tigernut flours substitution on the antioxidant, physicochemical and pasting properties of wheat-based composite flours

Y.A. Bamigbola1, O.O. Awolu* and I.B. Oluwalana1

Abstract: Wheat based composite flour was substituted with plantain and tigernut flours in order to enhance its protein, fibre, minerals, antioxidants and resistant starch contents. The proximate and compositions were optimized using optimal model design of response surface methodology. The independent variables were wheat flour (60–77%), plantain flour (92–37%) and tigernut flour (3–10%). The optimum blends, that is, blends with overall best protein, fibre and minerals contents were chosen for subsequent analyses (functional properties, amylose and amylopectin contents, resistant starch, dietary fibre, pasting characteristics and antioxidant properties). The control was 100% wheat flour. From the results, the optimum blends obtained were runs 2 (70% wheat, 20% plantain and 10% tigernut flours), 13 (77% wheat, 20% plantain and 3% tigernut flours) and 15 (65.66% wheat, 29% plantain and 5.33% tigernut flours) with overall best ash, fibre, protein and mineral contents. Substitution of wheat flour with plantain and tigernut flours reduces the swelling capacity and foaming capacity while there are no significant \((p \leq 0.05)\) differences in the oil absorption capacity at all levels of substitution. Substitution of wheat flour with plantain and tigernut however

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PUBLIC INTEREST STATEMENT

Several people in developing and underdeveloped countries are nutritionally deficient resulting in diseases ravaging the populace. The development of nutritionally balanced food is meant to combat the diseases. Bread is a ready-to-eat baked product available to underdeveloped and developed countries. The consumption of wheat-based baked products has resulted in celiac diseases for people who are gluten intolerant. Substitution of wheat with plantain and tigernut is meant to address this problem in addition to enhancing the nutritional quality of bread since bread is readily available to both the rich and the poor. This substitution will enhance the protein, fibre, minerals, antioxidants and resistance starch contents of bread making them functional and health promoting.
increases the water absorption capacity. In addition, substitution of wheat flour with plantain and tigernut flour above 30% significantly ($p \leq 0.05$) increases the antioxidant properties and insoluble dietary fibre. However, 100% wheat flour had better pasting characteristics. The study therefore showed that substitution of wheat flour with plantain and tigernut flour (30% and above) greatly enhanced its nutritional quality while the 100% wheat flour was better in terms of pasting characteristics.

Subjects: Food Analysis; Nutraceuticals & Functional Foods; Processing; Product Development

Keywords: antioxidants; composite flour; optimization; pasting characteristics; plantain flour; tigernut flour; wheat flour

1. Introduction
There is an increasing demand for convenient, ready-to-eat baked products made from wheat flour partially or fully substituted with cereals, legumes and tubers in order to enhance their nutritional quality (Awolu, Oluwaferanmi, Fafowora, & Oseyemi, 2015; Awolu, Omoba, Olawoye, & Dairo, 2016; Awolu, Osemeke, & Ifesan, 2016; Omoba, Awolu, Olagunju, & Akomolafe, 2013). Plantain (Musa paradisiaca) is rated as the fourth most important food in the world after rice, wheat and maize, with production in regions like Southern United States, the Carribbean, Central America, Colombia, Uganda and Nigeria. In Nigeria and many African countries, plantains are used as an inexpensive source of calories (Akubor, Adamolekun, Oba, Obari, & Abudu, 2003). In terms of nutritional importance, plantain constitutes a rich energy source with carbohydrate accounting for about 22–32% of the fruit weight. It is also a good source of dietary fibre, beta carotene, calcium, iron, potassium, Vitamins B6.

Tigernut (Cyperus esculentus), though underutilized is valued for its nutritious starch content, resistant starch, dietary fibre and carbohydrate. The nut is rich in mineral contents such as sodium, calcium, potassium, magnesium, zinc and traces of copper. The flour has been established as a rich source of quality oil and contain moderate amount of protein (Oladele & Aina, 2007; Ukwuru, Ibeneme, & Agbo, 2011).

Response surface had been used in several process and products optimization (Awolu & Layokun, 2013; Awolu et al., 2015; Awolu, Omoba, et al., 2016; Awolu, Osemeke, et al., 2016; Omoba et al., 2013). Precisely, optimal mixture design of response surface methodology had been used in the statistical analysis and optimization of some nutritional properties of composite flours (Awolu et al., 2015; Awolu, Omoba, et al., 2016; Awolu, Osemeke, et al., 2016; Omoba et al., 2013).

This study aim to incorporate unripe plantain and tigernut flours into wheat flour in order to enhance its nutritional, functional and antioxidant characteristics. The effect of the addition of unripe plantain and tigernut flours on the wheat flour was also evaluated.

2. Materials and methods

2.1. Source of materials
Matured unripe plantain (false horn) bunches were obtained from the Research Farm of the Federal University of Technology, Akure, Nigeria, while dried tigernut seeds and wheat flour were obtained at Oja-Oba in Akure.

2.2. Preparation of plantain flour
Plantain flour was produced according to the method described by Akubor et al. (2003). Mature unripe plantain fingers were detached, peeled, washed, sliced and soaked in 1% ascorbic acid and 0.2% citric acid for 5 min to prevent browning. The slices were drained and oven dried at 60°C for 24 h, milled using locally fabricated hammer mill and sieved through 600 μm aperture size. The fine plantain flour was stored in sealed polyethylene bag at room temperature for further processing.
2.3. Preparation of tigernut flour

The method described by Akande and Oladokun (2009) was used with slight modification. Dry tiger-nuts were sorted to remove unwanted materials and washed until clean. The cleaned nuts were sundried for 48 h. The dried nuts were milled using attrition mill and sieved through 600 μm aperture size. The fine tigernut flour was packed and sealed in polyethylene bags until analyzed.

2.4. Proximate composition

Proximate composition, including moisture content, ash, crude fibre, crude protein, and crude fat of the composite flour were determined using AOAC (2005) methods. Carbohydrate content was evaluated by difference.

2.5. Mineral composition

Mineral element of each sample which include; Na, Ca, K, Fe, Zn and Cu were determined using the method described by Jones and Case (1990) and Isaac and Johnson (1975). Iron, calcium, zinc and copper were read using Atomic Absorption Spectrophotometer (SOLAR 929 Unicam A.A. Spectrophotometer, UK), while the Flame Photometer were used for sodium and potassium.

3. Determination of functional properties

3.1. Bulk density

Bulk density was determined using method described by Narayana and Narsinga (1992). About 25 g of sample was weighed into 100 ml graduated cylinder. The cylinder was tapped 100 times and the packed bulk density was also calculated as weight of sample per volume occupied (Equation 1).

\[
\text{Bulk Density} = \frac{\text{Weight of sample}}{\text{Volume occupied after tapping}}
\]  

(1)

3.2. Water absorption capacity

Water absorption capacity (WAC) of sample was determined using the method outlined by Diniz and Martin (1997) with some modifications. About 0.5 g of the sample was dissolved with 10 ml of distilled water in centrifuge tubes and vortexed for 30 s. The dispersions were allowed to stand at room temperature for 30 min, centrifuged at 3,000 rpm for 25 min. The supernatant was filtered with Whatman No. 1 filter paper and the volume retrieved was accurately measured. The difference between initial volumes of distilled water added to the sample and the volume obtained after filtration was determined. The results were reported as mL of water absorbed per gram of sample and calculated using Equation (2).

\[
\text{Water Absorption Capacity} = \frac{\text{Amount of water absorbed}}{\text{Weight of sample}}
\]  

(2)

3.3. Oil absorption capacity

Oil absorption capacity (OAC) was determined using the method of AOAC (2005). About 1 g of the sample (\( W_0 \)) was weighed into pre-weighed 15 ml centrifuge tubes and thoroughly mixed with 10 ml (\( V_1 \)) of refined pure groundnut oil using vortex mixer. Samples were allowed to stand for 30 min. The sample-oil mixture was centrifuged at 3,000 rpm for 20 min. Immediately after centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder, and the volume was recorded (\( V_2 \)). OAC (milliliter of oil per gram of sample) was calculated using Equation (3).

\[
\text{Oil Absorption Capacity} = \frac{V_1 - V_2}{W_0}
\]  

(3)

3.4. Foaming capacity and stability

Foaming capacity (FC) was determined in triplicate using the method described by Narayana and Narsinga (1992). Concentration of 1% of the sample was prepared in deionized water and adjusted to pH 7.4 with 1.0 N NaOH and 1.0 N HCl. A volume of 100 ml (\( V_1 \)) of concentrate suspension was
blended for 3 min using a high speed blender, poured into a 250 mL graduated cylinder, and the volume of foam ($V_F$) was immediately recorded. FC was calculated using Equation (4).

$$ FC = \left( \frac{V_F}{V_1} \right) \times 100 $$  \hspace{1cm} (4)

Foam stability (FS) was determined by measuring the fall in volume of the foam after 60 min as shown in Equation (5).

$$ FS = \left( \frac{V_F \text{ after 60 min}}{V_1} \right) \times 100 $$  \hspace{1cm} (5)

### 3.5. Determination of amylose and amylopectin contents

Amylose content was determined using method described by AOAC (1995). One hundred milligrams of milled sample was weighed into 100 ml volumetric flask. One milliliter of 95% ethanol and 9 ml 1 N NaOH was added and the sample was heated for 10 min in boiling water bath. It was cooled and made up to 100 ml volume. Five millilitres from the 100 ml was pipetted into another 100 ml volumetric flask. One milliliter of 1 N acetic acid and then 2 ml iodide solution was added to make up the volume to 100 ml mark. The flask was shaken and allowed to stand for 20 min and the absorbance at 620 nm determined. This was done by preparing a series of standard starch solution containing; 0, 20, 40, 60, 80 and 100 mg/ml of amylase. The absorbance of the standards at 620 nm was read and a standard graph was plotted. Amylose content of the sample was determined in reference to the standard curve and expressed on mg/ml basis. The value obtained was subtracted from 100 to obtain the amylopectin content.

### 3.6. Determination of resistant starch

The resistant starch (RS) content of the samples was determined by the direct method of Goñi, Garcia-Alonso, and Saura-Calixto (1997). Ground samples (100 mg) were incubated with a solution containing 20 mg pepsin at 40°C for 60 min to remove any protein. Tris-maleate solution containing 40 mg pancreatic $\alpha$-amylase was added and incubated at 37°C for 16 h to hydrolyze digestible starch (DS). The hydrolysates were centrifuged and the residues were solubilized and incubated with amyloglucosidase at 60°C for 45 min to hydrolyze the RS. The glucose content was measured using a glucose oxidase peroxidase kit. The RS content comprising fractions of RS types I, II and III was calculated as mg of glucose multiplied by 0.9.

### 3.7. Determination of dietary fibre

The dietary fibre of the composite flour was determined according to the method of AOAC (1995). One gram of defatted dried sample was weighed and subjected to gelatinization and thermamyl incubation at pH 6.0 and 100°C for 30 min. Incubation was carried out using Protease enzyme at pH 7.5 and 60°C for 30 min. This was followed by Amyloglucosidase incubation at pH 4.5 and temperature of 60°C for 30 min. The total, soluble and insoluble dietary fibres were carried out as described below:

### 3.8. Determination of total dietary fibre

The residue was precipitated with 4 ml of ethanol. The solution was then filtered and the residue washed with ethanol and acetone. The residue was dried and weighed to give total dietary fibre of the sample.

### 3.9. Determination of soluble dietary fibre

The solution was filtered (Through tittered crucible containing Celite). About 4 ml of ethanol was added to the filtrate and filtration was carried out. The residue was collected in petri dish and weighed to give soluble dietary fibre.
3.10. Determination of insoluble dietary fibre

The solution was filtered (through triturated crucible containing Celite). The residue was collected and washed with ethanol and acetone. The washed residue was collected in crucible and weighed to give soluble dietary fibre.

3.11. Determination of pasting properties

The pasting properties of the samples were assessed using the Rapid Visco-Analyser (Model RVA series 4; Newport Scientific Pty Ltd., Warriewood, Australia). A 3 g sample was dispersed in an aluminium canister containing 25 ml of distilled water. The samples were tested according to Standard Profile 1, where the flour-water suspension was held at 50°C for 1 min and then heated to 95°C, held for 10 min, and then cooled to 50°C and held for another 2 min. The starch viscosity parameters measured were pasting temperature, peak viscosity, breakdown viscosity, final viscosity, trough viscosity setback viscosity and peak time. The results were expressed as RVU for all of the parameters with the exception of pasting temperature, which is expressed in °C.

3.12. Determination of total phenolic content

Total Phenolic Content (TPC) was determined by Folin–Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventós, 1999) using gallic acid as standard. Fifty microliters of the aqueous extract solution containing 0.5 mg of aqueous extract was dispensed into a test tube, 50 μl of distilled water and 500 μl of Folin–Ciocalteu reagent were added respectively and shaken thoroughly. After 3 min, 400 μl of 7.5% sodium carbonate solution was added and the mixture was incubated at 45°C in a water bath for 40 min. Absorbance was measured at 765 nm against blank. The same procedure was repeated for all standard tannic acid solution (0.1 mg/ml). The blank is a mixture of 100 μl of distilled water, 500 μl of Folin–Ciocalteu reagent and 400 μl of 7.5% sodium carbonate. All tests were carried out in triplicate. The TPC was expressed as gallic acid equivalent per gram of sample (mg of GAE/g sample)

3.13. Determination of total flavonoid content

Total Flavonoid Content was determined by aluminum chloride colorimetric assay (Bushra, Farooq, & Muhammad, 2009) with slight modifications. 500 μl of methanol was added to 10 ml flask containing 500 μl of aqueous extract. To this 500 μl, 10% AlCl3 and 50 μl of 1 M CH3COOH was added respectively. The total volume was made up to 2,500 μl with distilled water. The solution was then incubated at room temperature for 30 min. Absorbance was read against blank at 415 nm with spectrometer.

3.14. Determination of 2,2-Diphenyl-picryl-hydrazyl (DPPH) assay

The hydrogen atom or electrons donating ability of the corresponding samples were measured from the bleaching of purple coloured methanol solution of DPPH. The spectrometric assay uses the stable radical 2,2-diphenyl picryl hydrazyl (DPPH) as a reagent (Burits & Bucar, 2000).

3.15. Statistical analyses

All analyses were carried out in triplicates and the results of the triplicates were expressed as mean of triplicate determinations ± standard deviation. The SPSS 16.0 for windows computer software package was used for one way analysis of variance (ANOVA) and the Pearson correlation coefficients significance of the differences was ascribed at \( p \leq 0.05 \). The difference in means was compared using the Duncan’s new Multiple Range test. Optimization and statistical analyses of proximate and mineral compositions was carried out using optimal mixture design of response surface methodology (Design expert 8.0.3.1 trial version by Stat-ease Minneapolis, USA).

4. Results and discussion

4.1. Proximate composition of composite flours

The result of the proximate composition of the composite flour is presented in Table 1. The moisture content of the flour formulations ranged from 5.63 to 6.93 g/100 g. The moisture content of the flour formulations was within the acceptable limit of not more than 10% for long term storage of flour.
Moisture content and water activity have been reported to have great effects on the keeping quality and shelf life of foods (Eke-Ejiofor & Owuno, 2012). The model (Special quartic and model terms (linear mixture components $A^2BC$, $AB^2C$ and $ABC^2$) is significant ($p \leq 0.05$). The $R^2$ and the adjusted $R^2$ values are 0.9777 and 0.9521 respectively which indicate good model fitting (Awolu et al., 2015). The final equation representing the effect of the variables on moisture is given in Equation (6). The equation showed that tigernut flour ($C$) with the highest coefficient (+8.47) had highest positive effect on the moisture. $AB^2C$ (wheat, plantain squared and tigernut) with the least coefficient ($-99.66$) had the least effect on moisture content.

$$\text{Moisture} = +6.31A + 6.30B + 8.47C + 0.33AB - 1.33AC - 2.01AC - 77.73A^2BC - 99.66AB, C$$ (6)

The ash content of the flour blends ranged from 1.16 to 1.69 g/100 g. Plantain and tigernut flour contributed largely to total ash content as both have higher ash content than wheat flour. The ash content of tigernut as reported by Oladele and Aina (2007) is 3.97% while that of plantain flour is 2.80% (Evanse Inyang & Ekop, 2015). Soft wheat flour, however, had ash content of 1.00% (David, Arthur, Kwadwo, Badu, & Sakyi, 2015). High ash content is indicative of more mineral elements in the flour blends which could be of immense benefits to the body. The $R^2$ and the adjusted $R^2$ values were 0.8618 and 0.7039 respectively. The 3D plot showing the interactions between the variables (wheat flour, plantain flour and tigernut flour) and response (ash) is shown in Figure 1d. The model (special quartic) and model terms (special quartic mixture, $AC$, $BC$) are significant ($p \leq 0.05$). The final equation representing the effect of the variables on ash is given in Equation (7).

$$\text{Ash} = +1.20A + 1.63B + 6.12C + 0.069AB - 6.38AC - 7.75BC + 3.30A^2BC - 25.24AB^2C - 15.81ABC^2$$ (7)

The fat content of the flour blends ranged from 2.63 to 4.98 g/100 g. Oluwalana, Oluwamukomi, Fagbemi, and Oluwafemi (2011) observed that fat content of plantain flour is between the range of 2.24–2.63% while 24.29% was found in tigernut as reported by Alegria-Toran and Farre-Rovira (2003). According to the report of David et al. (2015), wheat flour has fat content of 1.33%. Thus, tigernut flour might have contributed a higher percentage of the fat in the flour blends. Flours high in fats have

| Runs | A (%) | B (%) | C (%) | Moisture | Ash | Fat | Fiber | Protein | CHO |
|------|-------|-------|-------|----------|-----|-----|-------|---------|-----|
| 1    | 77.00 | 20.00 | 3.00  | 6.28     | 1.18| 3.00| 2.97  | 9.47    | 77.09|
| 2    | 70.00 | 20.00 | 10.00 | 6.93     | 1.68| 4.98| 3.17  | 9.34    | 73.98|
| 3    | 60.00 | 37.00 | 3.00  | 6.29     | 1.64| 2.67| 3.11  | 8.24    | 78.03|
| 4    | 70.00 | 20.00 | 10.00 | 6.86     | 1.69| 4.70| 3.22  | 9.10    | 74.40|
| 5    | 69.00 | 23.33 | 7.66  | 5.63     | 1.37| 3.96| 3.14  | 9.08    | 76.11|
| 6    | 60.00 | 37.00 | 3.00  | 6.29     | 1.64| 2.85| 3.04  | 8.25    | 77.92|
| 7    | 60.00 | 37.00 | 3.00  | 6.30     | 1.63| 2.77| 3.36  | 8.25    | 77.68|
| 8    | 65.87 | 25.87 | 8.25  | 6.36     | 1.55| 4.00| 3.17  | 8.88    | 76.52|
| 9    | 60.00 | 30.00 | 10.00 | 6.79     | 1.65| 4.47| 3.24  | 8.45    | 75.39|
| 10   | 68.50 | 28.50 | 3.00  | 6.38     | 1.25| 2.69| 3.26  | 8.88    | 77.59|
| 11   | 71.33 | 25.67 | 3.00  | 6.37     | 1.56| 2.63| 3.05  | 9.10    | 77.25|
| 12   | 73.50 | 20.00 | 6.50  | 6.46     | 1.16| 3.85| 3.25  | 9.36    | 75.90|
| 13   | 77.00 | 20.00 | 3.00  | 6.36     | 1.18| 2.70| 3.07  | 9.49    | 77.20|
| 14   | 60.00 | 33.50 | 6.50  | 6.48     | 1.29| 3.38| 3.48  | 8.30    | 77.05|
| 15   | 65.66 | 29.00 | 5.33  | 6.90     | 1.56| 3.50| 3.44  | 8.75    | 75.82|
| 16   | 60.00 | 30.00 | 10.00 | 6.59     | 1.54| 4.39| 3.38  | 8.45    | 75.63|

Table 1. Proximate composition (g/100 g) of composite flour blends

Notes: A = Wheat flour, B = Plantain flour, C = Tigernut flour, CHO = Carbohydrate.
been reported to be good as flavour enhancers and useful in improving palatability when incorporated in foods (Aiyesanmi & Oguntokun, 1996). The $R^2$ and the adjusted $R^2$ values were 0.9636 and 0.9580 respectively which signifies that the model is well fitted. The 3D plot showing the interactions between the variables (wheat flour, plantain flour and tigernut flour) and response (fat) is shown in Figure 1b. The final equation representing the effect of the variables on fat is given in Equation (8).

$$
\text{Fat} = +2.89A + 2.65B + 7.20C
$$

(8)

The crude fibre content of the flours ranged from 2.97 to 3.48 g/100 g. Oluwalana et al. (2011) reported that crude fibre of blanched and unblanched plantain is between 2.27 and 4.27%. Crude fibre of 0.51% was reported for soft wheat flour (David et al., 2015). Crude fibre helps in the prevention of heart diseases, colon cancer and diabetes (Slavin, Jacobs, & Marquart, 1997). The model (special cubic) and model terms (linear mixture components AB, AC, BC and ABC) are significant ($p \leq 0.05$). The $R^2$ and the adjusted $R^2$ values were 0.6462 and 0.4103 respectively. The 3D plot showing the interactions between the variables (wheat flour, plantain flour and tigernut flour) and response (crude fibre) is shown in Figure 1c while the final equation representing the effect of the variables on crude fibre is given in Equation (9).

$$
\text{Fibre} = +2.99A + 3.18B + 0.42C + 0.53AB + 5.09AC + 5.28BC - 6.24ABC
$$

(9)

The crude protein content ranged from 8.24 to 9.49 g/100 g for the flour samples. Plantain had been reported to contain between 3.15 and 4.61% protein (Adeniji, Sanni, Barimalaa, & Hart, 2007; Eleazu, Okafor, & Ikpeama, 2010) while the protein content of tigernut as reported by Oladele and Aina (2007) is 7.12%. The composite flour comprising wheat, plantain and tigernut flour had higher protein content as observed in this work. The $R^2$ and the adjusted $R^2$ values of 0.9927 and 0.9916 respectively signifies good model fitting. The 3D plot showing the interactions between the variables (wheat flour, plantain flour and tigernut flour) and response (protein) is shown in Figure 1a. The final equation representing the effect of the variables on protein is given in Equation (10).

$$
\text{Protein} = +9.49A + 8.24B + 8.76C
$$

(10)

The carbohydrate contents of the flour sample ranged between 73.98 and 78.03 g/100 g. The result showed that the flour blends are rich sources of carbohydrate. David et al. (2015), Evanson Inyang
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and Ekop (2015) and Oladele and Aina (2007) had reported wheat, plantain and tigernut flours respectively to be good sources of carbohydrate. The high carbohydrate content of the flour blends indicated that it could be used in managing protein-energy malnutrition, since there is enough quantity of carbohydrate to derive energy from in order to spare protein, so that protein can be used for its primary function of building the body and repairing worn out tissues rather than as a source of energy (Butt & Batool, 2010). The $R^2$ and the adjusted $R^2$ values were 0.9900 and 0.9786 respectively which connotes that the model is fitting as required. The model (special quartic) and model terms (linear mixture components, BC, $A^2BC$, $AB^2C$ and $ABC^2$) are significant ($p \leq 0.05$). The final equation representing the effect of the variables on carbohydrate is given in Equation (11).

\[
\text{Carbohydrate} = +77.14A + 77.88B + 66.95C - 0.070AB + 5.21AC + 8.79BC - 80.11A^2BC - 115.71AB^2C + 288.37ABC^2
\]  

(11)
4.2. Mineral concentrations of the flours

The mineral composition of the composite flour is presented in Table 2. Sodium ranges from 1.44 to 6.00 g/100 g. The $R^2$ and the adjusted $R^2$ values were 0.8403 and 0.6578 respectively. Arisa, Adelakun, Alamu, and Ogunfowora (2013) reported 1.05 mg/100 g of sodium for 100% plantain flour similar to value of 1.125 mg/100 g reported for plantain by Zakpaa, Mak-Mensah, and Adubofour (2010). The sodium content in wheat flour is 3.5 mg/100 g (Akubor & Ishiwu, 2013). Some of the important functions of sodium in the body are maintenance of water balance, transmission of nerve impulses, absorption and transportation of some nutrients; it has been recommended to be taken in reduced amounts.

Table 2. Mineral composition of composite flour blends (%)

| Runs | A (%) | B (%) | C (%) | Sodium | Calcium | Potassium | Iron  | Copper | Zinc |
|------|-------|-------|-------|--------|---------|-----------|-------|--------|------|
| 1    | 77.00 | 20.00 | 3.00  | 3.10   | 24.10   | 143       | 1.13  | 0.18   | 0.62 |
| 2    | 70.00 | 20.00 | 10.00 | 2.30   | 20.68   | 117       | 1.18  | 0.08   | 0.51 |
| 3    | 60.00 | 37.00 | 3.00  | 1.44   | 23.50   | 155       | 1.15  | 0.08   | 0.31 |
| 4    | 70.00 | 20.00 | 10.00 | 2.28   | 20.68   | 114       | 1.19  | 0.09   | 0.51 |
| 5    | 69.00 | 23.33 | 7.66  | 6.00   | 23.90   | 136       | 1.45  | 0.15   | 0.52 |
| 6    | 60.00 | 37.00 | 3.00  | 1.45   | 23.50   | 156       | 1.16  | 0.09   | 0.30 |
| 7    | 60.00 | 37.00 | 3.00  | 1.44   | 24.10   | 156       | 1.12  | 0.08   | 0.32 |
| 8    | 65.87 | 25.87 | 8.25  | 4.40   | 25.60   | 163       | 1.12  | 0.08   | 0.32 |
| 9    | 60.00 | 30.00 | 10.00 | 4.90   | 28.20   | 170       | 1.34  | 0.08   | 0.37 |
| 10   | 68.50 | 28.50 | 3.00  | 2.20   | 28.00   | 204       | 1.40  | 0.09   | 0.45 |
| 11   | 71.33 | 25.67 | 3.00  | 4.30   | 21.20   | 121       | 1.05  | 0.07   | 0.31 |
| 12   | 73.50 | 20.00 | 6.50  | 1.84   | 26.40   | 167       | 1.38  | 0.09   | 0.51 |
| 13   | 77.00 | 20.00 | 3.00  | 3.10   | 24.20   | 140       | 1.11  | 0.19   | 0.60 |
| 14   | 60.00 | 33.50 | 6.50  | 6.30   | 26.00   | 172       | 1.39  | 0.11   | 0.41 |
| 15   | 65.66 | 29.00 | 5.33  | 4.40   | 50.40   | 159       | 1.26  | 0.08   | 0.43 |
| 16   | 60.00 | 30.00 | 10.00 | 4.80   | 28.60   | 175       | 1.35  | 0.07   | 0.38 |

Notes: A = Wheat flour, B = Plantain flour, C = Tigernut flour, CHO = Carbohydrate.
quantity. High sodium intake may contribute to high blood pressure in salt sensitive individuals (Whitney & Rolfes, 2010). The composite flour in this work is a good source of low sodium food. The final equation representing the effect of the variables on sodium is given in Equation (12).

\[
\text{Na} = +2.95A + 1.58B - 10.55C + 3.44AB + 18.37AC + 35.98BC - 73.10A^2BC - 130.57AB^2C - 169.91ABC^2
\] (12)

The calcium composition of the flour blends is between 20.68 and 50.4 g/100 g. Wheat flour had lower calcium content (10.3 mg/100 g) as reported by Anna and Malgorzata (2011). Calcium is essential in maintaining total body health. Apart from keeping bones and teeth strong, it ensures proper functioning of muscles and nerves (Piste, Didwagh, & Mokashi, 2013). The \( R^2 \) and the adjusted \( R^2 \) values were 0.9508 and 0.8946 respectively. The Model (special quartic) and model terms (linear mixture components, \( A^2BC, AB^2C \) and \( ABC^2 \)) are significant \( (p \leq 0.05) \). The 3D plot showing the interactions between the variables and calcium content is shown in Figure 2a while the final equation representing the effect of the variables on calcium is given in Equation (13).

\[
\text{Ca} = +24.18A + 23.54B - 2.22C + 3.74AB + 33.47AC + 60.81BC + 933.93A^2BC + 2165.53A^2BC - 3531.33ABC^2
\] (13)

Potassium values ranged between 114 and 204 g/100 g. Potassium is required to maintain osmotic balance of body fluids, body pH, muscle regulation and nerve irritability, glucose absorption control, and enhanced normal retention of protein during growth (NRC, 1980). Potassium is also an important component of cell and body fluids that helps control heart rate and blood pressure by countering negative effects of sodium (USDA, 2009). The ANOVA showed that the model (cubic) and model terms (linear mixture components, \( AB, AC, BC, ABC, AB(A-B), AC(A-C), BC(B-C) \)) were significant \( (p \leq 0.05) \). The \( R^2 \) and the adjusted \( R^2 \) values were 0.9959 and 0.9897 respectively. The 3D plot showing the interactions between the variables potassium content is shown in Figure 2b while the final equation representing the effect of the variables on potassium is given in Equation (14).

\[
\text{K} = +141.31A + 155.70B - 6644.69C + 218.40AB + 12596.40AC + 12901.00BC - 15691.82ABC - 978.61AB(A-B) - 6610.82AC(A-C) - 7205.06BC(B-C)
\] (14)

The iron composition of the flour blends are between 1.05 and 1.45 g/100 g. The \( R^2 \) and the adjusted \( R^2 \) values were 0.7526 and 0.4698 respectively. The ANOVA result for the flour blends showed that
the Model (special quartic) and model terms (linear mixture component, AC, BC, and ABC$^2$) is significant ($p \leq 0.05$). Arisa et al. (2013) reported iron content of 1.25 mg/100 g in plantain flour and 0.65 mg/100 g was reported by Oladele and Aina (2007) as the percentage of iron in yellow tigernut. Iron content is important in contributing to the overall daily dietary intake of essential elements especially the micronutrients. The 3D plot showing interactions between the variables and iron is shown in Figure 2c. The final equation representing the effect of the variables on iron is given in Equation (15).

$$Fe = +1.31A + 1.14B \quad - \quad 1.6C + 0.47AB + 4.24AC + 4.69BC + 28.47A^2BC \quad - \quad 22.76AB^2C - 33.46ABC^2$$

(15)
The Zinc level of the flour blends was between 0.3 and 0.62 g/100 g. The $R^2$ and the adjusted $R^2$ values were 0.8520 and 0.6828 respectively. The 3D plot showing the interactions between the variables and Zinc content is shown in Figure 2d. Zinc plays a central role in the immune system, affecting a number of aspects of cellular immunity (Shankar & Prasad, 1998). The final equation representing the effect of the variables on zinc is given in Equation (16).

$$Zn = +0.59A + 0.32B + 0.27C - 0.39AB + 0.16AC + 19.22ABC + 0.050A^2BC - 28.39ABC^2$$  \(\text{(16)}\)

The copper content in the formulated blends ranged from 0.07 to 0.19 g/100 g. The $R^2$ and the adjusted $R^2$ values were 0.8685 and 0.7182 respectively. The ANOVA result for the flour blends showed that the Model (special quartic) and model terms (linear mixture component AB, A²BC,) were significant ($p \leq 0.05$). The final equation representing the effect of the variables on copper is given in Equation (17).

$$Cu = +0.18A + 0.088B + 0.092C - 0.25AB - 0.27AC - 0.023BC + 8.97A^2BC - 2.78AB^2C - 5.06ABC^2$$  \(\text{(17)}\)

### 4.3. Dietary fibre content of optimized flour blends

The results of total dietary fibre, soluble fibre and insoluble fibre for the composite flours are presented in Table 3. Total dietary fibre significantly ($p \leq 0.05$) reduced from 23.70 mg/100 g in the control (100% wheat flour) to 21.73 and 20.42 mg/100 g in samples 15 and 2 respectively. Total dietary fibre obtained for samples 15 and 2 was higher than that reported for 20% Lupine flour substitution for wheat (Hofmanová, Hrušková, & Švec, 2014).

The value of soluble dietary fibre reduced from 8.75 mg/100 g in the control to a range of 6.20–8.10 mg/100 g in the composite flours while an increase was observed in insoluble dietary fibre (IDF). Hofmanová et al. (2014) reported that all composite flours from wheat showed a considerable level of dietary fibre. The nutritional potential of dietary fibre could be examined on the basis of both insoluble and soluble dietary fibre contents and their ratio. Reyes-Caudillo, Tecante, and Valdivia-López (2008) observed that ratio 3:1 is recommended for insoluble:soluble dietary fibre. In this case,
sample 2 with highest tigernut content satisfied the recommendation. Sample 15 had ration next to
that of sample 2. It clearly showed that tigernut promotes IDF contents. IDF typically has a high
water binding capacity which results in the formation of softer stools that pass through the system
faster (Adejuyitan, Otunola, Akande, Bolarinwa, & Oladokun, 2009).

4.4. Functional properties of optimized flour blends

The results of functional properties of optimized flour blends are presented in Table 4. The bulk den-
sity ranged from 0.70 g/cm³ in the control (100% wheat flour) to a range of 0.63–0.90 g/cm³ in the
composite flour blends. Akubor et al. (2003) had reported bulk density of 0.71 g/cm³ for wheat flour.
Bulk density is used to determine heaviness of a sample and have direct influence on packaging re-
quirement and material handling (Karuna, Noel, & Dilip, 1996). Low bulk density of the flours may be
considered advantageous when determining transportation cost and space requirement; as less
space will be required for packaging of the flours.

There was significant ($p \leq 0.05$) increase in WAC from 87.15 ml/g in the control to a range of 92.60–
98.54 ml/g in the composite flours. It was however observed that WAC of the composite flour in-
creased with increase in plantain flour substitution and inclusion of tigernut flour. Kiin-Kabari,
Eke-Ejoifor, and Giami (2015) reported that WAC of wheat-plantain-bambara groundnut composite
flour with 30% plantain flour substitution was considerably high (81.40%). Kinsella (1976) reported
that the ability of food materials to absorb water is linked to its protein content. Adebowale, Adeyemi,
and Oshodi (2008) had observed that addition of soy-flour to plantain flour confers high water binding
capacity which, in turn improves the reconstitution and textural abilities obtainable from food flours
and thus, high WAC of flours is essential in food formulations where absorption of water is desirable.

| Table 3. Dietary fibre content of optimized flour blends (mg/100 g) |
|---------------------------------------------------------------|
| **Samples** | **Total dietary fibre** | **Soluble dietary fibre** | **Insoluble dietary fibre** |
| Control     | 23.70 ± 0.50a           | 8.75 ± 0.50a              | 14.95 ± 0.00b              |
| 2           | 20.42 ± 0.60a           | 3.90 ± 0.10a              | 16.51 ± 0.51c              |
| 13          | 24.08 ± 0.10a           | 8.10 ± 0.10a              | 15.98 ± 0.01c              |
| 15          | 21.73 ± 0.50a           | 6.20 ± 0.00a              | 16.00 ± 0.01c              |

Notes: Means of triplicate determinations ± S.D.
Means with different superscripts on the same column are significantly different at $p \leq 0.05$.
Legends: control–100% wheat flour.
Sample 2–70% wheat/20% plantain/10% tigernut.
Sample 13–77% wheat/20% plantain/3% tigernut.
Sample 15–65.66% wheat/29% plantain/5.33% tigernut.

| Table 4. Functional properties of optimized flour blends |
|--------------------------------------------------------|
| **Sample** | **Bulk density** | **WAC (ml/g)** | **OAC (ml/g)** | **Foam capacity (%)** |
| Control    | 0.70 ± 0.00a     | 87.15 ± 0.00a  | 82.00 ± 0.00a  | 19.00 ± 0.00a         |
| 2          | 0.69 ± 0.00a     | 95.00 ± 0.00a  | 81.60 ± 0.21a  | 12.50 ± 0.50c         |
| 13         | 0.90 ± 3.52a     | 98.54 ± 0.06a  | 81.60 ± 0.06a  | 15.00 ± 0.50b         |
| 15         | 0.63 ± 0.00a     | 92.60 ± 0.06a  | 82.60 ± 0.06a  | 11.50 ± 1.00b         |

Notes: Means of triplicate determinations ± S.D.
Means with different superscripts on the same column are significantly different at $p \leq 0.05$.
Legends: control–100% wheat flour.
Sample 2–70% wheat/20% plantain/10% tigernut.
Sample 13–77% wheat/20% plantain/3% tigernut.
Sample 15–65.66% wheat/29% plantain/5.33% tigernut.
WAC–water absorption capacity, OAC–oil absorption capacity.
There were no significant differences \((p > 0.05)\) in the OAC of the flour blends. The relatively high OAC of the samples is an indication that flavour and mouth feel may be well enhanced when the flours are used in food preparation that involve oil mixing like in bakery products (Fagbemi, 1999). Oluwalana et al. (2011) had also reported that water/oil binding capacity of proteins is an index of its ability to absorb and retain oil and this influences texture and mouth feel of foods like ground meat formulations, doughnuts, pancakes, baked foods and soups.

The FC ranged from 19.00% in the control to a range of 11.50–15.00% in the composite flours. This is an indication that inclusion of plantain and tigernut flours reduced the potential of wheat flour to foam. The values obtained for composite flours were however higher than those reported for wheat/plantain composite flour by Mepba, Eboh, and Nwoajigwa (2007). The low foam capacity may be attributed to the low protein content of the flour since foomability is related to the amount of solubilized protein and the amount of polar and non-polar lipids in a sample (Narayana & Narsinga, 1992). The composite blends may find less applications in food formulations where foaming is desirable like ice creams and beer (Nwosu, 2010).

4.5. Antioxidant properties of optimized flour blends
The results of the antioxidant properties of optimized flour blends are presented in Table 5.

There was an increase in TPC from 2.06 mg/g in the control to 2.24 and 2.43 mg/g in samples 2 and 13 respectively. It however decreased to 1.98 mg/g in sample 13. The increase in phenols at both 5 and 10% tigernut inclusion may be connected to relatively high amount of antioxidant content observed (Abaejoh, Djomdi, & Ndojouenkeu, 2006). Phenolic compounds act as antioxidants

Total Flavonoid content of the samples has values from 3.61 mg/g in the control to a range of 3.25–4.31 mg/g in the composite flours; with sample 2 having the highest and sample 13 the lowest values. Other functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, viruses and tumors (Okwu, 2004).

The high DPPH observed in all the samples may be attributed to high antioxidant potentials of the flours (Abioye, Ade-Omowaye, Babarinde, & Adesigbin, 2011; Adejuyitan et al., 2009). Antioxidant compounds in foods have been noted to play important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risks for chronic diseases including cancer and heart disease (Miller, Rigelhof, Marquart, Prakash, & Kanter, 2000).

| Samples   | Total phenolic content (mg/g) | Total flavonoid content (mg/g) | DPPH (%)       |
|-----------|------------------------------|-------------------------------|----------------|
| Control   | 2.06 ± 0.01c                 | 3.61 ± 0.02c                 | 78.88 ± 0.10c  |
| 2         | 2.43 ± 0.01a                 | 4.31 ± 0.02c                 | 81.98 ± 0.15a  |
| 13        | 1.92 ± 0.01c                 | 3.25 ± 0.22d                 | 77.92 ± 0.01f  |
| 15        | 2.24 ± 0.02d                 | 3.92 ± 0.04c                 | 79.44 ± 0.05c  |

Notes: Means of triplicate determinations ± S.D.
Means with different superscripts on the same column are significantly different at \(p \leq 0.05\).

Legends: control–100% wheat flour.
Sample 2–70% wheat/20% plantain/10% tigernut.
Sample 13 – 77% wheat/20% plantain/3% tigernut.
Sample 15–65.66% wheat/29% plantain/5.33% tigernut.
4.6. Starch properties of optimized flour blends

The result of the amylose, amylopectin and resistance starch of the flour blends are presented in Table 6. Oko, Famurewa, and Nwaza (2015) reported amylose value ranging from 40 to 70% for different plantain cultivars. Thus, high value of amylose content detected in samples 2 and 13 may be due to level of plantain substitution. The ratio of amylose to amylopectin in carbohydrate has important implication on food quality, industrial application and health. Straight–chain amylose forms a solid bond so that it is not easily gelatinized, whereas amylopectin is highly branched, available for enzymatic digestion with open structure (Foster-Powell, Holt, & Brand-Miller, 2002). It has been reported that an increase in RS content increases with increasing level of plantain flour substitution. Odenigbo, Asumugha, Ubbor, and Ngadi (2013) also observed that RS for unripe plantain flour falls between 7.40 and 17.80%. There is a growing interest in developing foods with increased RS contents. High level of RS in food decrease glycemic index and as such it can improve control of diabetes mellitus by altering the glycemic impact of ingested carbohydrate (Sajilata, Singhal, & Kulkarni, 2006).

4.7. Pasting properties of optimized flour blends

The pasting properties of wheat flour (control) and composite flour blends are presented in Table 7. The peak viscosity decreased from 162.10 RVU in the control to a range of 141.00–150.00 RVU in the composite blends. Peak viscosity is often correlated with the final product quality and also provides an indication of the viscous loads likely to be entered during mixing (Maziya-Dixon, Dixon, & Adebowale, 2004). Reduction in peak viscosity of the composite blends could be due to reduction in starch gelatinization.

Table 6. Amylose, amylopectin and resistant starch of optimized flour blends

| Samples       | Amylose (mg/ml) | Amylopectin (mg/ml) | Resistant starch (mg/ml) |
|---------------|-----------------|---------------------|--------------------------|
| Control       | 12.50 ± 4.17b   | 87.50 ± 4.17a       | 6.20 ± 0.00d             |
| 2             | 25.00 ± 5.56a   | 74.99 ± 5.56b       | 8.60 ± 0.00e             |
| 13            | 12.50 ± 1.39b   | 87.50 ± 1.39a       | 6.80 ± 0.00c             |
| 15            | 33.33 ± 5.56a   | 66.66 ± 5.55c       | 13.50 ± 0.05a            |

Notes: Means of triplicate determinations ± S.D.
Means with different superscripts on the same column are significantly different at p ≤ 0.05.
Legends: control–100% wheat flour.
Sample 2–70% wheat/20% plantain/10% tigernut.
Sample 13–77% wheat/20% plantain/3% tigernut.
Sample 15–65.66% wheat/29% plantain/5.33% tigernut.

Table 7. Pasting properties of optimized flour blends

| Sample       | Peak viscosity (RVU) | Breakdown (RVU) | Final viscosity (RVU) | Setback (RVU) |
|--------------|----------------------|-----------------|-----------------------|---------------|
| Control      | 162.10 ± 0.35a       | 119.10 ± 1.15a  | 219.90 ± 1.15c        | 124.90 ± 1.15c|
| 2            | 147.00 ± 1.15b       | 87.00 ± 1.15b   | 223.00 ± 1.15b        | 127.00 ± 1.15b|
| 13           | 141.00 ± 1.15b       | 64.00 ± 0.57a   | 239.00 ± 1.15b        | 132.10 ± 1.15a|
| 15           | 150.00 ± 0.57b       | 97.00 ± 0.57b   | 219.30 ± 1.15d        | 124.50 ± 1.15c|

Notes: Means of triplicate determinations ± S.D.
Means with different superscripts on the same column are significantly different at p ≤ 0.05.
Legends: control–100% wheat flour.
Sample 2–70% wheat/20% plantain/10% tigernut.
Sample 13–77% wheat/20% plantain/3% tigernut.
Sample 15–65.66% wheat/29% plantain/5.33% tigernut.
The breakdown viscosity significantly (p ≤ 0.05) decreased from 119.10 RVU in the control to range of 64.00–97.00 RVU in composite flours. Breakdown viscosity is a measure of degree of disintegration of granules of cooked starch. Higher breakdown viscosity results in higher starch instability (Dengate, 1984). This indicates that the control (100% wheat flour) with highest breakdown viscosity contains relatively unstable starch and the composite flours should exhibit higher degree of starch stability.

The values for setback value increased significantly (p ≤ 0.05) from 124.90 RVU in the control (100% wheat flour) to 132.10 RVU in sample 13. Unripe plantain flour has been reported to be high in amylopectin content and this may account for lower value of setback reported in sample 15 (Chinma, Igbobul, & Omotayo, 2012). Low set back value is an indication that the starch has a low tendency to retrograde or undergo syneresis during freeze thaw cycles (Fasasi, 2009; Ikujenlola & Fashakin, 2005).

5. Conclusion
Substitution of wheat flour with plantain and tigernut flour between 25 and 30% improved nutritional quality (in particular, protein, dietary fibre and minerals contents) of the composite flour. The WAC, oil absorption capacity, amylose and amylpectin contents; and antioxidant properties were also significantly (p ≤ 0.05) enhanced. In addition, the pasting characteristics of the composite flour were similar to 100% wheat flour. The composite flour will be useful as a potential replacement to 100% wheat flour in the production of baked products.

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