Science Development
2022; 3(1): 31-45
http://www.sciencepublishinggroup.com/j/scidev
doi: 10.11648/j.scidev.20220301.15

Activation of Cotyledon as Nutrients and Antioxidant Enhancer in *Oryza sativa*, *Dioscorea bulbifera* and *Phaseolus vulgaris* for Food Formulation

Judith Uchenna Chima[1, 2, *], Folusho Morenike David-Abraham[2], Laura Chioma Okpala[2]

[1]Department of Food Technology, Akanu Ibiam Federal Polytechnic Unwana, Unwana, Nigeria

[2]Department of Food Science and Technology, Ebonyi State University, Abakaliki, Nigeria

Email address:

judichelal@yahoo.com (J. U. Chima)

[*]Corresponding author

To cite this article:

Judith Uchenna Chima, Folusho Morenike David-Abraham, Laura Chioma Okpala. Activation of Cotyledon as Nutrients and Antioxidant Enhancer in *Oryza sativa*, *Dioscorea bulbifera* and *Phaseolus vulgaris* for Food Formulation. *Science Development*. Vol. 3, No. 1, 2022, pp. 31-45. doi: 10.11648/j.scidev.20220301.15

Received: December 28, 2021; **Accepted**: February 18, 2022; **Published**: February 28, 2022

Abstract: The impact of cotyledon activation on brown rice Rice (*Oryza sativa*), Aerial yam (*Dioscorea bulbifera*) and black turtle bean (*Phaseolus vulgaris*) was studied. Composite flours were prepared using cotyledon activated brown rice (GBR), aerial yam (AYF) and black turtle bean (GTB). The ratio of GBR: AYF: GTB used in the flour formulations were: 100GBR: 0AYF: 0GTB; 0GBR: 100AYF: 0GTB; 0GBR: 0AYF: 100GTB; 33.33GBR: 33.33AYF: 33.33GTB; 66.67GBR: 16.67AYF: 16.67GTB, 16.67GBR: 66.67AYF: 16.67GTB and 16.67GBR: 16.67AYF: 66.67GTB. Proximate composition, functional properties, antinutrients, minerals, vitamins, amino acid profile and antioxidant activity of the composite flours were determined using standard procedures. Protein efficiency ratio, essential amino acid index and biological value were calculated from the amino acid profile. Results showed ash content (2.72-3.20%), fibre (2.12-2.42%), protein (13.23-39.31%), tannin (0.973-1.120mg/100g), saponin (1.95-2.44 mg/g), oxalate (2.50-3.38mg/g), water absorption capacity (107.71–190.23%), oil absorption capacity (145.30-296.70%), water solubility index (16.05-17.78%), swelling capacity (230.10-250.50%), calcium (36.64-40.62mg/100g), phosphorus (84.84-88.93mg/100g), manganese (0.46-0.57mg/100g), magnesium (1741.20-1823.20mg/100g), vitamin B_1 (2.75-4.06mg/100g, B_2 (2.01-2.78 mg/100g, B_3 (2.20-4.83mg/100g), B_6 (2.63-3.98mg/100g) B_{12} (1.59-5.95mg/100g), essential amino acid index (70-99%), protein efficiency ratio (1.95–3.79), and biological value (70-96%). Nutrients and antioxidant properties were improved through the cotyledon activation process, level of antinutrients were safe and below toxicity level. The composite flour is invaluable as functional ingredient in food formulation.

Keywords: Cotyledon Activation, Nutritional, Antioxidants, Amino Acid, Brown Rice, Aerial Yam, Black Turtle Bean

1. Introduction

Cotyledon activation is a process that enhances the nutritional quality of food commodities. It is a process by which grains and tubers are subjected to hypogeal or epigeal germination for the metabolism of chemical components of the produce. The final product is usually better in nutrients and bioactive compounds. Currently, more attention is being paid to nutrients and bio-compounds in foods because of the vital role they play in ensuring healthy living. Cotyledon activation also improves the taste. Moreover, composite flour has been found to have better nutritional value with respect to minerals, vitamins, fibres, and proteins than flour from any specific cereal alone [1]. According to Igbabul *et al.* [2] composite flour blends should include plant materials that are readily available, culturally acceptable, affordable, and possesses good nutritional and functional potential. The use of composite flour would encourage the use of native or local plant species hence promote domestic agriculture production.

Legumes are incorporated into cereal or tubers in flour blends to increase the consumption and as a means to combat malnutrition among children. According to Food and Agricultural Organization in 2001, protein-energy malnutrition (PEM) was a nutritional disorder of worldwide significance, more prevalent in developing nations, with health and economic challenges [3]. Hence, it is of utmost importance to exploit the potentials of underutilized or relatively neglected high-protein legumes that are cheaper to improve the nutritional situation of the poor and contribute to the nation's food security strategy.

Rice (*Oryza sativa*) is an important food crop worldwide. It is the second most consumed cereal; hence a staple for more than three billion people, representing about half of the world's population [4]. Rice possesses good nutritional and some functional properties such as high expansion index and sparkling white colour [5]. Its hypoallergenic proteins, low calcium content, and non-gluten characteristics makes it useful in many value-added products especially for gluten intolerant individuals [6].

Rice is eaten as whole milled grains or as rice flour eaten with soup or stew (*tuwo shinkafa*) in Nigeria [7]. According to Wu *et al*. [8] consumption of germinated or sprouted rice is of better health and nutritional advantage than the ungerminated rice. In addition, there is increased interest in the application of germinated rice flour in the development of nutritious and healthy food products [9]. Rice is deficient in lysine. Legumes complement cereals as a good source of lysine [10, 11]. Moreover, legumes are rich in protein and micronutrients which are necessary for normal growth and healthy body development.

Leguminous plants provide protein and energy. Legumes are a cheap source of plant protein; as well as possess other health benefits [12]. As a staple food, legumes form a major part of the diets in Nigeria as well as other countries of the world [13].

Black bean, otherwise known as black turtle bean *(Phaseolus vulgaris)* is an underutilized legume grown for its immature pods and seeds which serve as food. Black turtle bean is consumed in different forms and used for the preparation of various diets in Nigeria [14]. Black beans are a good source of vitamins, minerals and protein [15]. The high protein content makes black bean alternative source of protein for food and industrial applications. Moreover, they are high in the essential amino acids such as leucine, lysine, tryptophan and arginine [16]. Hence, black beans would complement cereal (rice) in terms of their essential amino acids and amino acid values. It was also reported to be important in the management of some degenerative health problems [17]. Black turtle bean is rich in bioactive compounds; the major bioactive compounds found in black turtle beans include phenolic acids (ferulic acid, *p*-coumaric acid, sinapic acid, and gallic acid), flavonoids (kaempferol, quercetin, catechin, and proanthocyanidin), and anthocyanins (3-O-glucosides of malvidin, petunidin, and delphinidin) [18].

Aerial yam or air potato (*Dioscorea bulbifera*) is one of the less popular and highly underutilized yam species. Aerial yam bulbils are rich in nutrients and have many traditional, therapeutic, and pharmaceutical uses [19]. Aerial yam is high in complex carbohydrates, some B complex vitamins and some essential mineral elements [20]. Aerial yam has no gluten but possesses resistant starch which makes it a desired substitute in flour blend formulation with minimal risk of obesity, diabetes, celiac disease or other allergies [21]. Aerial yam is characterized by high functional property and antioxidant activity [19].

Cereal/legume complementation has received considerable attention. Yet complementing germinated brown rice with germinated black turtle bean and aerial yam has not received any research attention.

This study highlighted the impact of cotyledon activation on black turtle bean (*Phaseolus vulgaris*), aerial yam (*Dioscorea bulbifera*), and brown rice (*Oryza sativa*) in improving their protein, vitamins, and antioxidative characteristics; converting black turtle bean seeds, and aerial yam into flour blends for food production will increase their utilization as well as improve the nutrients of the resultant composite flour.

2. Materials and Methods

2.1. Source and Preparation of Materials

Rice paddy (long-grain B12) and aerial yams were purchased from accredited farmers at Amasiri, Ebonyi State, Nigeria. The black turtle beans (*akidi)*, was obtained from an accredited seller at Afikpo, Ebonyi State, Nigeria.

2.1.1. Cotyledon Activation of Brown Rice Flour (GBR)

The rice paddy was winnowed with a stainless tray, soaked in clean distilled water (1:3w/v) for 16 h. The water was changed at intervals of 6 h. Thereafter, it was drained and the grains spread on a jute bag. The hydrated rice grains were frequently watered and sprouted for 72 h. The metabolized rice was parboiled, dried in an oven (NAAFCO BS Oven, model: OVH-102 Nigeria) at 60°C for 10 h, dehulled with a commercial machine and ground to flour with a disc attrition mill (Globe p44, China) to pass through a 100 µm mesh size sieve. The flour was stored in an airtight plastic container as germinated brown rice flour (GBR) at ambient temperature until used.

2.1.2. Cotyledon Activation Black Turtle Bean Flour (GBB)

Black bean seeds of good grades were sorted, washed, and soaked in clean tap water for 16 h. The soak water was changed every 6 h to reduce microbial load. The seeds were drained and germinated for 24 h at room temperature in a wet jute bag. The sprouted seeds were kilned in an oven at 45°C for 12h to terminate germination and finally dried at an increased temperature 60°C for 10 h in an oven (NAAFCO BS oven, model: OVH-102 Nigeria). The dried germinated seeds were dehulled manually, ground with an attrition mill (Globe p44, China), thereafter passed through a 100µm mesh size and stored in a plastic can at ambient temperature as germinated black turtle bean flour (GBB) for further determinations.

2.1.3. Cotyledon Activation of Aerial Yam

The method of [22] with slight modifications was used. Sound aerial yams bulbils were selected, washed clean with potable water, and peeled with a stainless kitchen knife. Next, the aerial yams were manually sliced (2mm thickness) under potable water to prevent browning. Thereafter, the sliced aerial yams were drained with a perforated sieve and dried in a hot air oven (NAAFCO BS oven, model: OVH-102 Nigeria) at 60°C for 7 h. The dried chips were then ground into fine flour with an attrition machine (Globe p44, China), it was passed through a 100µm mesh size, and packaged in a plastic container as Aerial yam flour (AYF).

2.2. Flour Blends Formulation

Flour blends were formulated by mixing cotyledon activated brown rice (GBR), aerial yam (AYF), and black turtle bean (GBB) to generate seven samples. Seven ratios were used namely 100GBR: 0AYF: 0GBB; 0GBR: 100AYF: 0GBB; 0GBR: 0AYF: 100GBB; 33.33GBR: 33.33AYF: 33.33GBB; 66.67GBR: 16.67AYF: 16.67GBB, 16.67GBR: 66.67AYF: 16.67GBB and 16.67GBR: 16.67AYF: 66.67GBB.

2.3. Evaluation of Proximate Composition of the Flour Blends

The moisture, fat, fibre, ash, and protein contents were evaluated according to AOAC [23] while carbohydrate content was determined by a difference.

$$CHO\ (\%) = 100 - (moisture\ content + ash\ content + fat + crude\ protein) \tag{1}$$

2.4. Determination of Antinutritional Factors

2.4.1. Tannin

Tannin was evaluated using the method of AOAC [23]. Test sample (1g) was weighed into 100 ml (which was diluted to 10mg/ml) of distilled water and vigorously shaken. The mixture was incubated for half an hour at room temperature and then filtered through Whatman no. 4 grade of filter paper. An aliquot (1 ml) was poured into a 25 ml volumetric flask. To prepare the standard, 1 ml standard tannic solution (1 mg/ml tannic acid) and 1 ml distilled water were dispensed in another volumetric flask. Then, 1.25 ml of saturated sodium carbonate (Na_2CO_3) solution and 0.5 ml of Folin-C reagent were also added to each flask and volume made up to 25 ml and shaken vigorously. The solution was allowed to stand for 90 min, then filtered using Whatman no. 4 grade of filter paper, and the absorbance read at 760 nm against reagent blank.

2.4.2. Saponin

Saponin was evaluated using the spectrophotometric method [24]. Exactly 2g of the composite flour was measured into a 250ml graduated flask; Isobutyl alcohol (100 ml) was poured into the flask. The suspension was thoroughly shaken for 5 hours to obtain a homogenous mixture. This was passed through No 1 Whatman filter paper into 100ml volumetric flask containing 20ml of 40% saturated solution of magnesium carbonate ($MgCO_3$). The solution was further filtered using No 1 Whatman filter paper, a colourless solution was obtained. The filtrate (1 mL) was pipetted into 50ml volumetric flask also 2 ml of 5% iron (III) chloride ($FeCl_3$) solution was poured into it and then filled up to the mark with distilled water. The solution was kept for 30 minutes to develop the colour. Likewise, Saponin standard was prepared at different concentrations of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml. The absorbance was measured against the blank at 380nm. The concentration of saponin was read from the standard curve obtained from the standard concentrations.

2.4.3. Oxalate

Oxalate content was evaluated by titrimetric method [25]. One gram of the flour blend was measured into a beaker, it was extracted with 190 ml distilled water and 10 ml 6 M HCl. The mixture was boiled in hot water for 2 h and then filtered. The filtrate was then diluted to 250 ml with water; into 25 ml extract, 5 ml of 6M HCl was added and filtered. The precipitate was washed with hot water. The mixture of the filtrate and the wash water was titrated against conc. NH_4OH until a faint yellow colour appeared. The solution was heated to 90°C thereafter 10 ml 5% (w/v) $CaCl_2$ solution was introduced to precipitate the oxalate overnight. The precipitate was washed free of calcium with distilled water and then washed into100 ml conical flask with 10 ml hot 25% (v/v) H2SO4 and then with 15 ml distilled water. The solution was finally heated to 90°C and then titrated against a standard 0.05M KMnO4 until a faint purple solution persisted for 30 s. The oxalate was calculated as the sodium oxalate equivalent.

1ml of 0.05M KMn04=2mg sodium oxalate equivalent/g of sample (2)

2.4.4. Phytate

Phytate contents of the samples were evaluated according to the method of Vaintraub and Lapteva [26]. The sample was extracted with 2.4% HCl for 60 min and then centrifuged. Three milliliters of the extract was poured into one milliliter of wade reagent (0.03% $FeCl_3{\bullet}6H_2O$ and 0.3% sulphosalicyclic acid in distilled water). The absorbance of the mixed solution was read at 500 nm using a UV–visible Spectrophotometer (722 Spectronic 20D, England). The reading was deducted from that of the blank. The phytate content (mg/100 g sample) was estimated from the phytic acid standard calibration curve (5–35 mg/kg) which was prepared in the same way as the sample.

2.5. Determination of Functional Properties

2.5.1. Water Absorption Capacity (WAC)

Water absorption capacity of the samples was evaluated using the method of Adebiyi and Aluko [27] with slight modifications. Exactly 2 g of each test sample (W_1) was measured into a tube (weight of tube and the sample=W_2) and 20 mL of distilled water was added. The suspension was vigorously shaken for 1 min, left for 30 min, and then centrifuged (Botch Centrifuge, 140N, England) at 3000 × g for 30 min at room temperature. The supernatant was drained while the residue was re-weighed (W_3) to measure the water retained per gram of sample.

$$Water\ absorption\ capacity = \left(\frac{W_3-W_2}{W_1}\right) * 100 \quad (3)$$

Where W_3=weight of the tube + sample after centrifuging and decanting

W_2=weight of the tube + sample before water was added

W_1=weight of the sample

2.5.2. Oil Absorption Capacity (OAC)

Oil absorption capacity was evaluated according to the method of Adebiyi and Aluko [27] with slight modifications. Two gram of test sample (W_1) was measured into a tube (weight of tube and the sample=W_2) and 20 mL. Gino oil added. The mixture was thoroughly shaken for 1 min, kept still for 30 min, and then centrifuged (Botch Centrifuge, 140N, England) at 3000 × g for 30 min at room temperature. The supernatant was drained while the residue was re-weighed (W_3) to measure the oil retained per gram of the sample.

Oil absorption capacity was determined as shown in the equation;

$$Oil\ absorption\ capacity = \left(\frac{W_3-W_2}{W_1}\right) * 100 \quad (4)$$

Where W_3=weight of the tube + sample after centrifuging and decanting

W_2=weight of the tube + sample before oil was added

W_1=weight of the sample

2.5.3. Bulk Density

The method of Maninder *et al.* [28] was adopted. The sample was filled into a 30 mL measuring cylinder, and the bottom was mildly and continuously tapped on a laboratory bench until there was no reduction in volume at the 30mL mark.

$$Bulk\ Density = \left(\frac{Weight\ of\ sample(g)}{Volume\ of\ sample(ml)}\right) \quad (5)$$

2.5.4. Swelling Capacity and Solubility Index

Swelling capacity and solubility index were evaluated according to the method of Takashi and Sieb [29]. Two grams of the flour sample and 50 ml of distilled water were poured into a cylinder and shaken. The mixture was heated in a water bath (Julabo water bath 565 England) at 80°C for 15 min. The mixture was centrifuged at 3000 x g for 10 mins. The supernatant was poured out while the sediment was measured. Moisture content of the sediments gel was, evaluated to obtain the dry matter content of the gel.

$$SC = \left(\frac{Weight\ of\ wet\ mass\ sediment}{weight\ of\ dry\ matter\ in\ the\ gel}\right) * 100 \quad (6)$$

$$WSI=\left(\frac{Weight\ of\ dry\ solid\ after\ drying}{weight\ of\ the\ initial\ powder\ or\ flour}\right) *100 \quad (7)$$

2.6. Determination of Vitamin and Mineral Contents of Composite Flour

The vitamin contents of the flour blend samples were determined according to the method described by Antakali *et al.* [30] using HPLC (Shimadzu-UFLC Prominence), equipped with an autosampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A). The data were recorded using LC-solutions software.

The mineral contents of the flour blends were determined using Atomic Absorption Spectrophotometer as described by Adedeye and Adewoke [31] for the minerals.

2.7. Evaluation of Antioxidants Activity

2.7.1. 1, 1-Diphenylpicrylhydrazine (DPPH)

The 1, 1-diphenylpicrylhydrazine (DPPH) radical scavenging ability of samples was evaluated according to the modified method of Girgih *et al.* [32]. A 0.5 mL of the sample extract was added to 0.5 mL of the DPPH solution and allowed to stand in the dark for 30 min. The buffer was used in the blank assay while Glutathione (GSH) was the control. Absorbance was measured at 517 nm.

$$\%DPPH=x = \frac{\text{absorbance of blank}-\text{absorbance of sample}}{absorbance\ of\ blank}\ x\ 100 \quad (8)$$

Ferric reducing antioxidant property (FRAP)

The reducing activity of the samples was determined by the method of Benzie and Strain [33] with slight modifications. FRAP reagent was prepared by mixing 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, and 20 mmol/L $FeCl_3$ in the ratio of 5: 1: 1, respectively. Samples were prepared to 10 mg/ml using distilled water. A 20 µl aliquot of the sample extract was mixed with 100 µl of FRAP reagent in a test tube and absorbance measured at 593 nm. Iron II sulphate heptahydrate served as a standard. This was obtained by serial dilutions of 0.025 to 0.25 µM from 1 mM of Iron II sulphate. Iron reducing power of the flours was estimated from the standard curve of Iron II sulphate heptahydrate.

2.7.2. Metal Chelating Activities (MCA)

The iron chelating ability of the composite flours was measured by method of Xie *et al.* [34] with slight modification. The samples were hydrated to 5 mg/ml in distilled water and centrifuged at 3000 × g for 30 min at room temperature. (Bosh, TLD-500, England). Exactly 1 mL of the extract was added to 50 µl of 2 mM$FeCl_2$, 1.85 ml distilled water and then 100 µl of 5 mM Ferrozine. The mixture was vigorously shaken in a tube and kept at ambient temperature for 10 min. Thereafter, the absorbances were read at 562 nm using a spectrophotometer.

$$\%\ \text{metal chelating ability}=x = \frac{\text{absorbance of blank}-\text{absorbance of sample}}{absorbance\ of\ blank}\ x\ 100 \quad (9)$$

2.7.3. Total Phenolic Content

The total phenolic content (TPC) of the composite flour was evaluated by Folin–Ciocalteu method [35] with slight modifications. A standard curve was prepared using 25–350 mg/ mL gallic acid concentration in 50% (v/v) methanol. Flour samples were diluted with 50% methanol and vortexed. About 0.25 mL of Folin-Ciocalteu reagent was mixed with 0.25 mL of the sample solution and kept in the dark at ambient temperature for 5 min to react. Afterwards, 0.5 ml 20% sodium carbonate solution and distilled water (4 mL) were poured into the mixture. The contents were shaken and allowed to stand in the dark for 1 hr. The absorbance was read at 725 nm using a UV–visible spectrophotometer (722 Spectronic 20D, England). TPC was expressed as milligrams gallic acid equivalents (GAE) per gram of sample (mg GAE/g).

2.7.4. Total Flavonoid Content

The flavonoid content of the sample was evaluated using spectrophotometric method as described by Nabavi, *et al.* [36]. A 0.01 g of the test sample was mixed with 5 ml of extraction solvent and later diluted to 20 ml to give a concentration of 0.5 mg/ml. It was thereafter centrifuged at 3000 × g for 30 min at room temperature (Bosh, TLD-500, England). Using a pipette, 0.5 ml of sample solution was transferred into a test tube containing 4.5 ml distilled water. Also, 0.3 ml of 5% (w/v) $NaNO_2$, 0.3 ml of 10% $AlCl_3$ and 4 ml of 4% (w/v) NaOH were added and the mixture allowed to stand for 15 min. The absorbance was measured at 500 nm against the reagent blank. Similarly, the standard calibration curve was prepared. The flavonoid content in the samples were calculated by extrapolating the standard calibration curve and then expressed as milligram rutin equivalent per g of sample.

2.8. Determination of Amino Acid Profile

Amino acid content of the composite flour was evaluated as described by Siswoyo *et al* [37] using the Beckman Amino Acid Analyzer (model 6300, Beckman Coulter Inc., Fullerton, Calif., USA) after hydrolyzing the composite flour with 6 N HCl for 24 h. Sodium citrate buffers were used as steep gradients with the cation exchange post-column ninhydrin derivatization method. The cysteine and methionine contents were evaluated after performic acid oxidation while tryptophan was determined according to Spies and modified by Pianesso *et al* [38].

2.9. Determinations of Protein Quality

The predicted protein efficiency ratio (P-PER) of the flour blends were computed according to Alsmeyer *et al* [39].

$$\text{P-PER} = -0.468 + 0.454 \times \text{Leu} - 0.105 \times \text{Tyr} \quad (10)$$

Essential Amino Acid Index (EAAI) was determined according to the equation described by Steinke *et al.* [40].

$$\text{EAAI} = 8\sqrt{\frac{\text{Phenylal X Val X Threo X Isoleu X Meth X Histi X Lys X Leu)a}}{\text{Phenylal X Val X Threo X Isoleu X Meth X Histi X Lys X Leu)b}}} \quad (11)$$

Where, (Phenylal x Val x…….)$_a$ in test sample and (Phenylal x Val x……..)$_b$ content of the same amino acid in standard protein (%; casein), respectively.

Predicted biological values (BV) were calculated using the methods described by Oser [41].

$$\text{BV} = 1.09 \times \text{EAAI} - 11.7 \quad (12)$$

2.10. Statistical Analysis

Results were obtained in five replicates. The data was subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS 23.0) to determine the means, statistically significant differences ($p<0.05$) were separated using Duncan's Multiple range test.

3. Results and Discussion

3.1. Proximate Analysis of the Composite flour

The proximate analysis is presented in Table 1. The values of the moisture content ranged from 9.79 to10.44%. The values obtained in this study are comparable with the report (9.14-10.21%) of Olaniran *et al.* [42] on cassava, cowpea, and potato blends. The values met the recommended range (≤ 10%) for good storage hence implied good keeping and product quality.

The value of ash ranged between 2.72% and 3.20%. Lower values (0.98-1.81%) were recorded by Awolu and Osigwe [43] on rice, Kersting's groundnut (*Kerstingiella geocarpa*) and lemon pomace composite flour, and (0.62-1.69%) Bello *et al.* [44] on wheat, pigeon pea, and plantain composite flours. A higher value (6.54-7.85%) was recorded by Olaniran *et al.* [42] on cassava, cowpea, and potato composite flours. Ash content of the flours studied is higher than the daily recommended dietary allowance for ash in foods ≤ 0.05% [45].

The crude fibre ranged between 2.12% and 2.42%. The result indicated that the individual flours are a good source of fibre. The obtained values were comparable to the range of 2.52-3.78% reported by Awolu and osigwe [43] for rice, kersting groundnut, and lemon pomace flour blends and higher than values 0.42-1.13% for wheat, pigeon pea, and plantain flour blends [44]. Fibre gives a sensation of fullness, provides roughages, and assists in bowel movement. Furthermore, it is important in the management and prevention of degenerative diseases such as coronary diseases, colon cancer, and diabetes [46].

The range of 13.23%-39.31% was reported on protein. The high values recorded could be due to the percentage inclusion of germinated black turtle beans in the flour mix. High protein content of the composite flours suggested that the composite flours could provide needed dietary protein needed to combat protein deficiency in endemic countries.

Similar range of values (22-31%) was recorded on cassava, cowpea, and potato blends [42].

Fat content of the blends ranged from 1.09% to 1.40%. The range of values obtained compares with values (0.83-1.38%) recorded by Onwurafor *et al.* [47] on wheat, maize, and mungbean malt flour blends. Fat is vital in the diet as it enhances the absorption of fat-soluble vitamins. The low-fat content of the composite flours would promote good health and as well enhance the storability of the flours without spoilage through rancidity.

Carbohydrate content ranged from 44.03% to 77.02%. The carbohydrate content of the flour blends decreased with higher inclusion of black turtle bean. The values of carbohydrate recorded in this work is comparable to the range of values (62.03-72.09%) reported by Awolu and Osigwe [43] on rice, kersting groundnut, and lemon pomace flour blends. The carbohydrate content implied that the flour blends could be classified as an energy supplier.

Table 1. *Proximate result for Germinated Brown Rice, Aerial yam and Black Turtle Bean Flour Blends (%).*

GBR	AYF	GTB	Moisture	Ash	Fibre	Protein	Fat	Carbohydrate
100	0	0	$9.80^{b}\pm0.06$	$2.92^{c}\pm0.05$	$2.26^{bc}\pm0.01$	$13.23^{f}\pm0.32$	$1.37^{a}\pm0.02$	$70.41^{a}\pm0.30$
0	100	0	$9.80^{b}\pm0.04$	$3.20^{a}\pm0.04$	$2.42^{a}\pm0.07$	$13.55^{f}\pm0.60$	$1.23^{b}\pm0.01$	$69.81^{a}\pm0.45$
0	0	100	$10.44^{a}\pm0.44$	$2.72^{d}\pm0.05$	$2.12^{d}\pm0.07$	$39.31^{a}\pm0.85$	$1.40^{a}\pm0.02$	$44.03^{f}\pm1.24$
33.33	33.33	33.33	$9.90^{ab}\pm0.16$	$2.87^{c}\pm0.03$	$2.26^{bc}\pm0.00$	$22.28^{c}\pm0.04$	$1.09^{d}\pm0.01$	$61.61^{d}\pm0.16$
66.67	16.67	16.67	$9.96^{ab}\pm0.03$	$2.74^{d}\pm0.04$	$2.23^{c}\pm0.03$	$17.76^{d}\pm0.13$	$1.15^{c}\pm0.00$	$66.17^{c}\pm0.16$
16.67	66.67	16.67	$9.79^{b}\pm0.23$	$2.93^{bc}\pm0.01$	$2.27^{bc}\pm0.01$	$16.41^{e}\pm0.08$	$1.09^{d}\pm0.01$	$67.52^{b}\pm0.33$
16.67	16.67	66.67	$10.05^{ab}\pm0.01$	$2.99^{bc}\pm0.04$	$2.29^{bc}\pm0.04$	$32.63^{b}\pm0.04$	$1.23^{b}\pm0.01$	$50.83^{e}\pm0.04$

Values are means ± standard deviation (n=5). Means with the same superscript within the same column are not significantly different (p>0.05).
GBR-Germinated brown rice, AYF-Aerial yam flour, GTB-Germinated black turtle bean.

3.2. Anti-nutritional Content of Flour Blends

The results of the anti-nutritional content of the flour blends are shown in Table 2. Tannin content ranged from 0.973 to 1.120 mg/100g. Flour sample 100 AYF (1.120 mg/100g) had the highest value. However, the tannin contents were within the recommended safe level (2 – 5 g/day) for human consumption [48]. Low tannin values recorded may be due to the methods (soaking, germination, and dehulling) employed. Ghavidel and Prakash [49] reported that germination and dehulling reduced tannin content in cowpea, chickpea, green gram, and lentil. Tannin decreases protein quality by interfering with protein digestibility; they also bind iron and zinc making them unavailable [50]. It is responsible for astringent taste, induces browning (off-colour) in food products, could interfere with digestive processes and increase the risk of cancer [46].

Saponin varied between 1.95 and 2.44 mg/g. The inclusion of aerial yam resulted to higher saponin content in the composite flours. The saponin levels reported in this study could be considered safe. Saponin has a characteristic bitter taste, hemolytic activity, and cholesterol-binding properties.

Oxalate varied from 2.50-3.38 mg/g. The value was lower than values (90.04 – 117.05 mg/100g) obtained on wheat, pigeon pea, and plantain flour blends [44]. Flour sample 100 AYF (3.38 mg/100g) recorded the highest oxalate value. According to Adejumo *et al*.[46]. This antinutrient could form insoluble salts with trace elements such as zinc and calcium resulting in unavailability of the mineral for utilization in the body. High levels of oxalate in diets could be harmful to human health. The level of oxalate obtained is safe and below the toxicity level of 2 – 5 g/day [48].

The Phytate value ranged from 2.65 to 7.96 mg/100g. Awolu and Osigwe [43] reported higher values of 22.89 – 25.05 mg/100g for rice, Kersting groundnuts, and lemon pomace flour blends. The highest phytate content was observed in100AYF. The inclusion of aerial yam could have affected the phytate content of the flour blends.

High phytate content has been reported to adversely affect digestion as well as reduces the bioavailability of minerals in foods [50]. Phytate at low and high pH forms complexes with protein causing indigestion of food and flatulence [46]. Nonetheless, the phytate level of the composite flours was considered safe since the values were below the maximum tolerable dose (250 – 500 mg/100g) in the body [44].

Table 2. *Anti-nutritional properties of Germinated Brown Rice, Aerial yam and Black Turtle Bean Flour Blends.*

GBR (%)	AYF (%)	GTB (%)	Tannin (mg/100g)	Saponin (mg/100g)	Oxalate (mg/100g)	Phytate (mg/100g)
100	0	0	$0.973^{f}\pm0.005$	$1.951^{f}\pm0.011$	$2.500^{f}\pm0.100$	$2.652^{f}\pm0.011$
0	100	0	$1.120^{a}\pm0.002$	$2.435^{a}\pm0.016$	$3.377^{a}\pm0.125$	$7.957^{a}\pm0.012$
0	0	100	$0.988^{d}\pm0.003$	$1.990^{e}\pm0.006$	$2.617^{e}\pm0.076$	$5.555^{c}\pm0.059$
33.33	33.33	33.33	$1.022^{c}\pm0.002$	$2.070^{c}\pm0.013$	$2.730^{c}\pm0.020$	$5.146^{d}\pm0.091$
66.67	16.67	16.67	$0.981^{e}\pm0.001$	$2.000^{e}\pm0.005$	$2.607^{e}\pm0.035$	$4.434^{e}\pm0.059$
16.67	66.67	16.67	$1.073^{b}\pm0.003$	$2.213^{b}\pm0.009$	$2.983^{b}\pm0.080$	$5.935^{b}\pm0.081$
16.67	16.67	66.67	$1.018^{c}\pm0.001$	$2.036^{d}\pm0.012$	$2.670^{d}\pm0.026$	$5.232^{d}\pm0.081$

Values are means ± standard deviation (n=5). Means with the same superscript within the same column are not significantly different (p>0.05).
GBR-Germinated brown rice, AYF-Aerial yam flour, GTB-Germinated black turtle bean

3.3. Functional Properties of Composite Flours

The findings of the functional properties of the composite flours (table 3) showed that significant differences ($p<0.05$) existed among some flour samples in water absorption capacity, oil absorption capacity, water solubility index, bulk density, and swelling capacity. WAC ranged between 107.71% – 190.23%. The value (107.71%) for sample 33.33GBR: 33.33AYF: 33.33GTB was the least while the 100GTB had the highest value of 190.23%. The result obtained was comparable to the report (189.43 – 211.40%) of Ajayi *et al* [51] on rice and germinated cowpea flour blends. Water absorption capacity is ability of the food products to dissociate with water during the conditions when water is limiting as in dough. [52]. It also reflects the interaction of protein and water in food systems; therefore it is a function of the protein content of the food material [53]. The WAC of the composite flours suggests their suitability in baking application that requires water to enhance their handling characteristics.

The oil absorption capacity (OAC) ranged from 145.30 to 296.70%. Higher OAC reported in this study could be due to higher levels of germinated black turtle bean. Range of values 103.67 – 220.60% was reported by Ajayi *et al* [51] on rice and germinated cowpea flour blends while lower values (16.70 – 31.40%) were reported by Adamu and Akhere [54] for orange flesh sweet potato. OAC indicates the rate at which the protein binds to fat in food systems [55]. It is an important characteristic in food especially as flavor retainer and in enhancement of mouthfeel. Oil absorption capacity is an important quality attribute in bakery products, where fat absorption is desired [56]. The high oil absorption capacity of the composite flours evaluated suggests their potential application in sausage and other bakery products.

The water solubility index (WSI) of the composite flours ranged between 16.05 and 17.78%. The WSI determines the gradual molecular breakdown of starch granules and fibre during heat processing [57]. It indicates the level of starch degradation.

The result of the bulk density ranged from 0.53 to 0.56 g/ml. Bello *et al.* [58] recorded higher values (0.62 – 0.70 g/ml) in bulk density of sorghum, African yam bean, and soybean flour blends. Bulk density determines weight per unit volume of food sample. It is greatly influenced by particle size and the moisture content of the flour. Bulk density is an essential factor in transportation requirements, product handling, and material application in food industry [59]. The reduced bulk density suggested that more flour samples could be contained in a given space. It could be useful in weaning food preparation [60]. The flours could also find application in product development with no fear of retrogradation [44].

The swelling capacity ranged between 230.10 and 250.50%. The values were similar to values (270 – 295%) recorded for acha-amaranth flour blends [61]. According to Sodipo *et al.* [62] "swelling capacity is the volume of expansion of molecule in response to water uptake which is retained until the colloidal suspension is achieved or until further expansion and uptake are prevented intermolecular forces in the swelled particle". The high values reported on the flour blends in this study could be attributed to the presence of amylose and amylopectin in the flour due to the degradation of starch during germination of brown rice and black bean. The high swelling capacity of the composite flours implies that the flours could serve as a thickener in liquid food products [63].

Table 3. *Functional Properties of Germinated Brown Rice, Aerial yam and Black Turtle Bean Flour Blends.*

GBR (%)	AYF (%)	GTB (%)	WAC (%)	OAC (%)	WSI (%)	BD (g/ml)	SC (%)
100	0	0	$153.48^{b}\pm0.37$	$146.66^{f}\pm1.27$	$16.35^{c}\pm0.03$	$0.536^{d}\pm0.001$	$250.50^{abc}\pm3.45$
0	100	0	$148.41^{c}\pm0.75$	$145.30^{f}\pm0.91$	$16.05^{d}\pm0.01$	$0.559^{a}\pm0.002$	$244.32^{abcd}\pm0.76$
0	0	100	$190.23^{a}\pm3.24$	$296.70^{a}\pm1.15$	$17.78^{a}\pm0.01$	$0.528^{e}\pm0.003$	$258.78^{a}\pm3.56$
33.33	33.33	33.33	$107.71^{f}\pm0.95$	$193.80^{c}\pm0.62$	$16.34^{c}\pm0.01$	$0.557^{b}\pm0.003$	$230.10^{cd}\pm2.07$
66.67	16.67	16.67	$126.68^{d}\pm1.24$	$175.35^{d}\pm0.81$	$16.42^{b}\pm0.01$	$0.546^{c}\pm0.002$	$238.50^{abcd}\pm2.84$
16.67	66.67	16.67	$117.91^{e}\pm1.71$	$167.98^{e}\pm0.45$	$16.42^{b}\pm0.01$	$0.552^{b}\pm0.002$	$231.50^{cd}\pm2.54$
16.67	16.67	66.67	$121.04^{e}\pm1.11$	$238.72^{b}\pm0.45$	$16.47^{b}\pm0.01$	$0.562^{a}\pm0.003$	$235.90^{bcd}\pm2.53$

Values are means ± standard deviation (n=5). Values with different superscript within the same column are significantly different ($p<0.05$).
GBR-Germinated brown rice, AYF-Aerial yam flour, GTB-Germinated black turtle bean
WAC-water absorption capacity; OAC-oil absorption capacity; WSI-water solubility index; BD-bulk density
SC-swelling capacity

3.4. Vitamin Composition of Composite Flours

The finding of the vitamin content is presented in Table 4. Significant difference ($p<0.05$) existed among the flour blends. The 100% GTB flour had the highest vitamin content while 100% AYF had the lowest value in all the vitamins evaluated. The observed high B-complex vitamins of the composite flour could be due to the presence of these vitamins in germinated black bean and germinated brown rice flour. The pro-vitamin A content of the flour blends (2.95-4.97 mg/100 g) was lower than the values (6.23 mg/100g to 12.56 mg/100g) reported on pro vitamin A content of yellow maize-soy and jackfruit seed flours [55] but similar to the range of 2.54 – 4.15 mg/100g reported for blends of sorghum, African yam bean and crayfish flours [64]. Vitamin B_1 ranged from 2.75 to 4.06 mg/100 g. Babarinde *et al.* [65] recorded lower values of 0.23 to 0.26 mg/100g on blends of fonio and pigeon pea flour. On the other hand, Meka *et al.* [55] recorded higher values (6.13 mg/100g to 9.65 mg/100g) for yellow maize-soy and jackfruit seed flours. Thiamine is essential for effective coronary, nervous, and muscular functions [66]. Thiamine is necessary in the management of beriberi and the development of a healthy mental attitude in infants and young children [67]. The riboflavin had a range of 2.01-2.78 mg/100g. Vitamin B_2 is essential for good nutrition and healthy living essential for proper growth and development in infants and young children [68]. It is reported as an important part of the coenzyme, Flavin mononucleotide (FMN), and Flavin adenine dinucleotide (FAD), necessary for oxidation/reduction reactions and energy production [69]. The vitamin B_3 (niacin) content ranged between 2.20 and 4.83 mg/100g. Babarinde *et al* [65] reported a range of 1.58-2.21 mg/100 g for a blend of fonio and pigeon pea. Vitamin B_6 (pyridoxine) ranged from 2.63 to 3.98 mg/100g. Babarinde *et al* [65] reported lower values (0.27 – 0.47 mg/100g) for fonio and pigeon pea flour blends. Pyridoxine is reported to possess anticancer and a strong antioxidant activity [70]. According to FAO/WHO, (2004), it improves the nervous system and the formation of blood. The vitamin B_{12} (cobalamin) content flours ranged between 1.59 and 5.95 mg/100g. The values reported were higher than the range 0.30 mg/100g-1.60.mg/100g reported on yellow-maize, soybean, millet, and carrot flours [71].

Table 4. *Vitamin Content of Germinated Brown Rice, Aerial Yam and Black Turtle Bean Flour Blends (mg/100g).*

GBR (%)	AYF (%)	GTB (%)	Pro-Vit A	Vitamin B_1	Vitamin B_2	Vitamin B_3	Vitamin B_6	Vitamin B_{12}
100	0	0	$3.89^{c}\pm0.01$	$3.79^{b}\pm0.01$	$2.56^{b}\pm0.03$	$3.23^{c}\pm0.03$	$3.95^{a}\pm0.04$	$4.32^{c}\pm0.01$
0	100	0	$3.18^{d}\pm0.04$	$2.75^{f}\pm0.01$	$2.28^{c}\pm0.01$	$2.31^{d}\pm0.00$	$2.53^{f}\pm0.00$	$4.01^{d}\pm0.01$
0	0	100	$4.97^{a}\pm0.02$	$4.06^{a}\pm0.06$	$2.78^{a}\pm0.00$	$4.83^{a}\pm0.04$	$3.98^{a}\pm0.01$	$5.95^{a}\pm0.05$
33.33	33.33	33.33	$3.12^{e}\pm0.01$	$3.06^{d}\pm0.01$	$2.11^{d}\pm0.01$	$2.32^{d}\pm0.01$	$2.92^{c}\pm0.02$	$3.59^{e}\pm0.01$
66.67	16.67	16.67	$3.10^{e}\pm0.01$	$3.05^{d}\pm0.01$	$2.01^{f}\pm0.01$	$2.34^{d}\pm0.02$	$2.82^{d}\pm0.04$	$3.07^{f}\pm0.01$
16.67	66.67	16.67	$2.95^{f}\pm0.00$	$2.88^{e}\pm0.01$	$2.08^{e}\pm0.01$	$2.20^{e}\pm0.01$	$2.63^{e}\pm0.01$	$3.55^{e}\pm0.01$
16.67	16.67	66.67	$3.99^{b}\pm0.01$	$3.63^{c}\pm0.01$	$2.56^{b}\pm0.01$	$3.29^{b}\pm0.01$	$3.75^{b}\pm0.01$	$5.00^{b}\pm0.00$

Values are means ± standard deviation (n=5). Values with the same superscript within the same column are not significantly different (p>0.05).
GBR-Germinated brown rice, AYF-Aerial yam flour, GTB-Germinated black turtle bean.

Table 5. *Mineral composition of Germinated Brown Rice, Aerial Yam and Black Turtle Bean Flour blends (mg/100g).*

GBR (%)	AYF (%)	GTB (%)	Ca	P	Fe	Na	Mn	Cu	K	Zn	Mg
100	0	0	$37.77^{c}\pm0.05$	$87.63^{b}\pm0.04$	$0.22^{d}\pm0.001$	$36.81^{b}\pm0.07$	$0.46^{c}\pm0.011$	$2.12^{e}\pm0.002$	$1528.50^{f}\pm0.91$	$5.36^{j}\pm0.03$	$1741.20^{fg}\pm1.44$
0	100	0	$40.62^{a}\pm0.05$	$88.75^{a}\pm0.54$	$0.62^{a}\pm0.001$	$38.00^{a}\pm0.08$	$0.57^{a}\pm0.004$	$2.96^{a}\pm0.002$	$1619.10^{e}\pm1.90$	$6.77^{a}\pm0.02$	$1761.20^{ef}\pm1.35$
0	0	100	$36.64^{c}\pm0.36$	$86.34^{c}\pm0.00$	$0.29^{b}\pm0.001$	$35.64^{c}\pm0.81$	$0.49^{b}\pm0.001$	$2.63^{b}\pm0.001$	$1660.10^{c}\pm2.40$	$6.26^{b}\pm0.01$	$1787.00^{cd}\pm0.21$
33.33	33.33	33.33	$38.48^{bc}\pm0.05$	$86.41^{c}\pm0.02$	$0.20^{e}\pm0.001$	$36.15^{c}\pm0.28$	$0.48^{b}\pm0.003$	$2.45^{d}\pm0.013$	$1693.90^{b}\pm0.49$	$5.85^{e}\pm0.01$	$1795.20^{bc}\pm0.16$
66.67	16.67	16.67	$37.53^{c}\pm0.38$	$84.84^{d}\pm0.03$	$0.20^{e}\pm0.001$	$35.93^{c}\pm0.59$	$0.48^{b}\pm0.002$	$2.44^{d}\pm0.007$	$1637.00^{d}\pm0.71$	$5.81^{f}\pm0.00$	$1762.60^{ef}\pm0.45$
16.67	66.67	16.67	$37.99^{c}\pm0.78$	$86.32^{c}\pm0.18$	$0.29^{b}\pm0.001$	$35.54^{c}\pm0.44$	$0.49^{b}\pm0.001$	$2.57^{c}\pm0.016$	$1634.0^{d}\pm0.49$	$6.00^{cd}\pm0.01$	$1770.40^{de}\pm0.08$
16.67	16.67	66.67	$39.09^{b}\pm0.21$	$88.93^{a}\pm0.12$	$0.25^{c}\pm0.001$	$36.22^{c}\pm1.41$	$0.48^{b}\pm0.001$	$2.45^{d}\pm0.024$	$1739.20^{a}\pm0.74$	$5.98^{d}\pm0.00$	$1823.20^{a}\pm0.02$

Values are means ± standard deviation (n=5). Values with the same superscript within the column are not significantly different (p>0.05).
GBR-Germinated brown rice, AYF-Aerial yam flour, GTB-Germinated black turtle bean.
Ca-calcium;
P-phosphorous;
Fe-iron;
Na-sodium;
Mn-manganese;
Cu-copper;
K-potassium;
Zn-zinc;
Mg-magnesium.

3.5. Mineral Content of Composite Flours

The result of the mineral composition of the composite flour is presented in Table 5. Significant differences (p<0.05) existed among some of the flour samples. Calcium contents for the flour blends ranged from 36.64 to 40.62 mg/100g. This was lower than values 55.65 to 65.10 mg/100g reported for composite flour made of rice, kersting's groundnut, and lemon pomace [43]. On the other hand, Adegunwa *et al*. [56] reported lower values (2.27 – 5.63 mg/100g) for plantain–tiger nut composite flour. Blends with higher inclusion of germinated turtle bean recorded high calcium content. Babarinde *et al*. [65] also observed that the inclusion of pigeon pea resulted in higher values in calcium. Legumes are a good source of the mineral. Calcium regulates the acid-basic balance of blood, assists in teeth development of strong teeth and bones, and aids in blood clotting [50, 20]. Phosphorus content in the composite blends ranged between 84.84 and 88.93 mg/100g. Iron contents in the composite flour ranged between 0.20 and 0.62 mg/100g. Sodium content ranged from 35.54 to 38.00 mg/100g. Manganese values ranged from 0.46 to 0.57 mg/100. Copper contents in the composite flour ranged between 2.12 and 2.96 mg/100g. Potassium ranged between1528.50 and 1739.20 mg/100g. Zinc values ranged from 5.36 to 6.77 mg/10. Magnesium values ranged between1741.20 and 1823.20 mg/100g. Magnesium is the most abundant mineral evaluated.

Increased substitution with germinated black turtle bean caused a significant increase in magnesium, potassium, calcium, and phosphorous. While substitution with aerial yam increased iron, manganese, copper, and zinc content of the composite flour. The micronutrients were high and of nutritional significance. The flour blends would be a good source of mineral. Micronutrients (Zinc, iron, copper, calcium, sodium, potassium, etc.) are vital for cognitive development, bone and blood formation, and electrolyte balance [72].

3.6. Antioxidant Activity of the Composite Flour

Antioxidant capacity can be measured by total reducing power, hydroxyl radical scavenging activity, 1, 1-diphenylpicrylhydrazine (DPPH) radical scavenging activity, and FRAP, based on the reaction of the reagent with antioxidant compounds that are electron-donating or hydrogen radical producing.

The Antioxidant property of the flour blends, as evaluated by the DPPH radical scavenging method ranged from 31.33 to 67.40% (Table 6). The DPPH of the flour blends in this study (50.05 – 67.38%) is higher than the range of 3.29 – 10.39% reported on wheat-prickly pear and banana composite flour [73]. Values of 3.10 – 51.20% were reported on lentil-wheat composite flours [74]. The high DPPH value of the GBB is reflected in the flour mix whereby the flour blends that contained the highest percentage of GBB (16.67GBR: 16.67AYF: 66.67GBB) resulted in high DPPH radical scavenging activities. DPPH radical scavenging, especially those that have high GBB is an indication of their potential to donate protons faster to free radicals [75]. Higher levels of DPPH observed in 16.67GBR: 16.67AYF and 66.67GBB flour blends could be attributed to germinated black turtle bean. Antioxidant capacity is reported to be correlated to the bioactive compounds present in the sample [76].

Metal chelating activity of the individual flours and blends ranged from 28.05 to 70.96%. Furthermore, flour blend that contained 16.67GBR: 16.67AYF: 66.67GBB has the highest chelating activity while the mixtures 66.67GBR: 16.67AYF: 16.67GBB exhibited the least metal chelating activity. This implied that higher inclusion of GBB flour improved the metal chelating activity of the resulting flours. Iron is implicated in numerous oxidative reactions, resulting to the production of harmful radicals, such as the Haber-Weiss and Fenton reactions which are associated with oxidative stress in humans [77]. Hence; iron sequestration would inhibit destructive interactions with food nutrients. According to Hsu *et al.* [78] ferrous ions are effective pro-oxidant and index of metal chelating activity; a higher value of ferrous ion chelating capacity is an indication of excellent ability to bind the oxidation catalytic ferrous ion.

The FRAP of the flour blends in this study varied between 0.330 and 0.425 $mMolFe^{2+}$. Mahloko *et al.* [73] recorded a range of 0.65-0.71 $mMolFe^{2+}$ on wheat-prickly pear and banana composite flour while Awolu *et al.* [11] recorded higher values of 2.41 – 3.69 $mMolFe^{2+}$ on rice-based composite flours. The addition of GBB to either the AYF or GBR improved the FRAP contents of the resulting flours. Similarly, blending the three flours enhanced the FRAP activities of the resulting flour samples, especially when compared with a flour mix of single strength that contained only AYF or GBR. The FRAP assay determines the ability of antioxidant compounds to release electron/hydrogen ions thereby prevent the complex reaction involving free radicals [79].

The total phenolic content (TPC) of the individual flours and blends is shown in Table 1. The values ranged between 0.72 and 5.45 mgGAE/g. Bouhlal *et al.* [74] reported a range of 0.49 – 1.0 mgGAE/g on wheat-lentil composite flours. The highest phenolic content was obtained in 100AYF flour (5.45 mgGAE/g). The addition of the AYF to the GBR or the GBB improved the phenolic content of the resulting flour. Phenolic compounds contributes greatly to the antioxidant capacity of food materials; total phenolic content is shown to be directly connected with antioxidant activity [74]. It plays an important role in the management of degenerative diseases.

Table 6. *Antioxidant Properties of Germinated Brown Rice, Aerial yam and Black Turtle Bean Flour Blends.*

GBR (%)	AYF (%)	GBB (%)	DPPH (%)	M. C (%)	FRAP (mMolFe2+)	TPC (mgGAE/g)	TFC (mgRUTIN/g)
100	0	0	$31.33^{f}\pm0.63$	$28.05^{g}\pm0.54$	$0.303^{d}\pm0.002$	$0.72^{e}\pm0.09$	$38.71^{f}\pm0.40$
0	100	0	$55.08^{d}\pm1.46$	$36.88^{f}\pm0.63$	$0.306^{d}\pm0.003$	$5.45^{a}\pm0.076$	$68.63^{a}\pm0.42$
0	0	100	$61.86^{bc}\pm0.21$	$66.69^{b}\pm0.21$	$0.418^{a}\pm0.001$	$1.84^{d}\pm0.01$d	$52.17^{e}\pm0.03$
33.33	33.33	33.33	$63.52^{b}\pm0.11$	$62.26^{c}\pm0.15$	$0.384^{b}\pm0.001$	$2.25^{c}\pm0.12$	$56.74^{c}\pm0.31$
66.67	16.67	16.67	$51.70^{e}\pm0.62$	$49.68^{e}\pm0.29$	$0.353^{c}\pm0.001$	$1.54^{d}\pm0.07$	$50.76^{g}\pm0.07$
16.67	66.67	16.67	$60.98^{c}\pm0.81$	$52.49^{d}\pm0.02$	$0.356^{c}\pm0.008$	$3.45^{b}\pm0.08$	$62.91^{b}\pm0.37$
16.67	16.67	66.67	$67.38^{a}\pm0.16$	$70.96^{a}\pm0.04$	$0.425^{a}\pm0.010$	$2.07^{cd}\pm0.08$	$54.03^{d}\pm0.28$

Values are means ± standard deviation (n=5). Values with the same superscript within the column are not significantly different (p>0.05). GTB – Germinated black turtle bean.
GBR-Germinated brown rice, AYF-Aerial yam flour, GBB-Germinated black turtle bean
DPPH-1,1-Diphenylpicrylhydrazine
MC-metal chelating
FRAP-Ferric reducing ability power
TPC-total phenolic content
TFC-total flavonoid content

The total flavonoids (TFC) of the flours and the blends ranged from 38.71 to 68.63 mgRUTIN/g. This is higher than values (1.21 – 1.28 mgRUTIN/g) reported on rice enriched with Kersting's groundnut [43] as well as the range of values (0.01 – 0.59 mgRUTIN/g) reported by Bouhlal *et al*. [74] on wheat-lentil composite flours. The result also showed that flour mixtures that contained the highest quantities of AYF recorded the highest total flavonoid content of the samples, suggesting that aerial yam is a good source of flavonoids. Flavonoids are important polyphenolic components of foods and exhibit numerous biological activities (such as anti-inflammatory, anti-allergic and anti-carcinogenic activities) [43] Antioxidants (TPC and TFC) are reducing agents and counter the accumulation of free radicals in the body [11].

3.7. Amino Acid Profile of the Composite Flour

The result of the essential amino acid (table 7) showed that lysine ranged from 3.04 to 4.25 g/100 g. Lysine was highest in 100% germinated brown rice (100GBR) flour. The values reported in this study exceeded the FAO reference standards (3.00 g/100g) for adults [80]. The valine content ranged from 3.77 to 5.44 g/100 g protein. It met the FAO and WHO (3.50 g/100 g protein) reference value for infants and (1.3 g/100g) for adults. Phenylalanine ranged from 3.74 to 5.10 g/100g protein. The result was higher than FAO (2.8 g/100 g protein) standard. Substitution with aerial yam and black turtle bean led to high phenylalanine content in the flour blends. Leucine was the most abundant (6.30-10.21 mg/100g) essential amino acid followed by arginine (4.27-9.10 g/100g) which is essential for children. Leucine was highest (10.21 g/100g) in100AYF. All the flour blends had high leucine content; hence met the FAO standard (6.60 g/100 g protein) [81]. Leucine activates the release of insulin and is claimed to be the most important amino acid for building muscular mass. On the other hand, arginine is of health benefit to premature infants and individuals with severe catabolic stress; such as HIV/AIDS-diagnosed patients and subjects with symptoms of protein-energy malnutrition (PEM) [82]. Histidine in the formulated flour blends ranged between 2.26 and 3.00 g/100g and exceeded the FAO/WHO (1.9 g/100 g protein) reference value. Histidine enhances the growth, creation of body cells and tissues. Its deficiency causes anemia [83]. Tryptophan ranged from 0.89 to 1.44 g/100 g protein. The values obtained were higher than FAO standard (1.1 g/100 g protein) for children except in 100AYF and 16.67GBR: 66.67AYF: 16.67GTB.

Table 7. *Essential Amino Acid Profile of Germinated Brown Rice, Aerial yam and Black Turtle Bean Flour Blends (g/100g protein).*

GBR (%)	AYF (%)	GBB (%)	Lysine	Leucine	Isoleucine	Phenylalanine	Tryptophan	Valine	Methionine	Histidine	Arginine	Threonine
100	0	0	$4.25^{a}\pm0.21$	$6.30^{f}\pm0.14$	$3.60^{c}\pm0.14$	$3.85^{c}\pm0.07$	$1.44^{a}\pm0.05$	$4.45^{c}\pm0.07$	$2.15^{c}\pm0.07$	$2.30^{cd}\pm0.14$	$9.10^{a}\pm0.14$	$3.11^{c}\pm0.01$
0	100	0	$3.53^{b}\pm0.04$	$10.21^{a}\pm0.02$	$3.11^{e}\pm0.04$	$5.10^{a}\pm0.06$	$0.89^{f}\pm0.01$	$4.18^{d}\pm0.04$	$1.58^{f}\pm0.04$	$3.00^{a}\pm0.00$	$4.64^{d}\pm0.00$	$3.47^{b}\pm0.04$
0	0	100	$4.17^{a}\pm0.04$	$8.61^{b}\pm0.04$	$4.55^{a}\pm0.04$	$4.97^{a}\pm0.03$	$1.37^{b}\pm0.01$	$5.44^{a}\pm0.16$	$2.65^{a}\pm0.04$	$2.68^{b}\pm0.00$	$5.84^{b}\pm0.00$	$3.72^{a}\pm0.08$
33.33	33.33	33.33	$3.04^{c}\pm0.01$	$7.53^{d}\pm0.04$	$3.63^{c}\pm0.06$	$3.74^{c}\pm0.01$	$1.05^{e}\pm0.00$	$3.99^{de}\pm0.09$	$2.06^{d}\pm0.02$	$2.30^{cd}\pm0.03$	$3.74^{f}\pm0.06$	$2.68^{e}\pm0.01$
66.67	16.67	16.67	$3.33^{bc}\pm0.04$	$7.05^{e}\pm0.01$	$3.60^{c}\pm0.01$	$3.79^{c}\pm0.00$	$1.10^{d}\pm0.01$	$4.05^{d}\pm0.09$	$2.01^{d}\pm0.01$	$2.26^{d}\pm0.01$	$5.27^{c}\pm0.08$	$2.89^{d}\pm0.01$
16.67	66.67	16.67	$3.10^{c}\pm0.01$	$8.07^{c}\pm0.01$	$3.36^{d}\pm0.08$	$4.06^{b}\pm0.06$	$0.93^{f}\pm0.00$	$3.77^{e}\pm0.09$	$1.77^{e}\pm0.03$	$2.45^{c}\pm0.01$	$3.78^{f}\pm0.05$	$2.90^{d}\pm0.01$
16.67	16.67	66.67	$3.39^{b}\pm0.01$	$8.09^{c}\pm0.04$	$4.02^{b}\pm0.05$	$4.10^{b}\pm0.06$	$1.26^{c}\pm0.01$	$4.68^{b}\pm0.05$	$2.44^{b}\pm0.02$	$2.46^{c}\pm0.05$	$4.27^{e}\pm0.04$	$2.82^{d}\pm0.04$
RDA			5.8	6.6	2.8	2.8	1.2	3.5	2.2	1.9	2.0	3.4

Values are means ± standard deviation (n=5). Values with the same superscript within the column are not significantly different (p>0.05).
GBR=Germinated brown rice, AYF=Aerial yam flour, GTB=Germinated black turtle bean. *RDA Recommended Daily Allowance (United States Department of Agriculture, USDA 2018)

The non-essential amino acids of the composite flour are presented in Table 8. Tyrosine, cysteine, and alanine ranged from 2.84-4.25, 1.21-1.92, and 3.95 – 6.49 g/100 g of protein respectively. The glycine, serine, and proline content were 3.25-4.32, 2.10-4.24, and 3.55-4.37 g/100 g of protein respectively. Aspartic acid (6.54 and 7.91 g/100 g) and glutamic acid (11.11 to15.37 g/100 g) of protein were the most abundant nonessential amino acids. Glycine, alanine, arginine, and phenylalanine form part of amino acids that enhance growth and tissue healing [43]. The flour blends contained all the essential amino acids. The complementary effect of the blend improved the levels of the amino acids in germinated brown rice, aerial yam, black turtle bean flour blends with 16.67GBR; 16.67AYF; 66.67GBB having a better amino acid balance. Hence, the blends could be used as a protein and amino acid supplement in the formulation of food products.

Table 8. *Non-essential amino acids profile of germinated brown rice, aerial yam and black turtle bean flour blends (g/100 g crude protein).*

GBR (%)	AYF (%)	GBB (%)	Tyrosine	Cystine	Alanine	Glutamic acid	Glycine	Proline	Serine	Aspartic acid
100	0	0	$4.25^{a}\pm0.21$	$1.85^{a}\pm0.01$	$4.75^{c}\pm0.07$	$13.15^{b}\pm0.21$	$4.32^{a}\pm0.03$	$5.20^{a}\pm0.14$	$2.10^{d}\pm0.14$	$7.25^{b}\pm0.07$
0	100	0	$3.61^{b}\pm0.00$	$1.21^{d}\pm0.00$	$4.40^{d}\pm0.11$	$13.17^{b}\pm0.00$	$3.56^{c}\pm0.00$	$3.65^{d}\pm0.00$	$3.73^{b}\pm0.08$	$6.54^{d}\pm0.04$
0	0	100	$3.53^{b}\pm0.12$	$1.82^{a}\pm0.00$	$6.49^{a}\pm0.16$	$15.37^{a}\pm0.11$	$3.90^{b}\pm0.06$	$4.37^{b}\pm0.15$	$4.24^{a}\pm0.04$	$7.91^{a}\pm0.22$
33.33	33.33	33.33	$2.84^{d}\pm0.08$	$1.65^{b}\pm0.04$	$4.12^{e}\pm0.01$	$11.11^{c}\pm0.45$	$3.25^{e}\pm0.06$	$3.55^{de}\pm0.06$	$3.54^{bc}\pm0.01$	$7.12^{bc}\pm0.05$
66.67	16.67	16.67	$3.34^{bc}\pm0.18$	$1.65^{b}\pm0.04$	$4.20^{de}\pm0.14$	$11.33^{c}\pm0.65$	$3.65^{c}\pm0.06$	$4.08^{c}\pm0.09$	$3.22^{c}\pm0.07$	$7.19^{bc}\pm0.08$
16.67	66.67	16.67	$3.01^{c}\pm0.05$	$1.36^{c}\pm0.04$	$3.95^{f}\pm0.02$	$11.73^{bc}\pm0.09$	$3.37^{d}\pm0.04$	$3.52^{de}\pm0.05$	$3.57^{b}\pm0.00$	$6.94^{c}\pm0.01$
16.67	16.67	66.67	$2.92^{d}\pm0.16$	$1.92^{a}\pm0.02$	$4.99^{b}\pm0.02$	$12.21^{bc}\pm0.58$	$3.25^{e}\pm0.04$	$3.70^{d}\pm0.06$	$3.67^{b}\pm0.10$	$7.32^{b}\pm0.13$

Values are means ± standard deviation (n=5). Values with the same superscript within the same column are not significantly different (p>0.05).
GBR=Germinated brown rice, AYF=Aerial yam flour, GTB=Germinated black turtle bean.

3.8. Protein Quality of the Composite Flour

The EAAI for the germinated brown rice, aerial yam, black turtle bean flours, and their blends in this study ranged from 79 to 99% (Table 9). 100GTB had the highest EAAI. Germinated black turtle bean could have improved the essential amino acid index (EAAI) of the flour blends. It has been reported that a protein food product is of high nutritional quality if the essential amino acid index (EAAI) is more than 90%, termed to be good as food when the values are above 80% and considered inadequate for food material if it is below 70% [84]. According to Fang *et al.* [85], the higher the essential amino acid index, the more balanced the amino acid profile and the better the quality of the protein. All the flour blends had an EAAI value above 70. This implies that the blends of germinated brown rice, aerial yam, and germinated black turtle bean are good protein sources. The biological value (BV) for the flour blends ranged from 70 to 96%. Biological value determines the amount of protein from food that is absorbed into the proteins of the organism's body [84]. A protein food material with BV between 70 and 100% is considered good protein quality. All the flour blends had BV higher than 70%. The high biological value of the composite flour could be attributed to substitution with Black turtle bean. Most cereals and tubers are low in protein and some essential nutrients; hence blending with a legume such as black turtle bean which has high protein and essential amino acids, could improve their nutritional content.

Table 9. *Protein quality of germinated brown rice, aerial yam and black turtle bean flour blends.*

GBR (%)	AYF (%)	GBB (%)	EAAI (%)	BV (%)	PER
100	0	0	$83.76^{c}\pm0.02$	$79.60^{c}\pm2.33$	$1.95^{f}\pm0.09$
0	100	0	$87.20^{b}\pm0.01$	$83.35^{b}\pm0.82$	$3.79^{a}\pm0.00$
0	0	100	$99.06^{a}\pm0.01$	$96.28^{a}\pm0.89$	$3.08^{b}\pm0.01$
33.33	33.33	33.33	$80.31^{d}\pm0.01$	$75.84^{d}\pm0.71$	$2.66^{d}\pm0.01$
66.67	16.67	16.67	$80.36^{d}\pm0.00$	$75.89^{d}\pm0.01$	$2.38^{e}\pm0.03$
16.67	66.67	16.67	$79.86^{d}\pm0.01$	$75.34^{d}\pm0.86$	$2.88^{c}\pm0.01$
16.67	16.67	66.67	$88.45^{b}\pm0.01$	$84.71^{b}\pm0.81$	$2.90^{c}\pm0.00$

Values are means ± standard deviation (n=5). Values with the same superscript within the same column are not significantly different ($p>0.05$).
GBR=Germinated brown rice, AYF=Aerial yam flour, GTB=Germinated black turtle bean.
EAAI=Essential amino acid index
BV=Biological value
PER=Protein efficiency ratio.

Protein efficiency ratio (PER) is the ratio of weight gained by the body to the amount of the ingested protein; it evaluates the capacity of the protein to enhance growth [86]. The PER range (1.95 – 3.79) for the composite flour studied was comparable to the range (2.30 – 3.19) reported by Malomo and Abiose [81] for Masa flours. Substitution with germinated black turtle bean could have improved the protein efficiency ratio (PER) of the composite flour. Casein has PER of 2.5 while soybean has PER of 2.2 [87]. According to Friedman [88], PER lower than 1.5 indicates low protein quality while values higher than 2 are an indication of high protein quality. All the flour blends had PER higher than 2 except 100GBR which suggests good quality protein.

4. Conclusion

The research showed that activation of cotyledon of brown rice, and black turtle bean improved their nutrients and antioxidative characteristics which are advantageous as a source of high quality protein to the poor. The mineral and vitamin contents of the blends are adequate to supply some of the essential micronutrients needed for good health. Water and oil absorption capacities, swelling capacity, and bulk density also contributed to the quality of the flour blends. Some of the composite flours showed good amino acid balance, protein quality; and antioxidant activity. The flour blends could be useful in the development of functional food products. Therefore, further studies should be carried out on the application of the flour blends in development of breakfast flakes and cookies.

Conflicts of Interest

All the authors do not have any possible conflicts of interest.

Ethical Approval and Consent to Participate

Not applicable.

Availability of Data and Material

Not applicable.

Funding

This research was supported by the Tertiary Education Trust Fund (TETFUND), Nigeria.

References

[1] Hasmadi, M., Noorfarahzilah, M., Noraidah, H., Zainol, M. K. and Jahurul, M. H. A. (2020). Functional properties of composite flour: a review. *Food Research* 4 (6): 1820–1831.

[2] Igbabul, B. D., Iorliam, B. M., and Umana, E. N. (2015) Physicochemical and sensory properties of cookies produced from composite flours of wheat, cocoyam and African yam beans. Journal of Food Research. 4 (2): 150-151.

[3] FAO/WHO (2001). Human vitamin and mineral requirements. Report of a Joint FAO/WHO Expert Consultation, Food and Nutrition Division, Bangkok, Thailand: 7-8.

[4] Ebuehi, O. A. T., and Oyewole, A. C. (2008). Effect of cooking and soaking on physical, nutrient composition and sensory evaluation of indigenous and foreign rice varieties in Nigeria. Nutrition and Food Science, 38 (1): 15-21.

[5] Bryant, R. J. Kadan, R. S., Champagne, E. J., Veinyord, B. T. and Boykin, B. B. (2001). Functional and Digestive Characteristics of Extruded Rice Flour. *Cereal Chemistry* 78: 131-137.

[6] Gujral, H. S and Rosell, C. M (2004) Functionality of rice flour modified by microbial transglutaminase. *Journal of Cereal Science* 39 (2): 225-230.

[7] Falade, K. O., and Christopher, A. S. (2014). Physical, functional, pasting and thermal properties of flours and starches of six Nigerian rice cultivars. Food Hydrocolloids, 39: 41-50.

[8] Wu, F. F., Yang, N., Toure, A., Jin, Z. Y and Xu, X. M. (2013). Germinated brown rice and its role in human health. Crit Rev Food Sci Nutr, 53 (5): 451–463.

[9] Cornejo, F., Caceres, P. J., Martínez-Villaluenga, C., Rosell, C. M., and Frias, J. (2015). Effects of germination on the nutritive value and bioactive compounds of brown rice breads. Food Chemistry, 173, 298-304.

[10] Awolu, O. O., Morayo, P. O., Iyanuoluwa, O. F. and Funmilayo, G. O (2015) Optimization of the extrusion process for the production of readyto-eat snack from rice, cassava and Kersting’s groundnut composite flours. *LWT-Food Sci Technol* 64: 19–24. doi: 10.1016/j.lwt.2015. 05.025.

[11] Awolu, O. O., Omoba, O. S., Olawoye, O., and Dairo, M. (2017). Optimization of production and quality evaluation of maizebased snack supplemented with soybean and tiger-nut (Cyperus esculenta) flour. Food Science & Nutrition. 5 (1), 3–13.

[12] Amoldi, A., Zanoni, C., Lammi, C., and Boschin, G. (2014). The role of grain legumes in the prevention of hypercholesterolemia and hypertension. Critical Review in Plant Sciences, Vol. 34; Issue 1-3, p. 105-143.

[13] Boelt, B., Julier, B., karagic, D. and Hampton, J. (2014). Legume seed production meeting market requirements and economic impacts. Critical Reviews in Plant Sciences, Vol 34; Issue 1-3 p. 95-103.

[14] Okafor, D. C., Enwereuzoh, R. O., Ibeabuchi, J. C., Uzoukwu, A. E., Alagboso, S. O and Udenkwo, C (2015). Production of Flour types from black bean (*Phaseolus vulgaris*) and effect of pH and temperature on functional physio-chemical properties of the flours. *European journal of Food Science and Technology*. 3 (2): 64-84.

[15] Chima, J. U and Fasuan, T. O (2020) Antioxidant, nutritional, antinutrients and functional characteristics of black turtle bean (Phaseolus vulgaris): synergistic and antagonistic interrelationship of epigeal germination periods. https://www.emerald.com/insight/0034-6659.htm.

[16] Aremu, M. O., Audu, S. S., and Gav, B. L (2017). Comparative Review of Crude Protein and Amino Acid Composition of Some Leguminous Seeds Grown in Nigeria. *International Journal of Sciences*. 6 (8): 89-97.

[17] Reverri, E. J; Randolph, J. M; Steinberg, F. M; Tissa Kappagoda, C; Edirisinghe. I and Burton-Freeman, B. M (2015) Black Beans, Fiber, and Antioxidant Capacity Pilot Study: Examination of Whole Foods vs. Functional Components on Postprandial Metabolic, Oxidative Stress, and Inflammation in Adults with Metabolic Syndrome. Nutrients 7, 6139-6154; doi: 10.3390/nu7085273.

[18] Hayat, I., Ahmad, A., Masud, T., Ahmed, A., & Bashir, S. (2014). Nutritional and health perspectives of beans (Phaseolus vulgaris L.): an overview. Critical Reviews in Food Science and Nutrition, 54 (5), 580–92.

[19] Lawal, A. I and Akinoso. R (2019). Physical Properties, Proximate Composition and Antioxidant Activities of Aerial Yam (*Dioscorea bulbifera*) Bulbils Grown in Nigeria. *BIBLID*: 1450-7188. 50, 143-151.

[20] Olatoye, K. K and Aruenya, G. L (2019) Nutrient and phytochemical composition of flour made from selected cultivars of Aerial yam (*Dioscorea bulbifera*) in Nigeria. *Journal of Food Composition and Analysis* 79: 23–27.

[21] Aathira M and Siddhuraju P. (2017). Evaluation on suitability of differentially processed *D. bulbifera* tubers (Aerial and Underground) as alternative in composite flours for future food innovations. *International Journal of Food Science and Nutrition*.; 2 (6): 142-148.

[22] Ojinnaka, M. C., Odimegwu, E. N., and Ilechukwu, R. (2016) Functional properties of flour and starch from two cultivars of aerial yam (*Dioscorea bulbifera*) in South East Nigeria. IOSR *Journal of Agriculture and Veterinary Science* (IOSR-JAVS) e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 8 Ver. I: 22-25.

[23] AOAC (2005). Official Methods of Analysis, 18th ed. Association of official Analytical chemists Washington, D.C. USA.

[24] Brunner J. H (1994). “Direct Spectrosphotometric Determination of Saponin”. *Journal. Analytical Chemistry.* 34: 1314-1326.

[25] Falade O. S, Dare A. F, Bello M. O, Osuntogun B. O, and Adewusi S. R. A (2004). “Varietal Changes in Proximate composition and the Effect of Processing on the ascorbic content of some Nigerian Vegetables”. *Journal of Food Technology* 2: 103-108.

[26] Vaintraub, I. A. and Lapteva, N. A. (1988). “Colorimetric determination of phytate in unpurified extracts of seeds, and the products of their processing”. *Analytical Biochemistry*, 175, 227–230.

[27] Adebiyi, A. P., and Aluko, R. E. 2011. ‘Functional properties of protein fractions obtained from commercial yellow field pea (*Pisum sativum L.)* seed protein isolate’. *Food Chemistry*, 128 (4): 902-908.

[28] Maninder, K., Kawaljit, S. S and Narpinder, S (2007) Comparative study of functional, thermal and pasting properties of flours from different field pea and pigeon pea cultivars. Food Chem 104: 259–267.

[29] Takashi S. and Seib R. A. (1988). “Paste and gel properties of prime corn and wheat starches with and without native lipids”. *Cereal Chemistry*. 65: 474.

[30] Antakli, S., Sarkees, N. and Sarraf, T. (2015). Determination of vitamins on c18 column with particle size 3 μm in some manufactured food products by HPLC with UV-DAD/FLD detection. *International Journal of Pharmacy and Pharmaceutical Sciences* Vol 7, Issue 6.

[31] Adedeye, A. and Adewoke, K. (1992). Chemical Composition and Fatty acid Profiles of Cereals in Nigeria Food Chem., 44: 41-44.

[32] Girgih, A., Udenigwe, C., Li, H., Adebiyi, A., and Aluko, R. (2011). “Kinetics of enzyme inhibition and antihypertensive effects of hemp seed (Cannabis sativa L.) protein hydrolysates”. *Journal of the American Oil Chemists Society*, 88: 1767–1774.

[33] Benzie, I. F. F and Strain, J. J. (1999). “Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay”. *Analytical Biochemistry*, 239: 70-76.

[34] Xie, Z., Huang, J., Xu, X. and Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry*, 111: 370–376.

[35] Hoff, J., and Singleton, K. (1977). “A method for determination of tannins in foods by means of immobilized protein”. *Journal of Food Science,* 42 (6), 1566–1569.

[36] Nabavi, S. F., Nabavi, S. M., Ebrahimzadeh, M. A., Eslami, B., and Jafari, N. (2013). In vitro antioxidant and antihemolytic activities of hydroalcoholic extracts of Allium scabriscapum Boiss. & Ky. aerial parts and bulbs. *International J. of Food Properties*, 16 (4), 713–722.

[37] Siswoyo, T. A., Mardiana, E., Lee, K. O and Hoshokawa, K (2011). Isolation and characterization of antioxidant protein fractions from melinjo *(Gnetum gnemon*) seeds. *J. Agric. Food Chem*., 59: 5648-5656.

[38] Planesso, D., Neto, J. R., Da Silva, L. P., Goulart, F. R and Adorian, T. J (2015). Determination of Tryptophan requirements for juvenile silver catfish (*Rhamdia quelen*) and its effects on growth performance, plasma and hepatic metabolites and digestive enzymes activity. *Anim. Feed Sci. Technol*., 210: 172-183.

[39] Alsmeyer, R. H., Cunninghan, A. E and Happich, M. L (1974). Equations predict PER from amino acid analysis. *Food Technology,* 28: 34-40.

[40] Steinke, F. H., Presher, E. E and Hopkins, D. T (1980). Nutritional evaluation (PER) of isolated soy bean protein and combination of food proteins. *Journal of Food Science*, 45: 323-327.

[41] Oser, B. L (1959). An Integrated essential amino acid profile of the brain and eye of African giant punch kratd (*Crieetomys gambianus*). *Agric Bist. J. North Am,* 2: 368-375.

[42] Olaniran, A. F., Okonkwo, C. E., Owolabi, A. O, Osemwegie, O. O, and Badejo, T. E (2020). Proximate composition and physicochemical properties of formulated cassava, cowpea and potato flour blends. *IOP Conf. Ser.: Earth Environ. Sci.* 445 012042.

[43] Awolu, O. O and Osigwe, M. A (2019) Nutritional and Antioxidant Potential of Rice Flour Enriched with Kersting’s Groundnut (*Kerstingiella geocarpa*) and Lemon Pomace. *International Journal of Food Studies*. 8: 30–40.

[44] Bello, F. A., Oyeniyi, A. O. and George, I. O. (2019a) Chemical and Functional Properties of Wheat, Pigeon Pea and Plantain Composite Flour. *European Journal of Food Science and Technology*. 7 (4): 1-8.

[45] FAO/WHO (1998) Carbohydrates in human nutrition: report of a Joint FAO/WHO Expert Consultation, 14–18 April 1997, Rome. FAO Food and Nutrition Paper No. 66. Rome.

[46] Adejumo P. O., Adejumo A. O., Edebiri O. E and Olukoya F. O (2020). Effect of unripe banana and pigeon pea flour on the chemical, anti-nutritional and sensory properties of whole wheat-based cookies. *GSC Advanced Research and Reviews,* 04 (01), 017–023.

[47] Onwurafor, E. U., Uzodinma, E. O., Obeta, N. A and Akubueze, V. O (2020) Development and Quality Evaluation of Noodles from Wheat Flour Substituted with Maize and Mungbean Malt Flour. *Pak. J. Nutr.,* 19 (7): 337-343.

[48] Hassan, L. G., Umar, K. J., and Umar, Z. (2004). Antinutritive factors in tribulus terrestris (linn.) leaves and predicted calcium and zinc bioavailability. *Journal Tropical Bioscience*, 7, 33–36.

[49] Ghavidel, R. A and Prakash, J (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *Food Sci. and Technol.* 40: 1292–1299.

[50] Achy, J. Y., Koffi, P. K. B., Ekissi, G. S. E., Konan, H. K and Kouame, L. P. (2016). Assessment of Physico-chemical Properties and Anti-nutritional factors of flour from Yam (*Dioscorea bulbifera)* Bulbils in Southeast Cote D’ivore. *Int. J. Adv. Res*. 4 (12): 871-88.

[51] Ajayi F. F., Kusimo O. O, Henshaw F. O and Ogori A. F (2017). “Proximate Composition and Functional properties of Rice (Oryza Sativa L.) Germinated-Cowpea (Vigna Unguiculata L.) flour blends”. *Innovative Techniques in Agriculture* 1.1: 32-40.

[52] Oppong D, Eric A, Samuel O. K, Eric B, and Patrick S (2015). Proximate Composition & Some Functional Properties of Soft Wheat Flour. *Int’l J. of Innovative Research in Sci, Engineering and Technol.* 4 (2): 753–758. DOI: 10.15680/IJIRSET.2015.0402097.

[53] Tortoe, C, Dowuona, S, Akonor, P. T and Dziedzoave1, N. T (2017). Examining the physicochemical, functional and rheological properties in flours of farmers’ 7 key yam (*Dioscorea spp*.) varieties in Ghana to enhance yam production. *Cogent Food & Agriculture.* 3: 1371564.

[54] Adamu, O. A and Akhere, E. P (2020). Proximate Composition, Functionality, and Pasting Properties of Orange Flesh Sweet Potato and Red Bambara Groundnut Flour Blends for Snacks Formulation. *Asian Food Science Journal*, 17 (1): 38-47.

[55] Meka, E., Igbabul, B. D., and Ikya, J (2019). Chemical and Functional Properties of Composite Flours Made from Yellow Maize, Soybeans, and Jackfruit Seed. *International Journal of Research and Innovation in Applied Science (IJRIAS)* | 4 (11): 57-63.

[56] Adegunwa, M. O., Adelekan, E. O., Adebowale, A. A., Bakare, H. A and Alamu, E. O (2017). Evaluation of nutritional and functional properties of plantain (*Musa paradisiaca* L.) and tigernut (*Cyperus esculentus* L.) flour blends for food formulations, *Cogent Chemistry* 3: 1383707.

[57] Rashid, S., Rakha, A., Anjum, F. M., Ahmed, W., and Sohail, M. (2015). Effects of extrusion cooking on the dietary fibre content and water solubility index of wheat bran extrudates. *Internastional journal of Food Science and Technology,* 50 (7), 1533-1537.

[58] Bello F. A, Edeke J. E, Sodipo M. A (2019b) Evaluation of Chemical, Functional and Sensory Properties of Flour Blends from Sorghum, African Yam Bean and Soybean for Use as Complementary Feeding. *International Journal of Food Science and Biotechnology* 4 (3): 74-81.

[59] Ocloo, F. C. K, Bansa D, Boatin, R, Adom, T, Agbemavor, W. S (2010). Physicohemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds. *Agriculture and Biology journal of North America*. ISSN print: 2151-7517.

[60] Ayo, J. A and Gidado, F. E. (2017) Physicochemical, phytochemical and sensory evaluation of acha-carrot flours blend biscuit current. *Journal of Applied Science and Technology known as British Journal of Applied Science & Technology*. 25 (5): 1-15.

[61] Ayo, J. A and Okoye. E. (2020). Nutrient Composition and Functional Properties of Fonio (*Digetaria exilis*) and Amaranth (*Amaranthus cruentus*) Flour Blends. *Asian Food Science Journal*. 16 (3): 53-62.

[62] Sodipo, M. A., Lawal, O. M., Alabi, O. E., Solomon, O. O., Oluwamukomi, M. O and Oluwalana, I. B (2018). Physicochemical Properties and Amino Acid Profile of Extruded Products from Pearl Millet and Germinated Pigeon Pea. *Annals. Food Science and Technology*. 19 (2): 183-190.

[63] Siddiqua, A., Ali, M. S. and Ahmed, S (2019). Functional properties of germinated and non-germinated cereals: A comparative study. *Bangladesh J. Sci. Ind. Res.* 54 (4): 383-390.

[64] Egbujie, A. E. and Okoye, J. I. (2019) Quality Characteristics of Complementary Foods Formulated From Sorghum, African Yam Bean and Crayfish Flours. *Science World Journal.* 14 (2): 16-22.

[65] Babarinde, G. O, Adeyanju., J. A, Ogunleye, K. Y, Adegbola, G. M, Ebun, A. A, and Wadel, D.(2020) Nutritional composition of gluten-free flour from blend of fonio (Digitaria iburua) and pigeon pea (Cajanus cajan) and its suitability for breakfast food. *J Food Sci Technol* https://doi.org/10.1007/s13197-020-04393-7.

[66] Eze C. R., Okafor G. I., Omah E. C. and Azuka, C. E. (2020) Micronutrients, antinutrients composition and sensory properties of extruded snacks made from sorghum and charamenya flour blends. *African Journal of Food Science.* 14 (1): 25-31.

[67] Okaka, J. C., Akobundu, E. N. T. and Okaka, A. N. C. (2006). Food a nd Human Nutrition: An Integrated Approach, 2nd ed. Ocjanc o Academic Publishers, Enugu, Nigeria. Pp. 102-144.

[68] Okwu, D. E. (2004). Phytochemicals and vitamin content of indigenous spices of South-Eastern Nigeria. *Journal of Sustainable Agriculture and Environmental Sciences*; 6 (1): 30-37.

[69] Onimawo IA, and Asugo, S (2008) Effects of germination on the nutrient content and functional properties of pigeon pea flour. *J Food Sci Technol* 41: 170–174.

[70] Theodoratou, E., Farrington, S. M., Tenesa, A., McNeil, G., Cetnarskyj, R., Barnetson, R. A., Porteous, M. E., Dunlop, M. G and Campbell, H (2008). Dietary vitamin B_6 intake and the risk of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.*, 17, 171-182.

[71] Oyegoke, T. G., Adedayo E. O., Fasuyi F. O., and Oyegoke D. A (2020). Vitamin and Mineral Composition of Complementary Food Formulated from Yellow Maize, Soybean, Millet and Carrot Composite Flours. *International Journal of Science and Research (IJSR)*, 9 (2): 450–456.

[72] Oyarekua, M. A., 2010. Sensory evaluation, nutritional quality and antinutritional factors of traditionally co-fermented cereals/cowpea mixtures as infant complementary food. Agric. Biol. J. N. Am., 1: 950-956.

[73] Mahloko, L. M; Silungwe H; Mashau, M. E and Kgatla, T. E (2019). Bioactive compounds, antioxidant activity and physical characteristics of wheat-prickly pear and banana biscuits. *Heliyon* 5 e02479.

[74] Bouhlal O, Taghouti M, Benbrahim N, Benali A, Visioni A and Benba J (2019). Wheat-lentil fortified flours: health benefits, physicochemical, nutritional and technological properties. J. Mater. Environ. Sci. 10 (11): 1098-1106.

[75] He, R., Girgih, A. T., Malomo, S. A., Ju, X and Aluko, R. E (2013) Antioxidant activities of enzymatic rapeseed protein hydrolysates and the membrane ultrafiltration fractions. *Journal of functional foods.* 5 (1): 219-227.

[76] Kaur, M., Asthiri, B and Mahajan, G (2017). Variation in Antioxidants, Bioactive Compounds and Antioxidant Capacity in Germinated and Ungerminated Grains of Ten Rice Cultivars. *Rice Science*, 24 (6): 349î359.

[77] Zhang, Y.; Lee, E. T.; Devereux, R. B.; Yeh, J.; Best, L. G.; Fabsitz, R. R. and Howard, B. V (2006). Prehypertension, diabetes, and cardiovascular disease risk in a population-based sample: The strong heart study. Hypertens, 47: 410–414.

[78] Hsu, A. L., Murphy, C. T., Kenyon, C (2003). Regulating of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300, 1142-1145.

[79] Dorman, H. J. D., Peltoketo, A., Hiltunen, R., Tikkanen, M. J. (2003). Characterization of the antioxidant properties of deodorised aqueous extracts from selected Lamiaceae herbs. Food Chemistry, 83 (2), 255–262.

[80] FAO/WHO. (1990). Protein Quality Evaluation. Report of joint FAO/WHO Expert Consultation. Held in Bethesda, USA, 4-8 December, 1989. FAO, Rome, Italy.

[81] Malomo, A. A and Abiose, S. H (2019). Protein quality and functional properties of masa produced from maize, acha and soybean. *Food Research* 3 (5): 556-563.

[82] Arogba S. S, Akpala S. N, Amlabu E and Amodu L (2020) Sumain nutritional supplement: Formulation, physico-chemical and nutritional assessment of its flour. GSC Biological and Pharmaceutical Sciences. 10 (03), 137–149.

[83] Fasuan T. O, Asadu K. C, Anyiam C. C, Ojokoh L. O, Olagunju T. M, Chima J. U and Okpara K. O (2021a) Bioactive and nutritional characterization of modeled and optimized consumer-ready flakes from pseudocereal (Amaranthus viridis), high-protein soymeal and modified corn starch. *Food Production, Processing and Nutrition* 3: 12 https://doi.org/10.1186/s43014-021-00057-x.

[84] Okpala, L. C., Egbadon, L and Okoye, S. (2016). Physicochemical and Protein Quality of Noodles made with Wheat and Okara flour blends. *Pakistan journal of Nutrition* 15 (9): 829-836.

[85] Fang, Y., Xingjian, H., Conglan, Z. C., Mei, Z., Chao, H. and Hao, Y. (2018). Amino acid composition and nutritional value evaluation of Chinese Chestnut (Castanea mollissima Blume) and its protein subunit. *Royal Society of Chemistry*, 8, 2653–2659.

[86] Fasuan T. O, Asadu K. C, Ojokoh L. O, Anyiam C. C, Olagunju T. M, Okpara K. O and Chima J. U (2021b) Ready-To-Eat Flakes from Acha, Partially Defatted Sesame Meal and Modified Corn Starch: Modeling, Optimization and Characterization. *Food Sc and Nutri. Tech.* 6 (1): 1-12.

[87] Olagunju, A. I., Ekeogu, P. C and Bamisi, O. C (2020). Partial substitution of whole wheat with acha and pigeon pea flours influences rheological properties of composite flours and quality of bread. https://www.emerald.com/insight/0007-070X.htm.

[88] Friedman, M (1996). Nutritional value of proteins from different food sources. A review. J. Agric. Fd. Chem., 446-29.