EVALUATION OF THE NUTRITIONAL, ANTIOXIDANT AND PHYSIOCHEMICAL PROPERTIES OF RICE FLOUR FORTIFIED WITH THE NIGERIA NATIVE "IGBEMO" RICE BRAN FLOUR.

Abstract

The study evaluate the physicochemical, functional and nutritional properties of rice flour fortified with Nigeria native rice bran flour. Response surface methodology was used and a total of thirteen runs was generated. The four best runs were selected from the thirteen based on the composition of their crude protein and fibre content viz: run (1, 4, 8 and 10). The proximate composition of the selected runs in protein (7.33-9.47%) and crude fibre (0.79-4.99%) respectively. The functional properties of the flours were WAC (77.72-84.95%), OAC (110.73-127.81%), foaming capacity (5.00-5.90%), swelling capacity (201.00-271.00 g/ml) has the best result obtained in run 8 respectively. The anti-nutritional factor of the flours were oxalate (0.270-1.305 mg/g), tannin (1.060-1.941 mg/g), alkaloid (6.813-10.413 mg/g) and phytate (9.476-19.364 mg/g). Further analyses on the mineral components of the flour blends showed high amount in potassium, phosphorus, sodium, iron and also appreciable amount of both zinc and copper. More so, the antioxidant properties of the flour samples were Fe2+ chelation (0.270-1.305%), FRAP (3.261-8.689 mg AAE/g), DPPH (69-76%), ABTS (0.011-0.023%), total flavonoid (0.009-0.050%) and total phenol (2.310-4.544%). Bread was produced from the composite flour blends and subjected to sensory evaluation on a nine point hedonic scale with overall acceptability in the range of (5.80 -6.03), crumbiness (6.17 – 6.57) and crustiness (6.30 -6.63). The flours also showed high amount of insoluble fibres against soluble fibres. Hence, the flour blends could serve as a wheat flour substitute.

Key word: Wheat flour, rice flour, Igbemo rice bran, bread, Physicochemical, Functional properties, Nutritional properties, Mineral composition, sensory properties

INTRODUCTION

Food fortification is a proven, sustainable, cost-effective and high-impact solution to address micronutrient deficiencies. The term is the deliberate addition of micronutrient in food to increase nutritional stature of food to address nutritional deficiency disorders which

occurs due to poor diet. It is most productive, effective and long term approach used to enhance micronutrient status of malnourished communities (De Romana et al, 2003; Cozzolino et al., 2019). Wheat, rice and maize are the most commonly used cereals and they are popular with respect to fortification significances among researchers and stakeholders due to recognition, affordability and availability (Dijhkuizen, 2000).

Rice is one of the leading crops of world and is second only to wheat in term of production and food based uses. About 90% of world's rice is produced and consumed in Asia (Allgrove and Shaw 2015). About 870 million people are estimated to suffer from chronic undernourishment globally, the vast majority of whom live in developing countries where rice is closely associated with food security. Due to its dietary uses, higher digestibility and commercial importance; rice is considered as queen of cereals. But, due to the lose of mineral, vitamin and dietary fibre during milling it is limited in nutritional components. Fortification of rice is an effectual strategy to the tackle micronutrient imperfection.

Rice bran, a byproduct of rice kernel is obtained during rice milling (Mendel, 2013). Rice brans contains essential amino acids such as tryptophan, histidine, methionine, cysteine, and arginine. It also contains many health promoting bioactive compounds such as ϒ-Oryzanol, Ferulic acid (Chaudhari *et al*., 2018) and also rich in fibres. Rice bran have been in the development of food products such as noodles, bread and plantain-based dough meals (Odebode*et al*., 2017; Famakin*et al*., 2016; Oluwajuyitan and Ijarotimi, 2019). Rice bran can potentially serve as a viable source of protein, fat, and fibre that could have positive impacts on human health. Hence, this study the nutritional advantage of incorporating unfermented and fermented rice brans into rice flour.

2.0 Materials and methods

2.1 Materials

Rice bran (Oryza sativa Linn) was obtained from Igbimo Rice Processing Company, Aisegba, Ekiti state, Nigeria. Wheat flour were obtained from the Kings market, Akure, Ondo state, Nigeria. The Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Nigeria verified the authenticity of the food-related items.

2.2 Preparation of unfermented rice bran flour

Rice bran flour was processed using the method described by Oluwajuyitan et al. (2021). Rice bran flour was washed with distilled water, drained and oven dried in hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 55 oC for 12 h, milled (Laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and sieved through 150 µm sieve to obtain rice bran flour. The flour was packed in a plastic container, sealed and stored at room temperature (~27 oC) until required for use.

2.3 Preparation of fermented rice bran flour

Rice bran flour was processed into fermented rice bran by soaking in water for 3 days, drained and oven dried in hot-air oven (Plus11 Sanyo Gallenkamp PLC, Loughborough, Leicestershire, UK) at 55 oC for 12 h, milled (Laboratory blender (Model KM 901D; Kenwood Electronic, Hertfordshire, UK) and sieved through 150 µm sieve to obtain rice bran flour. The flour was packed in a plastic container, sealed and stored at room temperature (~27 oC) until required for use

2.4 Formulation of Flour Blends

Rice bran, fermented rice bran and Wheat flour, were blended together using response surface methodology to generate 17 runs which were subjected to preliminary analysis. Response surface methodology has been used in many other analysis (Awolu*et al*., 2016; Awolu and Oseyemi, 2016; Awolu, 2018) for the mixture of composite flours because of its ability to generate many variables, and also for its reliability in data generation and optimization.

2.5 Production of Bread Samples from Blends of Unfermented Rice Bran, Fermented Rice Bran and Wheat Flour

The bread samples were produced using the method of Bhatt et al. (2015). The straight dough method is the easiest of the dough-making methods where all the ingredients are mixed at the same time in the mixer. Firstly, the water, compressed yeast and sugar were mixed properly in a separate bowl. And was left for 10-15 min till it forms slump and bubbles, this indicates that yeast is activated. All the flours were mixed with water (60% w/w) along with sugar-yeast solution and other required ingredients (salt, gluten, hydrocolloid, improver, preservative, dry milk powder) in a planetary mixer to form homogeneous dough. During mixing the hydration was checked. 5-7 inches the dough was stretched to check the proper gluten formation.

After proper kneading for 10-12 min, the dough was manually punched and left for 10-15 min in bench, this is called bench rest where first fermentation takes place. In between the fermentation the dough was knock backed to expel the excess gas and to redistribute the food for the yeast. Usually the knock-back is done when 2/3 of the fermentation time is over.

Table 1: Formulation of Flour Blends.

Rice bran, fermented rice bran and Wheat flour blends for determination of proximate and functional properties are presented in Table 1.

Runs	Riceflour	Fermented RiceBranFlour	WheatFlour	Total
1	60.00	24.20	15.8	100
2	89.20	0.00	10.73	100
3	78.10	11.05	10.82	100

4	86.92	13.08	0.00	100
5	60.00	24.20	15.80	100
6	78.95	23.05	0.00	100
7	77.00	0.00	23.00	100
8	60.00	10.00	30.00	100
9	68.03	1.97	30.00	100
10	100.00	0.00	0.00	100
11	68.75	10.96	20.29	100
12	69.29	21.89	10.23	100
13	76.95	23.05	0.00	100

3.0 Determination of Proximate Composition of Rice Bran, Fermented Rice Bran and Wheat Flour Blends

The proximate composition (moisture content, crude fiber, crude fat, total ash, and crude protein contents) of the generated 13 runs from blends of rice bran, fermented rice bran, and wheat flour were determined as described by AOAC (2012). The methods of determination of the parameters are described as follows

3.0.1 Determination of moisture content of rice bran, fermented rice bran and wheat flour blends

The standard method of AOAC (2012) was used to determine the moisture contents of the samples.Clean Petri dishes with lids were labeled and dried in an oven at 100 ^{o}C for 30 minutes, cooled in a dessicator containing reignited CaO as desiccant, and weighed to a constant weight (W_1) using Mettler balance. Each sample (5.0g) was weighed into respective

Petri – dishes. The dishes and food samples were weighed again before drying (W_2). The petri – dishes and food samples were transferred into the oven (Galenkamp, size 3, hot box, London, UK) maintained at 105 ^{o}C for 3 h. The dishes and content were removed and quickly transferred into a dessicator containing CaO as dessicant to cool and re-weighed. The samples were returned into the oven and re – dried for further one hour, cooled and weighed. The procedure was repeated until a constant weight was obtained (W_3). Triplicate determinations were made on each sample.

Calculation

The moisture content of each sample was calculated as the difference in weights before and after drying to constant weights. Values were expressed as percentage moisture.

$$Percentage\ Moisture\ Content = \frac{Weight\ loss\ (W_2 - W_3)}{Weight\ of\ sample\ (W_1)}\ x\ 100 \qquad \text{Eqn. 3.1}$$

Where,

W_1 = weight of sample

W_2 = weight of sample + Petri dish

W_3 = weight of sample + Petri dish (constant weight after drying)

3.0.2Determination of crude fat content of rice bran, fermented rice bran and wheat flour blends

The crude fat content was determined by using soxhlet apparatus as described by AOAC (2012). Sample (5.0 g) was weighed into the thimble and fixed into the soxhlet extractor, n - hexane was used as the solvent. The n-hexane was poured into a round bottom flask and placed on the heating mantle. The extraction was done continuously for about 3½ hours after which the flask was cooled and disconnected. The thimble with sample was removed and dried to a constant weight in hot air oven at 50 ^{o}C. The difference between the weight of thimble before and after extraction was recorded in order to obtain the crude fat extracted. The percentage crude fat content was then calculated and expressed on dry weight basis.

$$Percentage\ Crude\ fat = \frac{Weight\ loss\ (W_2 - W_3)}{Weight\ of\ sample\ (W_1)}\ x\ 100 \qquad \text{Eqn. 3.2}$$

where,

W_1 = weight of sample

W_2 = weight of sample + filter paper

W_3 = weight of sample + filter paper (constant weight after drying)

3.3 Determination of crude protein content of rice bran, fermented rice bran and wheat flour blends

The crude protein content was determined by Kjeldahl method AOAC (2012).

Digestion stage: Each sample (1.0 g) was weighed into a Kjeldahl flask and 3.0 g of hydrated cupric sulphate (catalyst), 20 ml of sodium sulphate solution (Na_2SO_4) and 1.0 ml of concentrated sulphuric acid (H_2SO_4) were added to sample in the flask. The flask was clamped and heated inside a fume cupboard until the solution became colourless.

Distillation stage*:* The clear solution was cooled, diluted with distilled water made up to 100 ml. Ten (10) ml of the resulting solution was mixed with 5 ml of 40 % sodium hydroxide solution in a distillation flask and distilled to release ammonia

Titration stage*:* The resulting solution above was titrated with 0.1 ml hydrochloric acid (HCl). The titre value or end point at which the colour changed from green to pink was noted and the crude protein was calculated using the expression.

$$Crude\ Protein\ (\%) = \frac{14.01\ x\ 6.25\ x\ V\ x\ T\ x\ 100}{W\ x\ 10} \text{ Eqn. 3.3}$$

$Where$

$VF = Total\ volume\ of\ the\ digest = 100\ ml$

W = Weight of the sample digested

$T = Titre\ Value$

$10 = Aliquot\ volume\ distilled$

$6.25 = Conversion\ factor$

$M = Molarity\ of\ HCl\ in\ moles\ per\ 100\ ml\ (0.1M)$

$V = Volume\ of\ 0.1\ M\ HCl\ used$

3.0.4 Determination of total ash content of rice bran, fermented rice bran and wheat flour blends

The total ash content of the flour samples was determined as described by AOAC (2012). The crucibles used were washed, dried in the air oven (Galenkamp, size 3, hot box, London, UK), and allowed to cool in a dessicator. The crucibles were weighed and 1 g of the flour sample

was weighed into the crucible. The crucible and its contents were then transferred into a muffle furnace set at 550 ^{o}C.The ashing continued until no black speck or white grey ash was obtained. The crucibles were taken out and immediately covered and were placed in a desiccator to cool and later weighed.

$$Percentage\ of\ Total\ ash = \frac{Weight\ loss\ (W_2 - W_3)}{Weight\ of\ sample\ (W_1)}\ x\ 100 \qquad \text{Eqn. 3.4}$$

where,

W_1 = weight of sample

W_2 = weight of sample + crucible

W_3 = weight of sample + crucible (constant weight after drying)

3.0.5Determination of crude fibre content of rice bran, fermented rice bran and wheat flour blends

The crude fibre content was determined as described by AOAC (2012). Sample (5 g) was weighed (W_1), transferred to a fat extraction apparatus, extracted with light petroleum other. The sample was then transferred to a dry 500 ml conical flask and about 200 ml of boiling 1.25% sulphuric acid was added and brought to boil within 1 minute. The content was allowed to boil gently for 30 min, filtered through a muslin cloth, rinsed with distilled water and scraped back into flask using spatula. Similar procedure was followed using 1.25% sodium hydroxide after which it was rinsed once with 10% HCl, four times with distilled water and finally with ethanol. The treated sample was then sewage into pre-weighed and drained silica dish, oven dried for 12 h at 105 ^{o}C, cooled and weighed (W_2). The samples were finally ashed at about 550 ^{o}C for 2 h, cooled in a desiccator and reweighed (W_3).

$$Percentage\ of\ Crude\ fibre = \frac{Weight\ loss\ (W_2 - W_3)}{Weight\ of\ sample\ (W_1)}\ x\ 100 \qquad \text{Eqn. 3.5}$$

where,

W_1 = Initial weight of sample

W_2 = weight of sample + crucible before ashing

W_3 = weight of sample + crucible after ashing (constant weight after drying).

3.0.6Determination of carbohydrate contentof bran, fermented rice bran and wheat flour

The carbohydrate content was determined by difference. The percentage total carbohydrate was estimated to be equal to the sum of percentage moisture, protein, ash and fibre subtracted from 100 g.

$$\%Carbohydrate = 100 - (\%\,protein + \%fat + \%fibre + \%ash + \%\,moisture) \quad \text{Eqn. 3.6}$$

3.1 Determination of Functional Properties of Rice bran, Fermented rice branand Wheat Flour Blends

3.1.1 Determination of bulk density of rice bran, fermented rice bran and wheat flour blends

Bulk density was determined according to the method of Asoegwu*et al.* (2006). Samples were placed in a 25 ml graduated cylinder and packed by gently tapping the cylinder on the bench top 10 times from a height of 5 cm and the volume of the sample was recorded. The procedure was repeated three times for each sample and the bulk density was computed as g/ml of the sample.

$$Bulk\ density\ (g/ml) = \frac{Weig\,ht\ of\ sample\ \ (g)}{Volume\ \ of\ sample\ \ (ml)} \quad \text{Eqn. 3.7}$$

3.1.2 Water absorption capacity (WAC) of rice bran, fermented rice bran and wheat flour blends

Water absorption capacity (WAC) is an index of the amount of water retained within a food matrix under certain conditions and it was determined using a modified method by Adebowale *et al.* (2005). Ten (10) ml of distilled water was added to 1 g of sample weighed into a dry centrifuge tube and stirred vigorously. The resulting suspension was centrifuged at 3500 *x g* for 30 minutes. The supernatant was measured into a 10 ml graduated cylinder. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant.

$$WAC\ (\%) = \frac{initial\ \ volume\ \ added\ \ to\ sample - volume\ \ of\ \ supernatant}{volume\ \ of\ \ supernatant}\ x\ 100 \quad \text{Eqn. 3.9}$$

3.2.3 Oil absorption capacity (OAC) of rice bran, fermented rice bran and wheat flour blends

Oil absorption capacity (OAC) was determined using the method of AOAC (2012). 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 3.10

$$OAC \ \frac{V_1 - V_2}{W_0} \text{Eqn. 3.10}$$

3.2.4 Foaming capacity and stability (FC)of rice bran, fermented rice bran and wheat flour blends

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 (3.11)

$$FC = \frac{V_F}{V_1} x\ 100 \qquad \text{Eqn. 3.11}$$

Foam stability (FS) was determined by measuring the fall in volume of the foam after 60 min as shown in Equation (3.12).

$$FS = \frac{V_F\ after\ 60\ min}{V_1} x\ 100 \qquad \text{Eqn. 3.12}$$

3.2.5 Swelling capacity

The swelling capacity of each sample was calculated as a multiple of the original volume as done by Ukpabi and Ndimele (1990). One (1) gram of each flour sample was transferred into clean, dry graduated (50 ml) cylinder. The flour sample were gently leveled into it and the volume noted. Distilled water (10 ml) was added to each sample. The cylinder was swirled and allowed to stand for 60 min while the change in volume (swelling) was recorded every 15 min. The swelling index of the sample was calculated by the formula;

$$Swelling\ capacity\ (\%) = \frac{volume of\ sample\ after\ swelling}{volume\ of\ sample\ before\ swelling} x\ 100 \qquad \text{Eqn. 3.13}$$

3.3 Anti-nutrient Composition of Bread Produced from Selected Flour Blends

3.3.1Determination of oxalatecontent

The method described by Ukpabi and Ejidoh (1989) was used. Two gram (2 g) of flour blend sample was digested with 10 ml of 6 M HCl for one hour and made up to 250 ml in a volumetric flask. The pH of the filtrate was adjusted with concentrated NH_4OH solution until the colour of the solution changed from salmon pink colour to a faint yellow colour. Thereafter, the filtrate was treated with 10 ml of 5% $CaCl_2$ solution to precipitate the insoluble oxalate. The suspension was centrifuged at 2500 *x g*, after which the supernatant was decanted. The precipitate was dissolved in 10 ml of 20% (v/v) H_2SO_4 and the solution was made up to 300 ml. An aliquot (125 ml) was heated until near boiling point and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 s after which the burette reading was taken and used to estimate the oxalate content.

$$Oxalate\ (mg/g) = \frac{(titre\ \ value\ \ x\ volume\ \ of\ \mathrm{KMnO_4}\ x\ dilusion\ \ \ factor\ )/5}{Sample\ \ \ weig\ ht} \quad \text{Eqn. 3.14}$$

3.3.2. Determination of tannin content

This was done using the method of Medoua*et al.* (2007). Two (2 g) of eachsample was weighed into a 250 ml flask followed by addition of 200 ml of 0.004 $MK_3Fe(CN)_6$ and 10 ml of 0.008 M $FeCl_3$ in 0.008 M HCl. The flask was allowed to stand for20 min and stirred occasionally at 10 min interval and 1 ml aliquot was removed.To this aliquot was added 2 ml of 0.008 M $FeCl_3$ in 0.008M HCl and 10 ml of 0.0015 $MK_3Fe(CN)_6$. After adding the final reagent, the absorbance was then read at 720 nm after 30seconds against a blank.

$$Tannin\ (mg/g) = \frac{Absolute\ of\ the\ sample\ x\ concentration\ of\ the\ standard\ x\ Dilution\ factor}{Absorbance\ of\ the\ standard\ x\ sample\ size} \quad \text{Eqn. 3.15}$$

3.3.3 Determination of alkaloid content

The alkaloid content was determined gravimetrically (Haborne, 1973). Briefly, 5 g of each sample was weighed using a weighing balance and dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 h before it is filtered. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated

Ammonium hydroxide was added drop wise in order to precipitate the alkaloids.

A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60 ^{o}C for 30 min, transferred into desiccators to cool and then reweighed until a constant weight was obtained. The constant weight was recorded. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed. The experiment was repeated thrice for each food stuff sample and the reading recorded as the average of three replicates.

3.3.4. Determination of phytate content

The determination of phytate in sample was done using the method described by Abulude (2004). Eight (8 g) of flour blend samples was dispersed in 200 mlof 2% HCl and extracted. Following extraction, the dispersion was filtered and 50 ml of the filtrate was mixed with 10 cm^3 of 0.3% ammonium cyanide (NH_4SCN) and diluted with 107 ml of distilled water. The extract was titrated against 0.00195 g/mlof Ferric chloride solution until a brownish yellow colour persisted. Phytate content was estimated use the formula below:

$$Phytate\ Phosphorous = (Iron\ equivalent\ x\ 1.95\ g\ x\ titre)\ x\ 3.65\ g \quad \text{Eqn. 3.16}$$

3.4 Antioxidant Activities of Bread Produced from Selected Flour Blends

3.4.1. Determination of total flavonoid

Total flavonoid content was determined by aluminum chloride colorimetric assay (Bushra *et al*., 2009) with slight modification. About 500 µL, of methanol was added to 10 ml flask containing 500 µL of aqueous extract. To this 50 µL 10% $AlCl_3$ and 50 µL of 1M CH_3COOK was added respectively. The total volume was made up to 2500 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. (JENWAY 6305, United Kingdom). The flavonoid was calculated using quercetin as standard.

$$Total\ flavonoid\ content\ \left(mg\ QE/g\right) = \frac{Abs._{sample}\ \ x\ Conc._{standard}\ \ (mg/ml)}{Abs._{standard}\ \ x\ Conc._{sample}\ \ (mg/g)} \quad \text{Eqn. 3.17}$$

Abs $_{standard}$ is the absorbance of the solution containing 500 µL quercetin, About 50 µL 10% $AlCl_3$ and 1M CH_3COOK. Blank is the mixture of 500 µL of distilled water, 500 µL of methanol, 50 µL distilled water and 1M CH_3COOK.

3.4.2 Determination of total phenolic content

The total phenol content (TPC) was determined by Folin–Ciocalteu assay (Singleton *et al.*, 1999) using gallic acid as standard. Fifty microliter of aqueous extract of the food sample 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 into the test tube and shaken thoroughly, after 3 min, 400 µL of 7.5% sodium carbonate solution was added and the mixture was incubated at 45 ^{0}C in a water bath for 40 min. Absorbance was measured at 765 nm against blank. The same procedure was repeated to all standard gallic 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. The total phenolic content was expressed as gallic acid equivalent per gram of sample (mg of GAE/g sample) through the calibration curve of gallic acid and calculated as follows;

$$Total\ phenolic\ content\left(mg\ GAE/g\right) = \frac{Abs_{.sample}\ \ x\ Conc_{.standard}\ \ (mg/ml)}{Abs_{.standard}\ \ x\ Conc_{.sample}\ \ (mg/g)} \quad \text{Eqn. 3.18}$$

3.4.3 Ferric-reducing antioxidant property (FRAP)

The ferric reducing power of the samples was determined according to the modified method of Zhang *et al*. (2008). Experimental sample or Glutathione (GSH) was dissolved in 0.2 M phosphate buffer, pH 6.6; an aliquot (250 µL) was mixed with 250 µL of the buffer and 250 µL of 1% potassium ferricyanide solution. The mixture was thoroughly mixed using a vortex machine and heated at 50 °C for 20 min. After incubation, 250 µL of 10% trichloroacetic acid (TCA) was added followed by 50 µL of 0.1% ferric chloride dissolved in double distilled water and then 200 µL of distilled water was added. The solution was allowed to stand for 10 min at room temperature, after which it was centrifuged at 1000 × g for 10 min. An aliquot (200 µL) of the supernatant was transferred to a clear bottom 96-well plate and the absorbance was measured at 700 nm.

3.5.4 Iron (Fe^{2+}) Chelation

The metal chelating activity of the samples was determined using a modified method of Xie *et al*. (2008). Experimental samples and Glutathione (GSH) solution (final assay concentration of 1 mg/dL) were combined with 0.05 ml of 2 mM $FeCl_2$ and 1.85 ml distilled water in a reaction tube. Ferrozine solution (0.1 ml of 5 mM) was added and mixed thoroughly. The mixture was then allowed to stand at room temperature for 10 min from which an aliquot of 200 µL was removed and added to a clear bottom 96-well plate. A blank experiment was also conducted by replacing the sample with 1 ml of distilled water. The absorbance of blank (Ab) and sample (As) at 562 nm were measured using a spectrophotometer and the metal chelating activity of the sample was compared to that of GSH. The percentage chelating effect (%) was calculated using the following equation:

$$\text{Metal } (Fe^{2+}) \text{ chelating activity } (\%) = \left(1 - \frac{A_{517}\ of\ sample}{A_{517}\ of\ blanck}\right) \text{X } 100 \qquad \text{Eqn. 3.19}$$

3.4.5. ABTS radical scavenging activity

The ABTS scavenging ability of the sample extract was determined according to the method described by Re *et al*. (1999). The ABTS was generated by reacting a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM/L), final concentration in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. About 0.2 ml of the appropriate dilution of the extract was then added to 2.0 ml of ABTS solution and the absorbance was read at 732 nm after 15 minutes. The ABTS scavenging activity was calculated using the following equation:

$$ABTS^{*}\ scavenging\ ability\ (\%) = \frac{Abs._{ref} - Abs._{sample}}{Abs._{ref}}\ x\ 100 \qquad \text{Eqn. 3.20}$$

$$ABTS^{*}\ scavenging\ ability\ (mmol/g) = \frac{Per_{sample}\ x\ Conc._{standard}}{Per_{standard}\ x\ Conc._{sample}}\ x\ TMW \qquad \text{Eqn. 3.21}$$

TMW = Molecular mass of Trolox (264.32 g/mol)

3.4.6. DPPH radical scavenging assay

The scavenging effect of the samples on 2, 2- Diphenyl-1-picryhydrazyl (DPPH) free radical was measured according to the method of Aluko and Monu (2003). Each sample (10 mg) was dissolved in 1 ml of buffer (0.1 M sodium phosphate buffer, pH 7.0 containing 1% (w/v) Triton X-100). DPPH was dissolved in methanol to a final concentration of 100 µM. Samples (100 µl) were mixed with 100 µL of the DPPH solution in the 96-well plate to a final assay concentration of 1 mg/ml and incubated at room temperature in the dark for 30 min. The absorbance values of the blank, Glutathione (GSH) (control) and samples were measured at 517 nm. The control consisted of sodium phosphate buffer in place of the samples while Glutathione (GSH) was used as the positive control. The percentage DPPH radical scavenging activity of the samples were calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{517}\ of\ sample}{A_{517}\ of\ blanc}\right) \text{x } 100 \qquad \text{Eqn. 3.22}$$

3.5 Determination of Mineral Composition of Bread Produced from Selected Flour Blends

3.51 Mineral analysis of samples

Sample preparation

The mineral elements of the bread samples were determined using dry ash methods as described in AOAC (2012). Each seed flours (1.0 g) was weighed into crucibles and transferred to a muffle furnace and ashed at 550 ^{o}C until all the carbon was burnt off and the crucibles plus ash were transferred into different desiccators to cool after which 0.1M HCl solution (10 ml) was added to the crucible to break up the ash and leach the metals. The crucible was washed three times with 0.1M HCl and made up to 100 ml with deonized water.

Determination of mineral elements of the samples

The mineral contents of the samples under study were carried out using *Atomic Absorption Spectroscopy*(*AAS*). The following elements were analysed calcium, magnesium, copper, zinc, and iron. Standard stock solution were prepared for each metal using suitable metal salts of each metal to prepare a standard curve (AOAC, 2012).

Sodium and potassiumdetermination: Flame photometer was used to determine the concentrations of the elements in the food samples.The standard solutions were prepared separately using sodium chloride and potassium chloride for sodium and potassium determinations respectively. The standard solutions were measured from the flame photometer and the value obtained was plotted against the strength of various solutions. The sodium and potassium content were determined from the flame photometer. The values were plotted in the respective standard value to read the original values of the concentration of the elements (AOAC, 2012).

Phosphorus determination: The phosphorus was determined using Vanodo-molybdate method. To series of 100 ml volumetric flasks 0.0, 2.5, 5.0, 7.5, 11.0, 15.0, 20.0, 30.0, 40.0, 50.0 ml of the standard phosphate solution was made acidic by addition of 2 ml nitric acid (2:1). After which 25 ml of the Vanodo-molybdate reagent was added. The solution was diluted to the mark, mixed thoroughly and allowed to stand for 10 min, while the optical density was measured at 47 mu (AOAC, 2012).

***Sample preparation*:** The mineral elements of the bread samples were determined using dry ash methods as described in AOAC (2012). Each seed flours (1.0 g) was weighed into crucibles and transferred to a muffle furnace and ashed at 550 ^{o}C until all the carbon was burnt off and the crucibles plus ash were transferred into different desiccators to cool after which 0.1M HCl solution (10 ml) was added to the crucible to break up the ash and leach the metals. The crucible was washed three times with 0.1M HCl and made up to 100 ml with deonized water.

***Determination of mineral elements of the samples*:** The mineral contents of the samples under study were carried out using *Atomic Absorption Spectroscopy*(*AAS*). The following elements were analysed calcium, magnesium, copper, zinc, and iron. Standard stock solution were prepared for each metal using suitable metal salts of each metal to prepare a standard curve (AOAC, 2012).

Sodium and potassiumdetermination: Flame photometer was used to determine the concentrations of the elements in the food samples.The standard solutions were prepared separately using sodium chloride and potassium chloride for sodium and potassium determinations respectively. The standard solutions were measured from the flame photometer and the value obtained was plotted against the strength of various solutions. The sodium and potassium content were determined from the flame photometer. The values were

plotted in the respective standard value to read the original values of the concentration of the elements (AOAC, 2012).

Phosphorus determination: The phosphorus was determined using Vanodo-molybdate method. To series of 100 ml volumetric flasks 0.0, 2.5, 5.0, 7.5, 11.0, 15.0, 20.0, 30.0, 40.0, 50.0 ml of the standard phosphate solution was made acidic by addition of 2 ml nitric acid (2:1). After which 25 ml of the Vanodo-molybdate reagent was added. The solution was diluted to the mark, mixed thoroughly and allowed to stand for 10 min, while the optical density was measured at 47 mu (AOAC, 2012).

3.6 Dietary Fibre Composition of Bread Produced from Selected Flour Blends

The dietary fibre of the composite flour was determined according to the method of AOAC (2012).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:

3.6.1 Determination of soluble fibre content

The solution was filtered (Through trittled 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

3.6.2Determination of insoluble fibrecontent

The solution was filtered (through trittled 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.

3.7 Glycaemic index of Bread Produced from Selected Flour Blends

In vitro glycemic index of bread samples were determined according to Goni et al. (1997). Fifty milligrams of freeze-dried sample was incubated with 1 mg of pepsin in 10 ml HCl-KCl buffer (pH 1.5) at 40 °C for 60 min in a shaking water bath. Digested samples were diluted with 2.5 ml phosphate buffer (pH 6.9), and then, 5 ml of α-amylase solution (in phosphate

buffer) was added. The mixture was incubated at 37 °C in a shaking water bath, and 0.1 ml was taken from each flask every 30 min from 0 to 3 h and boiled for 15 min to inactivate the enzyme. Sodium acetate buffer (0.4 M, pH 4.75) was added, and the residual starch was digested to glucose by adding 3 ml α-glucosidase and incubating at 60 °C for 45 min. Glucose concentration was quantified by adding 200 ml of dinitrosalicylic acid color reagent. The reaction mixture was stopped by boiling the mixture in a water bath for 5 min and then cooled to room temperature. The mixture was further diluted by addition of 5 ml distilled water and centrifuged at 1,200 g. The supernatant was collected, and the absorbance was read at 540 nm using spectrophotometer. The rate of starch digestion was expressed as the percentage of starch hydrolyzed per time.

3.13 Sensory Attributes of Bread Produced from Selected Flour Blends

Thirty (30) assessors were randomly selected among the students and staff from Food Science and Technology Department, Federal University of Technology, Akure, Nigeria. The panel members were assigned individually to well illuminate laboratory booths and the bread samples were served at 40 ^{o}C coded with random three digits. Sample attributes (appearance, texture, taste, aroma, etc.) were rated on a scoring scale of 1 to 9, where 1 = dislike extremely and 9 = like extremely (Olapade *et al*., 2012).

3.14 Statistical Analysis

All data were carried out in triplicates. Statistical analysis were carried out using statistical package for social science (SPSS) version 21.0 for windows. The results were presented as Means ± Standard deviation, and statistical difference between the means was determined using one-way analysis of variance (ANOVA) while means were separated at $p<0.05$ significant difference using New Duncan Multiple Range Test (NDMRT).

4.0 RESULTS AND DISCUSSION

4.1 Proximate Composition of Flour Blends

The proximate composition of the flour samples is presented in Table 4.1. The moisture content of the selected runs samples ranged from 8.74% to 9.67%. The highest value of moisture content was observed in the 10th run at 9.76% which has 100:0:0 of rice flour, fermented rice bran flour and wheat flour respectively while the 1st run has 8.74% from 60:24.20:15.8 of rice flour, fermented rice bran flour and wheat flour respectively had the

lowest value. There was no significant ($p \leq 0.05$) difference between the 1st run, the 8th run and the 12th run. While a significant ($p \leq 0.05$) difference was observed between the 4th run (which has 86.92:13.08:0 of rice flour, fermented rice bran flour and wheat flour respectively) and the 10th run (which has 100:0:0 of rice flour, fermented rice bran flour and wheat flour respectively). The reduction in moisture content observed in the flour blends might be attributed to the addition of rice bran, as rice bran increases moisture decreases. This might be as a result of decrease in starch properties of the flour blends, as starch engages in water retention (Yusuf *et al*., 2018).

The result of the moisture content from the wheat and amaranth seed, brewers' spent grain and apple pomace composite flour is 5.2 – 9.1% (Awolu*et al*., 2016) and 4.655-9.385% from Bambara groundnut and amaranth grain-based composite flour (Awolu and Olokunsusi, 2017) was in the same range with this study. More so, the value from the study were also lower than the values of 21-23% from wheat flour supplemented with stabilized undefatted rice Bran (Ameh *et al*., 2013). However, the moisture content values were higher than the values of 3.95 to 5.57% of cocoyam-based composite flour (Awolu, 2018), 2.69 to 5.94%. Cocoyam, Bambara groundnut and cassava starch composite flour (Awolu and Oseyemi, 2016).

Moisture content plays an important role is the shelf life of food product. Flour products with low moisture content hinders bacteria and mold growth there by making them have longer shelf life period (Ijarotimi, 2012; Oluwajuyitan 2021). Furthermore, all the moisture content of the selected run samples were within the acceptable limit of not more than 10% respectively (SON, 2007; Awolu, 2018).

The protein content of the flour samples ranged from 7.33% to 10.08%. The highest value was observed in run 8 at 10.08% from 60:10:30 of rice flour, fermented rice bran flour and wheat flour, respectively. while run 4 has 7.33% the lowest value from 86.92:13.08 of rice flour and fermented rice bran flour. There was significant ($p \leq 0.05$) difference observed across all samples. The addition of fermented rice bran and the wheat flour could have contributed to the increase in protein content of the composite flour. Whole wheat flour has been reported to contain between 8 to 13% protein content (Chandra and Samsher, 2013; Irakli *et al*., 2015; Olagunju, 2019). Rice bran has also been reported to contain high amount of protein 10– 16% protein (Bodie *et al*., 2019).

The protein content observed in this study was lower than the value of 16-19% from plantain, defatted soybean, rice-bran and oat-bran flour reported by Oluwajuyitan*et al.*, (2021). Also the protein content observed in this study was similar to the values of 9.9 to 11.5% of wheat and amaranth seed, brewers spent grain and apple pomace composite flour (Awolu*et al*., 2016). Likewise, it was higher than the fermented and unfermented potato flour of 5.68 and 8.34% respectively (Ayo-Omogie, 2021).

Fermentation also plays a role in nutritional content as they aid in the breakdown of macro molecules into simpler forms (Ayo-Omogie and Ogunsakin, 2013; Ayo-Omogie*et al.*, 2021 Sharma *et al*., 2020). Essential nutrients, like proteins, are needed for biochemical activities, building and repair of new tissues in the organs of the body (Wu, 2016).

The crude fibre content composition of the generated runs flour samples ranged from 4.99% in the 1st run and 0.79% in the 10th run. The highest value was observed in run 1 (4.99%) which has the highest fermented rice bran content of ratio 60:24.20:15.8 of rice flour, fermented rice bran flour and wheat flour respectively. while run 10 (0.79%) had the lowest value which comprises of 100% rice flour only. There was significant difference ($p \leq 0.05$) observed across all samples. The result showed that the addition of fermented rice bran contributed to the increase in fibre content of the composite flour. Rice bran has also been reported to contain high amount of fibre content (Friedman, 2013; Bodie *et al*., 2019).

Siriam*et al*. (2011) reported similar occurrence and concluded that the addition of 5, 10 and 15% rice bran increased the fibre content ratio in bakery products respectively. Fermentation process has been observed to play a role in nutritional content as they aid in the breakdown of macro molecules such as crude fibre into simpler forms (Ayo-Omogie and Ogunsakin, 2013; Ayo-Omogie*et al.*, 2021 Sharma *et al*., 2020). The fibre content observed in this result was in range with the recommended value of 5g/day according to Omoba*et al*., (2013). The crude fibre of this result was higher than the value of 0.7-3.9% reported for wheat and amaranth seed, brewers' spent grain and apple pomace composite flour (Awolu*et al*., 2016), similar in range to the value of 2.1–5.4 % reported for plantain, defatted soybean, rice-bran and oat-bran flour (Oluwajuyitan*et al.*, 2021).

This result was also higher than the value of 1.04-2.26% of both fermented and unfermented potato flour reported by Ayo-Omogie, (2021). More so, the value was

Samples	Moisture	Total Ash	Crude Protein	Crude Fat	Crude Fibre	Carbohydrate

also higher than the value of 1.57 to 3.41% wheat flour supplemented with stabilized undefatted rice bran (Ameh *et al*., 2013). Fibre serves as an advantage as it helps with gastro intestinal disorder aiding in bowel movement and cardiovascular diseases and accelerated transit time. (Awolu*et al*., 2016). Furthermore, the American Society for Nutrition states revealed evidence, that consumption of foods rich in fiber is associated with a reduced risk of obesity, type 2 diabetes, cardiovascular disease (Cho *et al*., 2013).

Table 4.1: Proximate Composition of Flour Blends

1	8.74± 0.05[f]	4.63±0.04[a]	9.47±0.02[e]	4.12±0.01[bc]	4.99±0.01[a]	68.06 ± 0.01[k]
2	9.73± 0.01[b]	0.65±0.02[j]	8.86± 0.02[f]	2.31 ± 0.03[j]	0.71±0.01[h]	77.75 ± 0.04[c]
3	9.24± 0.03[c]	2.33±0.01[f]	9.08±0.03[d]	3.38± 0.01[e]	3.01±0.01[d]	72.98 ± 0.04[f]
4	9.12± 0.03[d]	2.32±0.01[a]	7.33± 0.02[j]	3.08± 0.01[h]	3.05±0.02[d]	75.11 ± 0.01[d]
5	8.67± 0.05[g]	4.62±0.01[b]	9.46±0.04[c]	4.14± 0.01[b]	4.97±0.04[a.]	68.15 ± 0.13[k]
6	8.35± 0.02[h]	4.32±0.02[i]	7.33± 0.01[j]	4.03± 0.02[d]	4.88±0.02[b]	71.11 ± 0.02[h]
7	9.92± 0.01[a]	0.71± 0.01[h]	8.03± 0.01[i]	2.47 ± 0.01[i]	0.78±0.01[g]	78.11 ± 0.01[a]
8	8.78± 0.05[f]	2.42±0.01[e]	10.08±0.05[a]	3.26± 0.01[f]	2.95±0.02[e]	72.53 ± 0.11[g]
9	8.99± 0.01[e]	0.54±0.01[k]	9.01±0.01[e]	2.34 ± 0.01[j]	1.27±0.04[f]	77.87 ± 0.04[b]
10	9.76± 0.02[b]	0.87±0.01[h]	8.49±0.02[h]	2.02± 0.04[k]	0.79±0.02[g]	78.08 ± 0.03[a]
11	9.22± 0.02[c]	1.75±0.03[g]	9.61±0.01[b]	3.14± 0.01[g]	3.00±0.02[de]	73.28 ± 0.03[e]
12	8.78± 0.04[f]	4.18±0.04[d]	8.78±0.03[g]	4.09± 0.02[c]	4.74±0.05[c]	69.45 ± 0.01[j]
13	8.40± 0.01[h]	4.25±0.02[c]	7.34± 0.01[j]	4.91± 0.02[a]	4.90±0.01[b]	70.21 ± 0.01[i]

Means(±SEM)with different alphabetical superscripts in the same column are significantly differentatp≤0.05

4.2 Functional Properties of Flour Blends

The functional properties of the generated flour blends run samples are presented in Table 4.2.The bulk density of the selected runs samples ranged from 0.88 g/ml to 1.10 g/ml. Bulk density is used as an indicator of packaging properties of foods product. Thakur *et al*., (2016) stated that the lower the bulk density of a food product the better for its packaging. The result showed that sample 12 which comprises of 69.29:21.89:10.23 of rice flour, fermented rice bran flour and wheat flour respectively.

had the lowest result of 0.88 g/ml, followed by sample 1 with 60:24.20:15.8 of rice flour, fermented rice bran flour and wheat flour respectively has 0.90 g/ml. The inclusion of fermented bran might have attributed to the decrease in bulk density observed due to fact that bran is lighter than starch and therefore takes less space. Furthermore, Low bulk density could be an advantage in the formulation of weaning formulas where high nutrient density to low bulk is desired meaning that the lower the values of bulk density of a particular food product, the higher the amount of flour particles that can stay together which in turns increases the energy content that could be derived from such diets (Ayo-Omogie, 2013). The values reported for this result were similar to the values of 0.77-0.81 g/cm^3 reported for sweet potato-wheat flour (Ayo-Omogie, 2021). Chandra and Samsher, 2013 also reported a similar bulk density values for wheat flour (0.762 g/cc) and rice flour (0.914 g/cc) respectively. More so, Ayo-Omogie, 2021 reported that relatively high bulk density flours values are suitable for production of baked foods due to their ability to absorb more fat during baking process.

The water absorption capacity (WAC) of the selected runs flour samples ranged from 77.72% to 84.95%. The values reported for this study were higher than the value of 1.6-1.80 (g/g) reported for wheat and amaranth seed, brewers' spent grain and apple pomace composite flour (Awolu*et al*., 2016). However lower than the values of wheat flour (140%) and rice flour (192%) respectively (Chandra and Samsher, 2013) and also lower than the values of 190.00-300.00 g/g reported for Wheat: Cardaba banana flour (Ayo-Omogie and Odekunle, 2017).

Water absorption capacity is an indication that flours have the ability to retain water. This attribute is directly related to the amount of starch found in the flour. The higher the starch content in the flour sample the more its ability to retain water due to its hydrophilic constituent (Awolu*et al*., 2016). Furthermore, Noor Aziah *et al*., (2012) stated that the higher number of hydroxyl groups found in fibre structure could potentially allow more water interactions through hydrogen bonding in fibre-rich flours. Therefore, the flour blends could

be useful in bakery products where hydration to improve handling is desired. Furthermore, Awolu, (2018) reported that if the water absorption and retention capacity of a flour is higher, the better it performance in baked products texture.

The oil absorption capacity (OAC) of the selected runs flour samples ranged from 110.69% to 127.95%. The values of this study were higher than the reported value of 124% rice flour but lower than the value of 146% wheat flour respectively for different composite flours (Chandra and Samsher, 2013). Furthermore, this study was higher than the values of (2.507-3.066 g/g) reported for Bambara, amaranth grain based composite flour (Awolu and Olokunsusi, 2017) However, the past findings of Ayo-Omogie and Odekunle, (2017) who reported higher oil absorption capacity value of (106.67-200.00 g/g) in wheat, cardaba flour. Oil absorption capacity has been attributed to the physical entrapment of oil (Hasmadi*et al*., 2020).

Oil absorption capacity depends on several constituents of the flour most importantly its protein content. For instance, Oil absorption capacity of a flour is carried out by the availability of proteins in the flour which bind to fat by capillary attraction. Therefore, more non-polar amino acids of the protein are exposed to the fat which in turn enhance hydrophobicity of the flours to absorb oil (Awolu*et al*., 2016). Oil absorption capability is required in most food applications because fat content act as flavor retainer and increases mouth feel, such as in bakery products, wherein required a flavour retention and improvement of palatability (Hasmadi*et al*., 2020).

The foaming capacity of the sample ranged from 5.00 in run 1 to 5.90 in run 8. The highest foaming capacity was observed in run 8 with the ratio 60:10:30 of rice flour: fermented rice bran flour: wheat flour respectably. The foaming capacity observed in this study was higher than the values of whole wheat flour 1.94% and whole rice flour 0.98% reported by Chandra and Samsher, (2013). This study was also higher than the values 1.0-5.65% reported for optimized cocoyam-based composite flour comprising cassava starch (Awolu and Oseyemi, 2016). The foaming capacity measures the amount of interfacial area created by protein during foaming (Zhu *et al*., 2017). Flours can produce foams due to surface-active proteins thereby the protein dispersion may cause a lowering of the surface tension at the water air interface, thus increase in foaming will be observed due to protein activity which forms a continuous cohesive film around the air bubbles in the foam (Hasmadi*et al*., 2020). The

Runs	Bulk density (g/ml)	WAC (%)	OAC (%)	Foaming Capacity (%)	Solubility (%)	Swelling Capacity (g/ ml)

increase in foaming capacity observed in this study might be attributed to the combination of both flours with the addition of rice brain which might have increased its protein content.

The swelling capacity of the sample ranged from 201 in run 12, 69.29:21.89:10.23 of rice flour, fermented rice bran flour and wheat flour respectively and 271 in run 8 has 60:10:30 of rice flour, fermented rice bran, wheat flour which is the highest swelling capacity observed. The swelling capacity observed in this report were higher than the values of 13-14% (g/ml) reported for composite flour, consisting wheat and amaranth seed, brewers' spent grain and apple pomace (Awolu*et al*., 2016). More so, this result was higher than the report of Chandra and Samsher, (2013) for both wheat flour (17.60 ml) and rice flour (15.20ml) respectively. Also higher than the values of 4.5 to 8.5% reported for optimized cocoyam-based composite flour comprising cassava starch (Awolu and Oseyemi, 2016). Swelling capacity is important parameter in processing and maintaining structure of different food products like bakery products during processing and also after processing. The ability of the flours to swell depends on the presence of the flexible protein molecules which may decrease the surface tension of water, particle size of the flour, unit operation involved (Chandra and Samsher, 2013; Ayo-Omogie and Ogunsanki, 2013; Awolu*et al*., 2016).

Table 4.2: Functional Properties of Flour Blends

1	0.90 ± 0.02	79.49 ± 2.62	110.69 ± 0.24	5.00 ± 0.21	2.00 ± 0.02	220.00 ± 3.00
2	0.99 ± 0.01	82.61 ± 0.24	134.90 ± 2.12	5.80 ± 0.22	5.30 ± 0.21	260.00 ± 4.00
3	0.89 ± 0.01	80.53 ± 0.22	115.80± 0.32	5.20 ± 0.01	5.00 ± 0.04	261.00 ± 2.00
4	0.91 ± 0.03	79.07 ± 1.36	119.64 ± 0.41	5.30 ± 0.22	2.30 ± 0.02	210.00 ± 2.01
5	0.91 ± 0.02	79.55 ± 1.32	111.00 ± 0.31	5.00 ± 0.23	2.00 ± 0.01	220.00 ± 2.00
6	0.89 ± 0.02	78.30 ± 0.34	104.60 ± 0.22	5.10 ± 0.22	1.80 ± 0.21	193.00 ± 1.00
7	0.91 ± 0.01	83.11 ± 0.41	128.55 ± 2.21	5.20 ± 0.41	6.60 ± 0.03	275.00 ± 2.00
8	1.07 ± 0.23	84.95 ± 0.32	127.81 ± 1.82	5.90 ± 0.02	6.60 ± 0.22	271.00 ± 2.00
9	1.08 ± 0.01	83.07 ± 0.34	127.47 ± 1.82	5.60 ± 0.01	7.00 ± 0.26	278.00 ± 2.00
10	1.10 ± 0.21	80.21 ± 0.33	123.04 ± 2.41	5.40 ± 0.22	2.60 ± 0.23	246.00 ± 4.00
11	0.99 ± 0.02	81.96 ± 1.26	124.00 ± 2.23	6.10 ± 0.24	4.20 ± 0.21	255.00 ± 3.00
12	0.88 ± 0.02	77.72 ± 2.21	110.73 ± 2.21	5.10 ± 0.21	4.00 ± 0.21	201.00 ± 1.00
13	0.90 ± 0.21	78.12 ± 0.41	105.13 ± 3.21	5.20 ± 0.23	1.80 ± 0.01	193.00 ± 2.00

4.3 Anti-nutritional Composition of Selected Flour Blends.

The results for the anti-nutritional content of the selected composite flour samples are presented in Table 4.3. Oxalate concentration of the selected composite flour ranged from 0.070 mg/ g to 1.305 mg/ g. In comparison, the oxalate concentration of the selected sample B (with 86.92:13.08:0 of rice flour, fermented rice bran flour and wheat flour respectively) and D (with 69.29:21.89:10.23 of rice flour, fermented rice bran flour and wheat flour respectively) showed no significant ($p \leq 0.05$) difference. However, there was significant ($p \leq 0.05$) difference across all samples.

Tannin concentration of the selected composite flour samples ranged from 1.060 mg/g to 1.941 mg/g. Statistically, the tannin concentration of the selected flour sample shows no significant ($p \leq 0.05$) difference between sample A, B and D respectively but there was significant ($p \leq 0.05$) difference observed between sample C and E. The Alkaloid content ranged from 8.013 mg/g to 10.413 mg/g. There is significant ($p \leq 0.05$) difference across the samples observed.

Phytate concentration of the formulated composite flour ranged from 9.476 mg/g to 19.364 mg/g. Statistically, the phytate concentration of the formulated composite flour of sample C (with 60:10:30 of rice flour, fermented rice bran flour and wheat flour respectively) shows no significant difference ($p \leq 0.05$) from the sample D (with 69.29:21.89:10.23 of rice flour, fermented rice bran flour and wheat flour respectively). Similar values for phytate (14-18 mg/g), tannin (1.67-3.75 mg/g) and oxalate (0.36-1.54 mg/g) content have been reported for whole wheat flour substituted with acha and pigeon pea flour (Olagunju, 2019).

However, the tannin and phytate content observed in this study were lower than the values for tannin (1-4 mg/g) phytate (12-35 mg/g) reported for cocoyam-based composite flour comprising cassava starch (Awolu and Oseyemi, 2016) Furthermore, the content observe in phytate may be attributable to the fact that phytates are generally found in fibre-rich foods such as wheat bran, rice bran, whole grains, and legumes (Bodie *et al*., 2019; Olagunju, 2019) and its presence is associated with reduced mineral absorption. Phytates, oxalates, and tannins are known to adversely interfere with mineral bioavailability by forming insoluble salts with Zn, Ca, and Fe, and thus preventing their absorption and impair protein digestibility (Samtiya*et al*., 2020). The anti-nutrient content of the flour may not be detrimental to health as the values are far lower than the 80 mg/g critical limit reported by Malomo*et al*. (2011). Studies have reported that various processing methods like dehusking, sprouting, blanching, milling, soaking, fermentation, baking, cooking, usually brings about reductions in anti-

nutritional composition of food products (Ijarotimi and Kenshinro 2012; Temesgen, 2013; Samatiya*et al*., 2020).

Table 4.3:Anti-nutritional Composition of Selected Flour Blends

SAMPLE	Oxalate(mg/g)	Tannin(mg/g)	Alkaloid(mg/g)	Phytate(mg/g)
A	1.305±0.06^{a}	1.514±0.26^{ab}	8.838±0.23^{b}	19.364±0.58^{a}
B	0.855±0.06^{b}	1.287±0.19^{ab}	8.013±0.23^{c}	12.392±0.12^{c}
C	0.675±0.06^{c}	1.941±0.38^{a}	10.413±0.23^{a}	13.596±0.58^{b}
D	0.900±0.00^{b}	1.419±0.29^{ab}	8.288±0.23^{bc}	13.712±0.00^{b}
E	0.270±0.00^{d}	1.060±0.24^{b}	6.813±0.23^{d}	9.476±0.58^{d}

Means ± Standard deviation with different superscripts in the same column are significantly different ($P<0.05$)

Key: A = (Rice Flour 60.00% + Fermented rice bran 24.20% + Wheat flour 15.80%); B = (Rice Flour 86.92% + Fermented rice bran 13.08%); C = (Rice Flour 60.00% + Fermented rice bran 10.00% + Wheat flour 30.00%); D = (Wheat flour 100%); E = (Rice flour 69.29% + Fermented rice bran 21.89% + Wheat flour 10.23%)

4.4 Antioxidant Activities of Selected Flour Blends

The results of antioxidant properties of the selected flour blends are presented in Table 4.4. The total flavonoid ranged from 0.009 g/mg to 0.050 g/mg. There was significance ($p \leq 0.05$) difference among the samples. However, there was no significant ($p \leq 0.05$) difference between sample A with 60:24.20:15.80 of rice flour, fermented rice bran flour and wheat flour respectively and sample E (with 100:0:0 of rice flour, fermented rice bran flour and wheat flour respectively).

The total phenol ranged from 2.31 mg/g to 4.544 mg/g. There was significant ($p \leq 0.05$) difference across the samples except for sample A and E which showed no significant ($p \leq 0.05$) difference. The ability of the selected flour to scavenge free radical against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The free radical scavenging ability of the flour samples ranged from 69.97% to 84.67%. Comparatively, free radical scavenging ability of the formulated composite flour has no significant ($p \leq 0.05$) difference between sample A, D and E but there was difference ($p \leq 0.05$) between sample B and C respectively. The ability of formulated composite flour samples to scavenge free radical against 2, 2-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay is also presented in table 4.4. The free radical scavenging ability of the formulated flour samples had sample C has 0.023% while sample B has 0.011% had the least free radical scavenging ability. Similarly, with the DPPH ability there was no significant ($p \leq 0.05$) difference between sample A, D and E respectively but there was significant ($p \leq 0.05$) difference between sample B and C.

The Fe^{2+}chelation antioxidant power of the formulated composite flour samples and control flour samples is presented in Figure 4.4. The ability of the formulated composite flour samples to reduced Fe^{3+} to Fe^{2+} had its peak reducing ability at 1.305% in sample A and the least reducing ability at 0.270% in sample E. Comparatively, reducing ability of the formulated composite flour has no significant ($p \leq 0.05$) difference between the samples, however sample A and C showed no difference.

The ability of the selected composite flour samples to reduce $FeCl_3$ solution had its peak reducing ability at 8.689 mg AAE/g in sample A and the least reducing ability at 3.261 mg AAE/g in sample C. Similarly, with the DPPH and the ABTS ability of the samples there was no significant ($p \leq 0.05$) difference between sample A, D and E respectively but there was

significant ($p \leq 0.05$) difference between sample B and C. This finding could be attributed to phytochemicals, bioactive proteins that were present in these selected flour samples. For instance, the addition of rice bran could have uplifted the anti-oxidant activities of the samples. Rice bran contains several bioactive components such as Ferulic acid, ϒ Oryzanol, Inositol hexaphosphate, Compesterol, β-Sitosterol, Linoleic acid, α-Tocopherol, Tocotrienol, Salicylic acid, Caffeic acid, Caumaric acid, Tricin (Chaudhari *et al*., 2018). Tocopherol for example has been highlighted as an antioxidant against free radicals (Oladipo *et al*., 2018). Chowdhury *et al*. (2019) reported that treatment with ferulic acid, could effectively combat reactive oxygen species production as well as restore protein carbonylation, lipid peroxidation, and antioxidant activities. The antioxidant results of this findings were higher than past results such as DPPH of 49-59% reported for wheat and amaranth seed, brewers' spent grain and apple pomace composite flour (Awolu*et al*., 2016). Furthermore, this finding was also higher than the values of DPPH (49-57%) and ABTS (0.0172-0.0182 TEAC/g) but similar in the values of FRAP (1.66-11.29 mg AAE/g) but lower in Fe^{2+} chelation abilityvalue (50.86-63.430 mg/ml for plantain flour enriched with tigernut (*Cyperusesculentus*) and defatted soybean (*Glycine max*) (Oluwajuyitan and Ijarotimi, 2019). Therefore, phytochemicals such as Flavonoids, polyphenols, ϒ-orizanol and tocopherols.

Table 4.6: Antioxidant Activities of Bread Produced from Selected Flour Blends

Sample	Total Flavonoid (g/mg)	Total Phenol (mg/ g)	FRAP (mg/ml)	Fe^{2+} Chelation (%)	ABTS (%)	DPPH (%)
A	0.023±0.02^{c}	2.808±0.34^{c}	6.537±0.04^{b}	1.305 ± 0.06^{a}	0.020±0.06^{b}	76.602±0.72^{b}
B	0.009±0.21^{d}	2.312±0.32^{d}	3.261±0.30^{c}	0.855 ± 0.06^{b}	0.011±0.07^{c}	69.978± 2.32^{c}
C	0.050±0.01^{a}	4.544±0.32^{a}	8.689±0.91^{a}	0.675 ± 0.06^{c}	0.023±0.03^{a}	84.679± 0.04^{a}
D	0.032±0.34^{b}	3.592±0.08^{b}	6.017±1.12^{b}	0.900 ± 0.72^{b}	0.021±0.04^{b}	75.632± 0.04^{b}
E	0.021±0.21^{c}	2.31 ± 0.28^{c}	6.311±0.04^{b}	0.270 ± 0.21^{d}	0.020±0.31^{b}	75.641± 1.69^{b}

Means ± Standard deviation with different superscripts in the same column are significantly different ($P<0.05$)

Key: A = (Rice Flour 60.00% + Fermented rice bran 24.20% + Wheat flour 15.80%); B = (Rice Flour 86.92% + Fermented rice bran 13.08%); C = (Rice Flour 60.00% + Fermented rice bran 10.00% + Wheat flour 30.00%); D = (Wheat flour 100%); E = (Rice flour 69.29% + Fermented rice bran 21.89% + Wheat flour 10.23%

4.5 Mineral composition Bread Produced from Selected Flour Blends

The mineral compositions of the selected flour blends runs are presented in Table 4.4. The mineral compositions of the flour blends had potassium as the most abundant elements with

values ranging from 1183.00 mg/kg in run 12 to 4906.00 mg/kg in run 1, while copper had the least concentration with values ranging from 3.20 mg/kg in run 12 to 4.20 mg/kg in run 10 respectively. The values of Sodium ranged from 113.50 mg/kg in run 8 to 119.90 mg/kg in run 1. The beneficial effect of low sodium is beneficial in the control of arterial hypertension which requires an increase in potassium intake (Grillo *et al.*, 2019). Notably, Potassium in high amount has been reported to increase iron utilization in the body and both sodium and potassium are required to maintain osmotic balance of the body fluids, the body pH, regulation of muscle and nerve irritability, control glucose absorption, and also enhance normal retention of protein during growth (Omoba and Omogbemile, 2013).

The concentration of calcium in the selected flour bends runs ranged from 112.00 mg/kg in run 10 to 238.00 in run 1 respectively. The recommended dietary intake for calcium varies in relation to age group between 250-400 mg in young children, 960mg in female, 1200 mg in male and 1160 mg in lactating women. Adequate calcium balance is important throughout life, especially among children less than 2 years old, during puberty and adolescence, pregnant, lactating, and postmenopausal women, as well as elderly men. (Alasfoor*et al*., 2008).

The concentration of zinc in the selected flour blends runs ranged from 29.10 mg/kg in run 4 to 34.10 mg/kg in run 1. The role of zinc has been highlighted in growth and the immune system (Roohani*et al.,* 2013). The required dietary intake for Zinc is 15 mg for men and 12 mg for women (Omoba and Omogbemile, 2013) and minimum recommendation 9 mg/day dietary zinc according to the National Institute of Health, (2021). Hence the present result shows that the flour samples have appreciable amounts of zinc than the recommended daily intake.

Iron (Fe) are essential elements for almost all living organisms and is required for optimal human growth, also participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour *et al*., 2014). The level of iron ranged from 19.00 mg/kg in run 10 to 32.00 mg/kg in run 8. Phosphorus is needed by the body to repair tissue cells and to build strong bones and teeth (Emebu and Anijika, 2011). The values ranged from 108.60 mg/kg in run 10 to 475.00 mg/kg in run 1 respectively.

Copper is a micro-nutrient needed in trace amount as it plays an essential role towards the formation of red blood cells as it is a cofactor of many redox enzymes, including

ceruloplasmin, the most abundant copper dependent ferroxidase enzyme and maintaining nerve cells and the immune system (Filippini *et al*., 2018). The copper values ranged from 3.20 mg/kg in run 12 to 4.20 mg/kg in run 10. The values of copper reported for this study is above the daily recommended intake of 1.0-1.5 mg/day and below the tolerable intake of 10mg/day respectively (Filippini *et al*., 2018).

Table 4.5 Mineral composition of Bread Produced from Selected Flour Blends

Samples	K	Na	Ca	Zn	Fe	Cu	P
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
A	4906.00	119.90	238.00	34.10	23.00	3.27	475.00
B	2870.00	116.00	141.00	29.10	22.10	3.30	410.00
C	2420.00	113.50	118.00	30.30	32.00	3.30	392.00
D	1183.00	115.90	112.00	33.00	19.00	4.20	108.60
E	4125.00	118.70	198.30	33.70	22.00	3.20	440.00

Means ± Standard deviation with different superscripts in the same column are significantly different ($P<0.05$)

Key: A = (Rice Flour 60.00% + Fermented rice bran 24.20% + Wheat flour 15.80%); B = (Rice Flour 86.92% + Fermented rice bran 13.08%); C = (Rice Flour 60.00% + Fermented rice bran 10.00% + Wheat flour 30.00%); D = (Wheat flour 100%); E = (Rice flour 69.29% + Fermented rice bran 21.89% + Wheat flour 10.23%).

4.6 Soluble and Insoluble Fibre Content of Selected Flour Blends

The total soluble and insoluble dietary fibre content of the samples are presented on Table 4.8. The soluble dietary fibre content of the flour samples ranged from 17.75% in sample D to 36.63% in sample B. The highest value was observed in samples that have the highest ratios of both rice flour and rice bran which are samples B, C and A respectively. Likewise,

the insoluble dietary fibre content ranged from 82.15% in sample D to 61.87%. Similarly, the highest value was observed in ratios with highest ratios of both rice flour and rice bran. There was significant ($p \leq 0.05$) difference among the samples. The soluble and insoluble fibres of this findings were higher than the values of (0.92-3.74%) soluble and (28.09% to 41.23%) insoluble fibre for plantain and moringa flour reported by Badejo *et al*., (2017).

Dietary fibre belongs to a group of polysaccharides that are non-starch and lignin. They are unique because they are resistant to digestion and are poorly absorbed in the small intestine but ends up being fermented in the large intestine. Insoluble fiber cannot be dissolved in water, therefore it helps speeds the movement of food through the digestive tract which may help prevent, colon cancer and constipation. On the other hand, soluble fiber helps maintain a healthy cholesterol level, normalize blood sugar levels in diabetics and may even help reduce blood pressure (Alam *et al*., 2014; Badejo *et al*., 2017). The high increase in insoluble fibre and soluble fibre could have come from the addition of rice bran into the flour mix as they have been reported to be high in fibres (Friedman, 2013; Chaudhari *et al*., 2018; Bodie *et al*., 2019; Vidriales*et al*., 2020).

Table 4.6: Soluble and Insoluble fibre of Selected Flour Blends

SAMPLE	Soluble fibre (%)	Insoluble fibre (%)
A	25.595 ± 2.16^{bc}	73.975 ± 1.59^{b}
B	36.630 ± 2.19^{a}	61.870 ± 1.63^{d}
C	30.835 ± 2.24^{ab}	67.840 ± 2.35^{c}
D	17.750 ± 2.94^{d}	82.150 ± 2.80^{a}
E	21.430 ± 1.64^{cd}	78.070 ± 1.36^{ab}

Means ± Standard deviation with different superscripts in the same column are significantly different (P<0.05)

Key: A = (Rice Flour 60.00% + Fermented rice bran 24.20% + Wheat flour 15.80%); B = (Rice Flour 86.92% + Fermented rice bran 13.08%); C = (Rice Flour 60.00% + Fermented rice bran 10.00% + Wheat flour 30.00%); D = (Wheat flour 100%); E = (Rice flour 69.29% + Fermented rice bran 21.89% + Wheat flour 10.23%)

4.7 Blood Glucose Trend and Glycaemic Index (GI) of Selected Flour Blends

The blood glucose concentrations and glycaemic index of Albino Wista rat fed with the flour samples and glucose are presented in Tables 4.7 and 4.8 respectively. The blood glucose concentration was observed over 180 min after ingestion of the selected flour samples were recorded. The result indicated that the increase in glucose response across the samples were minimal compared to the glucose sample. For instance, sample which showed the greatest increase among other selected flour samples only showed 17% increase as compared to glucose which showed approximately 174% increase in glucose at 30 min while sample D only showed increase at 4%. The slight rise in blood sugar in sample B and D could be attributed to their mixture variation which showed higher rice flour percentage.

Foods with Glycemic Index (GI) values greater than 70 are high GI foods, GI between 56 and 69 are intermediate GI foods, while foods with GI lower than 55 are lower GI foods. All the flour samples have lower GI. This result is in agreement with the existing GI classification of food products whereby both rice and wheat has been classified as high GI food (Atkinson *et al*., 2008; Olagunju 2019).

Interestingly the addition of rice bran could have reduced or delay the breakdown of these composite as they are known to contain large amount of fibre (Bodie *et al*., 2019) which are not digested hence give rise to an intermediate and low glycaemic index.

The observed result is similar to that of Olagunju, (2019) who reported similar value of (44.88~54.87%) for Whole-grain wheat flour, acha flour, and pigeon pea composite flours but lower than the values of (36.05~ 42.95%) for plantain, tigernut and soybean flour (Oluwajuyitan and Ijarotimi, 2019). This study shows that an intermediate and low

glycaemicindex of this flour samples could be useful in diabetic patient as they do not increase the blood sugar level radically when compared with high GI foods.

Table 4.7: Blood Glucose (min) Trend of Selected Flour Blends

	Flour samples					
Time (min)	**A**	**B**	**C**	**D**	**E**	**Glucose (Control)**
0	90	89	98	110	62	80
30	85	104	79	114	68	219
45	93	113	84	121	94	165
60	101	125	110	125	127	143
120	102	97	121	135	111	133
180	108	96	101	119	109	103

Key: A = (Rice Flour 60.00% + Fermented rice bran 24.20% + Wheat flour 15.80%); B = (Rice Flour 86.92% + Fermented rice bran 13.08%); C = (Rice Flour 60.00% + Fermented rice bran 10.00% + Wheat flour 30.00%); D = (Wheat flour 100%); E = (Rice flour 69.29% + Fermented rice bran 21.89% + Wheat flour 10.23%).

Table 4.8: Glycaemic Index (%) of Selected Flour Blends

Sample	**Area Under Curve**	**Glycemic Index**
A	9720	49.32

B	11250	57.08
C	10890	55.25
D	12150	61.64
E	11430	57.99
Control (Glucose)	19710	100.00

Key: A = (Rice Flour 60.00% + Fermented rice bran 24.20% + Wheat flour 15.80%); B = (Rice Flour 86.92% + Fermented rice bran 13.08%); C = (Rice Flour 60.00% + Fermented rice bran 10.00% + Wheat flour 30.00%); D = (Wheat flour 100%); E = (Rice flour 69.29% + Fermented rice bran 21.89% + Wheat flour 10.23%).

4.8 Sensory Attributes of Bread Produced from Selected Flour Blends

The sensory attributes of the samples are presented in Table 4.8. The appearance of the bread samples ranged from 6.33 in sample A to 7.23 in sample C, while that of the control (commercial bread) showed the highest ($p \leq 0.05$) value in appearance (8.37) than the experimental bread samples. However, sample C (7.23) was observed to be higher than the other experimental bread samples. This could be attributed to the inclusion of 30% wheat flour in the sample. Samples A, D and E showed no significant ($p \leq 0.05$) difference between them while samples B, C and E also showed no significant ($p \leq 0.05$) difference.

The aroma of the bread samples ranged from 5.43 in sample D to 5.83 in sample C. Similarly, the highest value ($p \leq 0.05$) was also observed in the commercial bread (CB) sample (8.13). There was no significant ($p \leq 0.05$) difference between the experimental bread samples in terms of aroma. The taste attributes of the experimental bread samples ranged from 4.43 in E to 5.53 in C. Similarly, the highest value ($p \leq 0.05$) was also observed in the commercial bread (CB) sample (7.97). The significant ($p \leq 0.05$) difference that was only observed in the bread samples are shown in sample C, D and E respectively.

The crumbness (6.17 in C to 6.57), crustiness (6.30 in C to 6.63 in A) and the overall acceptability (5.80 in B to 6.03 in A) of the experimental bread samples did not show any

Samples	Appearance	Aroma	Taste	Crumb colour	Crust colour	Overall acceptability

significant difference ($p \leq 0.05$) between themselves but were lower than the commercial bread sample ($p \leq 0.05$) in crumbness (8.07), crustiness (8.10) and overall acceptability (8.43) respectively.The difference between the overall acceptability of the experimental bread samples and the commercial bread sample could be attributed to the familiarity with the commercial product as evidence could be seen in experimental bread sample C which showed similarities with the commercial bread due to its increase in wheat variation.

The overall acceptability of this result was similar to that of sweet potato-wheat bread (4.20-8.00) reported by Ayo-Omogie*et al*., (2021), wheat bread supplemented with stabilized undefatted rice bran (5.55-7.20) reported by Ameh *et al*., (2013), wheat bread substituted with rice bran (5.3-6.2) reported by Irakli *et al*., (2015). Bodie *et al*., (2019) highlighted that the quality of food is improved with the use of rice bran especially color, appearance, taste, and texture. The on-going consumer concern for better health and eating habits render rice bran an optimal nutritional and dietary supplement for overall health maintenance in food use (Issara and Rawdkuen, 2017;

Iriondo-DeHond, 2018).

4.8 Sensory Attributes of Bread Produced from Selected Flour Blends

A	6.33 ± 1.47^{c}	5.70 ± 1.48^{b}	5.07 ± 1.48^{bc}	6.63 ± 1.52^{b}	6.63 ± 1.63^{b}	6.03 ± 1.10^{b}
B	6.57 ± 1.33^{bc}	5.50 ± 1.43^{b}	4.93 ± 1.44^{bc}	6.40 ± 1.59^{b}	6.43 ± 1.52^{b}	5.80 ± 1.13^{b}
C	7.23 ± 1.25^{b}	5.83 ± 1.33^{b}	5.53 ± 1.33^{b}	6.17 ± 1.82^{b}	6.30 ± 1.25^{b}	5.93 ± 1.11^{b}
D	6.43 ± 1.52^{bc}	5.53 ± 1.33^{b}	4.43 ± 1.30^{c}	6.40 ± 1.63^{b}	6.40 ± 1.71^{b}	6.03 ± 1.30^{b}
E	6.87 ± 1.20^{bc}	5.53 ± 1.33^{b}	5.33 ± 1.71^{b}	6.57 ± 1.28^{b}	6.57 ± 1.70^{b}	5.83 ± 1.21^{b}
F	8.37 ± 0.67^{a}	8.13 ± 0.78^{a}	7.97 ± 1.59^{a}	8.07 ± 0.78^{a}	8.10 ± 0.61^{a}	8.43 ± 0.68^{a}

Means ± Standard deviation with different superscripts in the same column are significantly different ($P<0.05$)

Key: A = (Rice Flour 60.00% + Fermented rice bran 24.20% + Wheat flour 15.80%); B = (Rice Flour 86.92% + Fermented rice bran 13.08%); C = (Rice Flour 60.00% + Fermented rice bran 10.00% + Wheat flour 30.00%); D = (Rice flour 69.29% + Fermented rice bran 21.89% + Wheat flour 10.23%); E = (Wheat flour 100%); F= Control (Commercial bread).

Conclusion

The study established that partial substitution of wheat flour with rice flour and fermented rice bran flour contained appreciable amount of essential protein, increased fibre content and low moisture content for longer storage of the flour. Bread samples made from the composite flour also showed good attributes in protein and fibre content. The flour samples also exhibited good functional properties and low antinational content.

Reference

Allgrove J, Shaw NJ (2015). A practical approach to vitamin D deficiency and rickets. Endocrine Development; 28:119–33.

Medoua, G. N., Mbome, I. L., Agbor-Egbe, T. and Mbofung, H. (2007). Influence of fermentation on some quality characteristics of trifoliate yam (*Dioscorea dumetorum*) hardened tubers. *Food Chemistry*, 107(3):1180-1186.

Malomo, S. A, Eleyinmi, A. F, Fashakin, J. B. (2011). Chemical composition, rheological properties and bread making potentials of composite flours from breadfruit, breadnut and wheat. *African Journal Food Science* 5, 400-410.

Abbaspour, N., Hurrell, R., &Kelishadi, R. (2014). Review on iron and its importance for human health. Journal of research in medical sciences: *the official journal of Isfahan University of Medical Sciences*, 19(2), 164–174.

Awolu, O. O. (2018). Rheological evaluation of cocoyam-bambara groundnut-xanthan gum composite flour obtained from the optimization of its chemical composition and functional properties. *Rheology: Open Access*, 2, 118.

Awolu, O. O., &Olokunsusi, E. Y. (2017). Optimisation and Evaluation of the Effect of Bambara Groundnut Addition on the Nutritional Quality and Functional Properties of Amaranth Grain-Based Composite Flour. Acta Universitatis Cibiniensis. Series E: *Food Technology*, 21(2), 43-52

Awolu, O. O., &Oseyemi, G. F. (2016). Physicochemical and rheological properties of optimized cocoyam-based composite flour comprising cassava starch. Acta Universitatis Cibiniensis. Series E: *Food Technology*, 20(2), 65-84.

Awolu, O. O., Osemeke, R. O., &Ifesan, B. O. T. (2016). Antioxidant, functional and rheological properties of optimized composite flour, consisting wheat and amaranth seed, brewers' spent grain and apple pomace. *Journal of Food Science and Technology*, 53(2), 1151–1163.

Awolu, O. O., &Oseyemi, G. F. (2016). Physicochemical and rheological properties of optimized cocoyam-based composite flour comprising cassava starch. Acta Universitatis Cibiniensis. Series E: *Food Technology*, 20(2), 65-84.

Ameh, Michael &Gernah, D. &Igbabul, Bibiana. (2013). Physico-Chemical and Sensory Evaluation of Wheat Bread Supplemented with Stabilized Undefatted Rice Bran. *Food and Nutrition Sciences*, 4(09), 43-48.

AOAC. (2012). Association of Official Analytical Chemist. Official Methods of Analysis of the Analytical Chemist International, 18th ed. Gathersburg, MD USA.

Asoegwu S. N., Ohanyere, S. O., Kanu, O. P. and Iwueke, C. N. (2006). Physical properties of African oil bean seed (*Pentonclethranacrophylla*). *Agricultural Engineering International Journal*, 44(1): 1- 6.

Alam Ariful, Jahangir Alam, Abdul Hakim, Obidul Huq, Golam Moktadir. (2014). Development of fiber enriched herbal biscuits: A preliminary study on sensory evaluation and chemical composition. *International Journal of Nutrition and Food Sciences*, 3(4), 246-250.

Adebowale, Y. A., Adeyemi, I. A. and Oshodi, A. A. (2005a). Functional and physico-chemical properties of flour of six mucuna species. *African Journal of Biotechnology*, 4(12): 1461-1468.

Abulude, F. O. (2004). Effect of Processing on Nutritional Composition, Phytate and Functional Properties of Rice (*Oryza sativa* L) Flour. *Nigerian Food Journal*, 22(2): 97-104.

Alasfoor, D., Rajab, H., Al-Rassasi, B., & Abdu, S. (2008). Food Based Dietary Guidelines Technical background and description Task force for the development and implementation of the Omani Food Based Dietary Guidelines. www.fao.org/ag/humannutrition/195420561250979706400b7cc8ca7366cc07c0.pdf accessed September 21st, 2021.

Ayo-Omogie H. N. (2021). Gluten-Reduced Sweet Potato-Wheat Bread: Influence of Fermented Sweet Potato Flour Addition on Bread Quality and Dough Rheology, Journal of Culinary Science & Technology, 19(3), 87-213

Ayo-Omogie, H. N. and Odekunle, O. Y. (2017). Substituting Wheat Flour with Banana Flour: Effects on the Quality Attributes of Doughnut and Cookies. *Applied Tropical Agriculture* 22 (2):134 – 137.

Ayo-Omogie, H. N., Jolayemi, O. S., Chinma, C. E., (2021). Fermentation and blanching as adaptable strategies to improve nutritional and functional properties of unripe Cardaba banana flour, *Journal and Agriculture and Food Research*, 6, 100-214.

Ayo-Omogie, Helen &Ogunsakin, Regina. (2013). Assessment of Chemical, Rheological and Sensory Properties of Fermented Maize-Cardaba Banana Complementary Food. *Food and Nutrition Sciences*. 04(08), 844-850.

Badejo, Adeboye P. Adebanjo A. B Osunlakin, Ademola Famakinwa, Atinuke O. Idowu & Tayo N. Fagbemi | (2017) Analyses of dietary fibre contents, antioxidant composition, functional and pasting properties of plantain and Moringa oleifera composite flour blends, Cogent Food & Agriculture, 3:1, 1278871.

Bodie, A. R, Micciche, A. C, Atungulu, G. G, Rothrock, M. J. Jr., and Ricke, S. C. (2019) Current Trends of Rice Milling Byproducts for Agricultural Applications and Alternative Food Production Systems. *Front. Sustain. Food Syst*. 3:47.

Bushra, S., Farooq, A. and Muhammad, A. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules* (14): 2167-2180.

Chaudhary, R. C. (2003). Specialityrices of the world: Effect of WTO and IPR on its production trend and marketing. *Journal of Food Agriculture and Environment*, 1(2), 34-41.

Chowdhury, S., Ghosh, S., Das, A. K., & Sil, P. C. (2019). Ferulic Acid Protects Hyperglycemia-Induced Kidney Damage by Regulating Oxidative Insult, Inflammation and Autophagy. *Frontiers in Pharmacology*, 10, 27.

Cho, S., Lu, Q., Fahey, GC., Klurfeld, DM. (2013) Consumption of cereal fiber, mixtures of whole grains and bran, and whole grains and risk reduction in type 2 diabetes, obesity, and cardiovascular disease. *The American Journal of Clinical Nutrition*. 98:594–619.

Cozzolino M, Mangano M, Galassi A, Ciceri P, Messa P, Nigwekar S (2019). Vitamin K in chronic kidney disease. Nutrients.11:168.

Chandra, S. and Samsher, L. (2013) Assessment of Functional Properties of Different Flours. African Journal of Agricultural Research, 8, 4849-4852.

De Romaña DL, Lönnerdal B, Brown KH (2003). Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide. American Journal Clinical Nutrition. 78:279–83.

Dijhkuizen P. (2000). Processed complementary foods in the WFP. Food and nutrition bulletin.21:62–4.

Emebu, P. K. and Anijika, J. U. (2011). Proximate and mineral composition of kale (Brassica oleracea) grown in delta state, Nigeria. *Pakistan Journal of Nutrition*, 10, 190-194.

Famakin, O., Fatoyinbo, A., Ijarotimi, O.S., Badejo, A.A. and Fagbemi, T.N. (2016), "Assessment of nutritional quality, glycaemic index, antidiabetic and sensory properties of plantain (musa paradisiaca)-based functional dough meals",
. *Journal of Food Science and Technology*, 53(11), 3865-3875.

Friedman, W. (2013). Rice brans, rice bran oils, and rice hulls: composition, food and industrial uses, and bioactivities in humans, animals, and cells. *J. Agric. Food Chem*. 61, 10626–10641.

Filippini, T., Cilloni, S., Malavolti, M., Violi, F., Malagoli, C., Tesauro, M., Bottecchi, I., Ferrari, A., Vescovi, L., &Vinceti, M. (2018). Dietary intake of cadmium, chromium, copper, manganese, selenium and zinc in a Northern Italy community. *Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS)*, 50, 508–517.

Goni, L., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. Nutrition Research, 17, 427–437.

Grillo, A., Salvi, L., Coruzzi, P., Salvi, P., &Parati, G. (2019). Sodium Intake and Hypertension. Nutrients, 11(9), 19-70.

Harborne JB (1973). Phytochemical Methods, Chapman and Hall, London, pp. 11-21.

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

Ijarotimi, O. S and Keshinro, O. O. (2012). Formulation and nutritional quality of infant formula produced from germinated popcorn, Bambara groundnut and African locust

bean flour. *Journal of Microbiology, Biotechnology and Food science*, 1(6), 1374-1404.

Irakli M. Samanidon V F, Katsantonis D, Papadoyannis N I. (2015). Phytochemical Profiles and Antioxidant Capacity of Pigmented and Non-pigmented Genotypes of Rice (Oryza sativa L.). Cereal research communication.

Iriondo-DeHond, M., Miguel, E., and Del Castillo, M. D. (2018). Food byproducts as sustainable ingredients for innovative and healthy dairy foods. *Nutrients*, 10, 1358–1384.

Issara, U., and Rawdkuen, S. (2017). Rice bran: a potential of main ingredient in healthy beverage. *International Food Reserve Journal.* 23, 2306–2231.

National Institutes of Health (NIH). (2021) Zinc, Fact Sheet for Health Professionals. https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/accessed September 21st, 2021

Noor Aziah AA, Ho LH, Noor Shazliana AA, Rajeev B (2012). Quality evaluation of steamed wheat bread substituted with green banana flour. *International Food Reserve Journal.*19 (3), 869-876.

Olagunju A. I. (2019). Influence of Whole Wheat Flour Substitution and Sugar Replacement with Natural Sweetener on Nutritional Composition and Glycaemic Properties of Multigrain Bread. Preventive nutrition and food science, 24(4), 456–467.

Olapade, A. A., Oluwole, O. B. and Aworh, O. C. (2012). Physico-chemical properties and consumer acceptance of instant cowpea (Vigna unguiculata) powder for complementary food. *African Journal of Food Science and Technology*, 3(4): 102 – 106.

Oladipo, G. O., Nlekerem, C. M., Ibukun, E. O., and Kolawole, A. O. (2018). Quail (Coturnx japonica) egg yolk bioactive components attenuate streptozotocin

induced testicular damage and oxidative stress in diabetic rats. European journal of nutrition, 57(8), 2857–286.

Odebode, F. D., Ekeleme, O. T., Ijarotimi, O. S., Malomo, S. A., Idowu, A. O., Badejo, A. A., Adebayo, I. A. and Fagbemi, T. N. (2017), "Nutritional composition, antidiabetic and antilipidemic potentials of flour blends made from unripe plantain, soybean cake, and rice bran", Journal of Food Biochemistry, 42(4), 12447-12457.

Oluwajuyitan, T. D., &Ijarotimi, O. S. (2019). Nutritional, antioxidant, glycaemic index and Antihyperglycaemic properties of improved traditional plantain-based (Musa AAB) dough meal enriched with tigernut (Cyperus esculentus) and defatted soybean (Glycine max) flour for diabetic patients. *Heliyon*, 5(4), e01504.

Oluwajuyitan, T. D., Ijarotimi, O. S., Fagbemi, T. N., & Oboh, G. (2021). Blood glucose lowering, glycaemic index, carbohydrate-hydrolysing enzyme inhibitory activities of potential functional food from plantain, soy-cake, rice-bran and oat-bran flour blends. Journal of Food Measurement and Characterization, 1-9.

Omoba and Omogbemile. (2013). Physicochemical Properties of Sorghum Biscuits Enriched with Defatted Soy Flour. *British Journal of Applied Science & Technology*, 3(4), 1246-1256.

Re, R., Pellegrin, N., Proteggente, A., Pannala, A., Yang, M and Rice-Evans, C. (1999). Antioxidant activity applying an improve ABTS Radication decolourization assay.*Free Radical Biology and Medicine*. 26(1): 1231-1237.

Roohani, N., Hurrell, R., Kelishadi, R., & Schulin, R. (2013). Zinc and its importance for human health: An integrative review. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 18(2), 144–157.

Sairam, S., Gopala Krishna, A. G., Urooj, A. (2011) Physico-chemical characteristics of defatted rice bran and its utilization in a bakery 478- product. *Journal Food Science Technology*, 48, 483.

Samtiya, M., Aluko, R. E., and Dhewa, T. (2020). Plant food anti-nutritional factors and

their reduction strategies: an overview. Food Production, Processing and Nutrition, 2(1).

Sharma, R., Garg, P., Kumar, P., Bhatia, S. K., & Kulshrestha, S. (2020). Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods. *Fermentation*, 6(4), 106.

Singleton, V. L., Orthofer, R and Lamuela-Raventos, R. M. (1999). Analysis of Total Phenols and other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods Enzymology*, 299(10):152-177.

SON (2007): Nigerian Industrial Standard for biscuits. ICS: 664.68, Standard Organization of Nigeria.

Temesgen, M. (2013). Nutritional Status of Ethiopian Weaning and Complementary Foods: A Review. 2, 621.

Thakur, P.P. Pawar, V.S. and Shere, D.M. (2016). Material balance, proximate and functional analysis of green banana flour prepared by cabinet drying method. *Food Science Research Journal* 7 (2), 190-194.

Ukpabi, A. and Ejidoh, E. O. (1989). Experimental Procedures for Food and Water Analysis, *San Press Publishers*, Enugu, Nigeria. 89-94.

Vidriales, M. A., Peña-Rodríguez, A., Tovar-Ramírez, D., Elizondo-González, R., Barajas-Sandoval, D. R., Ponce-Gracía, E. I., Quiroz-Guzmán, E. (2020). Effect of rice bran fermented with Bacillus and Lysinibacillus species on dynamic microbial activity of Pacific white shrimp (Penaeus vannamei). *Aquaculture*, 532, 735958.

Xie, Z., Huang, J., Xu, X. and Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry*, 111(2): 370–376.

Wu, G. (2016). Dietary protein intake and human health. *Food & Function*, 7(3), 1251–1265.

Yusuf, M., Halilu, M., & Filli, K. B. (2018). Influence of Extrusion Variables on Proximate Composition Some Nutrient and Antinutrient Contents of Dakuwa Extrudates Produced from Blends of Sorghum (Sorghum bicolour L) Groundnut (Arachis hypogea L) and Tigernut (Cyperus esculentus). *Current Journal of Applied Science and Technology*, 26(4): 1-20.

Zhu, S.M., Lin, S.L., Ramaswamy, H.S., Yu, Y. and Zhang, Q.T. (2017). Enhancement of functional properties of rice bran proteins by high pressure treatment and their correlation with surface hydrophobicity. *Food Bioprocess Technology*, 10, 317–327.

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