RESEARCH ARTICLE

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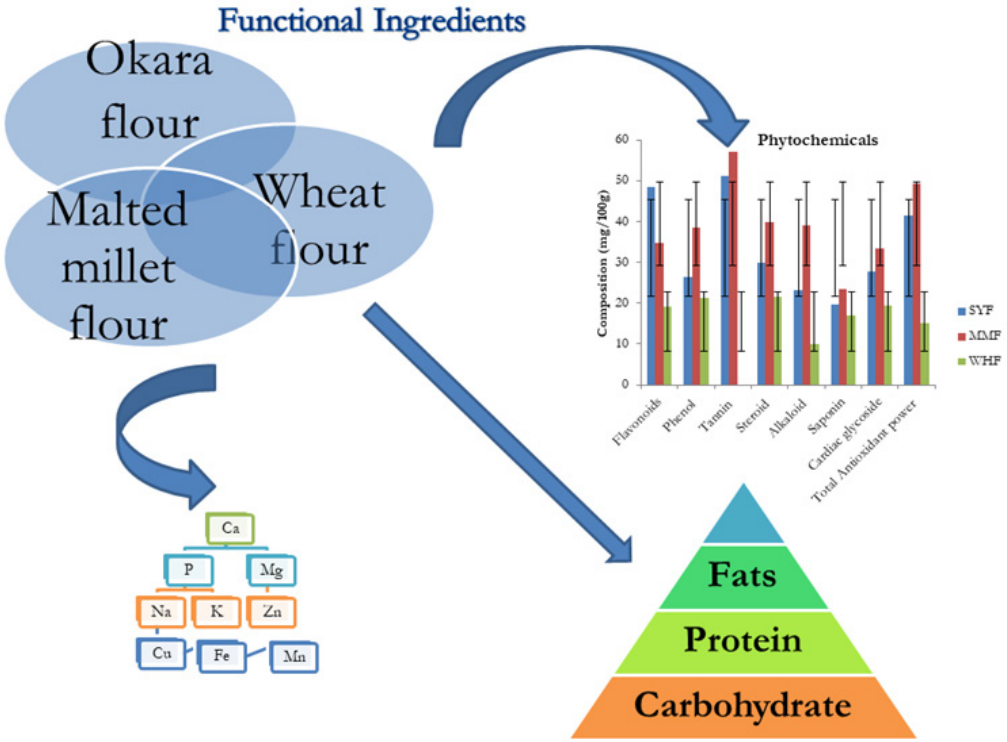

Highlights

- Functional ingredients are natural food components with health-promoting benefits.
- Nutritional composition of wheat, malted millet, and *okara* flour were evaluated
- Phytochemical screening of flour extracts revealed the presence of alkaloids, flavonoids, saponin, steroids, phenols, cardiac glycosides, and terpenoids.
- *Okara* contains significantly the highest dietary fiber than malted millet and wheat flours
- Total phenol and flavonoid contents were significantly higher in malted millet and *okara* flour, respectively

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NUTRITIONAL AND PHYTOCHEMICAL CHARACTERIZATION OF SELECTED FLOURS AS POTENTIAL FUNCTIONAL FOOD INGREDIENTS IN BAKING

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Functional food components are potentially beneficial components found naturally in foods or added to them as functional ingredients. This study sought to characterize the nutrients and phytochemical composition of soy residue "*okara*", malted millet, and wheat flour as natural sources of functional components. Chemical and phytochemical composition of the flours was carried out according to the standard analytical methods. Results showed that crude protein and fat were significantly higher ($p < 0.05$) in *okara* (26.83% and 19.00%) than malted millet (8.14 and 4.67%) and wheat flour (16.42% and 7.12%), respectively. A significant difference existed in the dietary fiber contents of the flours with '*okara'* exhibiting a substantial amount of soluble and insoluble dietary fiber ranging from 3.85 -15.34% and 5.54 – 19.46%, respectively. The mineral profile revealed *okara* as having an appreciable amount of calcium, phosphorus, and sodium than malted millet and wheat flour. The phytochemical screening of the flour extracts revealed the presence of alkaloids, flavonoids, saponin, steroids, phenols, cardiac glycosides, and terpenoids. *Okara* showed significantly ($p < 0.05$) highest flavonoid content than other flours while, on the other hand malted millet displayed significantly highest phenolic content. The study revealed that *okara*, malted millet and wheat flours are rich sources of both health-promoting minerals and phytochemicals, which possess nutritional and therapeutic benefits.

Keywords: *Okara*; Malted millet; Wheat flour; Phytochemical; Composition; Functional ingredient

INTRODUCTION

Functional ingredients are bioactive compounds that can be used in the development of functional food products. These bioactive compounds can be obtained from a variety of sources such as primary produce, marine sources, microorganisms and inorganic raw materials. Another significant source of bioactive compounds is from food processing waste such as in the hulls and fruit peels, and medicinal plants (Galanakis, 2021). Functional ingredients are natural food components that have health-promoting benefits and are added to foods for additional health benefits aside from basic nutritional functions. Naturally, plant foods contain functional food components that are also known as bioactive components in addition to those, which are traditionally considered macronutrients and micronutrients. These physiologically active compounds are referred to as 'phytochemicals'. Phytochemicals are biologically active naturally occurring chemical compounds found in plants, which provide health benefits for humans. Plant-based products such as fruits, vegetables, and whole grains contain high phytochemical contents that may act as natural antioxidants (Liu, 2004). They contribute to plant colours, aroma, and flavor. They are not essential nutrients and are not required by the human body for sustaining life, but have important health-promoting properties such as disease-preventing and or disease-fighting properties. These biologically active components are carotenoids, phenolic compounds (flavonoids, phytoestrogens, phenolic acids), phytosterols and phytostanols, tocotrienols, organosulfur compounds (allium compounds and glucosinolates), non-digestible carbohydrates (dietary fiber and prebiotics). They elicit their beneficial effects through their antioxidant properties (Adetuyi and Ibrahim, 2014; Kumar *et al.,* 2016). They are associated with the prevention of certain chronic diseases, including cardiovascular diseases (CVDs), cancer, diabetes, osteoporosis, vision diseases, constipation, and diverticulosis and diverticulitis, which have become intense in Western countries. Moreover, they help in lowering the risk of colon cancer, moderating post-prandial blood glucose responses resulting in improved insulin sensitivity, reduction of total and low-density lipoprotein (LDL)-cholesterol, regulating appetite, and enhancement of sodium and fluid balance. Hence, these phytochemicals could either be in the form of nutraceuticals or functional foods. Nutraceuticals are products isolated or purified from foods/ plants and generally sold in medicinal forms; on the other hand, functional foods represent conventional foods or dietary components with additional health-promoting benefits beyond basic nutrition.

The consumers' demand for healthy food products with the addition of various functional ingredients has significantly been on the increase. This has led to the development of

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an array of functional food products including functional baked products, dairy, nutritious beverages/juices, and meat products etc. as a way to battle with nutrient deficiency (Galanakis, 2021). Extensive studies have been documented on plant foods that have inherent biological components capable of health-promotion and disease-preventing properties (Rao, 2003; Xiao and Li, 2020).

Millet (*Pennisetum glaucum*) is an important drought-resistant crop among other major cereals such as rice, maize, etc. grown predominantly in the semi-arid tropics of Africa. Millets are unique cereal grains containing significant health-promoting components such as dietary fiber, resistant starch, minerals, vitamins, and phytochemicals that include phenolic compounds making it a good source of nutraceutical and functional food ingredients (Taylor and Duodu, 2015). Studies have shown that simple food processing such as malting of whole cereal grains could result in some biochemical modifications such as solubilization of carbohydrates and protein, the bioavailability of minerals, and reduction of the anti-nutrient contents such as phytates, tannin, etc. and consequently improve the nutritional quality of the grain. Traditionally, malted millet is utilized in infant food formulation, nutritious food blends, and also in nutritious beverage preparation (Najdi Hejazi *et al.,* 2016; Patel and Verma, 2015).

Soybean *(Glycine max*) and soy products are relatively cheap sources of proteins that are widely recognized for their substantial nutritional and excellent functional properties. 'Okara' is one of the by-products obtained during the processing of soybean; it is an insoluble residue obtained from tofu or soymilk processing that is either discarded or underutilized as animal feed or as agro-waste. Even though it was considered as having little market value, it is a very rich source of soy fiber and protein. Several reports have shown that soy fibers exhibit myriads' physiological benefits like the regulating effect on blood lipids, glucose metabolism, and nutrient absorption (Annison *et al.*, 1993). 'Okara' has a high nutritive value because of its high-quality protein, fat, carbohydrates, and fiber content (Wickramarathna and Arampath, 2003; Rinaldi *et al.,* 2000). According to Li *et al.* (2012), dietary fiber (42.4 - 58.1%) was found to be the predominant component of *okara* with a substantial amount of protein (15.2 - 33.4%) and fat (8.3 - 10.9%). Increased awareness of the significance of dietary fiber in the human diet has spurred research interests in '*okara*' utilization in foods, especially for high fiber foods and dietary supplement that has been linked to prebiotic activity, reduction of cholesterol levels, anti-inflammatory and anti-carcinogenic effects in the gastrointestinal tract, and increased fecal bulk (Tripathi and Shrivastava, 2017; Mateos-Aparicio *et al.,* 2010). Therefore, in recent times, dietary fiber is considered an important functional food ingredient in food products such as baked goods, beverages, meat, confectioneries, and pasta (Porcel *et al.,* 2017). Moreover, evidence-based research studies on the utilization of soy *okara* in bread and the nutritional properties of *okara* have also been reported with a focus on its amino acid profiles, isoflavones content, and antioxidant activities (Kumar *et al.,* 2016; Jimenez-Escrig *et al.,* 2009). Given this, the utilization of soy *okara,* and malted millet as functional food ingredients could be explored in the development of nutritious and functional food products for healthy nutrition. Therefore, this study sought to characterize the nutritional and phytochemical composition of soy residue *okara*, malted millet, and wheat flour.

MATERIALS AND METHODS

Source of raw materials

The Wheat Flour (*Triticum aestivum*) used was commercial baker's grade wheat flour milled by Nigeria Flour Mills (Golden Penny, Nigeria), Soy-bean; TGX 15 variety (*Glycine max),* millet (*Pennisetum glaucum*) were procured from a local market in Lagos. The chemicals and all organic reagents used in this study were procured from Sigma, Poole, UK and BDH Laboratory Supplies, UK, and they were of analytical grade.

Processing of malted millet flour

Malting of millet was carried out according to the procedure suggested by Adebiyi *et al. (*2016) with necessary modifications. Cleaned millet grains *(Pennisetum glaucum)* were steeped in tap water (1:3, w/v) for 24 h at room temperature (30-32^{o}C). After steeping, the grains were drained and spread on perforated trays lined with muslin cloth and malted at 34± 2^{o}C for 48 h. After 48 h, the sprouted grains were washed and then spread uniformly on a stainless steel tray and dried in a cabinet dryer (Mitchell Dryers Ltd. Denton Holmes Carlisle, CA2 SDU, England) at 60^{o}C for 5 h. The dried malts were devegetated to remove the vegetative parts(rootlets and shoots). Thereafter, the malted grains were milled in a disc attrition mill to obtain fine malted flour which was packaged in hermetically –sealed bags (Ziploc brand) and stored at ambient temperature for further analyses.

Preparation of soybean residue (*okara*) flour

The soybean seeds *(Glycine max)* were sorted, cleaned, and washed by floatation to remove all the foreign materials, damaged grain, and sand debris. The cleaned beans were blanched in hot water for 30 min at 100^{o}C and dehulled. The dehulled cotyledons were washed with hot (100^{o}C) water twice and wet-milled using 5l of water to 1kg of beans. The slurry obtained was mixed and filtered through a muslin cloth to remove the milk and recover the pulpy residue called *okara*. The fresh pulpy *okara* was dried using a cabinet dryer (Mitchell Dryers Ltd. Denton Holmes Carlisle, CA2 SDU, England) at a temperature of 70^{o}C for 12 h. The dried *okara* was milled in a disc attrition mill to obtain the flour and sieved through a 0.25 mm pore-sized sieve. *Okara* flour was then packaged in hermetically – sealed bags (Ziploc brand) and stored at ambient temperature for further analyses.

Preparation of Flour Extracts

The flour sample (15 g) was weighed into a beaker containing 200 ml of methanol, and was agitated in a mechanical shaker for 24 h. Thereafter, it was then filtered and evaporated to dryness under reduced pressure in a rotary

evaporator. The concentrated extract was re-dissolved with methanol to a concentration of 10 mg/ml and stored in the refrigerator until analysis. All the analyses were carried out in triplicates.

Determination of Proximate Composition

The proximate composition of the flour blends was determined using standard methods of AOAC (2010). The samples were analyzed for moisture, crude fiber, crude protein, ash, and crude fat, whereas carbohydrate (CHO) was calculated by difference.

Moisture content

The moisture content of the sample was determined by the oven-dry method as described by AOAC (2010). About 5 g of the sample was accurately weighed in a pre-weighed moisture pan and placed in a hot air oven and dry for 4 h at 105± 1°C. The dish with the sample was cooled in desiccator and weighed. This was repeated until a constant weight was obtained.

The moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{W2\text{-}W3}{W2\text{-}W1} \times 100$$

Where, W1-weight of empty Petri dish

W2 –weight of petri dish + sample

W3 – weight of petri dish + dried sample

Crude Protein Contents

Protein content was determined using the Kjeldahl method, according to the procedure of AOAC (2010). Concentrated H_2SO4 (12 mL) and two tablets of selenium catalyst were put into a Kjeldahl digestion flask containing 1 g of the sample. The flask was placed in the digester in a fume cupboard, switched on, and digested for 45 min to obtain a clear colorless solution. The digest was distilled with 4% boric acid, and 20% sodium hydroxide solution was automatically metered into it in the distillation equipment until the distillation was completed. The distillate was then titrated with 0.1 mol/L HCl until a violet color was formed, indicating the endpoint. A blank was run under the same condition as with the sample. The conversion factor is 6.25.

Total nitrogen content was then calculated using the equation

$$\text{Nitrogen (\%)} = \frac{(\text{Titre-Blank}) \times 14.008 \times \text{Nomality}}{\text{weight of sample}} \times 100\ \%$$

% Crude protein = % Nitrogen × 6.25

Crude fiber determination

The crude fiber was determined using the method described by AOAC (2010). The sample was treated with sulphuric acid to dissolve carbohydrates and subsequently with sodium hydroxide to separate fibrous matter which was filtered out and ignited.

About five (5) grams of sample were weighed into a 500ml Erlemeyer flask and 100ml of trichloroacetic acid (TCA) digestion reagent is added. It was then brought to boiling and refluxed for exactly 40 min. The flask was removed from the heater, cooled, and filtered through a 15.0cm No 4 Whatman paper. The residue was washed with hot distilled water and with methylated spirit. The filter paper with the sample was transferred into a porcelain crucible and dried in an oven overnight at 100˚C and weighed. The sample was cooled in desiccator, weighed, then ashed in a muffle furnace at 550°C for 4 h, and reweighed again after cooling.

$$\text{Crude fiber (\%)} = \frac{W1 - W2}{\text{Sample weight}} \times 100$$

Where, W2 = Weight of crucible + fiber + ash,

W1 = Weight of crucible + ash,

Crude Fat content determination

Crude fat was determined by the soxhlet method using the soxtec model apparatus as described by AOAC (2010). In the soxhlet method of fat estimation, lipids are extracted from the food by continuous extraction with petroleum ether. About 2 grams of the sample was weighed and wrapped up in a filter paper and was then placed in the extraction thimble. The extraction unit was cleaned, dried in an oven, and cooled in a desiccator before weighing. A 25 ml of petroleum ether was measured into the flask for the extraction of the fat. After extraction, the solvent was evaporated by drying in the oven. The flask and the content were then cooled in a desiccator and weighed. The fat content was calculated as follows:

$$\text{Fat content (\%)} = \frac{W3\text{-}W2}{W1} \times 100$$

Where, W1- weight of the sample (g)

W2- weight of empty cup (g)

W3- weight of the cup + extracted oil (g)

Ash content determination

Five grams of dried sample were taken in a weighed silica crucible in triplicate. The crucible was placed on a burner and heated till the material was completely charred. Then it was placed in the muffle furnace (Gallenkamp, SG93/11/888) and heated to 550° - 600° C for 3 h until a light grey ash is observed and constant weight was obtained. The crucible was transferred to a desiccator to cool it to room temperature and the weight of the crucible was recorded. The ash content of the sample was estimated using the following equation.

$$\text{Ash (\%)} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where, W1- weight of the empty crucible (g),

W2- weight of the crucible + food before ashing (g)

W3- weight of crucible + ash (g)

Determination of Dietary fiber

The samples were analyzed for soluble and insoluble dietary fiber fractions according to AOAC method 999.43, an enzymatic gravimetric procedure. Samples containing a high level of sugar were extracted with 85% ethanol to remove most of the sugar. Residues were suspended in MES-TRIS buffer and digested sequentially with heat-stable –amylase at 95-100°C, protease at 60°C and amyloglucosidase at 60°C. Enzymes digested were filtered through fritted glass crucibles. Crucibles containing insoluble dietary fiber were rinsed with dilute alcohol

followed by acetone and dried overnight in a 105°C. The filtrate was mixed with four times volume of 95% ethanol to precipitate material that was soluble in the digest. After 1 hour, the precipitates were filtered through tarred fritted glass crucibles. One of each set of duplicate fiber residues and soluble fiber residue was ashed in a muffle furnace at 525°C for 5 h. Total dietary fiber was calculated as the sum of soluble and insoluble dietary fiber.

Mineral analysis

Calcium, zinc, magnesium, iron, copper, and manganese of the test samples were determined by the AOAC (2010) method using an Atomic Absorption Spectrophotometer (AAS) (PYE Unicon, UK, model SP9). The potassium and sodium contents were determined by flame photometer while phosphorus was determined using the colorimetric method using phosphorvanadomolybdate as described by AOAC (2010). All the determinations were carried out in triplicates.

Phytochemical screening of the flour extracts

The preliminary test for the presence of alkaloids, flavonoids, saponins, tannins, steroids, and cardiac glycosides was carried out according to standard methods.

Test for alkaloid: Extracts were dissolved in dilute hydrochloric acid (27%) and filtered. Wagner's Test: filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown/ reddish precipitate indicated the presence of alkaloids (Joshi *et al.,* 2013).

Test for Flavonoids: Five (5) ml of dilute ammonia solution was added to 5 ml of the extract solutions followed by the addition of concentrated H_2SO4. A yellow coloration observed in each extract indicated the presence of flavonoids (Adeoye *et al.,* 2018).

Test for Saponins: Froth test: Five (5) ml of the extract solution was shaken vigorously for a stable persistent froth. The frothing was mixed with olive oil and shaken vigorously. The formation of emulsion indicated the presence of saponins in the samples (Adeoye *et al.,* 2018).

Test for tannins: Ferric chloride test: Extracts (1g) were dissolved in 20 ml of distilled water and filtered. Three drops of 10% of ferric chloride were added to the extract solutions and observed brownish-green or a blue-black coloration, which signified the presence of tannins (Magadula and Tewtrakul, 2010).

Test for steroids: A 5 ml sample of the extract was added to 2 ml acetic anhydride and 2 ml H_2SO4. The colour changed from violet to blue or green indicating the presence of steroids (Animesh and Dipankar, 2017).

Test for terpenoids: Salkowski test: A 5 ml sample of the extract was mixed in 2 ml of chloroform, and 3 ml concentrated H_2SO4 was carefully added along the sides of the test tube to form a layer. The formation of a reddish-brown coloration at the interface indicated the presence of terpenoids (Animesh and Dipankar, 2017).

Test for Cardiac glycoside (Keller-Killiani test)

About 0.5 g extract was shaken with distilled water (5 ml) and glacial acetic acid (2 ml) containing a few drops of ferric chloride was added, followed by H_2SO4 (1 ml) along the side of the test tube. The formation of a brown ring at the interface gives a positive indication of cardiac glycoside and a violet ring may appear below the brown ring (Joshi *et al.,* 2013).

Quantitative Estimation of Alkaloids

The alkaloid content of the samples was determined according to the procedure described by Abdullahi *et al*. (2020). A 5 g of the sample extracts were taken into a 250 ml beaker, and 200 ml of 10% acetic acid in ethanol was added, covered, and allowed to stand for 4 hr. The mixture was filtered and then concentrated over a water bath until ¼ of the initial volume was obtained. Thereafter, a few drops of concentrated HN_4OH were added to the extract until a complete precipitate was formed. The whole mixture was allowed to settle and the precipitate was collected and washed with dilute HN_4OH and then filtered. The residue produced was dried and weighed as an alkaloid.

Quantitative Estimation of Saponins

The saponins contents of the sample extracts were determined according to the procedure described by Obazelu *et al.* (2021). 5ml of the test extract was dissolved in a solution of methanol/ water in a ratio of 1:1. They were further dissolved in 80% methanol and 2ml ethanol was added, properly mixed, and heated in a water bath at 60°C to warm gently for 10 min. The solutions were filtered and the absorbance was measured at 544 nm against a reagent blank using a UV spectrophotometer.

Quantitative Estimation of Steroids

1g of flour extracts in a 10 ml volumetric flask was mixed with 2 ml of 4N H_2SO4 and 2 ml of 0.5% iron (III) chloride was added. Thereafter, 0.5ml of 0.5% potassium hexacyanoferrate (III) solution was added, and the resultant mixture was heated in a water bath maintained at a temperature of 70°C for 30 min with occasional shaking. Thereafter, they were filtered and the absorbance was measured at 780 nm using a UV spectrophotometer against the reagent blank (Obazelu *et al.*, 2021).

Quantitative Estimation of Total Cardiac Glycosides

In this test, 10% of extract samples were mixed with 10 mL of freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). The mixture was allowed to stand for 1 h. This was followed by dilution with 20 mL of distilled water and the absorbance was measured at 495 nm using a UV spectrophotometer (Obazelu *et al*., 2021).

Quantitative Estimation of Total Tannins

5 g of the extract samples were weighed, and about 50 ml of distilled water was added and shaken for 1 h and filtered into a volumetric flask and made up to the mark. Subsequently, 5 ml of the filtrate was pipetted into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance of the mixture was measured at 120 nm within 10 min (Obazelu *et al.,* 2021).

Determination of total phenolic content

The concentration of total phenol content was determined by Folin-Ciocalteau colorimetric method (McDonald *et al.,* 2001) using gallic acid as a standard following the method described by Slinkard and Singleton (1977). A 0.5 ml sample of extract and 0.1 ml of Folin- Ciocalteu reagent (0.5 N) were mixed and incubated for 15 min. Thereafter, 2.5 ml sodium carbonate solution (7.5% w/v) was added and further incubated for 30 min at room temperature. The absorbance of the solution was measured on a UV- visible spectrophotometer (Milton Roy Spectronic 601, USA) at 760 nm. The result was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

Determination of total flavonoid contents

The total soluble flavonoid of the extract was determined using the aluminum chloride colorimetric method using ascorbic acid as the standard (Chang *et al.,* 2002). One milliliter of sample solution (100µg/ ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 min and the absorbance of the reaction mixture was measured on a UV- visible spectrophotometer (Milton Roy Spectronic 601, USA) at 415 nm. The calibration curve was prepared by preparing ascorbic acid solutions at various concentrations in methanol.

2.11. Statistical Analysis

Analyses were performed in triplicates. The data were statistically evaluated using analysis of variance (ANOVA) using a statistical package for social sciences (IBM SPSS Statistics Version 20) and mean values were separated using Duncan's Multiple Range Test (DMRT) with a significance level of $p < 0.05$. Correlation analysis was obtained using bivariate correlations and expressed as Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

Chemical composition of the functional flour samples

The chemical composition of *okara*, malted millet, and wheat flours is presented in Table 1. There was a significant difference ($p < 0.05$) in the moisture content of the flour samples. It ranged between 9.35 and 8.72% with the highest moisture content observed in wheat flour and the least value in *okara.* The moisture content obtained in malted millet flour in this study is higher than that reported by Adebiyi *et al.* (2017). However, the results obtained by all the flours are lower than the minimum value of 10/100g moisture content for flour (Ihekoronye and Ngoddy, 1985). This is an indication that the flours will have a longer storage time. The ash contents of the flour samples were 0.95, 1.05, and 1.14%, respectively with the highest value obtained in wheat flour, followed by malted millet flour and *okara* flour having the least value. Crude protein and crude fat were significantly higher ($p < 0.05$) in *okara* than in malted millet and wheat flour. Proteins are considered essential biologically active compounds needed for body-building and the repair of worn-out tissues. The crude fat content of *okara* observed in this study is higher than the value reported by Wickramarathna and Arampath (2003). The crude fiber contents obtained were 3.43, 2.54 and 2.49% with the highest value obtained in *okara* flour, followed by malted millet flour while wheat flour had the least value. The carbohydrate contents revealed that malted millet flour had a significantly highest level, followed by wheat flour, and the least value was obtained in *okara* flour. It was also observed that *okara* exhibited significantly highest ($p < 0.05$) soluble, insoluble and total dietary fiber contents than other flour samples. Soluble dietary fiber ranged between 15.34 and 3.85% with the highest obtained in *okara*, followed by malted millet flour and wheat flour having the least value. However, the highest insoluble dietary fiber was obtained in *okara* flour, followed by wheat flour, and the least value was obtained in malted millet flour. The total dietary fiber contents of the flour samples varied significantly and it ranged between 34.80 and 9.82% with *okara* flour having the highest and malted millet flour recording the lowest. The high concentration of crude fiber in *okara* could be attributed to its insoluble dietary fiber equally observed. Insoluble dietary fiber cannot be dissolved in water unlike soluble dietary fiber (Wardlaw and Hampl, 2007). Several studies have demonstrated that the consumption of soluble dietary fiber can significantly reduce blood cholesterol and help stabilize blood glucose levels while the consumption of insoluble dietary fiber helps to protect against colon cancer and other bowel disorders of the intestinal tract (O'Shea *et al.,* 2012; Peressini and Sensidoni, 2009). To this end, the Academy of Nutrition and Dietetics recommends daily consumption of 38 and 25 g of fiber by adult men and women respectively (Dahl *et al.,* 2015). However, reports have shown that consumers had not met the required daily amount of dietary fiber; therefore, the need to complement this shortfall is of utmost public health concern. The study revealed that *okara* flour consumption could contribute significantly to the recommended daily intake of dietary fiber.

Minerals Composition

The mineral composition of the flour samples is presented in Table 2. The minerals investigated were calcium, magnesium, zinc, manganese, copper, phosphorus, iron, sodium, and potassium. The results show that calcium contents were 53.50 mg/100g, 19.56 mg/100g, and 27.13 mg/100g in *okara*, malted millet, and wheat flour respectively. The calcium content of soy *okara* was significantly ($p < 0.05$) higher than the malted millet and wheat flour. Similarly, *okara* flour contained significantly ($p < 0.05$) higher magnesium content than malted millet and wheat flour. The study also revealed that *okara* flour contained a significantly ($p < 0.05$) higher phosphorus content of 37.85mg/100g, followed by wheat flour containing 27.73 mg/100g and malted millet flour of 26.66 mg/100g, though there was no significant difference between the malted millet flour and wheat flour. Conversely, wheat flour displayed significantly ($p < 0.05$) highest iron content (2.23 mg/100g) than *okara* (1.52±0.01 mg/100g) and malted millet flour (0.94 mg/100g). The highest content might be attributed to the fact that wheat

flour had undergone refinement and fortification of certain micronutrients such as iron. Iron is an essential micronutrient in the formation of hemoglobin, oxygen, and electron transport in the human body (Kalagbor and Diri, 2014). It is needed for a wide range of metabolic activities in man and is essential for growth. Zinc content was found to show wide variations of 15.94, 4.73, and 3.52 mg/100g in *okara,* malted millet, and wheat flour, respectively showing significantly ($p < 0.05$) highest value in *okara* and least value in wheat flour. This observed higher content in *okara* flour could be a premise for its usage in combination with other flours that are limiting in zinc for the development of complementary foods for infants and possibly be employed in food formulation for school feeding programs. This is because it has been reported that zinc deficiency is considered a risk factor for disease infection and has been associated with severe malnutrition, increased disease conditions, and mental impairment (Wardlaw 2004; Mannay and Shadaksharaswany, 2005). Zinc is an essential component of a large number of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It plays an important role in cell division, protein synthesis, and the growth of humans. It is an essential mineral critical for infants, children, adolescents, and pregnant women (Ackland and Michalczyk, 2016). Sodium contents differed significantly ($p < 0.05$) among the functional ingredients with *okara* flour having the highest value of 19.96 mg/100g, followed by malted millet flour and wheat flour having a significantly lowest value of 1.10 mg/100g. Sodium is the principal cation in extracellular fluid in the body and is an essential nutrient necessary for the maintenance of plasma volume, acid-base balance, the transmission of nerve impulses, and normal cell function (WHO, 2012). Similarly, significant differences ($p < 0.05$) were observed in the potassium contents of the functional ingredients; *Okara* flour displayed the highest potassium content (2.92 mg/100g), followed by malted millet flour (1.21mg/100g) and wheat flour (0.58 mg/100g). This result aligns with the findings of Sladjana *et al.* (2014). Potassium is required to maintain the osmotic balance of the body fluids, and the pH of the body, regulate muscle and nerve irritability, control glucose absorption, and enhance the normal retention of protein during growth (Omoba *et al.,* 2013). Magnesium works as a cofactor (critical to Vitamin D activity) to regulate protein synthesis, muscle and nerve function, blood glucose regulation, and blood pressure regulation. It also plays a role in bone development and in the transport of calcium and potassium ions across cells, which is important for nerve impulse conduction, muscle contraction, and normal heart rhythm thus reducing the risk of a heart attack (Mathew and Panonnummal, 2021). Phosphorus is another essential macronutrient required for proper bone and teeth growth and development, as part of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), which are critical for genetic framework, protein synthesis and helps in energy metabolism (Yellavila *et al.,* 2015). Manganese content was found to display wide variations of 0.84, 0.48, and 0.25 mg/100g in *okara*, malted millet, and wheat flour respectively showing significant differences ($p < 0.05$) between the functional ingredients. Manganese (Mn) is also an important trace element that functions as an activator of several enzymes which protect cells from free radicals attack, regulation of glucose homeostasis, and also function in bone growth and development and the formation of prothrombin with vitamin K (Samsel and Seneff, 2015). The results from the mineral analysis showed that the flours would contribute substantially to the recommended dietary requirements for minerals.

Qualitative and quantitative screening of phytochemical constituents

Table 3 depicts the results of qualitative phytochemical screening of the methanolic extracts of *okara*, malted millet, and wheat flour. The results revealed the presence of alkaloids, flavonoids, saponin, tannin, steroids, phenols, cardiac glycosides, and terpenoids except in wheat flour tannin was absent.

Figure 1 shows the phytochemical composition of the methanolic extracts of functional flour samples. Significant ($p < 0.05$) differences existed in the phytochemical composition of the samples. The flavonoid content of the flour extracts ranged between 48.42 and 19.02 mg/100g. Results indicated that *okara* flour had a significantly ($p < 0.05$) highest flavonoid content, followed by malted millet flour, and wheat flour had the least value. Malted millet flour showed a significantly ($p < 0.05$) highest phenol content than *okara* flour and wheat flour had the least value. Malted millet flour exhibited higher tannin content compared to *okara* while it was not detected in wheat flour extract. Results indicated that malted millet flour had significant ($p < 0.05$) phenol and tannin content than *okara* and wheat flour. This is confirmed by the report of Rao and Muralikrishna, (2002) who documented that the main polyphenols present in cereals are phenolic acid and tannins while flavonoids are present in small quantities. The result showed that malted millet flour contained significantly highest measures of steroid, alkaloids, saponin, and cardiac glycoside which ranged from 39.73 to 21.50 mg/100g, 39.00 to 10.02 mg/100g, 23.56 to 17.00 mg/100g and 33.36- 19.32 mg/100g, respectively. Alkaloids are a class of naturally occurring organic compounds that have many health-promoting properties such as antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic potentials. They elicit these properties by scavenging free radicals or binding with catalysts of the oxidative reactions and as such prevent the initiation of some degenerative diseases (Kaur and Arora, 2015). Cardiac glycosides are a class of organic compounds known as steroids. They are one of the abundant phytochemicals that are naturally found in plants with numerous health-promoting effects (Firn, 2010). This type of steroid is useful in treating and resuscitating patients suffering from cardiac arrest and rhythm disorders as well as being a powerful destroying agent against growing tumor cells. Vladimir and Ludmila (2001) also documented that they help to increase the output force of the heart and increase its rate of contraction by acting on the cellular sodium-potassium ATPase pump. Terpenoids are the most numerous products found in many plants and several studies confirmed that they exhibit potent analgesic, and antibiotic effects as well

Table 1: Chemical composition (%) of selected functional flours: "*okara"*, malted millet and wheat flour.

Sample	Moisture Content	Ash	Crude protein	Crude fat	Crude fiber	CHO	IDF	SDF	TDF
SYF	8.72±0.06^{c}	3.95±0.01^{c}	26.83±0.42^{a}	10.20±0.95^{a}	5.43±0.28^{a}	44.87±0.72^{c}	19.46±0.23^{a}	15.34±0.08^{a}	34.80±0.31^{a}
MMF	9.16±0.08^{ab}	2.05±0.03^{b}	8.14±0.16^{c}	2.67±0.28^{b}	3.54±0.23^{b}	74.44±0.53^{a}	5.54±0.10^{c}	4.28±0.10^{b}	9.82±0.16^{c}
WHF	9.35±0.03^{a}	1.14±0.01^{a}	12.42±0.06^{b}	2.10±0.03^{c}	2.48±0.03^{c}	72.51±0.06^{b}	4.61±0.09^{b}	3.85±0.12^{c}	8.46±0.16^{b}

Values are means of triplicate determination. Means in the same column with different superscripts differed significantly ($p<0.05$).
SYF- *Okara* flour, MMF- Malted millet flour, WHF- Wheat flour
CHO- Carbohydrate, IDF- Insoluble dietary fiber, SDF- Soluble dietary fiber, TDF- Total dietary fiber

Table 2: Mineral Composition (mg/100g) of selected functional flours *okara,* malted millet and wheat.

Minerals	SYF	MMF	WHF
Calcium (Ca)	53.50±0.08^{a}	19.56±0.03^{c}	27.13±0.03^{b}
Magnesium(Mg)	22.40±0.02^{a}	12.20±0.01^{b}	4.60±0.02^{c}
Zinc (Zn)	15.94±0.11^{a}	4.73±0.04^{b}	3.52±0.03^{c}
Manganese (Mn)	0.84±0.00^{a}	0.48±0.00^{b}	0.25±0.00^{c}
Copper (Cu)	0.16±0.00^{a}	0.14±0.00^{a}	0.11±0.00^{b}
Phosphorus(P)	37.85±0.05^{a}	26.66±0.06^{b}	27.73±0.02^{b}
Iron (Fe)	1.52±0.01^{b}	0.94±0.02^{c}	2.23±0.04^{a}
Sodium (Na)	19.95±0.00^{a}	10.74±0.00^{b}	1.10±0.20^{c}
Potassium (K)	2.92±0.00^{a}	1.21±0.01^{b}	0.58±0.01^{c}

Values are means of triplicate determination. Means in the same row with different superscripts differed significantly ($p<0.05$).
SYF- *Okara* flour, MMF- Malted millet flour, WHF- Wheat flour

Table 3: Qualitative phytochemical Screening of methanolic extract of selected flours.

PHYTOCHEMICALS	SYF	MMF	WHF
Flavonoids	+	+	+
Phenol	+	+	+
Tannin	+	+	-
Steroid	+	+	+
Alkaloids	+	+	+
Saponin	+	+	+
Terpenoids	+	+	+
Cardiac glycoside	+	+	+

+ denotes present – denotes absent
SYF--- *Okara* flour, MMF- Malted millet flour, WHF- Wheat flour

as anti-inflammatory effects (Ludwiczuk and Georgiev, 2017). Saponins play an important therapeutic role in hypolipidemic, antioxidant, and anticancer activities. They also work in synergy with cardiac glycosides in the treatment of heart failure and cardiac rhythm disorders. In the same vein, the highest total antioxidant power was observed in malted millet flour, followed by okara and wheat flour having a significantly ($p < 0.05$) lowest value. Flavonoids have been shown to possess myriads of health benefits such as the ability to act as antioxidants as they help in scavenging free radicals, inhibiting inflammation and tumor growth; helps to boost immunity and production of detoxifying enzymes in the body (Ghasemzadeh *et al.,* 2011). Flavonoids and tannins are important polyphenol compounds because of their significant anticancer activity. According to Nicoletti, *et al.* (2013), phenolic compounds have health-promoting benefits as they prevent and reduce the risk of chronic diseases such as cardiovascular diseases, diabetes, and cancers and this has been strongly associated with their antioxidant properties. They are also radical scavengers by exhibiting inhibitory effects on mutagenesis and carcinogenesis of various neurodegenerative diseases and cancers in humans (Tanaka *et al.,* 1998). The results of this study offer useful data on selected functional flours' potential in functional food product development.

Pearson correlation matrix of phytochemical and mineral element composition is shown in Table 4. Correlation analysis was obtained using bivariate correlation in which the correlation coefficient obtained indicates a higher, positive correlation between the flavonoid contents and all the mineral elements except iron which is negatively correlated ($r = - 0.58$). However, only Copper (Cu) and sodium (Na) showed strong, positive, and significant ($p < 0.05$) correlations with total flavonoid content having $r = 0.997$ and 0.999 respectively, indicating that the flour rich in total flavonoids also contain a higher level of sodium and copper. Results also showed a strong positive correlation between alkaloids and total phenolic contents ($r = 0.98$), indicating that higher alkaloids level is associated with increased total phenolic content. Gan *et al.,* (2017) who established a positive correlation between alkaloids and total phenolic contents, corroborated this finding. Tannin also exhibited a strong positive correlation with the total flavonoid and phenolic content of the flour sample ($r = 0.84$ and $r = 0.79$) respectively. This was in line with the findings of Zakaria Khiya *et al., (*2021) who reported a strong correlation between total phenolic content and total condensed tannin in *Salvia officinalis* leaves. Generally, the correlation coefficient obtained between the total phenolic content and the minerals was weak except for iron ($r = -0.96$), which exhibited a strong negative correlation, implying that a low level of iron in flour samples is associated with higher total phenolic content of the flour samples, which might be linked to contributing to their antioxidant property. These positive correlations suggest that the phytochemicals and mineral elements of the flour samples could contribute to their health-promoting effects and antioxidant property.

CONCLUSIONS

The outcome of the study displayed nutritional and phytochemical profiles of *okara*, malted millet and wheat flours and showed that the flours are good sources of proximate compositions and mineral elements that are highly beneficial to the normal functioning and well-being of human beings. The phytochemical screening of malted millet, *okara*, and wheat flour revealed the presence of flavonoids, phenols, alkaloids, steroids, saponin, cardiac glycoside, and tannin (except wheat flour). In addition,

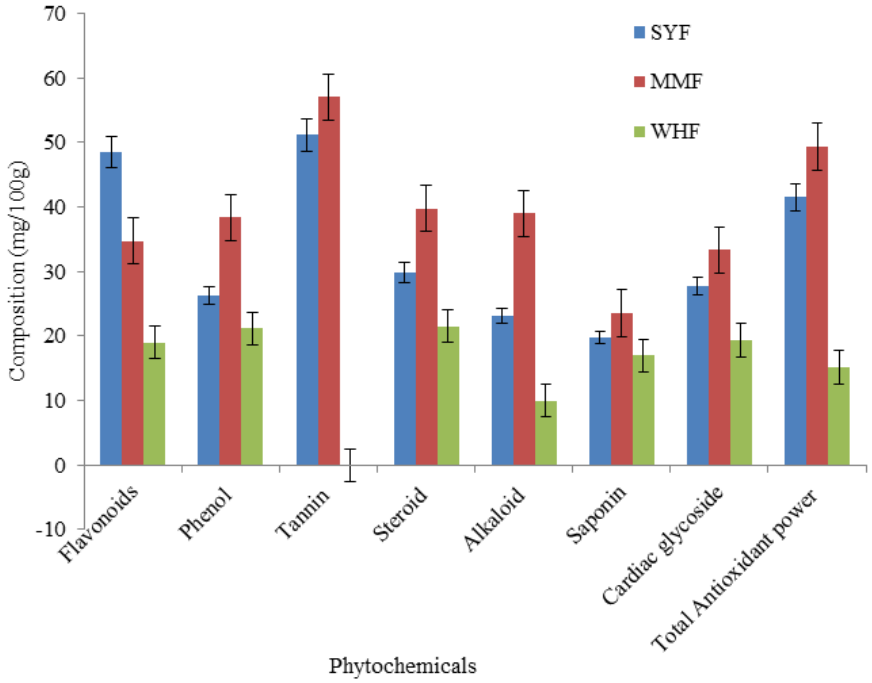

Figure 1: Phytochemical composition (mg/100g) of methanolic flour extracts. Bars are mean values ± SD (n=3).

Table 4: Pearson Correlation Matrix of phytochemical constituent with mineral elements of the functional flour.

	TFC	*TPC*	*Tannin*	*Alkaloids*	*Ca*	*Mg*	*Zn*	*Mn*	*P*	*Cu*	*Fe*	*Na*	*K*
TFC	1												
TPC	0.32595	1											
Tannin	0.838103	0.788899	1										
Alkaloids	0.489827	0.983866	0.886113	1									
Ca	0.712797	-0.43073	0.214792	-0.26232	1								
Mg	0.992349	0.206735	0.76434	0.378441	0.793937	1							
Zn	0.88939	-0.14228	0.496022	0.037097	0.954585	0.939027	1						
Mn	0.986224	0.165077	0.736321	0.338866	0.818995	0.999101	0.952758	1					
P	0.796086	-0.31265	0.337068	-0.13767	0.991905	0.864714	0.98469	0.885226	1				
Cu	0.997165*	0.396159	0.876773	0.554037	0.658003	0.980246	0.852472	0.970982	0.748294	1			
Fe	-0.57639	-0.96042	-0.92885	-0.99476	0.162295	-0.47109	-0.13906	-0.43327	0.035686	-0.63624	1		
Na	0.999657*	0.301094	0.823538	0.46684	0.73091	0.995241	0.901051	0.990216	0.811654	0.994854	-0.5548	1	
K	0.954908	0.030565	0.638348	0.208894	0.888894	0.984259	0.985014	0.990865	0.939869	0.929861	-0.30778	0.962352	1

*Correlation is significant at the 0.05 level (2 tailed).

okara and malted millet flours were able to deliver a substantial amount of bioactive components: phenols, flavonoids, and dietary fibers with high total antioxidant power. In the light of scientific data obtained from this investigation, *okara* and malted millet flours could be considered as potential health-promoting food ingredients and could be used as an option for applications either as composites or singly in functional food development and dietary supplement to suffice the emerging healthy food choices.

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DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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