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Micronutrient and Antinutrient Content of some Nigerian Edible flours: promoting healthy dietary alternatives

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Abstract
Nigerians eat predominantly high-starch meals however, due to the association between these and non-communicable diseases, dietary alternatives have been proposed. Research has linked the consumption of whole grain cereal products with reduced incidence of chronic diseases, such as diabetes, cardiovascular disease, and certain cancers. Although cereal products are rich in minerals and vitamins, their bioavailability may be limited by their antinutrient content. This study aimed at determining the micronutrient and antinutrient content of some flours made from some cereals - finger millet (*Eleusine coracana*), black fonio (*Digitaria iburua*), and rye (*Secale cereale*) and also cassava (*Manihot esculenta*). The cereal samples were purchased from local markets, sorted, washed, dried and prepared for analyses; the cassava flour was purchased as a finished product. The determination of macro and micro minerals, vitamins and anti-nutrients, was carried out after sample digestion with the use of Atomic Absorption Spectrophotometry. Laboratory results were analysed using Microsoft Excel and Statistical Package for Social Sciences (SPSS). The results show that rye flour had significantly ($p<0.05$) higher content of all the 8 minerals analysed, except calcium. The flours had considerable amounts of all the vitamins analysed, with finger millet having significantly ($p<0.05$) higher content of vitamin A (3.84mg/100g), vitamin E (1.91±0.01mg/100g), vitamin K (4.17±0.01mg/100g) and the B vitamins. Rye flour also had a significantly higher content of antinutrients – oxalate, phytate, lecithin, tannin and phenols though the values were all within safe limits. All the flours studied have significant content of essential micronutrients which are even more than in the popularly consumed starchy cassava flour. Hence, the use of these cereal alternatives in making swallows should be encouraged.

Keywords: micronutrients, antinutrients, diet, alternatives, and cereals

Introduction

Non-communicable diseases (NCDs) have been reported to be responsible for about 70% of deaths worldwide and these can be modified with nutritional interventions (Noorfarahzilah *et al*., 2014). Influencing public nutrition is a key task in the global strategy for the prevention of chronic diseases. Due to the nutrition transition as a result of globalization, there is a need for an improved healthy diet. The formulation of single flours and sometimes composite flours from high-fiber cereal grains and low glycemic index foods such as wheat, sorghum, and oats, has been proven beneficial for the prevention and management of diabetes and hypertension (Noorfarahzilah *et al*., 2014).

The past decade has seen tremendous advancement in understanding the role of nutrition in health promotion and disease prevention. The relationship between dietary intake and chronic diseases (such as diabetes and obesity) has come under scrutiny (Venkat-Narayan *et al*., 2006). In Nigeria, NCDs are estimated to account for 24% of total deaths and the probability of dying between the ages of 30 and 70 years from the four main NCDs (cancer, diabetes, cardiovascular diseases, and chronic respiratory disease) is 20% (World Health Organization, 2014). In some African countries such as Namibia and Mauritius, NCDs cause over 50% of all reported adult deaths, implying that NCDs will soon be a leading cause of ill health, disability and premature death in the Region (World Health Organization, 2020.). With the increase in the incidence rates of diet-related NCDs, there has been an emphasis on the prevention/management of diseases using dietary intervention (Venkat-Narayan *et al*., 2006).

It has become necessary to explore other less-popular dietary alternatives in a bid to proffer solutions to the various diet-related diseases. Some of these cereals are more common in the middle-belt region of the country Nigeria. They are usually transported for commercial purposes to other parts of the country where they are used as food either for children or adults. Flour derived from the cereals in this study - finger millet (*Eleusine coracana*), black fonio (*Digitaria iburua*), and rye (*Secale cereale*), form the staple diets of the indigenes of these regions. They also hold an indispensable role in the diets of some of the indigenes of the areas where they are predominant. These cereals serve beyond mere sustenance, and provide essential ingredients used in a variety of culinary traditions, from the preparation of bread to the creation of wholesome porridges and the thickening of stews.

On the other hand, cassava (*Manihot esculenta)* has emerged over the years as a dominant staple of primary or secondary importance in many developing countries of the humid and sub-humid tropics in Africa and beyond. Since it can withstand drought, it is sometimes a nutritionally strategic famine reserve crop in areas of unreliable rainfall especially in Nigeria. Cassava is a starchy staple whose roots are very rich in carbohydrates, a major source of energy. Cassava flour is used for the production of foods such as noodles, breakfast cereals, cookies, breads, cakes, pastries, muffins and doughnuts (Akinlonu, 2011). Cassava flour has a similar chemical composition as wheat flour except for protein (2.08% vs 14.78%) and crude fibres (2.24% vs 0.51%), Ciacco and D'Appolonia (2018) indicated that 5 to 15% of the cassava flour may be substituted for wheat flour without serious detrimental effect on bread quality. Although cassava roots are rich in calories, they are grossly deficient in proteins, fat, and some of the minerals and vitamins. Consequently, cassava flour appears to be of lower nutritional value than cereals, legumes, and even some other root and tuber crops, such as yams (Latham, 2019).

Nutrition is further compounded by the presence of anti-nutritional factors in foods. These compounds, such as phytates, tannins, and oxalates, can interfere with nutrient absorption, potentially contributing to instances of malnutrition (Nkafamiya and Igbabul, 2018). Notably, while essential to the composition of edible flours, these compounds paradoxically may impede the bioavailability of crucial nutrients (when the antinutrients are present in high concentrations), prompting concerns about the nutritional adequacy of some cereal flours. However, many strategies have been employed to combat the problem of malnutrition and also food insecurity. These strategies are either long-term or short-term and are usually designed to enhance the energy and nutrient density of cereal-based flours, increase the production and consumption of micronutrient-dense foods, incorporate enhancers of micronutrient absorption, and reduce antinutrient (especially phytate) content of cereals.

Consequently, this research sought to determine the micronutrient (macro and microelements), vitamin (fat soluble and water soluble) and antinutrient content of some unpopular cereal flours and cassava flour in Nigeria.

Materials and Methods

Ethics

For this study, a waiver was obtained from the Faculty Animal Research Ethics Committee (FAREC-FBMS), Faculty of Basic Medical Sciences, University of Calabar, Cross River State on the 7th of July, 2023.

Collection of Samples

The four samples were purchased from three different locations in Nigeria: cassava flour (Calabar), rye (Jos), black fonio, and finger millet (Benue). All samples were conveyed to the Department of Human Nutrition and Dietetics, University of Calabar, Calabar for sample preparation. The cereal samples (finger millet, black fonio and rye) were selected, washed using clean water and dried under the sun for three days. The sun-dried grains were grinded into a fine powder using a miller (Retsh ZM 200 miller, Germany). The purchased cassava flour was also opened and put in a clean ziplock bag. The flours were placed and sealed in labelled Ziploc bags and sent to the laboratory for analysis.

Digestion of clay samples

The samples were digested according to Hu (2014). Accurately 5g of clay samples were weighed into a 250 cm^3 conical flask and moistened with a few drops of water to prevent sputtering; then 3cm^3 of 30% H_2O_2 was added and left to stand for 60 min until the vigorous reaction ceased. About 75 cm^3 of 0.5mol/dm^3 solution of HCl was added and the content was heated gently at low heat on the hot plate for 2 hr. The digest was allowed to cool and then filtered into a 50 cm^3 standard flask. The content was then diluted to a 50 cm^3 mark with the same acid solution. Triplicate digestions of each sample together with a blank were carried out.

Experimental Procedure

-Determination of Micronutrients - Minerals and Vitamins

A portion of the sample (0.5g) was weighed into a 500ml volumetric flask, 15ml HNO_3, and 5ml of 70% Perchloric acid ($HCIO_4$) wasadded to the flask. The flask was placed on a hot plate in the fume cupboard to digest for clarity. The solution was transferred to a 100ml volumetric flask and diluted to volume with water. Aliquots of dilutions of this sample were then aspirated into the air-acetylene flame of the WFX 320 (AAS) Atomic Absorption spectrometer to determine the elements. The elements were conventionally reported through graphic extrapolations with standard pure metals. The macro minerals Mg, Ca, Na, K and micro minerals Fe, Zn, Mn, and Se, were determined using the standard methods of AOAC International (2010). The AOAC (2010) method using the colourimeter was adopted for the determination of vitamin A. AAS was also used to determine vitamins D, E and K by taking absorbance readings at the appropriate wavelengths.

Anti-nutrient determination

Standard laboratory methods were used to determine the five antinutrients analysed in this study. To about 1g of the sample was added 75ml of 1.5N H_2SO_4 and the solution was carefully stirred using a magnetic stirrer for 1 hour before being filtered using What-man No. II filter paper; 25ml of the extract was collected and titrated when hot against 0.1N $KMnO_4$ solution to a faint pink colour end point.

Oxalate = (titre value x 0.9004) mg/g

Phenol contents in the extracts were determined by the modified Folin-Ciocalteu method as described by Kupina *et al.* (2018). An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VS spectrophotometer.

Tannin content was determined using the method described by AOAC (2005). Five grams of sample was dispersed in 50ml of distilled water and shaken. The mixture was allowed to stand for 30 minutes at 28°C before it was filtered through Whatman No. 42 grade filter paper; 2ml of the extract was dispersed into a 50ml volumetric flask. Similarly, 2ml standard tannin solution (tannic acid) and 2ml of distilled water were put in separate volumetric flasks to serve as standard and a reagent was added to each of the flasks and then 2.5ml of saturated Na_2CO_3 solution was added. The content of each flask was made up to 50ml with distilled water and allowed to incubate at 28°C for 90 minutes. Their respective absorbance was measured in a spectrophotometer at 260nm using the reagent blank to calibrate the instrument at zero.

Lecithin concentration was determined using the spectrophotometric method. The sample was first prepared by dissolving 1g in a 100ml distilled water flask, then 5ml of the sample solution was measured inside a 50ml flask before adding 3ml of a colour reagent. The colour reagent is a combination of glycerophosphocholine and phosphodiaterase reagents; the content was mixed and placed in the water bath and incubated at 37°C for 10 min. The absorbance of the sample and standard solutions of phospholipids were taken at a wavelength of 595nm.

Phytate content was determined using the spectrophotometric method as described by AOAC (2005). Half a gram (0.5g) of the sample was weighed into a 500ml flat bottom flask. The flask was placed in a shaker and the sample was extracted with 2.4% HCl for 1 hour. The aliquot was filtered and 5ml of the filtrate was pipetted and diluted to 25ml with distilled water; 15ml of NaCl was added to 10ml of the diluted sample and this was passed through an amplet resin (200 – 400 mesh) to elude inorganic phosphorus then 15ml of 0.7M NaCl was added to the solution which was mixed on a vortex mixer for 5 sec. The mixture was then centrifuged for 10 min and the supernatant was read at 520nm wavelength in UV spectrophotometer. The phytate concentration was read off from a standard curve prepared with standard inositol phytate and the value was expressed in mg/l00g using the formula:

$$\text{Phytate (mg)} = \frac{\text{conc. of phytate (mg/100g) from standard curve} \times \text{dilution factor}}{\text{Weight of sample}}$$

Data analysis

Laboratory analytical results were compiled, entered into the computer, and analyzed using a Microsoft Excel 2013 spreadsheet and expressed as mean ± SEM. Statistical analyses – Analysis of Variance (ANOVA) and T-tests were carried out using Statistical Package for Social Sciences (SPSS version 20.0) and significance was accepted at $p< 0.05$.

Data availability Statement

The results supporting the findings of this study are published in this article. Any additional information can be made available by the authors, on request.

Results and Discussion

Results

Mineral composition of the edible flour

In Table 1, the results of the determination of four macro (Ca, Na, K, and Mg) and four microelements (Fe, Zn, Mn and Se) are reported. Potassium was the most predominant among the elements analysed with concentrations ranging from 145.78 ± 0.02mg/100g (cassava flour) to 152.52 ± 0.15mg/100g (rye flour). It is worthy of note that rye flour had significantly (P=0.001) higher content of all the macro and micro minerals analysed except for calcium. Black fonio flour had the highest content of calcium 54.94 ± 0.02mg/100g while cassava flour had the least - 48.70 ± 0.09mg/100g. Rye flour had a significantly (P=0.001) higher content of iron 1.06 ± 0.01mg/100g while finger millet flour had the least (0.79 ± 0.01mg/100g). Similarly, rye flour had the highest content of zinc (1.27 ± 0.02mg/100g) while finger millet four had the lowest concentration 0.86 ± 0.33mg/100g.

The fat-soluble and water-soluble vitamin content of the edible flour

The results of the fat-soluble and water-soluble vitamin analyses are presented in Table 2. The data shows that the flours had considerable amounts of the vitamins analysed in this study. From general observations, finger millet flour had significantly (P=0.001) higher concentrations of most of the vitamins. Vitamin A and K were the most predominant fat-soluble vitamins in the flour samples, with finger millet flour having the highest content of vitamin A (3.84mg/100g) and rye flour having the least (2.85 ± 0.01mg/100g). Vitamin K content ranged from 3.63 ± 0.01mg/100g in rye flour to 4.17 ± 0.01mg/100g in finger millet flour. Vitamin B_9 (folate) values were the highest of all the vitamins and again, finger millet flour recorded a significantly (P=0.001) higher value of 38.78 ± 0.02mg/100g while cassava flour had the least (34.63 ± 0.03mg/100g).

Antinutrient content of the edible flour

From the results shown in Table 3, the oxalate and phytate seemed to generally have the highest concentrations among the antinutrients analysed. It is also worthy of note that rye flour had significantly (P=0.001) higher content of all the five antinutrients. Oxalate content ranged between 1.35 ± 0.01mg/100g (finger millet flour) and 1.77 ± 0.03mg/100g (rye flour). Phytate was least in cassava flour (1.25 ± 0.01mg/100g) and highest in rye flour (1.62mg/100g). Black fonio flour had the lowest lecithin concentration (0.89%) while rye flour had a significantly (P=0.001) higher concentration of lecithin (1.87 ± 0.03%.

Discussion

The cereal flour and cassava flour had good content of the minerals evaluated in this study. Potassium, which had the highest concentration in all the four edible flours, is known for its role in the transmission of nerve impulses and muscle contractions including those of the myocardium (D'Elia *et al*., 2011).). Low-potassium diets can lead to hypertension and hypokalemia (Campanozzi *et al*., 2015). Furthermore, the sodium-to-potassium ratio appears to be more strongly associated with Blood Pressure (BP) than either sodium or potassium alone (Iwahori *et al*., 2017). Studies have shown that higher sodium and lower potassium intakes are associated with higher BP (>140/90 mm Hg), whereas high potassium intake is associated with low BP (He and Macgregor, 2009; Cook *et al*., 2007; Jackson *et al*., 2014). The cereal flours had relatively lower sodium and higher content of potassium (as recommended by WHO) which is desirable for heart health. The effect of potassium on the state of bone tissue and its calcium content is essential. It was established that increased consumption of potassium-rich fruits and vegetables reduces the acidity of the diet and helps maintain calcium in the bones, preventing its leaching and, therefore, osteoporosis (Jehle *et al*., 2013). Calcium is essential for developing and maintaining healthy bones and teeth and assists in blood clotting, muscle contraction, nerve transmission, and oxygen transport (Lovis, 2018). Calcium was also present in considerable amounts in the flour samples with black fonio flour recording the highest value. The WHO/FAO recommended intake is 400-500 mg per day of calcium for adults and 1200 mg per day for children. Hence, the cereal flours would contribute significantly to meeting an individual's RDA for calcium. The sodium and magnesium content in rye flour was significantly higher than in other flour samples. According to Vega *et al.* (2010), these macronutrients are found in sufficient quantities in rye grains for adequate human nutrition.

Iron (Fe) is an essential micronutrient both for plants and humans. Graham *et al*. (2007) found that the average Fe concentration in wheat grain is 3.5 mg/100g. This is slightly higher than the Fe results obtained in this study. The Fe content of commercial wholemeals is said to be higher than that of white flour and the data showed that 73% of the Fe content is lost during the milling process. The recommended daily Fe intake is 8 mg per day (Dietary Reference Intake, 2004) and is based on the average Fe content of varieties, which can be reached by the consumption of at least 220 g of whole cereals. Fe is very important for the formation of haemoglobin in the red blood cells which helps deliver oxygen from the lungs to body tissues, transport electrons in cells, and synthesis of iron-containing enzymes that are required to utilize oxygen (O_2) for the production of cellular energy (Kharkar and Ratnaparkhe, 2013). Similarly, the average Zn concentration in wholegrain wheat in various countries is between 2.0 to 3.5 mg/100g (Cakmak *et al*., 2004) which is similar to the values obtained in this research for zinc content of the cereal flours. Research reports that different types of white flour and semolina have significantly lower Zn content than whole cereals. The highest Zn content (1.28mg/100g) in this study, was still too low to meet daily human requirements. It is recommended that the Zn concentration in wholegrain needs to be increased up to 4.4mg/100g), assuming 250 g per day intake of wholegrain flour to reach the recommended daily Zn intake (11 mg per day, Dietary Reference Intake, 2004). Selenium is mostly in protein-bound form in cereal grain, therefore it is more evenly distributed in the kernel, and a higher proportion is stored in the endosperm, compared to other minerals (Lyons *et al*., 2005). Se is not an essential element for plants, and its concentration in grains correlates with plant-available selenium in soils (Spadoni *et al*., 2007). The soil pH, the microbial activity, the content of organic materials and the soil texture are all important parameters that affect the concentration of available Se in soil (Stroud *et al*., 2010).

Vitamins essential for humans are classified into fat-soluble (Vitamins A, D, E and K) and water-soluble vitamins (B complex and C). The results reveal that vitamin A content in finger millet flour was significantly ($p<0.05$) higher than the other flour samples. Vitamin A promotes growth/development, prevents night blindness and strengthens corneal structure and immune function in children. Vitamin D content was higher in black fonio flour compared to the other flours. Vitamin D promotes bone health in children (Nadar and Uday, 2021). The results from this study also agree with Kulp and Ponte (2020), who reported that cereals also contain appreciable amounts of vitamin E. The edible flours also showed good content of vitamin K which functions in synthesizing various proteins that are needed for blood clotting and the building of bones.

Out of the five anti-nutrients analysed in this study, oxalate seems to be the most predominant in all four flour samples, closely followed by phytate. Anti-nutrients at high concentrations may cause reduced nutrient bioavailability. Most of the anti-nutritional factors become ineffective by simple processing methods such as heating, soaking, germination or autoclaving. Anti-nutrients in foods are known to inhibit the proper absorption and utilization of essential nutrients thereby decreasing bioavailability. In sensitive people, small amounts of oxalates can result in burning in the eyes, ears, mouth, and throat; large amounts may cause abdominal pain, muscle weakness, nausea, and diarrhoea (Popova & Mihaylova, 2019). Oxalates reduce calcium and magnesium utilization by binding them and making them indigestible; the negative effect of oxalates on humans depends on the levels (Nguyen *et al*., 2018). The phytate content in rye flour underscores the importance of processing techniques to reduce antinutrient levels and enhance nutrient bioavailability (Nkafamiya and Igbabul, 2018). Cereal grains constitute a major source of dietary nutrients all over the world. Although cereals are deficient in some basic components (e.g. essential amino acids), fermentation may be the most simple and economical way of improving their nutritional value, sensory properties and functional qualities.

Limitation of the Study: The main limitation of this study was insufficient time to produce composite flours and baked products from these unpopular cereals. This will be looked into subsequently.

Conclusion

Cereal flours are richer in micronutrients than cassava flour, notably potassium- which has a significant protective effect on cardiovascular health. These flours can be suggested as alternatives in the dietary management of diabetes, obesity and hypertension, as similar studies show that these cereals are also low in fat and high in useful dietary fibre. Hence, the use of these cereals to make swallows that accompany our Nigerian soups should be encouraged especially for people who seek to cut down on their intake of starchy foods. Rye flour had the highest concentrations of all the minerals but also had the highest concentrations of the antinutrients (although the values were all within safe limits). Rye can be incorporated into baking and production of nutrient-dense snacks for combatting micronutrient deficiencies.

Conflict of interests: The author hereby declares that no conflict of interests exists.

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Table 1: Mineral composition of the edible flour

Flours	Ca (mg/100g)	Na (mg/100g)	K (mg/100g)	Mg (mg/100g)	Fe (mg/100g)	Zn (mg/100g)	Mn (ug/g)	Se (ug/g)
Cassava	48.70 ±0.09	35.84 ±0.02	145.78 ±0.02	26.44 ±0.32	0.85 ±0.01	1.28 ±0.14	0.82 ±0.03	1.54 ±0.01
Finger millet	52.69 ±0.05*	38.45 ±0.03*	148.77 ±0.02*	27.66 ±0.13*	0.79 ±0.01*	0.86 ±0.33	0.84 ±0.01	1.92 ±0.01*
Black fonio	54.94 ±0.02*[,a]	37.74 ±0.06*[,a]	148.96 ±0.27*	31.74 ±0.06*[,a]	0.85 ±0.00[a]	1.24 ±0.01	1.09 ±0.01*[,a]	2.19 ±0.01*[,a]
Rye	49.51 ±0.15*[,a]	41.33 ±0.04*[,a,b]	152.52 ±0.15*[,a,b]	34.70 ±0.10*[,a,b]	1.06 ±0.01*[,a,b]	1.27 ±0.02	1.16 ±0.01*[,a,b]	2.32 ±0.01*[,a,b]

**** Values are presented as mean ±SEM, n = 3. * = significantly different from cassava flour at p<0.05***
a = significantly different from finger millet flour at p<0.05 b = significantly different from black fonio flour at p<0.05

Table 2: Vitamin composition of the edible flours in mg/100g

Flours	Vitamin A	Vitamin D	Vitamin E	Vitamin K	Vitamin B_1	Vitamin B_2	Vitamin B_3	Vitamin B_9
Cassava	3.42 ±0.00	0.87 ±0.02	1.84 ±0.00	3.83 ±0.01	0.13 ±0.00	0.10 ±0.00	0.46 ±0.00	34.63 ±0.03
Finger millet	3.84 ±0.00*	1.29 ±0.01*	1.91 ±0.01*	4.17 ±0.01*	0.57 ±0.00*	0.32 ±0.00*	0.84 ±0.00*	38.78 ±0.02*
Black fonio	3.61 ±0.01*[,a]	1.33 ±0.01*[,a]	1.45 ±0.00*[,a]	3.91 ±0.01*[,a]	0.47 ±0.00*[,a]	0.32 ±0.00*[,a]	0.76 ±0.00*[,a]	36.41 ±0.01*[,a]
Rye	2.85 ±0.01*[,a,b]	0.93 ±0.01*[,a,b]	1.68 ±0.00*[,a,b]	3.63 ±0.01*[,a,b]	0.38 ±0.00*[,a,b]	0.13 ±0.00*[,a,b]	0.48 ±0.00*[,a,b]	35.63 ±0.11*[,a,b]

**** Values are presented as mean ±SEM, n = 3. * = significantly different from cassava flour at p<0.05***
a = significantly different from finger millet flour at p<0.05 b = significantly different from black fonio flour at p<0.05

Table 3: Antinutrient composition of the edible flours

Flours	Tannin (mg/100g)	Oxalate (mg/100g)	Phytate (mg/100g)	Phenol (mg/100g)	Lecithin (%)
Cassava	0.76 ±0.00[a]	1.59 ±0.01[c]	1.25 ±0.01[a]	0.65 ±0.00[a]	1.26 ±0.00[b]
Finger Millet	1.19 ±0.01[b]	1.35 ±0.01[a]	1.31 ±0.01[b]	0.72 ±0.00[b]	1.34 ±0.00[c]
Black Fonio	1.35 ±0.00[c]	1.53 ±0.01[b]	1.47 ±0.01[c]	0.78 ±0.00[c]	0.89 ±0.00[a]
Rye	1.49 ±0.01[d]	1.77 ±0.03[d]	1.62 ±0.00[d]	0.85 ±0.00[d]	1.87 ±0.01[d]

**** Values are presented as mean ±SEM, n = 3. * = significantly different from cassava flour at p<0.05***
a = significantly different from finger millet flour at p<0.05 b = significantly different from black fonio flour at p<0.05