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Proximate analysis, mineral element and total phenolic contents of some commonly consumed grains in Nigeria

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Abstract

Grains amongst other plant foods are known to be rich sources of phenolic compounds which are known to exhibit high antioxidant activity. In this study, eight selected grains; polished rice (*Oryza sativa*), *ofada* rice (a peculiar rice specie cultivated in Ofada, Ogun State, Southwestern Nigeria), a wheat specie -*Triticum aestivum*, a millet specie - *Eleusine coracana*, two maize subspecies (white and yellow subspecies) - *Zea mays subsp*., and two sorghum subspecies (red and white subspecies) -*Sorghum bicolor subsp.*, were analysed for the total phenolic content with the standard spectrophotometric method of analysis using garlic acid as a standard. In addition to this, mineral content and proximate composition (% ash, moisture, fat and protein) of each grain was also determined. Results revealed a total phenolic content range of 132 µg GAE/g and 2166 µg GAE/g for *ofada* rice and wheat respectively. Grains with high mineral contents (wheat and millet) as well as high nutritional values were observed to have the highest total phenolic content. This study provides needed information on benefits of common Nigerian grains, in terms of their nutritional composition, phenolic content, and levels of essential trace minerals.

Keywords: grains, mineral content, proximate composition, total phenolic content

1. Introduction

The high nutritional values associated with the consumption of grains are largely due to their anti-oxidative properties (Agbor, *et al.*, 2011). Anti-oxidants play vital role in the human body as they help neutralize the effect of free radicals, also known as reactive oxygen species (ROS), generated as by-products when oxygen is used up in the human body, especially the human brain (Betteridge, 2000; Sell and Eckel, 2009). When these free radicals are produced, they attack body cells leading

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to increase in oxidative chemical modification of lipids, DNA, and proteins in various tissues. A balance between free radicals and antioxidants is necessary for proper physiological activity. However, if the number of free radicals produced exceeds the antioxidant level in the human body, a condition known as oxidative stress occurs. Free radicals thus adversely alter biological molecules and trigger several human diseases (Lobo *et al.*, 2010; Osawa and Kato, 2005)

Foods obtained from plants have phenolic compounds, which are secondary metabolites synthesized in plants. These phenolic compounds possess biological properties such as: antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, cardiovascular protection, as well as cell proliferation activity (Montecucco *et al*., 2011). The main antioxidants in plant are the phenolic acids, flavonoids and tannins (Han *et al.*, 2007). Other naturally occurring antioxidants present in foods obtained from plants are selenium, lignans, vitamins and some minerals (Rochfort and Panozzo, 2007). The combinative effects of these compounds generally enhance antioxidative properties of such food items.

There are several methods of determining antioxidant activities, as antioxidants are a diverse group of compounds with different reactivity towards different reactive oxygen species (Adom *et al.*, 2005). Antioxidant activities have been reported to be determined using Phosphomolybdenum antioxidant assay (Prieto *et al*., 1999; Wan *et al.*, 2011), DPPH (1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) free radical scavenging antioxidant assay (Villano *et al.*, 2007), and Ferric reducing antioxidant power (FRAP) assay (Fasahat *et al.*, 2012; Yu et al., 2002). Another method is to determine the total phenolic content as an indicator of the presence of antioxidant activity, since the beneficial effects of phenolic compounds are largely as a result of their antioxidant activity (Kim and Yoon, 2014).

In Nigeria, few reports exist for phenolic content of plant foods, specifically grains. Amongst these few, is the work by Agbor *et al.* (2011) that evaluated the phenolic content of some beverages, vegetables and processed cereals. Odukoya *et al.* (2007) as well as Oboh and Akindahunsi (2004) reported the phenolic content of some green leafy vegetables. Similarly, Azeez *et al.* (2012) and Olayiwola *et al.* (2013) determined antioxidant activity of some vegetables and fruits obtained locally in South Western Nigeria. Studies from other countries include works of Basu *et al.*, (2012); Liu and Yao (2007); Qui *et al.*, (2010) and Wu *et al.*, (2004) in India, China, Canada, and USA respectively.

The antioxidant activity of grains has not been extensively studied in Nigeria and there is dearth of reports available. Few existing reports centred on antioxidant activities of other plant foods like vegetables, fruits and beverages as well as processed cereals. Considering that there are different species of plant crops in various parts of the world, it is necessary to conduct a study of this kind, to communicate the need for consumption of grains in Nigeria. This work aimed to determine and compare the total phenolic contents, minerals and proximate compositions of eight commonly consumed grains in Nigeria, in order to know the beneficial effects from consumption of the grains in Nigeria. To statistically test the synergistic effects of proximate composition and mineral content on the antioxidant properties (phenolic content) of Nigerian grains.

2. Materials and methods

2.1 Samples and sample milling

Eight grain types, *viz.*, wheat, (*Triticum aestivum*), millet (*Eleusine coracana*), polished rice (*Oryza sativa*), *ofada* rice (a special of blend of African rice (*Oryza glaberrima*) and polished Asian rice cultivated in Ogun State, Nigeria), maize (white and yellow) (*Zea mays*) and sorghum (red and white) (*Sorghum bicolor*) were purchased from Agege market in Lagos State, South Western Nigeria. The whole grains were milled with Perten laboratory mill 120 and stored in air tight containers.

2.2 Proximate Analysis

Proximate composition of the grains which includes: ash content, moisture content, protein, and fat, was determined using method similar to Indrayan *et al.* (2005). For ash content, 3 g of each grain sample was weighed into crucibles and heated in muffle furnace for about 5-6 hours at 550 °C. Samples were cooled in a desiccator and weighed till a constant weight was obtained. Ash content was calculated using the final sample weight against the blank and converted to a percentage value by multiplying by 100. Moisture content was determined by weighing 2 g of each sample in a flat-bottom flask, which was kept for 16 hours in an air oven at 100-110 °C.

Crude fat was estimated after Soxhlet extraction of 2 g moisture free sample of each grain with n-hexane at 60-80 °C for 8 hours. Each sample was tightly packed in a Whatman no. 40 filter paper and placed in the extractor vessel of the Soxhlet apparatus. After 8 hours of several siphoning process, the extract in the round bottom flask of the apparatus was carefully transferred into a pre-weighed beaker and evaporated to dryness. Increase in weight of beaker gave crude fat. Determination of total nitrogen (crude protein) was conducted using the Kjeldahl method. The sample was digested in sulfuric acid using K_2SO_4/ $CuSO_4$ as a catalyst. Nitrogen which is converted into NH_3 is distilled, trapped in boric acid and titrated with H_2SO_4.

2.3 Determination of Mineral Content

Seven minerals (Zinc (Zn); Manganese (Mn); Potassium (K); Calcium (Ca); Iron (Fe); Magnesium (Mg); Copper (Cu)) were analysed in the grains using Atomic Absorption Spectrometry (AAS) (AA 200, Perkin-Elmer Germany). After digestion of 2 g samples of each grain in a clean digestion flask with 20 ml of HCl: HNO_3 (3:1 v/v). Calibration was performed by analysing prepared working standard solutions of each element prior to the determination of mineral contents of our grain samples. A blank sample was also analysed following the same procedure.

2.4 Extraction of milled grains and estimation of total phenolic content

Two grams of each milled grain was extracted in 20 ml aqueous methanol (80%, v/v) with continuous shaking (mechanical shaker IKA HS 260 Basic) for 4 h at room temperature. This was later centrifuged at 2700 rpm for 20 min using a REMI CM 12 Plus Bench Top Centrifuge. The extracts were filtered and stored in the fridge prior to analysis for their total phenolic contents. The amount of total phenolic content of crude extracts was determined according to Folin-Ciocalteu procedure described by Fasahat *et al. (*2012), with slight modification. Briefly, 1.0 ml of the tested samples was introduced into test tubes; 1.0 ml of Folin-Ciocalteu reagent and 1 ml of sodium carbonate (7.5%) were added and the mixture made up to 10 ml with distilled water, this was followed by centrifuging for 20 min at 2700 rpm. Absorbance at 700 nm was measured on UV-visible spectrophotometer (Genesys 10 UV thermospectronic). The total phenolic content was expressed as gallic acid equivalents (GAE) in microgram per gram (µg/g) of grain. Gallic acid solutions (20, 40, 60, 80 and 100 µg/ml) were prepared from 1000 µg/ml stock solution prepared by dissolving 10 mg of gallic acid in 10 ml of 80% methanol.

2.5 Statistical Analysis

Multivariate analysis (ANOVA, cluster, principal component analysis (PCA) and linear regression) of data was carried out on SPSS 15.0 for windows version. ANOVA was conducted to test the statistical significance in mineral concentrations per grain sample and per element analysed. Cluster analysis was conducted to identify the presence of similar patterns in the grain samples. PCA was done to decipher the source of observed variations and their significance by reducing the variables in smaller numbers called principal components. Linear regression was performed to determine the extent to which the predictor variables could explain the variance observed in the dependent variable.

3. Results and discussion

3.1 Proximate analysis, minerals and Total phenolic content (TPC)

3.1.1 Proximate composition

Nutrient composition of white maize, yellow maize, red sorghum, white sorghum, *ofada* rice, millet, polished rice and wheat are presented in the Table 1.

Table 1: Percentage moisture, ash, protein, fat and carbohydrate content of grain samples

Grains	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
White maize	9.31±1.28	1.69±0.02	9.96±0.08	3.07±0.02	75.95±2.02
Yellow maize	8.58±0.50	1.35±0.01	5.83±0.06	3.64±0.22	80.60±4.14
Red sorghum	8.26±0.20	1.76±0.02	2.24±0.12	2.82±0.04	84.92±4.62
White sorghum	8.46±0.09	1.26±0.04	4.75±0.10	2.79±0.21	82.74±4.32
Ofada rice	8.13±0.02	0.66±0.02	4.08±0.18	1.56±0.11	85.57±3.02
Millet	10.17±2.48	3.03±0.06	11.23±0.15	4.73±0.09	70.84±2.01
Polished rice	9.46±0.11	0.72±0.02	3.20±0.19	1.68±0.03	84.94±3.07
Wheat	7.47±0.19	1.83±0.02	14.81±0.28	2.40±0.15	73.49±2.42

Mean ± S.D of 3 replicate determinations of each sample

The moisture content ranged from 7.47 % for wheat to 10.17 % for millet. The results obtained for rice (polished and *ofada*) in this study were below the ranges reported by Fasahat *et al.* (2012) for different rice species from Malaysia. The moisture content in all grains were within limit (not higher than 14 %), this therefore indicates long storage life for all studied Nigerian grains. Protein content varied among grains (Table 1). Wheat had the highest protein content, approximately 14.8 %, followed by millet (11.6%). Polished rice and red sorghum exhibited the lowest level of protein (3.2 % and 2.2 %) respectively. High protein content of millet-based drink over sorghum-based drink has been reported by Ajiboye *et al.* (2014). The protein content trend observed by Ajiboye *et al.* (2014) was similar to what was observed in this study for whole millet and sorghum grains, implying that both processed and whole grains have similar proximate composition distributions. In this study, all investigated grains had low fat contents (1.56 - 4.73 %), however millet had the highest fat content of the studied grains. Ash contents were all below 2 % for all grains. The range obtained is comparable with previous reports (Sompong *et al.*, 2011; Yodmanee *et al.*, 2011).

3.1.2 Mineral content of grains

Generally, of the seven mineral elements analysed, concentrations of calcium (Ca) was the highest for six (6) of the grains (Table 2), however concentration of potassium (K) was the highest in millet and wheat. Millet had the highest iron content (37.95 µg/g) while wheat, maize, *ofada* rice showed intermediate iron content, sorghum and polished rice exhibited low iron content. Wheat appeared to be rich in manganese and potassium (28.39 and 957.19 µg/g),

red sorghum rich in copper (5.14 µg/g) and yellow maize rich in zinc and calcium (5.93 and 1004.69 µg/g). The observed variations were in agreement with the work of Mohammed and Ahmad (2014). However, ANOVA comparing the concentrations of all minerals in each of the samples of grain analysed revealed that there was no statistical difference in their mineral contents, thus implying that all grains have statistically comparable mineral content irrespective of their type. On the contrary, comparing the concentrations of each mineral in all grains revealed a statistically significant difference based on mineral type.

Table 2: Minerals and phenolic content of grains

Grains	Minerals (µg/g)							TPC
	Fe	Cu	Mn	Zn	K	Mg	Ca	µg GAE/g
White maize	22.53	0.37	8.04	1.96	393.4	210.3	628.8	322.4± 19.3
Yellow maize	27.96	1.29	5.32	5.93	101.3	307.2	1004.7	436.72 ± 1.42
Red sorghum	18.76	5.14	9.18	2.45	198.0	290.3	505.0	1701.67 ± 36.8
White sorghum	18.69	0.37	3.15	2.69	272.2	299.7	667.2	514.73±4.15
Ofada rice	20.34	0.64	3.39	2.53	94.6	162.8	926.6	132.17±11.3
Millet	37.95	0.44	3.59	3.61	923.8	473.1	667.5	1869.5±5.35
Polished rice	16.53	0.18	2.42	1.41	344.1	281.7	928.6	294.47± 1.87
Wheat	26.28	0.13	28.39	2.11	957.2	343.8	713.8	2165.67±5.67

3.1.3 Phenolic content of grains

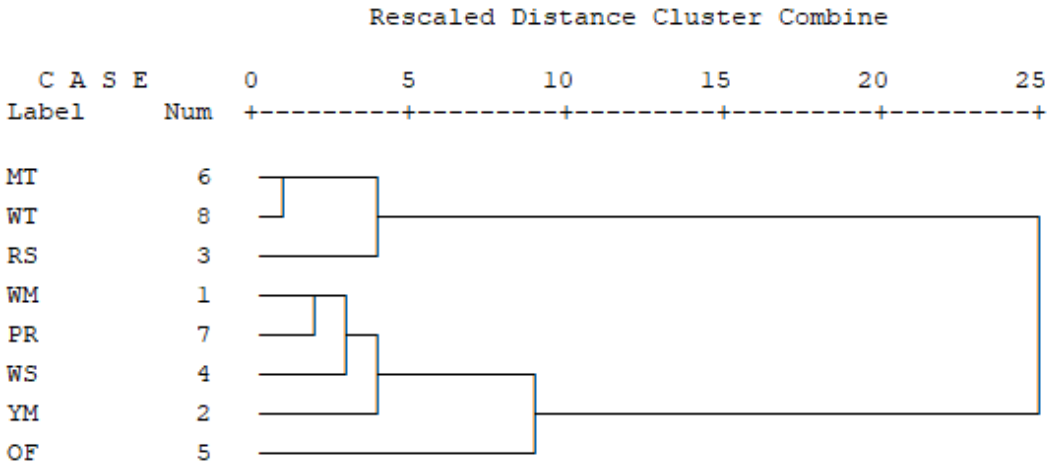
Generally, food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions (Kim and Yoon, 2014). It has been recognized that phenolic compounds show antioxidant activity and their effects on human nutrition and health are considerable. There was a wide variation in the amount of total phenolics in the grains ranging from 132.17$\pm$11.3 to 2165.67 $\pm$ 5.67 µg/g (GAE) (Table 2).

Table 3: Comparison of total phenolic content (TPC) in grains from this study with other previous reports

Reference (Country)	Grain type	TPC (µg GAE/g of grain
This study (Nigeria)	Wheat (1 genotype)	2166
Beta *et al.*, 2005 (Canada)	Wheat (6 genotypes)	1300 – 5300
Yu *et al.*, 2002 (USA)	Processed wheat	203- 524
This study (Nigeria)	Millet (1 genotype)	1870
Zhang *et al.*, 2017 (China)	Millet (6 cultivars)	1172- 1205
Moore *et al.*, 2006 (USA)	Millet (20 genotypes)	2700 – 3500
This study (Nigeria)	Maize (2 genotype)	322-437
Bacchetti et al. 2013 (Italy)	Maize (2 genotypes)	1154- 1755
Adom and Liu, 2002 (USA)	Maize (1 genotype)	360
This study (Nigeria)	Rice (2 genotype)	132-295
Fasahat *et al.*, 2012 (Malaysia)	Rice (2 genotype)	320-1580
This study (Nigeria)	Sorghum (2 genotype)	515-1702
Mami-Soulem *et al.*, 2013 (Algeria)	Sorghum (1 genotype)	313
Irondi et al. 2019	Sorghum (1 genotype)	72-73

The amount of total phenolic content of the investigated grains arranged in descending order is: wheat >millet > red sorghum > white sorghum > yellow maize > white maize > polished rice >ofada rice. The phenolic content in polished rice from this study is lower than that reported by Fahasat *et al.* (2012) (Table 3), for Thailand rice but in range with cross bred rice variants from Malaysia. The phenolic content in white maize was comparable to values reported for phenolic content in corn by Adom and Liu in (2002) while yellow maize was slightly higher. Worthy of note is the fact that the phenolic content in wheat from this study was about six times higher than reported by Yu *et al.* (2002). The low phenolic content level reported by Yu *et al.* (2002) was attributed to the fact that the wheat was processed, indicating that processed grains have significantly lower phenolic content than whole grain. On the basis of phenolic content, results revealed that whole wheat is likely to have the most potent antioxidant activity among the grains studied. Cluster analysis was conducted to explain the similarities among grains based on the studied parameters (TPC, minerals and proximate composition). Clustering the observations (grains) (Figure 2) revealed that millet (MT), wheat

(WT), and red sorghum (RS) showed great similarity. The second major cluster comprises of white maize (WM), white sorghum (WS), yellow maize (YM) and polished rice (PR). However, ofada rice (OR) was least similar to other grains at distance of about 10 (Figure 1).



Key: **MT**- millet; **WT**- wheat; **RS**- red sorghum; **PR**- polished rice; **WS**- white sorghum; **YM**- yellow maize; **OF**- ofada rice

Figure 1: Dendogram clustering grains based on their phenolic, minerals and proximate compositions

3.2 Effect of mineral content and proximate composition on total phenolic activity of grains

To deduce meaningful patterns and variance in minerals, proximate composition and total phenolic content (TPC) of grains, principal component analysis (PCA) and regression analysis were conducted.

PCA revealed that the variables with high positive component scores in principal component 1 (PC 1) (which explains the greatest variation within the data set) were K > TPC > protein > Mn > ash > Mg > Fe > fat. Potassium had closest positive component score with TPC in PC1, followed by protein content. It could be inferred that of the 7 minerals and proximate compositions, K and protein are the most closely related to total phenolic content (TPC) (Figure 2). K and Protein also had the closest component scores to TPC in principal component 2 (PC2), indicating that TPC in grains are strongly associated with their K and protein contents. Variables with negative scores in PC1 were Ca, Zn, and Cu, carbohydrate and moisture content. Cu and carbohydrate were the only two variables that contributed negatively to the component scores of both PC1 and PC2. From the PCA result, it could be deduced that total phenolic content (TPC) is correlated with both mineral and nutrient contents, however more with mineral content. To further affirm this inference, linear regression was performed to determine the variance that could be explained by the two variables (K and protein content) that had the closest component scores to TPC. Two linear regression analyses were performed using TPC

as the dependent variable in both cases. In the first instance, K was made the predictor variable and for the second regression analysis protein was used as the predictor variable. The result revealed $R^2 = 0.57$ (at significant level of 0.03) when K was the predictor variable, this implied that K could account for 57% of the variance in TPC. On the other hand, $R^2 = 0.31$ (at significant level of 0.15) when protein was the predictor variable, this implied that protein content could account for 31% of the variance in TPC. From the regression results, it could be interpreted that K was a significant (p-value = 0.03) predictor of TPC.

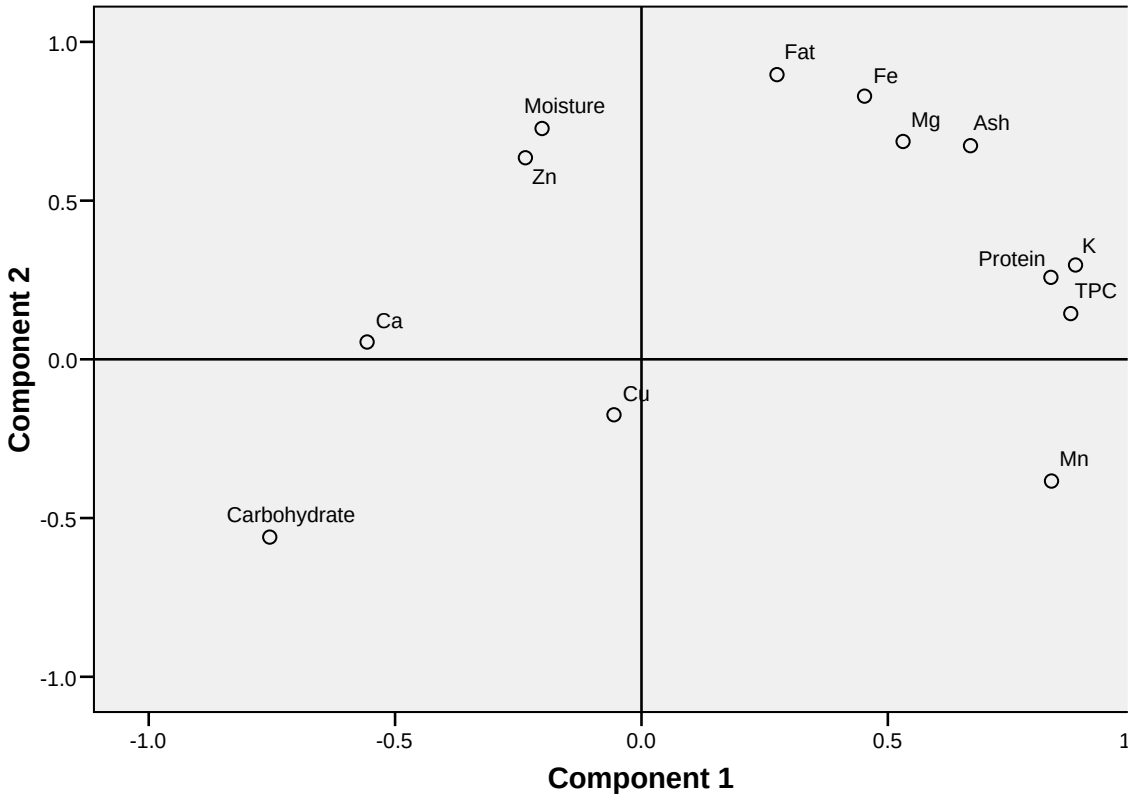


Figure 2: Component plot for two components comparing variables in grains

4. Conclusion

Phenolic content of studied grains varied, but was found to be significantly correlated with the minerals and nutritional composition, as grains (wheat and millet) with high mineral content as well as high nutritional values had highest total phenolic content. The role of whole grain products in nutrition and health has been scientifically documented. Bioactive substances occur in grains at different concentrations and identities depending on their species. In this study, sorghum, millet, and wheat were found to contain reasonable levels of dietary fibre and antioxidant properties. Incorporation of such materials into bakery products would enhance their nutritional and physiological properties. This study also showed that total

phenolic content of grains exhibited greater relationship with mineral content than proximate composition.

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