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NUTRIENT COMPOSITIONS AND ZINC-BIOAVALABILITY ESTIMATION (IN-VITRO) OF THE EDIBLE TROPICAL CEREALS

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ABSTRACT

Nutritive value and Zn-bioavailability estimation of the edible tropical cereals were investigated in this study. White and yellow maize (Z*ea mays*), brown and white sorghum (*Sorghum bicolor (L). Moench*), and yellow and brown millet (*Pennisetum glaucum*) of the cereal samples were air-dried and milled into powder. Subsequently, the samples were analyzed for their proximate, mineral composition and calculated [phytate]/[Zn], [Ca]/[(Phytate] and [Ca][Phytate]/[Zn] molar ratios were used in predicting the effect of phytate on zinc bioavailability.

The result revealed that brown sorghum was significantly higher (P<0.05) in protein content (12.5 ± 0.3%) while yellow millet had the least value. Millet (yellow and brown) had the highest carbohydrate content in all the cereal grains analyzed. Moreover, yellow maize (13.5 $\pm$ 0.1 %) had the highest crude fat content while white sorghum (5.5 ± 0.3 %) had the least. The macro-elements (Na, K, Mg and Ca) contents of the cereals were significantly higher (P<0.05) than the micro-element (Fe, Zn, Pb, Cu and Ni). Furthermore, yellow (58.1 $\pm$ 0.16 mg/g) and white (52.7 $\pm$ 0.15 mg/g) maize had the highest phytate content. The calculated [Ca][phytate]/[Zn] molar ratios indicated that the phytate contents of the cereals may reduce zinc bioavailability to a critical level. Moreover, values obtained in the present study for [phytate][Zn] molar ratios for the cereals were far above 15.0. The high value of phytate content in the cereals is an indication of the critical factor for the decrease in the bioavalability of Zn. However, cereals grains such as sorghum (Brown & White and Yellow maize) are good sources of minerals but low in protein yet with substantial nutritional value, it can avert the case of malnutrition.

KEYWORDS: Cereals, Molar Ratio, Nutrient Composition, Phytate, Zn-Bioavalability

INTRODUCTION

The world's population dependence much on coarse, unrefined cereals grains, which contain high contents of phytic acid, a potent inhibitor of zinc absorption, combined with recurrent infectious episodes, is thought to be the basis of wildspread Zinc deficiency in developing countries (Brown *et. al.,* 2004 and Hess *et. al.,* 2009). Zinc is an essential trace element in human nutrition and its deficiency is major public health threat worldwide. Among the micronutrient malnutrition situations afflicting the human population, its deficiency is of major concern not only because of the serious health consequence it may have, but also because of the number of people affected worldwide particularly in Africa (Kayode, 2006).

Zinc is also an essential component of a large number (>300) of enzyme participating in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids as well as in the metabolism of other micronutrients.

It stabilizes the molecular structure of cellular components and membranes and in this way contributes to the maintenance of cell and organ integrity (Hambridge *et. al.,* 1987). Furthermore, Zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms (Hambridge *et. al.*, 1987).

It also plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998). The clinical features of several zinc deficiency in humans are growth retardation, delay sexual and bone maturation, skin lesions, diarrhoe, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in immune system, and the appearance of behavioural changes (Hambridge *et. al.*, 1987).

Cereals nutritional qualities are dictated mainly by their chemical compositions and the presence of anti-nutritional factors, such as phytate. Phytates are present in whole-grain cereals and legumes and in smaller amounts in other vegetables. They have a strong potential for binding divalent cations and their depressive effect on zinc absorption has been demonstrated on humans (Sandstrom, 1989). The molar ratio between phytates and zinc in meals or diets is a useful indicator of the effect of phytate in depressing zinc absorption. At molar ratios above the range of 6-10, zinc absorption starts to decline; at ratio above 15, absorption is typically less than $15^0/_0$. The effect of phytate is, however, modified by the source and amount of dietary proteins consumed (Sandstrom and Lonnerdal, 1989).

MATERIALS AND METHODS

Sample Collection

Two varieties each, of some edible tropical cereals namely; white and yellow maize (*Zea mays*), Brown and white sorghum (*Sorghum bicolor (L) Moench*), and yellow and brown millets (*Pennisetum glaucum*), were purchase from Waso market in Ogbomoso metropolis, Oyo state in Nigeria. The authentication of the sample was carried out by Dr G.O Adesina of the Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso in Oyo State, Nigeria. The cereals were hand-picked to separate the spoiled portion or inedible portion from the edible portion. Consequently, the prepared samples were kept under a room temperature for analyses. All chemical used were analytical grade and the water used was glass distilled.

Sample Preparation

The raw samples were air-dried to reduce the moisture content using LEEC drying cabinet (LEEC Ltd., Private rd., No 7. Colwick. Nottingham.UK) and are milled into powder using a Waring heavy duty blender (Waring Products Division, New Hartford. Connecticut. USA). Subsequently, the powdered samples were subjected to proximate analysis, mineral composition and Zn-bioavalability analyses.

Proximate Analysis

Proximate analysis was determined by the method of Association of official Analytical chemist (AOAC, 1990).

Mineral Composition

Appropriate hollow cathode lamp was fixed (i.e according to the element that was to be determined). The Atomic Absorption spectrophotometer was set and allowed to stabilize using the standard solutions. The stock standard for each mineral was 10ppm. The standard for each metal was aspirated into the flame as well as the sample and the respective concentration was read while the absorbance of the standards was noted. The concentration of the minerals in mg/100g was subsequently calculated (AOAC, 1990).

Phytate Content Determination

Phytate was determining according to the method of wheeler and Ferrel (1974). Sample (4.0g) were soaked in 100ml of 2% HCl for 3 hrs and then filtered through whatman No. 2 filter paper. Then, 25ml of the filtrate was placed in conical flask and 5ml of 0.3% ammonium thiocynate solution was added after which 53.5ml of distilled water was added. This was titrated against a standard Iron (III) chloride solution containing 0.00195g of iron/ml until a brownish yellow colour persisted for 5min. The phytate content was expressed as mg/g phytate in the sample.

Zn Bioavailability Determination

Approximately 2g of each powdered sample was dry-ashed at 500^0c. The Zn and Ca contents were determined on aliquots of the solution of the ash by established atomic absorption spectrophometry procedures using a perkin-Elimer absorption spectrophotometer (Model 372) (Perkin - Elimer, 1982). Lanthanum chloride (1%) was added to both samples and standard solutions for calcium determination overcome phosphorus interferences. Calculation of [Phytate][Zn], [Ca][phytate], [Ca][Phytate]/[Zn] molar ratios were used in prediction of Zn bioavailability in the cereals product (Ferguson, *et al.*, 1988).

Statistical Analysis

The result of the replicates were pooled and expressed as mean $\pm$ standard error (SE). A one way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significant was accepted at $P \leq 0.05$.

Table 1: Proximate Composition of Some Edible Tropical Cereals Expressed in Percentage (%)

Sample	Crude Protein	Crude Fat	Crud Fibre	Ash	CHO	Moisture
Maize – Yellow -	$10.7^e \pm 0.5$	$13.5^f \pm 0.1$	$1.3^b \pm 0.2$	$1.0^b \pm 0.1$	$62.0^b \pm 0.3$	$11.5^f \pm 0.5$
White -	$9.6^d \pm 0.2$	$8.0^e \pm 0.2$	$2.2^c \pm 0.3$	$0.5^a \pm 0.2$	$69.2^d \pm 0.2$	$10.5^d \pm 0.3$
Sorghum – Brown	$12.5^g \pm 0.3$	$11.5^g \pm 0.3$	$4.3^h \pm 0.1$	$2.0^d \pm 0.1$	$58.9^a \pm 0.4$	$10.8^e \pm 0.4$
White	$11.2^f \pm 0.2$	$5.5^b \pm 0.3$	$3.4^e \pm 0.4$	$1.0^b \pm 0.2$	$69.5^d \pm 0.6$	$9.5^b \pm 0.1$
Millet – yellow	$5.3^a \pm 0.3$	$9.0^f \pm 0.1$	$3.4^f \pm 0.2$	$1.5^e \pm 0.0$	$71.8^f \pm 0.4$	$9.0^a \pm 0.2$
Brown	$5.9^b \pm 0.4$	$7.5^d \pm 0.2$	$3.6^g \pm 0.3$	$2.0^d \pm 0.1$	$70.8^e \pm 0.3$	$10.3^c \pm 0.2$

Data represent the mean of triplicate readings.
Values with the same uppercase superscripts letter along the same column are not significantly different (*P*>0.05).

Table 2: Mineral Composition of Edible Tropical Cereals (Mg/100g)

Sample	Mg	Fe	Pb	Ca	Na	Ni	Zn	K	Cu
Maize -									
Yellow	$17.71^f \pm 0.02$	$6.65^d \pm 0.03$	$1.65^f \pm 0.01$	$13.20^d \pm 0.07$	$13.10^f \pm 0.02$	$0.25^d \pm 0.03$	$2.23^e \pm 0.01$	$57.15^d \pm 0.04$	$0.03^e \pm 0.02$
White	$13.78^c \pm 0.04$	$3.25^c \pm 0.00$	$1.25^d \pm 0.04$	$11.00^c \pm 0.02$	$10.41^e \pm 0.02$	$0.45^f \pm 0.01$	$2.24^e \pm 0.01$	$50.05^c \pm 0.06$	$0.05^b \pm 0.01$
Sorghum -									
Brown	$26.93^h \pm 0.03$	$7.40^e \pm 0.03$	$1.50^e \pm 0.01$	$15.0^f \pm 0.02$	$13.10^g \pm 0.01$	$0.30^e \pm 0.01$	$1.52^{bc} \pm 0.01$	$74.20^g \pm 0.03$	$0.10^c \pm 0.00$
White	$23.44^g \pm 0.03$	$9.20^f \pm 0.04$	$0.95^c \pm 0.04$	$14.25^e \pm 0.02$	$9.45^c \pm 0.03$	$0.20^d \pm 0.01$	$1.49^b \pm 0.03$	$64.60^e \pm 0.03$	$0.10^c \pm 0.00$
Millet -									
Yellow	$15.07^d \pm 0.05$	$3.05^b \pm 0.01$	$0.00^a \pm 0.00$	$8.85^a \pm 0.03$	$4.85^a \pm 0.02$	$0.15^c \pm 0.01$	$1.75^d \pm 0.01$	$66.20^f \pm 0.04$	$0.05^b \pm 0.01$
Brown	17.02e $\pm$ 0.01	$3.15^{bc} \pm 0.02$	$0.95^c \pm 0.03$	$13.75^d \pm 0.03$	$5.92^b \pm 0.02$	$0.05^b \pm 0.01$	$1.72^d \pm 0.02$	$75.50^h \pm 0.06$	$0.51^d \pm 0.01$

Data represent the mean of triplicate readings.
Values with the same uppercase superscripts letter along the same column are not significantly different (*P*> 0.05).

Table 3: Phytate Content in the Edible Tropical Cereals (Mg/G)

Sample	Phytate (mg/g).
Maize - Yellow	$58.1^{h} \pm 0.16$
- White	$52.7^{g} \pm 0.15$
Sorghum - Brown	$14.7^{a} \pm 0.12$
- White	$37.8^{f} \pm 0.12$
Millet - Yellow	$25.9^{b} \pm 0.12$
- Brow	$26.5^{c} \pm 0.06$

Date represent the mean of triplicate readings.
Values with the same column are not significantly different (*P*> 0.05).

Table 4: Estimation of Zn Bioavailability in the Edible Tropical Cereals (Mol/Kg)

Sample	(Phytate)/(Zn)	(Ca)/(Phytate)	(Ca)(Phytate)/(Zn) (mol/kg)
Maize - Yellow	148.3	0.5	$6.4^{e} \pm 0.01$
White	135.0	0.4	$4.9^{c} \pm 0.01$
Sorghum - Brown	46.0	2.2	$2.3^{a} \pm 0.02$
White	116.0	0.8	$5.3^{d} \pm 0.03$
Millet - Yellow	80.0	0.7	$2.3^{a} \pm 0.01$
Brown	82.0	1.1	$3.6^{b} \pm 0.02$

Date represent the mean of triplicate values.
Values with the same uppercase superscript latter along the same column are not significantly different (*P*> 0.05).

RESULTS AND DISCUSSIONS

Plant foods, including grains play an important role (in recent years) in our diet as functional foods and nutraceuticals, since they are good sources of dietary fibre, proteins, energy, minerals, vitamins and antioxidants required for human health (charalampopoulos *et al.,*2002).

Table 1 shows the results of the proximate composition of the cereals; the crude protein content of the cereals is generally low when compared with legumes like soya bean (35%) and (cowpea) (22.7%) (Oshodi, 1993). However, brown sorghum had high content of protein (12.53%) followed by the white sorghum (11.12%), yellow maize (10.7 $^{o}/_{o}$) and white maize (9.6$^{o}/_{o}$) but, there was a significant decrease (P<0.05) in the values of both varieties of millet (yellow (5.3$^{o}/_{o}$) -brown (5.9$^{o}/_{o}$). Also, carbohydrate content ranged from 71.76% (yellow-millet) to 58.93% (brown-sorghum), which is an indication that cereals are good source of calories in human diets. The moisture contents of the cereals which ranges from [9.0 (yellow millet) – 11.5 (yellow maize)] fall within the range reported for varieties of cowpeas (Ukhun and Ifebigh, 1988; Aletor and Aladetimi, 1989) and this might be advantageous in terms of the self life of the products. However, the ash, crude fibres and crude fat contents are significantly decreased (P<0.05). The ash and crude fibre of the cereals were generally lower than that of cultivated yam tubers (Akindahunsi and oboh, 1998) and cassava products (Oboh and Akindahunsi, 2003. Oboh *et al.,* 2002). Nevertheless, the ash content of the cereals is within the same rage for the cereals grains reported by uwaegbute and Nnanyelugo (1989), and Fashakin (1989.)

The results of mineral composition of the various cereals expressed in mg/100g revealed that macro-elements content (Na, K, Ca and Mg) were significantly higher (P<0.05) than that of the micro-elements content (Fe, Pb, Ni, Zn and Cu) (table 2). However, maize (yellow and white) had the highest Ca and Na contents when compared with other cereals analyzed while sorghum (brown and white) had the highest Mg and Mn content. The Mg, K, Na and Ca of the cereals are within the same range with that of cassava products (Oboh and Akindahunsi, 2003).

The antinutrient (phytate) content of the cereals is generally low when compared to that of unfermented cassava product (Oboh and Akindahunsi, 2003). Phytate is a very stable and potent food component chelators and it is considered an antinutrient by virtue of its ability to chelate divalent metals and prevent their absorption from the stomach after consumption (Oboh *et al.*, 2003). Phytate is known to decrease the bioavalability of minerals in the the body, especially Ca, Zn, Fe and Mg. (Agte *et al.*, 1999, Oboh *et al.*, 2003., Bhandari and Kawabata, 2004). However, the effect of the phytate content on the zinc bioavailability in the cereals is shown in Table 4. [Ca][Phytate]/[Zn] ratio was found to be a measure of zinc bioavalability than [phytate]/[zn] ratio (Oboh and Amusan, 2009). The calculated [phytate]/ [Zn] molar ratio for the cereals were far above 15.0, which is considered as the critical value for reduced zinc bioavailability (Fergusson *et al.*, 1988), this suggests that the level of phytate present in the cereal could lead to reduction in bioavailability of Zn. Mineral, Protein and Starch solubility digestion and absorbtion was also reported to be affected by phytate (Umar, 2005). On the other hand, phytate is an anti-carcinogen that protects against colon cancer and it is known to be a potent antioxidant that inhibits Fenton reactions leading to lipid peroxidation and inhibition of polyphenol oxidase (Agte *et al.*, 1999). Also the calculated [Ca]/[Phytate] molar ratios for the cereals were well below 6. Wise (1983) has suggested that the solubility of phytate and proportion of zinc bound to the complex depend on the dietary Ca levels. In his model, phytate precipitation is not complete until dietary (Ca):(Phytate) molar ratios attain a value of approximately 6:1.

At lower ratios, phytate precipitation is incomplete, causing some dietary zinc to remain in solution (Oboh *et al.*, 2003). The proportion remaining in solution increases with decreasing Ca: phytate molar ratio. However, calculated [Ca][Phytate]/[Zn] molar ratio is a better index for predicting Zn bioavailability due to a kinetic synergism which exists between (Ca) and (Zn) ions resulting in a Ca : Zn : phytate complex, which is less soluble than the phytate complex formed by either ion alone (Oberleas, 1973). The calculated molar ratios for the various cereals were far above 0.5mol/kg; which is considered as the critical level for reduced Zn bioavailability (Davis and warrington, 1986; Oboh *et al.*, 2003). This reduced Zn bioavailability may be due to the fact that Zn present in the cereals are generally low and/or the Ca contents of the cereals are not high enough to create a sparing effect on Zn from the phytate. (Oboh *et al.*, 2003)

CONCLUSIONS

All varieties of cereals (maize, sorghum and millet) analyzed were good sources of nutrients, and also contained useful quantities of minerals which would contribute to the recommended dietary allowances (RDA) but were low in protein yet with substantial nutritional values, it could avert the case of malnutrition. However, high value of the phytate content in both varieties of all cereals analysed was an indication of the critical factor for the reduced Zn bioavalability.

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