Original Research Article

The anti-nutritional effect of phytate on zinc, iron and calcium bioavailabilities of some cereals staple foods in Zaria, Nigeria

ABSTRACT

Aim of Study: To evaluate the effect of phytate on the bioavailabilities of zinc, iron and calcium of some cereals staple foods in Zaria, Nigeria.

Study Design: Experimental

Place of Study: Department of Biochemistry, Faculty of Sciences, Ahmadu Bello University Zaria, Nigeria.

Methodology: *Tuwon surfafen masara* was prepared by first milling the processed maize. *Tuwon masara* and Pap were prepared using standard local preparation methods. Phytic acid content was determined according to the method described by Reddy **(mention year)**; The minerals were determined using atomic absorption spectrophotometry; AOAC 1990. Data was analysed with one way ANOVA and differences were considered significant at $P = .05$

Results: The ratios of Phytate:Ca, Phytate:Fe, Phytate:Zn for Tuwon surfafen masara were found to be 0.0026, 0.197 and 0.429 respectively. While that of Tuwon masara was 0.0044, 0.127 and 0.376 respectively. Accordingly pap showed a phytate to mineral ratios of 0.0025, 0.043 and 0.162 for Phytate:Ca, Phytate:Fe and Phytate:Zn respectively. The ratios of Phytate:Ca, Phytate:Fe and Phytate:Zn for local rice was found to be 0.0075, 0.110 and 0.625 respectively. While that of foreign rice was 0.0031, 0.046 and 0.266 respectively. The phytate to mineral ratios of all the staple foods in the present study falls below the critical values of >0.24, >1 and >18 for Phytate:ca, Phytate:Fe and Phytate:Zn respectively which indicate good bioavailability.

Conclusion: The result obtained showed that the bioavailability of Ca, Fe and Zn in Tuwon surfafen masara, tuwo, Pap, local rice and foreign rice in Zaria, Nigeria is not affected by their phytic acid contents.

Keywords: Bioavailability; Phytic acid; Calcium; Iron; Zinc

1. INTRODUCTION

Malnutrition results from imbalance between the body's needs and intake of nutrients which can lead to symptoms of deficiency, toxicity or obesity [1]. It includes moderate and severe malnutrition. Malnutrition can result from inadequate food intake, malabsorption, abnormal systemic loss of nutrients due to diarrhea, hemorrhage or renal failure, infection and addiction to drugs [2].

Anti-nutritional factors are compounds which reduce the nutritional utilization or bioavailability of nutrient in food substances and they play a vital role in determining the use of plants as human food and animals feed. Apart from the cyanogenic glycosides, food poisoning arising from other anti-nutritional factors has not been properly addressed in most part of the developing world [3]. People have died out of ignorance, poverty and inadequate nutritional information. There are reports from time to time of death after consuming some certain species of beans even after cooking. This is known to be due to the presence of toxic plants secondary metabolites also refer to as anti-nutritional factors [3]. It is well established that plants generally contain these compounds obtain from fertilizers, pesticides and several naturally occurring compounds [4]. Some of these plants secondary metabolites are known to be highly biologically active [5]. Among them is phytic acid whose effect on the bioavailability of some minerals is the main focus of the present study.

Phytic acid chelates metal ions, especially zinc, iron, and calcium, but not copper [6], forming insoluble complexes in the gastrointestinal tract that cannot be digested or absorbed in humans because of the absence of intestinal phytase enzymes [7], Phytate also complexes endogenously secreted minerals such as zinc [8] and calcium [9], making them unavailable for reabsorption into the body. At present, studies on the effect of phytate on the bioavailability of iron, zinc, and calcium on cereals staple foods commonly consumed in Zaria, Nigeria has not been reported. The aim of the present study is to investigate the possible anti-nutritional effect of phytate on these mineral elements. This will serve as indicator of the need either to include phytate-reducing local cereal processing method and/or fortification with minerals.

Minerals, classified as micronutrients are needed by our body in small amounts. Deficiency in minerals, however, can have a major impact on health such as anemia and osteoporosis that commonly occur in both developed and developing countries [10]. The deficiency of calcium result in abnormalities in the bones and teeth, subnormal growth, low milk and egg production, depressed appetite and fertility is also likely to be impaired. Studies in animals have clearly shown that iron deficiency has several negative effects on some important functions [11]. Physical working capacity in rats has been shown to be significantly reduced in iron deficiency which is especially valid for endurance activities [12, 13]. Result obtain from zinc supplementation studies showed that a low zinc status in children not only affects growth but is also associated with increased risk of severe infectious diseases [14]. Episode of acute diarrhea, respiratory tract infection and malaria has also been reported in severe zinc deficiency. Decrease in severity, duration, and reductions in incidence of diarrhea have been reported in a group of zinc supplemented children.

3. Materials and methods – Leave the space before mentioning of the units

3.1 Samples collection. The rice and maize used in this work were bought from Samaru market, Zaria.

3.2 Chemicals and reagents. Concentrated sulphuric acid ($H_2S0_{4)}$, concentrated nitric acid ($HN0_3$), perchloric acid ($HCl0_4$), ammonium thiocyanate (NH_4SCN), hydrochloric acid (HCl), Iron (iii) chloride ($FeCl_3$), and deionized water.

3.3 Sample Preparation. *Tuwon surfafen masara* was prepared by first milling the processed maize using a milling machine to produce maize flour, the maize flour was added to boiling water with continuous stirring until a thick paste is formed which was allowed to cool at room temperature for 3 hrs. *Tuwon masara* was prepared using the same method describe above only that the corn used in this case was not processed. Pap was prepared by first soaking the corn for 8 hrs and milled, the milled corn was filtered and the filtrate was collected in a container and allowed to stand for 24 hrs, the clear supernatant was discarded and the thick lower portion that settled was then added to boiling water with continuous stirring to form a thick paste (Pap). Local and foreign rice were washed and cooked at 100^oC for 45 min. About 200 g each of the food samples were oven dried and grinded to powder for laboratory analysis.

3.4 Determination of Phytate. Phytic acid content of food samples was determined according to the method described by Reddy et al. [15]; Exactly 40 g each of the powdered samples were soaked in 100 mL of 2% hydrochloric acid for 5 hrs and filtered. 25 mL of the filtrate was taken into a conical flask and 5 mL of 0.3% ammonium thiocynate (NH_4SCN) was added, the mixture was titrated with a standard solution of $FeCl_3$ (0.25M), until a brownish yellow colouration appear and persisted for 5 min. From the titre values obtained, phytic acid content was calculated from the relation; 1 mL of 0.25 M $FeCl_3$ = 6.606 mg of phytate according to the method of Reddy et al. [15].

3.5 Determination of Minerals (Ca, Zn and Fe). The minerals were determined using atomic absorption spectrophotometry [16].

3.5.1 Digestion of Samples. Exactly 500 mg each of the powdered samples were digested with 10 mL Conc. HNO_3, 4 mL of 60% $HCl0_4$, and 1 mL of Conc. H_2S0_4. After cooling each of the digest was diluted with 50 mL of deionized water. It was then filtered and the filtrate was made up to 100 mL in a glass volumetric flask with deionized water [16]. All the minerals were determined from the triple acid-digested samples by atomic absorption spectrophotometer (AAS). The AAS analysis was carried out in NARICT Zaria, Nigeria and Central Laboratories Complex Bayero University Kano, Nigeria. Exactly 1 mL each of the digested samples was analysed using AAS for the various elements mentioned, and their respective absorbance recorded. The various concentrations in part per million (ppm) of the minerals was extrapolated from their standard calibration curves.

3.5.2 Preparation of Standard Curve for Various Elements. A stock solution of calcium was prepared by dissolving 2.49 g of dried calcium carbonate in 1000 mL volumetric flask and the solution made up to the mark with distilled water. This solution contained 1000 ppm Ca^{2+}. From this stock solution, calcium standard solutions were prepared and their absorption was taken, a standard curve of absorbance against concentration for calcium was plotted. A stock solution containing 1000 ppm of Fe^{2+} was prepared by dissolving 1 g of pure iron wire in 10 mL of concentrated nitric acid, boiled in a water bath and diluted to 1000 mL. Standard solutions were prepared and absorption taken and a standard curve for iron were plotted. Similarly, stock solution containing 1000 ppm of Zn was prepared by dissolving 2.0 g of $ZnCl_2$ in 1000 mL volumetric flask and the volume was made up to the mark. Standard solutions were prepared and absorptions readings were taken and a standard curve of Zn was plotted.

3.6 Statistical analysis. The data obtained were expressed as means ± SD and analyzed with SPSS version 17. One way analysis of variance (ANOVA) with subsequent Bonfferoni's *post hoc* test was carried out. Differences were considered significant at $p \leq 0.05$. Data was presented as tables as appropriate.

4. Results and Discussion –leave the space before mentioning of the unit

The term "bioavailability" is defined as the proportion of an ingested nutrient in food that is absorbed and utilized through normal metabolic pathways [17]. Environmental conditions (climate, soil, and irrigation), fertilizer applications, and stage of maturation influence the phytate content of cereals, legumes, and oleaginous seeds [18]. Hence the need to determine the phytic acid levels of food consumed in a specific region. Many techniques are used to determine the bioavailability of minerals in foods with respect to the anti-nutritional effect of phytic acid, one of the methods is by measuring the mole ratio of phytate to minerals in the food samples [19]. Foods with ratios above the critical levels have a poor bioavailability for the mineral. The result obtained in the present study showed that the phytic acid content of local rice (9.90 mg/100g) was greater than all the other foods analysed, Pap has the lowest phytic acid content of (2.76 mg100/g) (Table 1)

Table 1: Phytate and mineral contents of commonly consumed staple cereal foods in Zaria, Nigeria

Samples	Phytate and mineral elements (mg/100g of sample)			
	Phytate	**Calcium**	**Iron**	**Zinc**
A	4.40 ± 0.95^{d}	100.70 ± 2.49^{cd}	1.90 ± 0.41^{d}	1.02 ± 0.00^{bcd}
B	6.05 ± 0.95^{ac}	83.68 ± 2.43^{e}	4.04 ± 0.41	1.58 ± 0.20^{ae}
C	2.76 ± 0.95^{ade}	66.66 ± 2.45^{ae}	5.46 ± 0.40	1.68 ± 0.00^{ae}
D	9.90 ± 1.65^{abce}	79.42 ± 2.46^{ae}	7.60 ± 0.41^{a}	1.56 ± 2.10^{ae}
E	5.50 ± 0.95^{cd}	105.00 ± 22.50^{bcd}	10.00 ± 0.31^{a}	2.04 ± 0.00^{abcd}

Data presented are means ± SD and analysed using one way ANOVA followed by Bonfferoni's *post hoc* test. Values with the same superscript to that of the samples differ significantly at (P = .05) along a column. Sample A; Tuwon surfefen Masara, Sample B; Tuwon masara, Sample C ; Pap, Sample D; Local rice, and Sample E; Foreign Rice

According to Gibson et al. [20] soaking is one of the food preparation methods that can reduce phytic acid content. This could be the reason why Pap has the lowest phytic acid content (its preparation involves soaking of the maize for 8 hour). According to Gibson et al. [20], unrefined cereals and legumes have a high content of phytate, a potent inhibitor of mineral absorption compared to their refined counterpart. This is most likely the reason why Tuwon masara and local rice (unrefined) demonstrate significant (P = .05) high phytic acid content compared to Tuwon surfafen masara and foreign rice (refined) respectively (Table 1). In general the phytic acid levels obtained in the present study are lower than those previously reported for cereal foods [21, 22], this could be attributed to differences in geographical locations [18] and food processing methods [20]. Foreign rice showed the highest levels of calcium, iron and zinc; 105, 10 and 2.04 mg/100g respectively (Table 1). The levels of calcium are lowest in Pap (66.66 mg/100g) similarly; Tuwon Surfafen Masara records the lowest levels of iron (1.90 mg/100g) and zinc (1.02 mg/100g). The low levels of these minerals demonstrated by Pap and Tuwon surfafen Masara could be attributed to the food processing methods in their preparation which has to do with soaking and refinement respectively. The highest ratio for phytate:ca was demonstrated by Local rice (0.0075) and the lowest ratio was demonstrated by Pap (0.0025) (Table 2).

Table 2: Phytate and minerals mole ratios of commonly consumed staple cereal foods in Zaria, Nigeria

Sample	Phytate:Ca	Phytate:Fe	Phytate:Zn
Tuwon surfafen masara	0.0026	0.197	0.429
Tuwon masara	0.0044	0.127	0.376
Pap	0.0025	0.043	0.162
Local Rice	0.0075	0.110	0.625
Foreign Rice	0.0031	0.046	0.266

According to Emeta et al. [22] phytate:calcium molar ratios less than 0.17 are indicators of good bioavailablity. The phytate:ca mole ratios obtained are far lower than the critical levels of 0.17 which showed that the bioavailability of calcium is not affected by phytic acid in the food samples analysed. According to Hurrell [23], phytate:Fe mole ratios less than one (1) are desirable for iron bioavailabilty. The result in table 2 showed phytate:Fe ratios of less than 1 for all the food samples with the highest ratio (0.197) demonstrated by Tuwon surfafen masara. Accordingly, this result showed that the bioavailability of iron is not affected by phytic acid. Similar result was obtained for the phytate:zn ratio which were all below the critical levels of 18 according to the classification of Hotz and Brown [24]. Local rice showed the highest levels of Phytate:zn of 0.625

5. Conclusion

All the food samples analysed showed a phytate to mineral mole ratios below the critical values and therefore the bioavailability of Ca, Fe and Zn is assured. The phytate content of the food samples is low this may be due to the effect of cooking and other processing during food preparation. The consumption of these cereal foods: Pap, *Tuwon masara*, *Tuwon surfaffen masara*, local rice and foreign rice in Zaria, Nigeria will not result in the deficiency of Ca, Zn and Fe in the body with respect to the anti-nutritional effect of phytate

Recommendations

Further work should be carried out to evaluate the effect of other anti-nutrient such as oxalate, tannins, saponins and metal chelators on the bioavailabities of Ca, Zn and Fe in cereal staple foods consumed in Zaria, Nigeria.

References

[1] Akintayo l, Adeyeye EI, Arogundade LA, Akintayo ET, Aisida OA, and Alao, PA. Calcium, zinc and phytate interrelationships in some foods of major consumption in Nigeria. Food Chemistry. 2000; 71(1): 435-441.

[2] Davidsson L. Approaches to Improve Iron Bioavailability from Complementary Foods. The Journal of Nutrition. 2003; 133(1): 1560S-1562S. 10.1093/jn/133.5.1560S.

[3] Soetan KO, Oyewole OE. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds. A review. African Journal of Food Science. 2009; 3(9): 223-231.

[4 Lestienne I, Icard-Verniere C, Mouquet C, Picq C. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. Food Chemistry. 2005; 89(3): 421- 425.

[5] Oberleas D, Harland BF. Phytate content of foods: effect on dietary zinc bioavailability. Journal of the American Dietetic Association. 1981; 79(4): 433-436.

[6] Egli I, Davidsson L, Zeder C, Walczyk T, Hurrell R. Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults. The Journal of Nutrition. 2004; 134(5):1077-1080.

[7] Iqbal TH, Lewis KO, Cooper BT. Phytase activity in the human and rat small intestine. Gut. 1994; 35 (9):1233–1236.

[8] Sandström B. Bioavailability of zinc. European Journal of Clinical Nutrition. 1997; 51 (Suppl 1): 117-119.

[9] Morris ER, Ellis R. Bioavailability of dietary calcium. In: Kies, C, ed. Nutritional bioavailabilty of calcium. Washington DC: American Chemical Society, 1985; 63–72. DOI:10.1021/bk-1985-0275.ch006

[10] Paul M, Insel R, Elaine Turner, Don Ross Jones, Bartlett L, 2004 - Nutrition – pg 644

[11] Gaël N, Myriam B, Arlette P, Sandrine M, Carole B, Bernard G, Mario S, Michèle S, Axel K, and Sophie V. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proceedings of the National Academy of Sciences. 2002; 99(7): 4596-4601; https://doi.org/10.1073/pnas.072632499

[12] Edgerton VR, Bryant SL, Gillespie CA, Gardner GW. Iron deficiency anemia and physical performance and activity of rats. The Journal of Nutrition. 1972; 102(3): 381-99.

[13] Finch CA, Miller LR, Inamdar AR, Person R, Seiler K, Mackler B. Iron deficiency in the rat. Physiological and biochemical studies of muscle dysfunction. The Journal of Clinical Investigation. 1976; 58(2): 447-53.

[14] Robert EB. Zinc Deficiency, Immune Function, and Morbidity and Mortality from Infectious Disease among Children in Developing Countries. 2001; 22(2): 155-160.

[15] Reddy NR, Sathe SK, and Salunkhe DK. Phytates in Legumes and Cereals, Advances in Food Research. 1982; 28(1): 1–92.

[16] AOAC. Official methods of analysis (15th ed.) 1990: Washington, DC: Association of Official Analytical Chemists (Method 925.10).

[17] Hurrell RF. Bioavailability a time for reflection. International Journal of Vitamin and Nutrition Research 2002; 72(1): 5–6.

[18] Reddy NR. Occurrence, distribution, content and dietary intake of phytate. In: Reddy, NR, Sathe, SK, eds. Food phytates. London: CRC Press, 2002: 30–32.

[19] Morris ER, and Ellis R. Usefulness of the dietary phytic acid/zinc molar ratio as an index of zinc bioavailability in rat and humans. Biological Trace Element Resesearch 1989; 19(1-2): 107-117.

[20] Gibson RS, Perlas LA, Hotz C. Improving the bioavailability of nutrients in plant foods at the household level. Proceedings of the Nutrition Society. 2006; 65(2):160–168.

[21] Gibson RS, Bailey KB, Gibbs M, Ferguson EL. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. Food and Nutrition Bulletin. 2010; 31(2 Suppl): 134-146.

[22] Umeta M, West CE, Fufa H. Content of zinc, iron, calcium and their absorption inhibitors in food commonly consumed in Ethiopia. Journal of Food Composition and Anaysis 2005; 18(1):803–817

[23] Hurrell RF. Phytic acid degradation as a means of improving iron absorption. International Journal of Vitamin and Nutrition Research. 2004; 74(6): 445–452

[24] Hotz C, Brown KH, eds. International Zinc Nutrition Consultative Group (IZiNCG) Technical Document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food and Nutrition Bulletin 2004; 25(1 Supp 2):199-203.