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RESEARCH ARTICLE

THE EFFECT OF INCLUSION OF CABBAGE AND POTATOES IN THE MAIZE AND BEANS COMPOSITE MEAL ON THE BIOAVAILABILITY OF IRON AND ZINC BY IN-*VITRO* METHOD

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ABSTRACT

The extent of bioavailability of iron and zinc from meals is of great health concern since these micronutrients play key role in mental performance and immune response in human beings. In this study the bioavailability of iron and zinc from maize and bean composite was investigated and found to at 13% for iron and 27.7% for zinc. The inclusion of cabbage enhanced the bioavailability of both to 98.8% and 83.9% respectively to the extent that the differences in the concentrations of iron and zinc in the non-digested and digested samples were not significant (iron, $p=0.12$: Zinc, $p=0.263$). There was a significant difference between concentration of iron and Zinc in the basic mixture when non-digested and digested were compared with $p= 0.004$ for iron and 0.012 for zinc respectively. In the samples where cabbage was included, there were no significant differences in the concentrations of iron and zinc between the non-digested and digested (iron, $p=0.12$, zinc, $p=0.263$) an indication of high bioavailability. The inclusion of potatoes reduced the bioavailability of iron and zinc to 76.86 % and 38.9% respectively. This is due to matrix effect where the potato introduces stronger physico-chemical interactions. The composite with maize, beans, cabbage and potatoes have a bioavailability of 76.2%, $p= 0.249$ for iron and 71.76%, $p = 0.405$ for zinc). This is an indication that cabbage has a higher enhancing effect than the potatoes. The generally high bioavailability of iron and zinc from a composite of beans and maize can be due to the interaction between the metal ions and tridentate amino acids in the beans where the chelate formed enhances their bioavailability. This interaction increases mucosal membrane permeability and lipo-solubility of the metal ions. The recommendation is that dietitians should educate people on best combination of foods that will enhance the bioavailability of not only iron and zinc but all micronutrients since they are of health importance to pregnant and lactating mothers, young children, adolescents and the elderly. Other alternatives include double fortification of salt with iron and zinc and bio-fortification.

INTRODUCTION

The sub clinical malnutrition may go un-noticed due to lack of physical manifestation in terms of observable clinical conditions. This condition is more prevalent in populations where the types of food eaten have low levels of micronutrients accompanied by low bioavailability of the micronutrients. Iron deficiency affects a large percentage of women and children in Kenya. Moderate to severe anemia is higher among pregnant women while older children, adult men and the elderly have lower but significant burden of mild anemia estimated at 20% (GOK, 1999). In the severe deficiencies in individuals, this may lead to reduced capacity for physical work due to inadequate oxidative mechanisms, depressed immune status, low birth weights, pre-natal mortality and obstetric complications, poor memory, poor learning and low attention span. The amount of iron absorbed from food in the intestinal tract is governed by food mixture among other factors (Madhavan, 2009).

In a healthy adult there is a general absorption of 5 to 10% from foods but this varies greatly depending on the type or nature of food eaten. The harem iron is absorbed at about 10 to 30 % while from plant food it is between 2 to 20 %. The presence of high quality protein, ascorbic acid, vitamin A, E and B complex enhance absorption of iron (Sharma, 2003). Zinc is found in a wide variety of foods especially nuts, seeds, legumes and whole cereals. However, the presence of inhibitors especially present in legumes (Sahequillo *et al.,* 2003) reduces its bioavailability. In Kenya high risk for zinc deficiency is at about 50% in children, men and mothers (GOK, 1999). Zinc deficiency can be characterized by delayed sexual development, short stature, anemia, enlarged liver and spleen and abnormalities in the skeletal maturation (Ploysangan, 1997). Degenerative changes associated with aging may be due to zinc deficiency. The absorption of zinc in the intestine can be reduced by as much as 10 fold where the is physico-chemical interactions and other dietary factors. Sub-clinical deficiencies of iron and zinc may be due to inadequate

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intake and large levels of inhibitors in the foods eaten (Donangel *et al.,* 2003).

Causes of Low Iron and Zinc Bioavailability from composite foods

A composite of maize and beans is a common food in Kenya, where it either just boiled or fried with addition of cabbage and/or potatoes. The bioavailability of iron and zinc from this type of composite is very poor and depends on a variety of factors (Castermiller and West, 1998).

(i) **Species of Compound**:This looks at nature of food components and their effect on bioavailability of the micronutrient. It includes the levels of phytates, polyphenols and Maillard reaction products which chelate the iron and zinc and reduces bioavailability (Lonnedal, 2000).
(ii) **Linkage:** The existence of iron and zinc as complexes in foods , controls rate and extent of hydrolysis by the intestinal enzymes and hence their bio-availability.
(iii) **Matrix:** The different types of components in the food composite forms a food base which may be a balance of enhancers and inhibitors. Such matrices can modify the bioavailability of the micronutrients.
(iv) **Enhancers of absorption:**These include ascorbic acid which enhance non-haem iron and zinc absorption due to antioxidant properties which convert ferric to ferrous iron which is more absorbable in the stomach lumen.

The interactions between the elements can lead to reduced bioavailability (Whiting, 1995). In the human body, the levels of existing micronutrients determine the absorption of the micronutrients from foods eaten (Turnlund *et al.,* 1990 and McKenna *et al.,* 1997). The low bioavailability of micro nutrients from legumes and cereals (Reddy *et al.,* 2000 and Gillooly *et al.,* 1983), can be improved by the addition of vegetables of the *Brassica* family to the composite meal. This class of vegetables have considerable amount of ascorbic acid which is an enhancer of bioavailability (Hallberg *et al.,* 1989) – (37g of cabbage provides about 50mg of ascorbic acid). Other method of enhancing levels of micronutrients in food is bio-fortification of the food crops (Welch, 2002 and Welch 2004). The technique of bio-fortification is a way of making available for absorption higher levels of the micronutrient, which would otherwise be low under normal circumstances (Torar *et al.,* 2005 and Hunt *et al.,* 2003). The physiology of the individual, male or female affects the extent of absorption of the various micronutrients from the foods eaten, so higher levels may not necessarily translate to higher bioavailability (Hunt *et al.,* 2000).

Bioavailability Methods

There are several models which have been used for the determination of iron and zinc bioavailability from foods. The Monsel model (Monsen *et al.,* 1978) requires the knowledge of the individual's iron and zinc stores in the body which is estimated from plasma ferritin and haemoglobin concentrations. Its limitation is that it does not take into consideration the inhibitory effect of the dietary ligands like phytate, tannin, caffeine e.tc. The FAO/WHO model (Hallberg, 1983) is an estimation based on the absorption of iron from typical meals. The meals are classified as low bioavailability, intermediate bioavailability and high bioavailability diets and it has correction factors for bioavailability due to levels of inhibitors. The Murphy model is a combined adaptation of the Monsen and FAO/WHO models. It calculates bioavailability for the entire day of food intake rather than from each individual meal. It has been used in Kenya successfully (Murphy, 2007). The artificial gut ($CaCo^{-2}$ cells) is an in-vitro laboratory model which simulates digestion in a human gut. The radioisotope methods use radio labeled isotopes of iron and zinc and can be done in-vivo and in-vitro with very high degree of accuracy (Turnlund, 2006). The in-vitro method adopted for this work simulates the human gut in a test tube. It is very efficient and effective but very simple (Svernberg *et al.,* 1998).

MATERIALS AND METHODS

Sampling and Sample preparation

The food samples were purchased from the local market based on the market measure (2Kg) except for cabbage which was bought as a head. The foods included Maize, Kidney Beans, Potatoes and Cabbage. 2Kg of each was bought from different lots in the market and mixed to get a composite sample from which the required weight for cooking(250g) was obtained. Mettler TC2000 (0.01g) balance was used in weighing and Non-stick Aluminium pans were used for cooking. The samples were ground using a Moulinex stainless steel grinder. The basic mixture consisted of 500g of maize and 250g of beans. This was boiled in deionised water till soft then dried in an air cooled oven at 150^{0}C for 12 hours to constant weight. The dried samples were stored in polythene bags. The other two composites included 250g each of cabbage and potatoes.

In total, there were four composite sets:

(i) Maize+beans mixture
(ii) Maize+beans+cabbage mixture
(iii) Maize+beans+potatoes mixture
(iv) Maize+beans+cabbage+potatoes mixture

A total of 15 samples were analyzed for each mixture.

Determination of Iron and Zinc

(i) Total Iron and Zinc 2.5g samples of dried composite from each of the four sets was mixed with 30ml deionised water, 2ml concentrated hydrochloric acid and 8ml concentrated nitric acid and digested for 3hours under reflux. This was then diluted to 50ml with deionised water and iron and zinc determined by atomic absorption spectrophotometer (AAS) using AOAC protocol (AOAC, 1998). The calibration curves were prepared using spectrosol standards.

(ii) Bio-available Iron and Zinc 2.5g samples were digested with pepsin solution containing physiological amounts of sodium, potassium, calcium, magnesium and phosphate for at 37^{0}C for 90 minutes. The pH was then adjusted to 5 for pancreatic digestion for another 30 minutes according to the method of (Svanberg, *et al.,* 1993). The supernatant was filtered through 45 µm filters and soluble iron and zinc determined by AAS using AOAC protocol. The calibration curves were prepared using spectrosol standards.

Statistical Analysis

The amount of iron and zinc in the none and digested composites were compared in order to determine the extent of bioavailability of the micronutrients. A total of 15 samples were analysed for each of iron and zinc. A two tailed t-test for the differences in the mean concentrations of iron and zinc in the non-digested and the digested samples. A p = value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 and 2 show the mean concentrations of iron and zinc in the non digested and digested composites. The statistical analysis show that there is a significant difference between the total concentrations of iron (p=0.004) and zinc (p= 0.012) in the basic mixture of maize and beans and the bio-available. This implies that more iron and zinc are retained in the food and not absorbed into the lumen (non-bioavailable). Beans contain phytates and polyphenols which are inhibitors (Glahn *et al*., 2005). In the basic mixture with cabbage, there is no significant difference between the digested and the undigested composite. The iron has a p = 0.12 and zinc p = 0.263.The basic mixture with potatoes has an iron p = 0.089 and zin p = 0.005, this indicates no significant difference in iron but significant difference in zinc levels of undigested and the digested mixture. In the basic mixture with potatoes and cabbage, the p = 0.249 for iron and 0.405 for zinc indicating no significant difference between the undigested and the digested mixture.

89% for iron and 56% for zinc. When both cabbage and potatoes were included, the bio-availabilities increased for iron to 76.2% and for zinc to 71.76%. This could be due the molecular bonds zinc forms with the matrix and also other interactions within the matrix that are different from those of iron. Zinc and iron are both first row transition metals but zinc has fixed oxidation state and hence bond formed is stronger to enzyme hydrolysis than that of iron which is capable of variable oxidation state. The bioavailability is also affected by amino acid-iron interaction with arginine, cysteine, histidine and lysine which may enhance bioavailability due to formation of tridentate chelates which are generally more bioavailable (Martinez-Torres *et al.,* 1970 and Vijayalashmi *et al.,* 2008). The other factor that influences bio-availability is the redox potential of the chelate formed. A valence change due to chelation alters the redox potential leading to change in membrane permeability and lipo-solubility (Smith, 1988). The composite meal of maize, beans, cabbage and potatoes although seems to be good for general micronutrient bioavailability due to the presence of large amount of ascorbic acid that is an enhancer of bioavailability, is still very poor in iron and zinc and may not meet the iron RDA requirements for women (15mg/day) and men (10mg/day)and zinc RDA requirements for women (8mg/day) and men (11mg/day) according to WHO guidelines. This can lead to anemia if no other iron sources are eaten. The addition of small amount of meat would improve the iron content and bioavailability (Engle-stone, 2005). The zinc content of the meal could be increased by inclusion of organ meats and nuts. To able to get a clearer picture, it is necessary to determine the levels of phytate, tannins, oxalates and fiber present in the composite meal blends. The best solution under the prevailing circumstances is dietary diversity.

Table 1. Iron (Fe) content in the composite foods

n = 15

	Sample	Feμg/100g (Total Fe) Non-digested	Fe μg/100g (Bioavailable Fe*) Digested	Bioavailability	p-value
1	Basic Mixture	1.290 ±0.21	0.167±0.03	13.0%	0.004
2	Basic Mixture + Cabbage	1.315±0.26	1.299± 0.25	98. 8%	0.12
3	Basic Mixture+Potatoes	1.301±0.27	1.000±0.29	76.86%	0.089
4	Basic Mixture+ Cabagge+Potatoes	1.322±0.23	1.008±0.34	76.2%	0.249

Table 2. Zinc (Zn) content in the composite foods

n = 15

	Sample	Feμg/100g (Total Zn) Non-digested	Fe μg/100g (Bioavailable Zn*) Digested	Bioavailability	p-value
1	Basic Mixture	1.867 ±0.32	0.518±0.04	27.7%	0.012
2	Basic Mixture + Cabbage	4.444±0.30	3.732± 0.3	83.9%	0.263
3	Basic Mixture+Potatoes	5.040 ±0.27	1.962±0.03	38.9 %	0.005
4	Basic Mixture+ Cabagge+Potatoes	7.476±0.04	0.169±0.044	71.76%	0.405

Conclusion

The bioavailability of micronutrients from composite foods is complex since there is interplay of several factors (Castermiller and West, 1998): Species of compound, molecular linkage, matrix in which the nutrient is incorporated, effectors of absorption and bioconversion and micronutrient interaction algorithms. All these factors may interact to either enhance or inhibit the bioavailability of the micronutrients. It was found that the bioavailability of iron in the composite is 13% and that of zinc 27.7% but on addition of cabbage, it increased to 76.86% for iron and 38.9% for zinc. When potatoes were added, the bio-availabilities reduce to

The higher the dietary diversity index or score of an individual, the more likely the individual will meet his or her iron and zinc requirements.

Recommendations

Sub optimal iron and zinc status in pregnant and lactating women, young children, adolescents and the elderly is of concern. Deficiency of iron and zinc can be eradicated by reducing the levels of dietary factors which reduce their bioavailability. This can be done through appropriate food processing methods like germination or fermentation of cereals and legumes and incorporating ascorbic acid rich foods like *Brassica* vegetables and tomatoes in the composite meals of maize and beans. The low levels of iron and zinc in the basic maize and beans mixture implies that the composite is not able to meet at least 50% of RDA for the elements iron(5.0 to 7.5mg/day) and zinc (4.0 to 5.5mg/day) for both men and women. Hence there is need to enhance the levels of both iron and zinc in the composite meal. This can be through the use of iron and zinc fortified cooking salt- double fortified salt.

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