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Bioaccessibility and risk assessment of essential and non-essential elements in vegetables commonly consumed in Swaziland

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ABSTRACT

The green leafy vegetables (*Mormodica involucrate, Bidens pilosa* and *Amaranthus spinosus)* are economic; seasonal; locally grown and easily available; easy to propagate and store; highly nutritious food substances that form an important component of diets. This study applies a physiology based extraction technique (PBET) to mimic digestion of these vegetables to determine the fraction of essential (Fe and Zn) and non-essential elements (Cd, Cr and Pb) that are made available for absorption after ingestion. Prior to the application of the PBET, the vegetables were cooked adopting indigenous Swazi cooking methods. Cooking mobilized most of the metals out of the vegetable mass, and the final substrate concentrations are: raw > cooked > supernatant for all the metals, and the order of average metal leaching was: Pb (82.2%) > Cr (70.6%) > Zn (67.5%) > Fe (60.2%) > Cd (53.6%). This meant that the bioavailable concentrations are significantly lower than in the original vegetable mass, if only the solid mass is consumed. Bioaccessibility was higher in the gastric tract than in the intestinal phases of the PBET for all the metals in all the vegetables. Risk assessment protocols employed on the non-essential elements (Cr, Cd and Pb) showed that the associated risks of ingesting metal contaminated vegetables are higher for children, than they are for adults, based on the target hazard quotient (THQ) index. However, the overall health risk associated with ingestion of these metals is low, for both children and adults, based on the HR index. Conclusively, this study expounds on the nutritional and risk benefits associated with ingesting naturally grown vegetables.

1. Introduction

Emerging health issues and general human wellness have a direct relationship to the quality of the food we eat. Amongst the many dietary requirements available, there are approximately 40 vitamins and minerals that are considered essential for physical and mental development, immune system development and metabolic processes in the human body (Kennedy et al., 2003). Some of these could be easily found in numerous traditional foods cultivated, particularly at the community level. Nonetheless, due to drastic changes in weather and climatic conditions coupled with erratic world economies, food production has dwindled over the years. This has resulted in reported malnutrition cases the world over, especially in developing countries. According to the Food and Agriculture Organisation (FAO), an estimated 3.7 million deaths are attributed to underweight in children, and a further 750,000–800,000 deaths due to deficiencies in iron (Fe), zinc (Zn) or vitamin A (Marshall, 2004).

Prevalence of HIV and related infections have intensified research into the provision of such necessary nutrients to the human body, for example through supplementation, fortification and dietary modifications (Kennedy et al., 2003). As a result, the pharmaceutical industry has made significant economic gains through the production of micronutrient supplements (Johns, 2003). This approach, unfortunately, has consequent challenges on food security, increases incidences of malnutrition due to associated costs, and further increases the cost of living that is already not affordable to a larger population in developing countries.

Therefore, different approaches have been proposed to counter this challenge, some of which have been applied with some degree of success. For instance, indigenous products, especially vegetables, have recently been advocated as good alternatives (Johns, 2003) since they are easily available; economic; have high nutritional values; easy to propagate and store; and have been used for generations without any reported side effects (Marshall, 2004).

However, the reported nutritional values of these indigenous vegetables may be exaggerated, and hence there is a need to further

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expand on the question of how much of the total nutritional content is available at the cellular level. This fraction is the bioaccessible component and according to Dean (2010), bioaccessibility refers to the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption. It is therefore not sufficient to report dietary values compared against Recommended Daily Intake (RDI) values, but also a determination of the bioaccessibility of vitamins and minerals in vegetables is critical before valid conclusions can be drawn on the nutritional status of each food package, or the associated risks thereof.

This study therefore investigates nutrient bioaccessibility in three indigenous vegetables commonly consumed in Swaziland namely; Bitter gourd (*Mormodica involucrate*), Tassel flower (*Amaranthus spinosus*) and Blackjack (*Bidens pilosa)*. They grow in the wild and usually as a weed in farms. The essential, as well as the non-essential elements may be present either as deposits on the surface of vegetables, or may be taken up by the crop roots and incorporated into the edible parts of plant tissues (Li et al., 2015). Their concentrations in the plant not only depend on the texture of the soil medium but also on the type and nature of the plant (Itanna, 2002). Consequently, leafy vegetables can accumulate much higher contents of some essential elements than other vegetables. The Physiology Based Extraction Technique (PBET) was used to determine the bioaccessible component of essential (Fe and Zn) and non-essential elements (Cd, Pb and Cr) in the selected vegetables. Preparation of the vegetables was based on the indigenous Swazi method of cooking vegetables, to minimize errors during sample preparations.

2. Experimental

2.1. Chemicals and apparatus

All chemicals and reagents were of analytical grade and were procured from Merck Chemicals (South Africa). Gastric solution was prepared by adding; 1.25 g pepsin, 0.5 g sodium malate, 0.5 g sodium citrate, 420 µL lactic acid and 500 µL acetic acid and made up to 1 L with de-ionised water, adjusting the pH to 2.5 with concentrated HCl (Intawongse and Dean, 2008).

2.2. Sampling

The indigenous common vegetables in this study are Bitter gourd (*Mormodica involucrate*), Tassel flower (*Amaranthus spinosus*) and Blackjack (*Bidens pilosa)*. Fresh samples were collected in the Middleveld region of Swaziland at Mfabantfu (25.5009°S, 31.3141°E), during the summer months (October).

2.3. Method of investigation

The investigation/study was divided into 3 sections. The first section determines the total concentrations of the elements before they are introduced into the simulated digestive system. The three samples were first cooked according to Section 2.3.1. The resultant residue and filtrate were analysed to determine total metal concentration (essential and non-essential) and the bioavailable component per sample.

The *in-vitro* PBET experiment consists of the other 2 sections - (1) a gastric sequential extraction, and (2) an intestinal simulated digestion, each carried out under simulated and controlled human digestion conditions (enzymes, pH and temperature) according to Intawongse and Dean (2008).

2.3.1. Sample preparation

Prior to analysis, vegetable samples were washed with de-ionised water to remove adhering dirt and then cooked according to traditional Swazi practice (Bwembya et al., 2007), as outlined forthwith: 200 g of the vegetable sample and 2 g salt were placed into a cast-iron three legged pot together with 200 mL of de-ionised water (DI) and allowed to boil for 20 min.

2.3.2. Determination of total elemental concentrations

2.3.2.1. Total metal analysis of uncooked samples. Sample digestion protocol was followed after Intawongse and Dean (2008). Prior to metal extraction, samples were dried at 70 °C until constant weight, and then pulverized. Triplicate samples of 1.00 g and 10 mL conc. HNO_3 were mixed together in digestion tubes. The mixture was heated to 95 °C on a heating block for 1 h. After cooling, 5 mL conc. H_2SO_4 was added and the mixture was heated to 140 °C until the onset of charring. After cooling, 5 mL conc. HNO_3 was added and heated to 180 °C. Further HNO_3 aliquots were added until the sample digest appeared clear. After cooling, 1 mL of 500 g/L H_2O_2 was added and the mixture heated to 200 °C until observed brown fumes disappeared. After cooling, 10 mL DI water and 0.5 mL conc. HNO_3 were added and heated until white fumes were observed. After cooling, 10 mL DI water and 1 mL 500 g/L H_2O_2 were added and heated to 240 °C until white fumes evolved from the solution. The digest was then cooled, filtered through a 0.45 µm glass fibre filter membrane, diluted to 100 mL volumetric flasks and analysed using atomic absorption spectrometry (AAS).

2.3.3. The Physiology-based extraction test (PBET)

2.3.3.1. The PBET in the Gastric Region. Extraction protocol was followed after Intawongse and Dean (2008). Triplicate samples of 0.5 g of the cooked, dry sample (oven-dried at 70 °C for 3 days) were placed into 50 mL screw-cap tubes and treated with 30 mL gastric solution. The mixture was then shaken at 100 rpm in a thermostatic bath maintained at 37 °C. After 1 h, the mixture was centrifuged at 3000 rpm for 10 min and a 5 mL aliquot taken and filtered through a 0.45 µm glass fibre membrane filter prior to AAS analysis. 5.0 mL of the gastric solution was backflushed into the sample container to retain the original solid: solution ratio.

2.3.3.2. The PBET in the intestinal region. 52.5 mg bile salts and 15 mg pancreatin were added to the tube, and the mixture adjusted to pH 7 with saturated $NaHCO_3$. The sample was then shaken at 100 rpm in a thermostatic bath maintained at 37 °C for 2 h. A 5 mL aliquot was removed and filtered. After a further 2 h another aliquot of 5 mL was obtained. This was used to check if small intestinal equilibrium was attained. The fractions obtained were analysed using a Varian Atomic Absorption Spectrometer (AAS).

2.3.4. Calculations of % bioaccessibility and risk assessment factors

Bioaccessibility measurements for Zn and Fe are reported as relative bioaccessibility expressed as a percentage and calculated per digestion according to the following equation (Oomen et al., 2002);

$$\%Bioaccessibility = \frac{metal solubilised from sample during digestion\,(\mu g)}{metal present in sample before digestion\,(\mu g)} x100\% \quad (1)$$

Risk assessment calculations are based on the total hazard quotient (THQ) and the health risk index (HRI) using reference dose values from US EPA (2000). These are represented by Eqs. (2) and (3), respectively (Zhuang et al., 2009):

$$THQ = \frac{CMxIRxEFxED}{BWxATxRfD} x10^{-3} \quad (2)$$

Where: *CM* is the concentration of the metal (mg/kg); *IR* is the ingestion rate (5 g/day); EF is the exposure frequency (180 days/year, assuming that these vegetables are only available half yearly, considering that other people store them for prolonged usage throughout the year); *ED* is the exposure duration (70 years, based on the current life expectancy); *BW* is the average body weight: 15 kg for children and 61.6 kg for adults (USEPA, 2000); *AT* is the averaging time (days/year); R_fD is the