

ASSESSMENT OF THE ESSENTIAL AND TOXIC ELEMENTS IN COMPLEMENTARY FOODS FOR UNDER FIVE CHILDREN IN TANZANIA USING EDXRF SPECTROSCOPY

Catherine Paschal and Najat K. Mohammed*

Department of Physics, P.O.Box 35063, University of Dar es Salaam,
Dar es Salaam, Tanzania. Tel. +255222410258

najat@udsm.ac.tz

ABSTRACT

In this study, the commonly used complementary foods (Unga wa Lishe) for children 0-5 years in Tanzania were analyzed for essential and toxic elements in order to assess their nutritional levels. 60 samples were purchased from shops in Dar es Salaam, Moshi and Arusha regions and analyzed using Energy Dispersive X-Ray Fluorescence Spectroscopy (EDXRF). The concentrations of the essential elements were referenced to the Recommended Nutrient Intake (RNI) values for 6-12 months old children while the levels of toxic elements were compared to the Maximum Tolerable Limits (MTL) set by WHO 2004. The nutrient intake for Fe, Zn, Cu and Ca obtained in the complementary foods were less than the RNI values of 11.6 mg/day, 8.3 mg/day, 0.56 mg/day and 500 mg/day respectively set by Joint FAO/WHO and Codex. On the other hand, the concentrations of the toxic elements Ni and As were higher than the MTL (0.2 µg/g and 11 µg/g, respectively) for these elements recommended by EC and WHO 2004, respectively. Further analysis of the complementary foods in Tanzania is needed to produce more data which will be a guide for the appropriate measures to reduce mineral malnutrition in Tanzania.

Keywords: Tanzania, complementary foods, children under five, mineral malnutrition, toxic elements

INTRODUCTION

Mineral deficiency is increasingly recognized as a prevalent and important nutrition problem in developing countries (WHO 2000, Nyaruhucha et al. 2006). Iron (Fe), Zinc (Zn) and Iodine (I) deficiencies are the most occurring mineral nutritional deficiencies in the world. UNICEF report of 2012 have shown that, 26%, of world children under five were stunted out of which 36% were from Africa; 16%, were underweight while 8%, were wasted (UNICEF 2012). Stunting which is defined as low height for age is taken as an indicator of chronic malnutrition whereas wasting which is low weight for height is a measure of acute malnutrition. The most recent report of UNICEF/WHO/WB 2015 indicated that more than one third of all stunted children under 5 in 2014 lived in Africa. In Tanzania,

the percentage of under five children suffering from severe wasting, wasting and stunting in 2014 are reported to be 0.9%, 3.8%, and 38%, respectively (UNICEF 2015).

Among the measures which have been devised to deal with the malnutrition in children is the use of complementary foods. This is recommended to begin at the age of six months when the child's birth weight is expected to double and breast milk is no longer sufficient to meet the nutritional needs of the growing child (WHO 2000, Monte and Gluglian 2004). FAO/WHO/UNICEF (1971) encouraged the use of local foods formulated at home as weaning foods for children above six months. According to UNICEF, the formulated foods must have high nutritional

value to supplement breastfeeding, must be accepted, be of low price, and should use local food items so that every mother can get access to them. Following this emphasis, many researchers in developing countries including Tanzania suggested formulae for complementary foods which are mixtures of legumes, cereals and sometimes with vegetables (Kimanya, et al. 2004, Mamiro et al. 2005, Lyimo et al. 2007, Muhimbula et al. 2011).

Many brands of complementary foods have been formulated and marketed by small scale industries all over the country. However, to the best of our knowledge, there is hardly any existing knowledge to guide quality assurance of the products. At the same time the formulae suggested by the scientists in Tanzania are said to be based mostly in eradicating Protein Energy Malnutrition (PEM) rather than mineral malnutrition (Mamiro et al. 2005, Muhimbula et al. 2011). This hypothesis is supported by the fact that statistics on stunting which is deficiency of Zn and anaemia which is a deficiency of Fe in Tanzania are still high (UNICEF 2015).

At the same time, the literature from elsewhere e.g. Meharg et al. 2008 and Ljung et al. 2011 suggest the existence of toxic elements in complementary foods for children. The toxic elements are introduced into the food through processing (Onianwa et al. 2001), methods used in farming or through food preparations (Ullah et al. 2010). At the same time, modernization of agriculture using chemical fertilizers and pesticides has been reported to create metal toxicity in staple foods (Pritchard et al. 2010). For instance, rice based food products intended for children in UK and Sweden were reported to contain concentrations of As above what is considered safe for consumption (Meharg et al. 2008, Ljung et al. 2011).

There is always a relationship between the uptakes of toxic elements with the essential elemental status in a body. Malnourished body usually absorbs more toxic elements than well nourished one. For instance, low dietary intake of Fe, Se and Ca may lead to more absorption of Pb and Cd in gastrointestinal track (Gordon 2003). Therefore it is always recommended that studies of nutrition should include reports on essential and toxic elements. This study has analyzed different brands of local made complementary food for children of age 0-5 years in Tanzania in order to assess their nutrition and toxic levels.

MATERIALS AND METHODS

Sample Collection

Complementary foods, *Unga wa Lishe*, samples were purchased from different shops in Dar es Salaam, Moshi and Arusha. Twenty samples were collected from each region making a total of sixty samples. The samples were selected by taking into consideration the popularity of the product in the local market. *Unga wa Lishe* from Dar es Salaam had a mixture of finger millet, sorghum, pearl millet, and soya protein; that from Moshi had maize, rice, finger millet, wheat, soy and groundnut while that from Arusha consisted of finger millet, maize, rice, groundnut and soya.

Sample preparation

In the laboratory, the *Unga wa Lishe* samples were dried overnight in an oven at a temperature of 50°C (Amour and Mohammed 2015). Since the samples were fine enough, they were sieved to obtain homogeneity with the same matrix as the reference material. 6 g of the flour from each sample were measured together with 1.35 g of binder and pulverized to obtain homogeneity. The samples were placed in the hydraulic pressing machine under a pressure of 20 tons to make the pellets. The pellets were of intermediate thickness ($m_{thin} < m < m_{thick}$) with the outer diameter of 32

mm. The pellets were finally placed in sample holders and inserted in EDXRF Spectroscopy machine for elemental analysis.

Quality assurance

The appropriate quality assurance procedures and precautions were taken to ensure the reliability of the results. Samples were carefully handled and sample holders

were properly cleaned. In order to verify the accuracy and precision of the proposed method, two Standard Reference Materials from the National Institute of Standards and Technology (NIST), SRM 1568a Rice Flour and SRM 1573a Tomato Leaves were analyzed with the samples and the results are as shown by Table 1.

Table 1: Plot of the experimental values against the certified values ($\mu\text{g/g}\pm\text{SEM}$) for two reference materials (SRM 1568a and SRM 1570a)

Element	Rice Flour (SRM 1568a)		Tomato Leaves (SRM 1573a)	
	Exp.	Cert.	Exp.	Reference.
Mg	540±11	560±20	11550	12000
Al			549	598
P	1529±28	1530±80		
Cl	245±1.2	300*		
K	1021±6	1280±8	25086	27000
Ca	119±1	118±6	49351	50500
Cr			1.44	1.99
Mn	18±0.5	20±1.6	275	246
Fe	8.0±0.5	7.4±0.9	309	368
Cu	2.2±0.1	2.4±0.3	4.17	4.7
Zn	15.3±0.1	19.4±0.5	28	30.9
As	0.3±0.03	0.29±0.03		
Br	6±0.5	8*		

* recommended value

Sample analysis

The elemental analyses of samples were conducted using a bench top energy dispersive X-ray spectrometer of Tanzania Atomic Energy Commission (TAEC) in Arusha. The machine which is operated by automated turbo-quant X-lab Pro™ software uses a 0.03 mm beryllium window X-ray tube with copper body anode and ceramic envelope with palladium target. The tube

was operated at a rate of 50 W and 50 kV voltage. The florescent X-rays were collected by a Si(Li) detector having a resolution (FWHM) at $\text{MnK}_\alpha \leq 160$ eV. A spectrum run for 20 minutes gave a good continuity statistics and resolution of the peaks.

Concentrations of elements in the samples were calculated by the inbuilt software

called X-lab ProTM with Turboquant (Tq 9232) algorithm for matrix effect correction (Schramm and Heckel 1998). The software corrects for the matrix effects (M_i) and the interference effects (K_i) basing on fundamental parameter methodology. The software corrects also for the background effect on a spectral line intensity (I_i), given as counts per second (cps). After all the corrections, the software converts the intensity into concentration of the element using Equation. (1) (Rousseau and Bouchard 2005).

$$C_i = K_i \times I_i \times M_a \quad (1)$$

where C_i is the concentration of a given element i ; M_a is the correction factor for matrix effects. K_i is the constant of proportionality; I_i is the intensity of the fluorescent radiation from the element i .

RESULTS AND DISCUSSION

The mean concentration values of elements obtained in *Unga wa lishe* from the three regions in Tanzania are presented in Table 2. The normality tests showed that 87 % of the elements in the overall data were not normally distributed. Only Mg and As

followed the normal distribution while the rest of the elements in the 60 samples followed neither normal nor lognormal distribution. Because of that, non-parametric tests were applied for statistical analysis of the data obtained in this work. The Kruskal-Wallis test was conducted to find the differences in means of the elements in the samples (significant value was taken as $p < 0.05$). As shown in Table 2, Cd was not detected in any of the samples while Pb was found in concentrations below MDL (0.6 $\mu\text{g/g}$) of the system used in this study. The Kruskal-Wallis test indicates that, except for As, the elemental mean concentrations differ significantly ($p < 0.05$) among the regions. The mean concentrations of As were similar in samples from all the three regions. Samples from Dar es Salaam had significantly ($p < 0.05$) higher mean concentration values of Mg, K, Ca, Mn, Fe, Ni, Cu and Zn than samples from the other two regions. The mean concentrations of P and Br were significantly ($p < 0.05$) highest in the samples produced in Moshi whereas samples from Arusha had highest mean concentrations of Cl and Se.

Table 2: Elemental composition ($\mu\text{g/g} \pm \text{SEM}$) of *Unga wa Lishe* samples from Dar es Salaam, Moshi and Arusha

Element	Dar es Salaam	Moshi	Arusha
Mg	1034.81±17.25	922.37±17.62	784.94±14.81
Al	161.70±3.20	78.00±9.80	56.20±9.00
P	2567.22±33.22	2922.73±28.28	2376.48±12.43
Cl	364.77±3.78	425.04±10.07	491.74±2.24
K	7149.62±145.92	4133.48±36.33	4158.14±22.37
Ca	1548.39±13.31	227.15±6.11	490.31±8.96
Mn	171.47±3.91	14.29±0.46	29.68±0.73
Fe	104.47±2.27	55.51±1.25	49.35±1.34
Ni	0.87±0.09	0.49±0.04	0.74±0.06
Cu	5.27±0.07	2.08±0.06	2.16±0.07
Zn	25.52±0.33	20.41±0.19	24.47±0.12

As	15.07±0.29	14.10±0.32	14.45±0.35
Se	0.14±0.01	0.28±0.03	0.33±0.04
Br	4.04±0.02	6.38±0.07	6.19±0.05
Cd	ND	ND	ND
Pb	< 0.3	< 0.3	< 0.3

ND: *Not Detected*

The difference of mean concentration of elements between the samples from the three regions could be attributed to the choice of food components and the preparation methods adopted by each region. It has been reported in the literature that, variation in whole grain contains of trace metals such as Fe, Mn, Mg, Cu, and Zn may be due to environmental condition such as whether during the cultivation, raining and levels of contamination (Demirozu and Saldamli 2002).

The mean concentrations of Fe in samples from all three regions were lower than those obtained by Mburu et al. (2012) and Obiajunwa et al. (2005) in children foods made from *Amaranth*, grain flour and Nigerian sesame seeds. On the other hand, the mean concentration of Zn, Cu and Ca obtained in this study were lower than the values obtained in complementary foods studied elsewhere (Compaoré et al. 2011, Ukegbu and Anyika 2012, Mburu et al. 2012, Ijarotimi and Keshinro 2013).

Breast milk can make a substantial contribution to the total nutrient intake of children between 6 and 24 months of age, particularly for protein and many of the vitamins. However, breast milk is relatively

low in minerals such as Fe and Zn, even after accounting for bioavailability (Dewey 2001). At 9-11 months of age, for example, the proportion of the Recommended Nutrient Intake (RNI) that needs to be supplied by complementary foods is 97% for Fe, 86% for Zn, 81% for P, 76% for Mg, and 72% for Ca (Dewey 2001). RNI is defined as the daily dietary intake of a nutrient sufficient to meet the requirements of nearly all apparently healthy individuals in a specific population group, usually by age and sex (WHO 2000).

Literature reviewed in this study showed that, in addition to consuming breast milk, a healthy six to seven month old African infant consumes approximately 65 g (dry weight) of complementary food per day (Fernandez et al. 2002, Solomon 2005, Onabanjo et al. 2009, Ukegbu and Anyika 2012). This value was used in this study to calculate (equation 2) (Cui et al. 2004) the contribution of the essential elements in the analyzed samples to RNI. The daily intake was then compared to the recommended value in Table 3.

Daily intake of metals (mg/day) = [65 g/day × sample metal concentration (mg/g)] (2)

Table 3: Amount (mg/day) of element that can be provided by the daily intake of 65g of *Unga wa Lishe* by children (6 – 12 months)

Element					% Contribution		
	Dar	Moshi	Arusha	RNI*	Dar	Moshi	Arusha
Fe	6.79	3.61	3.21	11.6	59	31	28
Cu	0.34	0.14	0.14	0.56	61	24	25
Zn	1.66	1.33	1.59	8.3	20	16	19
Ca	100.65	14.77	31.87	500	20	3	6
Se**	8.67	21.23	25.68	10	51	125	151

*: RNI for Older Infants and Young Children (Codex, 2013)

** : Concentration in $\mu\text{g/day}$

Table 3 shows the concentration of the essential elements with their Recommended Nutrient Intake (RNI). The concentrations are in mg/day except for Se which is a trace element recommended in $\mu\text{g/day}$. As Table 3 shows, the analyzed foods had concentration of Fe, which is essential element, in lower amounts than the recommended value of 11.6 mg/day set by Codex 2013. The lowest value is contributed by food samples from Arusha, which contributes to only 28% of the recommended value. Fe is necessary for production and functions of red blood cells and iron-containing enzymes. It is an essential part of haemoglobin (Hb), myoglobin and respiratory chain proteins, which are involved in the cellular respiration in mitochondria, catalyzing and participating in body energy production. As a result, Fe deficiency reduces a person's energetic efficiency and physical work capabilities (Haas and Brownlie 2001). Children with iron deficiency feel tired all the time, are less active, less attentive, less responsive and react poorly with their environment (Gordon 2003).

Reports have shown that Fe deficiency anaemia is still a problem in Tanzania. A study involving 448 under-five children in Mwanza revealed that 77.2% (346/448) of

the studied children had anaemia. Out of these 16.5%, 33% and 27.7% had mild, moderate and severe anaemia, respectively (Simbouranga et al. 2015). Iron Deficiency Anemia (IDA) can have an adverse effect on psychomotor and mental development in children, and increases the mortality and morbidity (Gibney et al. 2009).

The contributions to the daily intakes of Zn through the consumption of the foods analyzed in this study are lower than the RNI for Zn set by Codex 2013. As shown in Table 3 the contribution of Zn from *Unga wa Lishe* analyzed in this study is worse than the contribution of Fe. The maximum contribution of Zn is from samples prepared in Dar es Salaam which is only 20%. Zn is an essential mineral, vital to human growth and immune function (Shankar and Prasad 1998). A body Zn deficiency may result into a body being too susceptible to diseases. Worldwide, Zn deficiency is responsible for approximately 16% of lower respiratory tract infections, 18% of malaria and 10% of diarrhoeal disease (WHO 2002). Stunting in children, which is an indication of Zn deficiency, is still a concern in Tanzania. A recent report prepared jointly by UNICEF, WHO and World Bank (2015) reported that the percentage of children under five suffering from stunting in Tanzania is 38%.

The analyzed foods had concentration of the essential element Cu in lower amounts than the recommended value set by Codex 2013. Cu is an essential cofactor for oxidation-reduction reactions involving copper-containing oxidases. Cu enzymes regulate various physiologic pathways, such as energy production, iron metabolism, connective tissue maturation, and neurotransmission. In this study, the highest concentration of Cu was found in samples from Dar es Salaam which contributed to about 61% of the recommended value. Samples from Arusha and Moshi contribute to only 25% of the recommended value.

The contributions to the daily intakes of Se through the consumption of *Lishe* from Moshi and that from Arusha were higher than 100 percent of the RNI for Se set Codex 2013. Se has been reported to protect cell membranes from damage caused by the peroxidation of lipids, thereby decreasing the risk of cancer and disease of the heart and blood vessels (Kohrle et al. 2005). Se has also been described to decrease the effects of As, including clastogenicity, cytotoxicity, delayed mutagenesis and teratogenicity (Abernathy et al. 2003). This study shows that, the RNI for Se (17 µg/day) was met by *Lishe* from Moshi (21.23 µg/day) and Arusha (25.68 µg/day) by contributing 125% and 151% respectively.

Furthermore, the analyzed foods in this study had concentration of the essential element Ca in lower amounts than the recommended value set by Codex 2013. The highest contribution was that from the samples from Dar es Salaam which contributed to only 20% of the recommended value. Ca is a major structural element in bones and teeth (Heaney 2000). A chronically low calcium intake in the growing child may prevent the attainment of optimal peak bone mass. However, Ca can

be obtained in large quantities from the dairy products such as milk and yoghurt.

Table 4 compares the mean concentrations of toxic elements found in this study to the Maximum Tolerable Limits (MTL) set by International agencies. Ni and As were detected in all samples analyzed in this study. Ni has not been demonstrated to be essential for human body (EFSA 2006). The element has a property of replacing essential elements such as Zn and Cu in enzymes which inhibits their functions in the body. In this study, the mean concentration of Ni was found to be highest in the samples from Dar es Salaam than those from Moshi and Arusha but lower than that reported in maize grain in Rorya, Tanzania (Marwa et al. 2012). The mean concentrations of Ni in samples from all the three regions were higher than the Maximum Tolerable Limit (MTL) for Ni in food (0.2 µg/g) set by FAO/WHO 2001.

Arsenic (As) was reported in all samples analyzed in this study. Moreover, the values are higher than the Maximum Tolerable Limit for As of 11 µg/g set by WHO 2004. As has also been reported in childrens' foods elsewhere (Meharg et al. 2009, Signes-Pastor et al. 2008, Ljung et al. 2011). The presence of As in food is associated with the use of pesticides in farming and storing the crops (Abernathy et al. 2003). Early-life exposure to fairly low levels of inorganic As in drinking water has been associated with increased infant morbidity and mortality, as well as impairment of child development (Rahman et al. 2009).

Lead was found in concentrations below the minimum detection limit (MDL = 0.32 µg/g) of the instrument used in this study. However, the MDL value for Pb in this study is higher than the MTL for Pb in food set by Codex 2006.

Table 4: Comparison of mean concentrations ($\mu\text{g/g}$) of toxic metals in the *Unga wa Lishe* with the Maximum Tolerable Limits (MTLs) set by different International Organizations

Element	Dar	Moshi	Arusha	MTL	Organization
Ni	0.87 \pm 0.09	0.49 \pm 0.04	0.74 \pm 0.06	0.2	EC 2006
As	15.07 \pm 0.29	14.10 \pm 0.32	14.45 \pm 0.35	11	WHO, 2004
Cd	ND	ND	ND	0.5	Codex, 2006
Pb	<0.32	<0.32	<0.32	2	Codex, 2006

CONCLUSIONS

The present study has generated data on the essential and toxic elements in *Unga wa Lishe* samples and estimated the daily intake of those elements by children under five in Tanzania. The study has found presence of toxic elements Ni and As in concentrations higher than the MTL for these elements recommended by WHO 2004. The other toxic elements Cd was not detected in any of the analyzed samples while Pb was found in concentrations below the detection limits (0.32 $\mu\text{g/g}$) of the system used in this study. However, this value is larger than the MTL (0.2 $\mu\text{g/g}$) for Pb in food set by Codex 2006. Moreover, the concentrations of the essential elements in the complementary food were below the RNI standards set by Codex 2013. Although the number of data was not big enough to make a definite conclusion this study has realized that the locally produced complementary foods (*Unga wa Lishe*) for children under five in Tanzania do not provide the adequate amounts of the essential elements needed for their health growth. Hence, appropriate measures such as food fortifications and proper food planning are needed to reduce mineral malnutrition in Tanzania.

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