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Determination of Mn, Fe, Cu and Zn in indigenous complementary infant flour from Kenya by total-reflection x-ray fluorescence

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Abstract: Four dietary trace elements were determined in indigenous complementary infant flours collected from mothers in rural areas of Kenya using total-reflection x-ray technique. A high variability of these trace element levels were observed in the samples. The variability was dependent on type of ingredients used, the proportions of these ingredients in the sample and the origin of the samples. Further studies on bioavailability of trace elements in such kinds of complementary infant foods could be carried out to ascertain its viability of eliminating micronutrients deficiencies among infants and young children in developing countries.

Keywords: Indigenous Complementary Infant Flour, Dietary Trace Element, Total-Reflection X-Ray Fluorescence

1. Introduction

The need for adequate dietary trace elements during periods of rapid growth such as infancy and early childhood is crucial [1]. These elements are required for promotion of good growth, health, and behavioral development of an infant [2]. Consequently, infants tend to be at a higher risk to the effects of inadequate intake of dietary trace elements. Studies have shown that inadequate intake of dietary trace elements during infancy is the main cause of infant morbidity and mortality in developing countries [2]. For instance, zinc deficiency has been recognized to impair growth and immune function and its importance with regard to childhood infectious diseases has become better understood [3]. Likewise, deficiency of copper has been associated with decreased production of white blood cells and impaired functions of T and B lymphocytes [4] whose effects on the immune system are more pronounced in infants. Iron deficiency during the infancy stage has also been associated with delayed psychomotor development and changes in behavior [5].

Kramer and Kakuma [6] recommends exclusive breastfeeding up to the age of six months, thereafter, complementary feeding should be introduced. World Health Organisation [7] further recommends that the complementary foods should adequately provide all the nutrients requirements as the infant continues to receive breast milk up to the age of 24 months or more. These complementary infants foods are needed to fill the gap between the total nutritional needs of the child and the amount provided by the breast milk. Unfortunately, reports indicate that during this period, nutrition requirements of many infants are not met, leading to the onset of micronutrient malnutrition that is prevalent in children under the age of 5 years worldwide [8,9,10]. This challenge has been attributed to inappropriate complementary feeding practices, low quality of complementary foods and high cost of fortified nutritious complementary foods which is always beyond the reach of many families [11].

Strategies such as food diversification and modification of indigenous foods have been proposed to provide sustainable solutions to addressing trace element malnutrition in developing countries [12]. Cultivation of indigenous cereal crops such as sorghum and finger millet, which are rich in micronutrients and at the same time resistant to environmental factors such as drought have been encouraged [13]. In addition, several farmers have embraced the nutritional importance of legumes such as soya. Mothers in the developing countries will therefore more often modify these indigenous cereal crops and legumes to serve as complementary infant foods. This study sought to provide information on the concentrations of manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) in formulated indigenous complementary infant flour obtained from different mothers in three rural areas in Kenya.

2. Methods and Materials

2.1. Sample Collection

A total of 28 samples of complementary porridge flour for weaning infants between the ages of 6 months to 24 months were obtained in the year 2012 from mothers in Bomet, Sega and Turbo (Figure 1), areas among others in Kenya known for cultivation of indigenous food crops such as sorghum and millet [14, 15]. A community health worker known by the locals was assigned to the researcher by the officer in charge of a local health centre to assist in identifying the mothers who had infants within this age bracket. The ages were verified by looking at the births certificates or the baptismal cards. The sampling of the infant flour from the identified mothers depended on the willingness of the mothers to give out, at a small fee, part of the readymade flour. Other samples were bought from the local shops in these rural areas. The type of ingredients used varied as shown in table 2.

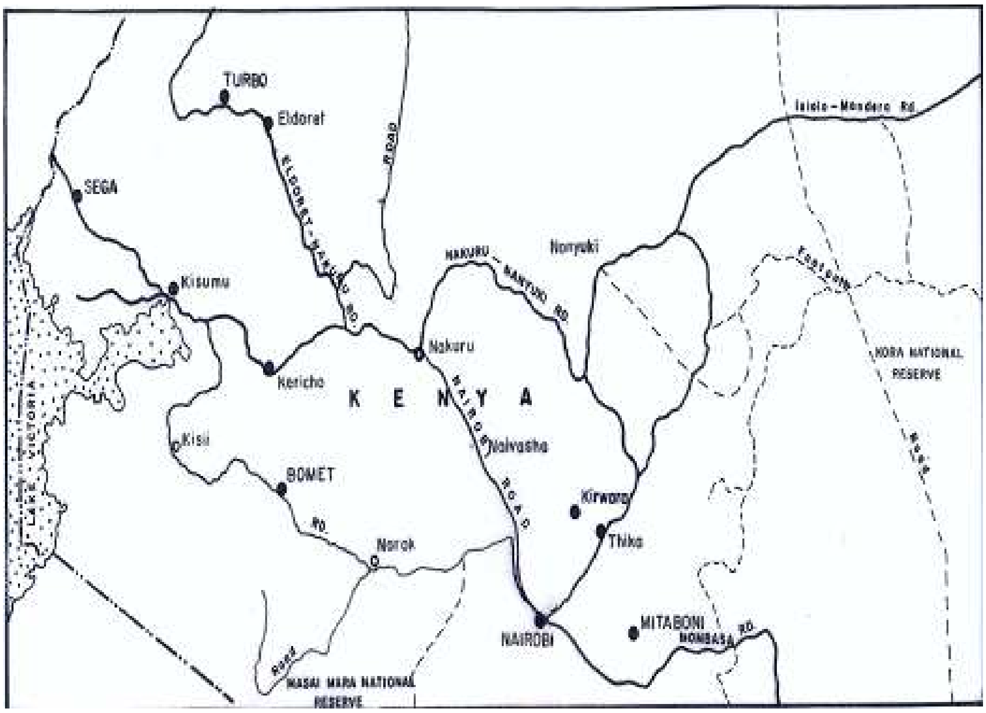

Figure 1. *A section of map of Kenya showing the sampling sites (Bomet, Turbo and Sega)*

All the samples were then packed in marked polythene bags and transported to Institute of Nuclear Science and Technology laboratory at the University of Nairobi for analysis.

2.2. Sample Preparation

A sub sample of approximately 1 g was transferred into a pre-cleaned flat-bottomed flask. Wet digestion in an open system using ANALAR nitric acid was then used to extract the elements from the sample matrix [16]. This analytical procedure was validated by subjecting certified reference material, NIM-GB10017, to the same analytical procedure. For each digested sample, three aliquots were prepared. 100 µl of Ga (2ng μl^{-1}), an internal standard, was added to 100 µl of each sample. 10 µl of each aliquot was then deposited onto a siliconized pre-cleaned polished quartz sample carrier and placed on a hot plate at 60^0C for approximately 3 minutes.

2.3. Determination of Mn, Fe, Cu and Zn in the Samples using TXRF

S2-Picofox TXRF spectrometer was used to determine the concentration of these elements. The spectrometer operated at 50 kV, 1 mA fitted with a Mo anode and Ni/C multilayer monochromator. The monochromatic beam produced impinged on the sample holder, which was placed at an angle less than 0.1^0 in the direction of the incident beam. The energies of the fluorescent X-rays emitted were then detected by Peltier cooled silicon drift detector with a resolution of 145 eV at MnK_α FWHM. Each of the samples was analyzed for 1000 s.

2.4. Quantification of the Elements

SPECTRA software was used to deconvolute the spectra and calculate the net intensities of the peaks using internal standardization. The internal standard was used to correct for any variations in sample deposition on the sample-reflector surface that could produce difference in regard to the resulting absolute sensitivity [17]. Since the sample was a thin film whose absorption and enhancement effects are negligible [18] with gallium as an internal standard, the concentration of the respective elements was calculated using (1)

$$C_i = \frac{C_{Ga} \times N_i \times S_{Ga}}{N_{Ga} \times S_i} \tag{1}$$

where

C_i is the concentration of element to be analyzed,

C_{ga} is the concentration of Ga,

N_i is the net peak area within the measurement spectrum of the element to be analyzed,

N_{Ga} is the net peak within the measurement spectrum of Ga,

S_{Ga} is the relative sensitivity of Ga, and

S_i is the relative sensitivity of element to be analyzed.

Equation (2) was then used to convert the elemental concentrations from mg l^{-1} to mg kg^{-1},

$$C_s = \frac{C_i \times V \times D}{W} \tag{2}$$

where

C_s is the sample concentration in mg kg^{-1},

C_i is the concentration of extract in mg l^{-1},

V is the volume of extract,

D is the dilution factor, and

W is the weight of the sample in the extract.

3. Results and Discussion

3.1. Validation of the Analytical Procedure

The experimental concentrations were compared with the certified values of the reference material and subjected to a one sample t-test [19]. The associated p-value and relative standard deviation for each element was calculated (table 1). The p-values at 95% confidence level for all the elements were greater than 0.05. These values were therefore not significantly different. In addition, the relative standard deviations (RSD) of the experimental values from the certified values were below 10% for all the elements of interest. This analytical procedure was therefore suitable for extraction of trace element of interest in the indigenous complementary infant flour from the selected rural areas.

Table 1. *Comparison of experimental and certified concentration of Mn, Fe, Cu and Zn in NIM-GBW 10017*

Concentrations in mg kg^{-1}				
Element	**Experimental (N=3)**	**Certified**	**p-values**	**RSD (%)**
Mn	0.45 ± 0.15	0.51 ± 0.17	0.190	6.3
Fe	7.6 ± 3.5	7.8 ± 1.3	0.945	2.6
Cu	0.48 ± 0.06	0.51 ± 0.13	0.564	5.9
Zn	31 ± 4	34 ± 2	0.307	8.8

3.2. Concentrations of Mn, Fe, Cu and Zn in the Samples

The concentrations of Mn, Fe Cu and Zn in the samples varied enormously (table 2). A cluster analysis was performed on these samples to establish the relationship between the samples (Figure 2). The input data was normalized and the Euclidean distance was used as a measure of similarity between the samples. Six clusters (P, Q, R, S, T, and U) were obtained. All the samples in cluster P were from Bomet region and finger millet was a dominant ingredient. In cluster R, samples B5, B6, T4 and T9 were maize samples. Samples B3 and T5, also found in this cluster had maize as one of the ingredient. However, T7, a maize sample was grouped with sample S8, whose ingredients were maize, sorghum and cassava in cluster U. Other samples in this cluster were sample S2 whose ingredients were maize and finger millet and sample S6 which had cassava, groundnuts and soya as the ingredients. Cluster S had 6 samples and the 4 of the samples had soya as a common ingredient. The ingredients of the four samples in cluster Q were maize and finger millet. Sample S3 was isolated.

The type of ingredients used, proportions of these ingredients in the samples and the origin of the samples may have had an influence on the variability of these trace elements in the samples hence the clusters. For instance sample B8 (found in cluster P) whose ingredient was only finger millet had the highest concentration of Mn. On the other hand, the concentration of Mn in maize samples (B5, B6, T4 and T9, all grouped in cluster R) was very low. Other samples that had finger millet as one of the ingredients had varied concentrations of Mn. Sample B8 was grouped in cluster P together with B1, B9 and B7 whose ingredients were maize and finger millet. However these 4 samples were collected from Bomet. A study done by Maina *et al.* [20] showed that finger millet had high concentrations of Mn. Other studies done by Maina [21] and Mohammed [22] showed that maize had very low concentrations of Mn. This indicates that finger millet is a good source of Mn while maize is a poor source of Mn. Consequently, the proportions of these two ingredients in the sample may have had an effect on the variability of Mn in the flours as observed in samples that had finger millet and maize as one of the ingredients.

Farmers in Bomet region use indigenous technical method to prepare land. This method involves the heating of the soils. Heating of soils at particular temperatures increases the PH of the soils [15], consequently improving the bioavailability of Mn in soils hence boosting the uptake of Mn by plants [23]. A study done by Nafuma *et al*. [15] showed that Mn in finger millet from Bomet region was high when the soils were burnt at 200^0 C. Compared to the high levels of Mn in sample B8, sample B2 which was grouped together with B8 in cluster Q and whose ingredient was also finger millet only had lower concentrations on Mn. Sample B2, a Famila brand, sold across the Kenyan markets was bought from the shops while the other samples were collected from the mothers who modified the available food to fit their needs.

Samples in cluster S had Fe concentrations ranging between 113 ± 4.8 mg kg^{-1} and 178 ± 20 mg kg^{-1} while samples in cluster T had comparably higher concentrations of Fe ranging between 290 ± 95 mg kg^{-1} and 376 ±57 mg kg^{-1}. The samples from these two clusters had either finger millet or soya or both as one of the ingredients. However, sample S9 whose ingredients were sorghum, maize and cassava also had very high concentrations of Fe. The concentration of Fe in the maize samples varied. The concentration of Fe in maize samples B5, B6 T4 and T9 ranged between 14.6 ± 1.6 mg kg^{-1} to 28.8 ± 3.2 mg kg^{-1} while sample T7 which was also a maize sample had Fe concentrations of 61.5 ± 5.6 mg kg^{-1}. Sorghum, finger millet [24] and legumes such as groundnuts [25] and soya [26] are considered to be good sources of Fe. Nevertheless, it is worth noting that high levels of Fe in flour may be as a result of contamination of Fe from grinding equipments during milling processes [27] or from soil residues and dust which have high levels of Fe may settle on the products during the drying process [28].

Table 2. *Mean concentrations (mg kg^{-1} ± 1 SD) of Mn, Fe, Cu and Zn in samples collected from the three regions of study*

Region	Label	Ingredients	Mn	Fe	Cu	Zn
Bomet	B1	Finger millet, Maize	174 ± 59	47.8 ± 14.8	3.27 ± 0.99	19.3 ± 6.4
	B2	Finger millet (Famila brand)	113 ± 2	46.6 ± 5.4	3.20 ±0.12	22.1 ± 0.7
	B3	Maize, finger millet and souring agent (Famila brand)	35.7 ± 5.5	47.3 ± 2.5	1.95 ± 0.17	14.5 ± 1.3
	B4	Maize, finger millet	132 ± 8	90.2 ± 7.2	1.56 ± 0.18	16.5 ± 0.5
	B5	Maize	2.49 ± 0.28	15.8 ± 1.8	0.73 ± 0.05	15.7 ± 0.5
	B6	Maize	1.96 ± 0.39	14.6 ± 1.6	0.72 ± 0.02	14.3 ± 0.3
	B7	Maize, finger millet	167 ± 5	112 ± 3	3.41 ± 0.20	22.5 ± 0.8
	B8	Finger millet	329 ± 28	31.2 ± 4.5	2.49 ± 0.18	23.2 ± 2.4
	B9	Maize, finger millet	200 ± 42	37.6 ± 12.0	4.00 ± 0.25	26.4 ± 0.6
Turbo	T1	Millet, soya, sorghum, maize	6.67 ± 0.03	158 ± 35	1.77 ± 0.05	18.5 ± 0.3
	T2	Sorghum, soya, fine maize meal	6.83 ± 0.14	126 ± 7	2.15 ± 0.19	20.1 ± 0.4
	T3	Millet, sorghum, soya, cassava, groundnuts, fine maize, fish powder, milk powder, green grams	13.0 ± 4.3	150 ± 12	1.85 ± 0.10	29.6 ± 1.3
	T4	Maize	2.47 ± 0.14	28.8 ± 4.9	1.82 ± 0.13	36.2 ± 1.7
	T5	Sorghum, finger millet, maize	46.5 ± 0.8	22.8 ± 3.2	1.53 ± 0.02	21.0 ± 0.6
	T6	Sorghum, finger millet, maize	78.1 ± 2.0	74.4 ± 1.8	2.17 ± 0.07	22.0 ± 0.2
	T7	Maize	11.2 ± 1.2	61.5 ± 5.6	4.08 ± 0.25	81.2 ± 0.4
	T8	Maize, finger millet	73.2 ± 2.1	58.7 ± 4.5	2.39 ± 0.09	16.7 ± 0.5
	T9	Maize	2.46 ± 0.15	17.7 ± 1.0	0.95 ± 0.02	17.2 ± 0.3
	T10	Maize, finger millet	46.2 ± 0.7	178 ± 20	2.17 ± 0.03	18.5 ± 0.2
Sega	S1	Maize, cassava, finger millet	33.8 ± 1.2	113 ± 4.8	2.15 ± 0.04	11.2 ± 0.2
	S2	Maize, finger millet	19.8 ± 0.3	136 ± 5	6.16 ± 0.30	87.7 ± 1.0
	S3	Groundnuts, soya, finger millet, sorghum, cassava	259 ± 1	246 ± 14	10.3 ± 0.3	49.0 ± 0.5
	S4	Soya, maize, finger millet	43.1 ± 1.6	202 ± 18	5.56 ± 0.21	66.1 ± 2.0
	S5	Soya, maize, finger millet	60.7 ± 4.6	330 ± 60	3.75 ± 0.11	21.1 ± 0.1
	S6	Cassava, groundnuts, soya	38.2 ± 3.1	196 ± 34	11.0 ± 0.2	97.1 ± 4.4
	S7	Soya, sorghum, cassava	86.5 ± 0.6	376 ± 57	5.53 ± 1.04	24.6 ± 1.0
	S8	Maize, sorghum, cassava	11.6 ± 0.7	85.0 ± 24.5	3.00 ± 0.39	68.5 ± 2.8
	S9	Sorghum, maize, cassava	22.2 ± 1.2	290 ± 93	3.99 ± 1.75	51.0 ± 6.9

Samples in cluster U had comparably higher concentration of Zn. The concentration of Zn in samples in the other clusters varied widely. For instance, Zn concentration in cluster Q ranged between 16.5 ± 0.5 mg kg^{-1} and 22.1 ± 0.2 mg kg^{-1} while in cluster S, Zn concentration ranged between 11.2 ± 0.2 mg kg^{-1} and 66.1 ± 2.0 mg kg^{-1}. A direct relationship between low Zn in soil and Zn deficiency in cereal crops has been observed [24]. It is estimated that nearly half of the soils in the world on which cereals are grown have low concentrations of Zn [29]. Since cereal grains have inherently low concentrations of Zn, growing them on Zn deficient soils aggravates Zn concentrations in them. Alloway [29] further noted that maize and sorghum cereals were highly susceptible to Zn deficiency. Moreover, removing of the outer layer of the cereals during the milling process also greatly reduces the level of Zn in cereal grains. A study done by Maina [21] and Bauernfeind [30] showed that milling process reduced the level of Zn in maize by over 70%.

Sample T7, a maize sample collected from Turbo region had relatively high concentrations of both Fe and Zn. These high concentrations of Fe and Zn were also noted in other samples from other regions collected during this research [31]. In study done by Musa *et al*. [24], it was noted that there was certain varieties of maize that could concentrate more Fe and Zn in the grain.

Compared to other elements, the concentration of Cu in all the samples was very low. However, samples B5, B6 and T9 whose ingredient was maize had lowest Cu concentrations while samples S3 and S6 which had groundnuts as one of the ingredients had higher concentrations of Cu. Groundnuts have been considered to be very good source of Cu [32]. On the other hand, low concentrations of copper in plants are closely related to copper deficiency in soils [33]. Furthermore, high soil pH decreases the uptake of Cu by plants. Cereal plants tend to be highly sensitive to the effect of Cu deficiencies in soils [34]. Therefore, crops that are planted on these types of soil will tend to have low concentrations of Cu. Deficiency of Cu has been noted to occur in many developed and developing countries [35].

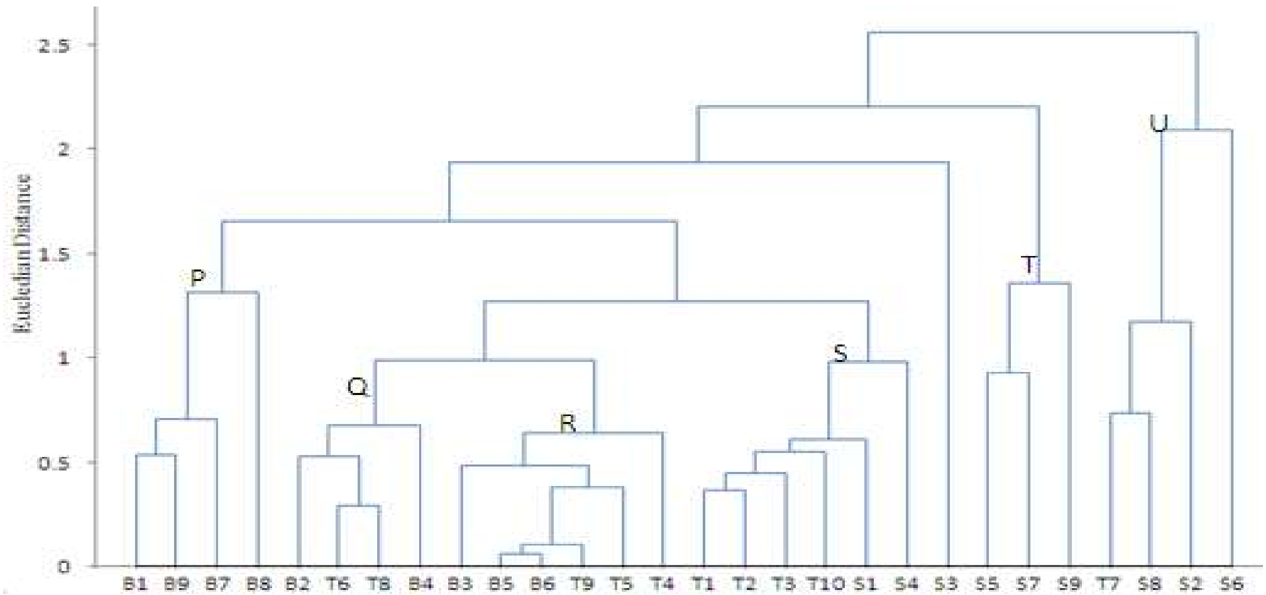

Figure 2. *A dendrogram showing the similarities between the samples*

Complementary infants foods are needed to fill the gap between the total nutritional needs of the child and the amount provided by the breast milk. For instance, at the age of 9 to 11 months, these foods should be able to provide 97% of iron, 86% of zinc [6] and 50% of Cu and 50% to 75% of manganese [36]. However, complementary foods based on cereals and legumes contain anti-nutritional elements such as phytic acids which may inhibit absorption of Fe, Mn and Zn [37]. On the other hand, cereal products provide about a third of the intake of Mn [38].

Excessive dietary intake of these trace elements may also have detrimental effects on infants and of main concern is Mn. Infants and young children tend to absorb more manganese from food and very little is excreted since the homeostatic mechanism is not yet fully developed [39]. Most of the Mn is excreted into the feces by the way of bile. Some studies have shown that insufficient biliary excretion may result in accumulation of Mn in the brain. Kriegler *et al* [40] observed that accumulation of Mn in the brain had a role in the pathogenesis of disease, especially neurotoxicity. Therefore, high retention of manganese in young infants combined with relatively high dietary intake of manganese has led to some concern about potential manganese toxicity [41].

4. Conclusion

This study indicates that there is a considerable variability of Mn, Fe, Cu and Zn in formulated indigenous complementary infant foods. There is a need therefore, to carry out studies on bioavailability of trace elements in such kinds of complementary infant foods to ascertain its viability of eliminating micronutrients deficiencies among infants and young children in developing countries.

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