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Zinc Levels in Raw and Blanched Slenderleaf sp. (*Crotalaria ochroleuca & Crotalaria brevidens*) Indigenous Vegetables

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Abstract

An estimated 20% of the world's population is at risk for zinc (Zn) deficiency. Micronutrient deficiencies are most prevalent in Africa and remain a major problem facing poor populations, whose impact is worsened by HIV/AIDS pandemic. Adequate zinc nutrition is essential for adequate growth, immunocompetence and neurobehavioral development. In Sub-Saharan Africa, Zn deficiency risk stands at 34.6% with 25.6% of its population having inadequate zinc intake. It is widespread in areas where diets lack diversity and it has been implicated as a contributing factor to stunting in approximately a third of children in low-income countries. In Kenya, it is a public health problem that about 50% of children under 6 years and 50% of women are affected. Zn deficiency rates are severe and pose severe consequences whose impact would translate into poor economic development and would set a vicious cycle effect that will take many generations to correct if left unchecked. It is important to examine zinc in the diet but its concentration in food varies depending partly on processing besides other factors. The objective of this study was to assess blanching as a food preparation method's implication to Zn levels in vegetables. Blanching is a method where vegetables are dipped in boiling water for around two minutes and removed to avoid over cooking. Zn levels in slenderleaf sp. Indigenous vegetable commonly found in the Lake Victoria Basin region is not known. An experimental study was carried out to analyze zinc levels in raw and blanched slenderleaf sp. (*Crotalaria ochroleuca & Crotalaria brevidens*) vegetables. Results indicated that blanching reduces Zinc levels in slenderleaf vegetables, however, the reductions are not significant; the levels after blanching are still vital. It is, therefore, recommended to minimize blanching time in order to reduce loss of the vital nutrient in slenderleaf sp. vegetables.

Keywords

Zinc Levels, Indigenous Vegetables, Slenderleaf, *Crotalaria ochroleuca*, *Crotalaria brevidens*, Blanching

1. Introduction

Zinc deficiency is widespread and affects the health and well-being of populations worldwide. The supply of nutritionally adequate foods to the global population is still challenging (Gudrun, Katja and Irmgard, 2013) [1]. While protein-energy malnutrition (PEM) is the most lethal form of malnutrition, micronutrient deficiency (MND) is the most serious threat to health and development of populations worldwide (WHO, 2004a) [2]. Trace mineral deficiencies affect populations in both developed and developing countries (IOM, 2001) [3]. "Hidden hunger", a deficiency of micronutrients, vitamins and minerals, affects as many as 3 billion people globally while 38% of children under five in Sub-Saharan Africa are stunted due to chronic malnutrition (Anonymous, 2013) [4]; (Mason and Garcia, 1993) [5]; (R.M. Welch, Combs-Jr. and Duxbury, 1997) [6]; (WHO, 1999) [7]. Studies indicate that the prevalence of stunting is positively correlated with the estimated prevalence of inadequate zinc intake. The mean prevalence of stunting in countries identified as being at low, moderate and high risk of inadequate zinc intake are 19.6%, 28.8% and 43.2%, respectively. In low- and middle-income countries, the mean estimated prevalence of inadequate zinc intake stands at 19.6% (Wessells, R.K. and Brown, H.K., 2012) [8]. Malnutrition and diet-related diseases leads to high social and economic costs at all income levels (FAO, 2013) [9], and most prevalent in Africa (UNICEF, 2004) [10]; (WHO, 2002b) [11]. Zinc deficiency is largely related to inadequate intake or absorption of zinc from the diet (Gibson, R.S., 1994) [12]; (WHO, 1996) [13]. Africa has plenty of neglected and underutilized crops rich in micronutrients important for good health (Abukutsa-Onyango, M., 2003) [14]; (Grivetti, L.E. and Ogle, B.M., 2000) [15]; (Oniang'o, R.K., Shiundu, M.K., Maundu, P. and John, S.T., 2005) [16].

There are more than 45,000 species of plants in Sub-Saharan Africa of which more than 1000 can be eaten and are the mainstay of traditional African diets (MacCalla, A.F., 1994) [17]. These plants are inexpensive, easily accessible and provide millions of African consumers with minerals needed to maintain health (Abukutsa-Onyango, M., 2003) [14]; (MacCalla, A.F., 1994) [17]; (Abukutsa-Onyango, M., Tushaboomwe, K., Onyango, J.C. and Macha, S.E., 2005) [18]; (FAO, 2003) [19]; (ICRAF, 2004) [20]. Indigenous diets can help alleviate nutrient deficiencies by increasing nutrient supplies (ICRAF, 2004) [20]; (Engle, L.M. and Altoveras, N.C., 2006) [21]. Despite the availability of minerals in underutilized crops, researchers in Kenya have concentrated on increased production and nutrient content of raw crops (Maundu, P.M., 1997) [22] with very little effort to explore the nutritive value of cooked crops (Habwe, F., 2008) [23]. Despite the availability of these crops, their nutritional value after cooking or processing are not documented.

Consumption of a broad range of plant species, in particular, those currently identified as "underutilized", can contribute to improved health and nutrition, livelihoods and household food security (Jaenicke, H. and Höschle-Zeledon, I., 2006) [24]. Among many micronutrients of public health concern is zinc, has become a matter of great interest concerning the nutritional value of diets (Luo, Y.W. and Xie, W.H., 2012) [25]. Incorporation of vegetables as a food source rich in micronutrients provides one strategy to sustainably improve the micronutrient status in the human body (Ali, M. and Tsou, C.S., 1997) [26]. Although nutritious and important for good health, other crops regarded as "underutilized" have not been exploited to ensure supply of micronutrients. The exploitation of underutilized crops thorough processing could provide information on whether this important micronutrient still exists in adequate amounts to meet the recommended intake levels even after processing. This could in the long run help minimize Zn deficiency. However, the ability of the processed slenderleaf sp. to supply adequate daily recommended Zn intake unknown.

Nutritional Underutilized Species (NUS) present tremendous opportunities for fighting poverty, hunger and malnutrition; their value in traditional foods and cultures can empower indigenous communities particularly women, therefore the time for action on NUS is now (Padulosi, S., Thompson, J. and Rudebjer, P., 2013) [27]. Underutilized crops including slenderleaf are cheap, readily available and culturally acceptable. However, it is not known if the two species of slenderleaf sp. (*Crotalaria ochroleuca* & *Crotalaria brevidens*) can still supply Zn after processing.

2. Methodology

2.1. Ethical Clearance

Approval for the study was obtained from the Ministry of Higher Education, and Maseno University School of Graduate Studies, Kenya.

2.2. Materials and Methods

All the reagents, chemicals and standards used in this work were from Aldrich supplied by Kobia Limited Nairobi. The methods used in this work for the various determinations of the samples are standard methods and all chemicals used for the study were of analytical grade.

2.3. Source of the Slenderleaf sp. Vegetables

Slenderleaf vegetables were procured at Luanda market located at 34˚36' East and 0˚ North at an altitude of about 1530 meters above sea level. The same amount (500 g) of each species (*Crotalaria ochroleuca* & *Crotalaria brevidens*) was procured from three different sellers at different locations of the market and mixed.

2.4. Laboratory Methods

An experimental study was carried out where the two species of slenderleaf sp. (*Crotalaria ochroleuca* & *Crotalaria brevidens*) were processed and analyzed for the Zn levels in both raw and processed vegetables. Slenderleaf vegetable preparation and processing were carried out at Maseno University Nutrition Department Foods Laboratory at 65% humidity and 23˚C. The procured crops were processed as follows: The two species of Slenderleaf indigenous vegetables were separately destalked and half of it dried under shade in readiness for elemental analysis. The remaining vegetables were washed under running double glass-distilled and deionized water to remove surface soil contamination. Vegetables were rinsed three times using deionized water to remove all the dirt. Each vegetable was processed separately (*C. ochroleuca* and *C. brevidens*). After washing, life processes were stopped by immediately blanching. The blanched vegetables were then dried and ground into powder using mortar and pestle and sieved by a 0.5 mm size sieve.

The following procedure was followed in blanching the vegetables

1) A large pan half-full of double glass-distilled water was brought to rapid boiling.

2) Clean raw vegetables were put in a wire basket and gently lowered in the boiling water.

3) Once the water began to boil again, vegetables were left in for two minutes.

4) The wire basket containing vegetables was removed from the boiling water and plunged into ice-cold water for two minutes to stop the cooking process.

5) The blanched vegetables were drained and dried under shade until completely dry then powdered in readiness for elemental analysis.

2.4.1. Sample Preparation for Elemental (Zn) Analysis

The procured vegetables were cleaned and made free from dirt, foreign matter and other stubbles; this was followed by washing using distilled and deionized water after which processing was carried out in readiness for analysis. Processing of the vegetables involved the following activities; fresh vegetable leaves were separated from roots, washed under running double glass-distilled and deionized water. Vegetables were then blanched, drained completely and dried under shade. All samples were then labeled as raw sample and processed sample. The dried samples (raw & processed) were ground into powder and then stored in clean and dried 100 ml polypropylene bottles for further processing. Digestion of food samples was carried out as follows: Food samples of 0.1 g were put in a beaker and 10 ml of tri-acid mixture (concentrated HNO_3, $HClO_4$ and H_2SO_4) in the ratio 3:1:1 was added. The mixture was heated on a hot plate at 105˚C until white fumes were observed (Lindsay, W.L. and Norvell, A.W., 1978) [28]. The digested samples were then filtered using a Watmann filter paper No. 42 into a 50 mL volumetric flask and topped up to the mark with distilled water and two drops of HNO_3 added for preservation. The digested sample was transferred into plastic bottles ready for analysis using the Atomic Absorption Spectrophotometer (AAS) (Varian AA240).

2.4.2. AAS Determination of Zinc

Zn element standard (1000 parts per million) was prepared as follows: A zinc stock solution (1000 ppm Zn^{2+}) was prepared by dissolving 4.697 g of $Zn(NO_3)_2 \cdot 6H_2O$ and made up to the mark in a 1000 mL volumetric flask with distilled water. A working standard was prepared from it through serial dilution. The spectrophotometer (Varian AA240) was operated under standard conditions using a wavelength specified for Zn (213.9 nm). The standard solution was aspirated into the AAS machine after placing the element light in place to generate light specific to Zn. The machine then drew a standard curve of absorption against concentration. Sample filtrate was then aspirated into the machine which in turn gave the sample's (solution) absorption. The standard curve was used to obtain the sample's concentration by dropping a line to the x-axis (concentration) from the y-axis (absorption data) (AOAC, 2000) [29].

2.5. Statistical Analysis

Data analysis was performed using ANOVA with critical significance levels set at $P \leq 0.05$. Paired samples *T-test* was used to compare zinc levels between raw and processed vegetables.

3. Results

Zinc Levels in Raw and Processed Selected Underutilized Crops

The two species of slenderleaf contained essential micronutrient (Zn). Data present the mean Zn concentration of the raw and processed vegetables. Processing reduced zinc concentration (Table 1). All data were based on dry weight.

Results indicate that, among the selected indigenous food crops, raw bitter slenderleaf (*C. brevidens*) recorded the highest mean zinc content of followed by raw mild slenderleaf (*C. ochroleuca*) (Table 1). Similar to raw crops, processed bitter slenderleaf (*C. brevidens*) recorded higher mean zinc content compared to mild slenderleaf (*C. ochroleuca*) (Table 1).

4. Discussion

Both species of slenderleaf (*Crotalaria brevidens* and *Crotalaria ochroleuca*)

Table 1. Mean Zn levels in raw and processed slenderleaf vegetables.

Sample	ZINC LEVEL (mg/g)			
	Raw	Processed	Mean difference	P-value
Bitter Slenderleaf (*C. brevidens*)	2.8 (mg/g)	2.7 (mg/g)	0.1 (mg/g)	**0.040***
Mild Slenderleaf (*C. ochroleuca*)	2.2 (mg/g)	2.0 (mg/g)	0.2 (mg/g)	**0.001****

There was negligible standard deviation ranging between 0.001 - 0.005, *-Significant, **-Highly significant.

indigenous vegetables recorded presence of Zn. This is because plant based food are good sources of Zn (IOM, 2001) [3]. Generally, this study reveals that processing by blanching reduces zinc content of slenderleaf vegetables. The reduction in Zn content was statistically highly significant in the mild slenderleaf (*C. ochroleuca*) and significant in the bitter slenderleaf (*C. brevidens*). This could be due to the fact that conditions of plants, food handling methods like processing and cooking affect their zinc content (IOM, 2001) [3]. Although statistically significant, the mean reductions in Zn content were minimal in the vegetables *C. brevidens* and *C. ochroleuca* at 0.1 mg/g and 0.2 mg/g respectively. These reductions could be due to leaching of Zn into the water during blanching of the vegetables. However, results by (Adeniji, T.A. and Tenkouano, A., 2008) [30] indicated that blanching had no significant effect on zinc contents in different whole flour made from some plantain and banana hybrids pulp and peel mixture. On the contrary, (Shashi, K.Y. and Salil, S., 1995) [31] noted that blanching and cooking resulted in significant improvement in Zn levels. According to (Achidi, U.A., Ajayi, A.O., Maziya-Dixon, B. and Bokanga, M., 2008) [32]; (Ahenkora, K., Kyei, M.A., Marfo, E.K. and Banful, B., 1996) [33], processing lead to a decrease in Zn content in the processed crops. This study concurs with these findings and further reveals that blanching results into leaching of Zn into the water used for blanching thus reducing Zn levels in vegetables.

5. Limitation of the Study

The elemental experimental method used can only be used of dry samples, therefore, it was not possible to determine whether the water used for blanching the vegetables contained zinc which could have leached from the vegetables during blanching.

6. Conclusion

The study has shown that the two species of Slenderleaf indigenous vegetables are good sources of Zn; however, processing has effect on the levels. Blanching leads to leaching of Zn into the water used for blanching thus resulting in reductions in mean Zn levels in slenderleaf vegetables.

7. Recommendations

Slenderleaf sp. indigenous vegetables are good sources of zinc and should, therefore, be promoted and included in our diets.

It is important to minimize blanching time and water temperature in order to minimize zinc loss in vegetables.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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