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Alteration in Trace Elements Concentration and Possible Copper Toxicity in Juiced Leafy Vegetables Commonly Consumed in Southwestern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author BAS designed the study. Author AKA performed the statistical analysis. Author GGD wrote the protocol. Author POA wrote the first draft of the manuscript. Authors POA and KTO managed the analyses of the study. Author BAS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the effects of juicing on four micro-minerals (Cu, Zn, Mn and Mo) contents of six different leafy vegetables commonly consumed in South western, Nigeria. Micro-minerals were determined using Atomic Absorbance Spectrophotometer (AAS). Among the fresh vegetables, highest level of micro-minerals was recorded in *Senecio biafrae* for Cu (9.13±0.21 mg/100 g dry weight); *Manihot esculenta* for Zn (2.01±0.17 mg/100 g dry weight); *Ipomoea batatas*

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for Mn (3.78±0.29 mg/100 g dry weight) and *Senecio biafrae* for Mo (35.00±0.00 µg/100 g dry weight). In juiced form, highest value of Cu was noted in *Launaea taraxacifolia* (21.43±1.58 mg/100 g dry weight); Zn in *Senecio biafrae* (3.24±0.26 mg/100 g dry weight), Mn in *Launaea taraxacifolia* (11.76±2.25 mg/100 g dry weight) and Mo in *Piper guineense* (44.00±0.00 µg/100 g dry weight). The least micro-minerals contents noted in juice fraction of vegetables are as follows: Cu, *Ipomoea batatas* (2.35±0.15 mg/100 g dry weight); Zn, *Launaea taraxacifolia* (0.36±0.01 mg/100 g dry weight); Mn, *Piper guineense* (2.68±0.27 mg/100 g dry weight) and Mo, *Ipomoea batatas* (0.002±0.00 mg/100 g dry weight). Increased concentrations of micro-minerals was observed in virtually all juice fractions when compared with their corresponding fresh vegetables with exception of *Ipomoea batatas,* where Zn and Mo contents reduced in the juice when compared with its corresponding fresh vegetables. It could be deduced that the juice fraction of vegetables contain more of trace-minerals in some of the vegetables. In addition, intake of 100 g dry weight of any of the vegetable (fresh form) does not lead to toxic intake of any of the minerals using Recommended Daily Tolerable Upper Intake Level (UL) as a standard. However, intake of the same corresponding dry weight in juice would lead to toxic level of Cu in all the vegetables except *Ipomoea batatas*; whereas, such amount would not lead to Zn, Mn and Mo toxicity.

Keywords: Microelements; vegetables; juicing; intake limit; toxicity.

1. INTRODUCTION

Regular consumption of vegetables especially the green as well as bright colour leafy ones has undeniable positive influence on health due to their phytochemical components which help to protect animals and human alike against several types of diseases especially the chronic ones [1]. Although, the mechanism by which vegetables decrease risk of diseases is complex and poorly understood nonetheless, many components of vegetables have been identified to contribute to the overall health benefit [1]. Vegetables may be edible roots, stems, leaves, fruits or seeds; each of these groups contributes to diet in their peculiar manner, which distinguishes one from another [2]. Leafy vegetable plants play a vital role in the diet of the inhabitants of the Southwestern Nigeria [3].

Reviews have reported that these leafy vegetables could be rich sources of viatmins and minerals, required for physiological stabilities and metabolic processes [2,3]. Although vitamins and minerals are readily available in vegetables, however, their concentrations vary with respect to their groups as well as other factors such as harvest time, environment or soil components and most especially genetic factors and plant's demand for such nutrients. While these minerals and nutrients are beneficial, most importantly the trace minerals, their impacts are greatly dependent on availability and concentration in the body [3]. Despite the essentialities of trace minerals, their inadequacy may lead to impaired growth while their excesses cause irreversible health complications or even death in few cases [3].

As it is well documented that no vegetable is rich in all minerals and/or sufficiently supply balanced amounts. In the same manner, while some vegetables may contain insignificant amount of certain micro-elements others may have them at high concentration [4,5]. Hence different processing methods have been shown to influence their concentration, bioavailability as well as toxicity [6].

Processing methods such as salting, drying, blanching, juicing among others have being reported as some of the earliest technologies used by man to his own advantage, affecting the outcome in term of availability of nutrients and non-nutrients alike [7,8]. Juicing, a processing method of concentrating nutrients from plant tissues such as fruit or vegetables has continued to attract significant interest because of the concentrated nutrients in the juice extract with a belief of having more concentrated nutrients in their natural state. Although, our previous studies revealed alterations in juice contents of vegetable such as oxalate, tannin and saponin, which may adversely affect the consumers [9-11]. Nevertheless, the presence of other phytochemicals may outweigh their benefits. Hence, there is need for a general screening of some trace elements, which their high intake may not be advisable. It is therefore expedient to evaluate such trace elements in vegetable juice in other to know how juicing affect the content of such trace elements.

2. MATERIALS AND METHODS

2.1 Sample Collection

The green leafy vegetables used for the study were bought from some major markets in Ogun and Lagos States, Nigeria. The samples' weights were within 1-5 kg; identified at the herbarium of the Plant Science and Zoology Department, Olabisi Onabanjo University, Ago-iwoye, Ogun State, Nigeria.

2.2 Sample Preparation

Vegetables leafs were destalked and washed to remove any possible contaminants. Juicing was achieved by using master chef juice extractor (model no: mc-J2101) to separate the juice and the pulp [10].

2.3 Quantitative Analysis of Trace Elements

10 g of the sample was oven dried at 100℃ until constant weight. The dried samples were ashed in furnace at 500℃ for 5 hours. The ashes were dissolved in HNO_3, the solution was filtered and analyzed according to AOAC (1990) [12] using Atomic Absorption spectrophotometer (Schimadzu, Model: AA-7000).

2.4 Statistical Analysis

The experimental design was completely randomized and data were analyzed by Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) at $p<0.05$, using the Statistical Package for Social Sciences (SPSS) version 16 for Windows.

3. RESULTS AND DISCUSSION

Requirement of microelements in the body though in trace amount are necessary because of their crucial role in metabolic processes such as immune processes, nervous coordination, pigmentation and other metabolic processes especially in redox reactions [13]. This study gave an overview of juicing effect on six leafy vegetables commonly consumed in Southwestern Nigeria on four micro-minerals on assumption of 100 g dry weight consumption and using the reference values recommended by Institute of Medicine (IOM), 2001 report as standard for Adequate Intake (AI) and Tolerable Upper Intake Limit (UL) for the four micro-minerals [14]. The Cu content in the six vegetables (fresh state) were within required AI while juicing concentrated all to a toxic level except *Ipomoea batatas* that maintained normal range. Effect of juicing on Zn was insignificant in all the leafy vegetables as the Zn level in fresh were below AI and also their corresponding juices had Zn content below the recommended AI limit. Juicing affects Mn content in differently. It increased its level in all juiced vegetables. In addition, their Mn level was within AI range. Mo content in all the leafy vegetable (fresh form) was below AI; and juicing could not concentrate Mo level in most of the vegetable juice to AI range except in *Manihot esculenta* and *Amaranthus sp*. Hence, consumption of 100 g dry weight of vegetables would not lead to toxic level of trace minerals either, in fresh or juice form except in Cu.

Table 1. Cu content in fresh and juiced leafy vegetables

Leafy vegetables	Fresh (mg/100 g dry weight)	Juice (mg/100 g dry weight)	Percentage difference (%)	Intake Limit [14] AI – UL Male: (0.9 – 10 mg/d) Female: (0.9 – 10 mg/d)	
				Fresh	Juice
Manihot esculenta	7.25±0.57^{b,c}	12.19±3.78^{b}	68.14	N	T
Launaea taraxacifolia	8.15±0.12^{b}	21.43±1.58^{a}	162.94	N	T
*Amaranthus sp**	6.44±0.23^{c}	20.47±1.97^{a}	217.86	N	T
Piper guineense	5.44±0.19^{d}	12.75±1.46^{b}	134.38	N	T
Ipomoea batatas	5.96±0.31^{c,d}	2.35±0.15^{c}	(60.57)	N	N
Senecio biafrae	9.13±0.21^{a}	20.36±3.33^{a}	123.00	N	T

**Local name (Yoruba) of vegetable; Results presented are mean ± SEM (n = 4); values in the same column with the same superscript are not significantly different from each other (P > 0.05); Bracket "()" indicate negative value. Adequate Intake (AI), and Tolerable Upper Intake Level (UL); Status of trace element compared to recommended dietary range are represented as: B (Below normal); N (Normal range); T (toxic level)*

Cu, one of the major cofactors of many enzymes, is required for cellular respiration, iron oxidation, pigment formation, neurotransmitter biosynthesis, antioxidant defense, peptide amidation, and connective tissue formation [15]. Its deficiency may adversely impair metabolism of cholesterol and glucose; blood pressure control and heart function; as well as mineralization of bones, and immunity because of loss of function of the Fe-Cu dependent proteins. Similarly, accumulation or excesses of this metal may result in unregulated oxidation of proteins, lipids, and other cellular components, causing tissue injury [15].

Vegetables are known rich sources of trace minerals like Cu. In this study the level present in different vegetables varies. *Senecio biafrae* (9.13 mg/100 g dry weight) had the highest Cu content among fresh vegetables. The least value was recorded in *Piper guineense* (5.44 mg/100 g dry weight) but *Piper guineense* Cu content was different from that of *Ipomoea batatas*. Ajiboye et al. [16] reported low content of Cu in *Senecio biafrae* (0.53 mg/100 g dry weight); a study carried out by Singh et al. [17] showed that *Amaranthus* sp. had significantly high level of Cu in fresh vegetable. Effiong et al*.* [18] reported similar values of Cu (2.62 mg/100 g dry weight) for *Piper guineense*. Many factors have been ascribed to be responsible for the variations of Cu contents in leafy vegetables some of which are genetic make-up [2], soil and enviroment conditions [4-6] as well as growth stage [19], among others. The increased Cu contents oberserved in most of the vegetables could be due to their fibre content [20] as well as acidity [21]. Despite these obvious variations, all the fresh vegetables contain Cu level that was within AI range per 100 g dry weight.

Like other processing methods, juicing is adopted mainly to concentrate nutrients and other phytochemicals. Though, expected to improve nutritional values on one hand may as well concentrate nutrients to a toxic level on other hand. Juicing effect increased Cu in most of the vegetables except for *Ipomoea batatas* that had 60.57% loss in Cu content when compared to its fresh counterpart. Among vegetable juice fraction, *Senecio biafrae* (9.13 mg/100 g dry weight) had the highest Cu content, though not different from *Launaea taraxacifolia* and *Amaranthus* sp*.* whereas, the least was noted in *Ipomoea batatas* (2.35 mg/100 g dry weight). The juicing effect concentrated Cu in virtually all the vegetables to level above upper limit of recommeded daily intake except in *Ipomoea batatas*. Brewer, [22] reported that Cu toxicity may induce ROS production – a risk factor in diseases of aging, particularly atherosclerosis and Alzheimer's disease. Therefore, consumption of equivalent amount of 100 g dry weight/day of juice extracts of most of these vegetables may result in Cu toxicity with aforementioned attendant problems. Unlike other juiced leafy vegetables, *Ipomoea batatas* Cu was within the safe range for consumption.

Zn is essential for biological functions such as growth, appetite, testicular maturation, skin integrity, mental activity, wound healing and immune-competence [23]. It is essential in metabolic activities of many metalloenzymes. Zn deficiency may result to skin lesion, growth retardation, impaired wound healing, anemia and immune deficiency [24]. Similarly, its excesses may results in several complications like alterations in gastroin that may immediately cause burning and pain in the mouth and throat, vomiting and later exhibits (acute pancreatitis symptoms) pharyngitis, esophagitis, hypocalcaemia, and elevated levels of amylase; impaired nervous imbalance and death at chronic level [25].

Variation was observed in Zn content in fresh leafy vegetables. *Manihot esculenta* had the highest Zn content (2.01 mg/100 g dry weight), whilst no difference was observed between *Amaranthus* sp. (1.75 mg/100 g dry weight), *Piper guineense* (1.73 mg/100 g dry weight) and *Manihot esculenta. Ipomoea batatas* (0.46 mg/100 g dry weight) and *Senecio biafrae* (0.42 mg/100 g dry weight) had equal level of Zn. The juice fraction of *Senecio biafrae* had the highest Zn level; though not difference from *Piper guineense.* While *Launaea taraxacifolia* (0.36 mg/100 g dry weight) had the least.

Among the fresh leafy vegetables, there are variations in Zn level. Notwithstanding, the Zn content per 100 g of the fresh leafy vegetables was below AI value. In addition, juicing effect though increased Zn contents in most of the vegetables surprisingly, could not sufficiently increase Zn level to meet the daily minimum AI of Zn if 100 g dry weight is consumed.

Mn, one of the main micro-minerals that have important role in human and other living organisms; as a component of enzymes involved oxidation reactions. Also, function essentially as antioxidant [26] and an activator of several other enzyme systems. Barminas et al. [27] stated that consumption of Mn within recommended daily allowance (RDA) value may substantially prevent adverse effects of dietary deficiencies of Mn on the central nervous systems and skeletal anomalies among children, and its deficiencies may affect bone, brain and reproductive systems [28]. In excessive intake, Mn may induce neurological disorder including decreased memory and concentration, fatigue, headache, vertigo, equilibrium loss, insomnia, Ménière's disease, trembling of fingers, muscle cramps, rigidity, alteration of libido, and sweating [28].

Mn content in all the fresh leafy vegetables investigated varies. *Ipomoea batatas* (3.78 mg/100 g dry weight) had the highest level among the leafy vegetables. Whereas, *Amaranthus sp* (1.15 mg/100 g dry weight) and *Launaea taraxacifolia* had lowest Zn content. In addition, among the fresh leafy vegetables investigated, three (*Ipomoea batatas*, *Manihot esculenta*, and *Senecio biafrae*) out of six fresh leafy vegetables contain Mn in amount that is within the recommended AI range considering 100 g dry weight. Others (*Launaea taraxacifolia*, *Amaranthus* sp., and *Piper guineense*) were below AI.

Juicing increased Mn level in all the leafy vegetables investigated. *Launaea taraxacifolia* (11.76 mg/100 g dry weight) contained the highest. Mn level in juice fraction of *Launaea taraxacifolia* (11.76 mg/ 100 g dry weight) was the highest. Whereas, no significant difference (p>0.05) was observed among other vegetable juices. The juicing effect seems to be moderate on virtually all the vegetables investigated because all the vegetables were within the AI range. That is, those that were within AI range in fresh form maintained the intake safe range. While, those others that were below the AI range increased to a level that is within AI.

Table 2. Zn content in fresh and juiced leafy vegetables

Leafy vegetables	Fresh (mg/100 g dry weight)	Juice (mg/100 g dry weight)	Percentage difference (%)	Intake Limit [14] AI – UL Male: (11 – 40 mg/d) Female: (8 – 40 mg/d)	
				Fresh	**Juice**
Manihot esculenta	2.01±0.17^{a}	1.26±0.22^{c}	(37.31)	B	B
Launaea taraxacifolia	0.77±0.01^{b}	0.36±0.01^{d}	(53.25)	B	B
Amaranthus sp*	1.75±0.16^{a}	2.20±0.20^{b}	25.71	B	B
Piper guineense	1.73±0.04^{a}	2.03±0.40^{b}	17.34	B	B
Ipomoea batatas	0.46±0.08^{c}	1.16±0.01^{c}	152.17	B	B
Senecio biafrae	0.42±0.05^{c}	3.24±0.26^{a}	671.43	B	B

**Local name (Yoruba) of vegetable; Results presented are mean ± SEM (n = 4); values in the same column with the same superscript are not significantly different from each other (P > 0.05); Bracket "()" indicate negative value. Adequate Intake (AI), and Tolerable Upper Intake Level (UL); Status of trace element compared to recommended dietary range are represented as: B (Below normal); N (Normal range); T (toxic level)*

Table 3. Mn content of fresh and juiced leafy vegetables

Leafy vegetables	Fresh (mg/100 g dry weight)	Juice (mg/100 g dry weight)	Percentage difference (%)	Intake Limit [14] AI – UL Male: (2.3 – 11mg/d) Female: (1.8 – 11mg/d)	
				Fresh	**Juice**
Manihot esculenta	2.78±0.36^{b}	3.51±0.87^{b,c}	26.26	N	N
Launaea taraxacifolia	1.69±0.11^{c}	11.76±2.25^{a}	595.86	B	N
Amaranthus sp*	1.15±0.02^{d}	2.65±0.25^{c}	130.43	B	N
Piper guineense	1.90±0.07^{c}	2.68±0.27^{c}	41.05	B	N
Ipomoea batatas	3.78±0.29^{a}	3.89±0.98^{b,c}	2.91	N	N
Senecio biafrae	2.73±0.17^{b}	4.78±0.58^{b}	75.09	N	N

**Local name (Yoruba) of vegetable; Results presented are mean ± SEM (n = 4); values in the same column with the same superscript are not significantly different from each other (P > 0.05); Bracket "()" indicates negative value. Adequate Intake (AI), and Tolerable Upper Intake Level (UL); Status of trace element conspired to recommended dietary range are represented as: B (Below normal); N (Normal range); T (toxic level)*

Table 4. Mo content of fresh and juiced leafy vegetables

Leafy vegetables	Fresh (mg/100 g dry weight)	Juice (mg/100 g dry weight)	Percentage difference (%)	Intake Limit [14] AI – UL Male: (0.045 – 2mg/d) Female: (0.045 – 2mg/d)	
				Fresh	Juice
Manihot esculenta	32.00±0.00^{b}	43.00±1.00^{a,b}	34.38	B	N
Launaea taraxacifolia	32.00±1.00^{b}	41.00±0.00^{b}	28.13	B	B
Amaranthus sp*	27.00±1.00^{c}	43.00±1.00^{a,b}	59.26	B	N
Piper guineense	18.00±0.00^{d}	44.00±0.00^{a}	144.44	B	B
Ipomoea batatas	26.00±0.00^{c}	2.00±0.00^{c}	(92.31)	B	B
Senecio biafrae	35.00±0.00^{a}	42.00±1.00^{a,b}	20.00	B	B

**Local name (Yoruba) of vegetable; Results presented are mean ± SEM (n = 4); values in the same column with the same superscript are not significantly different from each other (P > 0.05); Bracket "()" indicate negative value. Adequate Intake (AI), and Tolerable Upper Intake Level (UL); Status of trace element conspired to recommended dietary range are represented as: B (Below normal); N (Normal range); T (Toxic level)*

The nutritional essentiality of Mo is among the most important trace minerals, as it is an activator for three important enzymes - aldehyde dehydrogenase, sulfiteoxidase, and xanthine oxidase. Mo deficiency has been reported to inhibit growth and development, especially in prenatal and neonatal stages of development [29]. Low dietary intake of Mo has been linked and identified to be responsible for loss of Cu in the body via urine [30]. On the other hand, prolonged and excessive intake of Mo may result to the development abnormally high serum uric acid levels and increased cellular xanthine oxidase activity [29,30].

Similar to other trace minerals investigated in this study, variation in Mo level was observed among the fresh leafy vegetables. Although, none of the fresh leafy vegetables (at 100 g dry weight) contain the amount of Mo required to its daily AI. However, *Senecio biafrae* had highest concentration of Mo (35.00 µg/100 g dry weight) followed by *Manihot esculenta*, and *Launaea taraxacifolia* which had equal amount of Mo (32.00 µg/100 g dry weight) while the least was observed in *Piper guineense* (18.00 µg/100 g dry weight). Report has shown that Mo level in plants is species dependent. In addition, environmental contaminants especially tungsten (an anti-molybdenum) element may alter the uptake of Mo from the soil [31]. Although, general increase was observed in the level of Mo in all the leafy vegetables investigated after juicing. However, only two vegetable (*Manihot esculenta* and *Launaea taraxacifolia*) juices contain Mo in the required amount (at 100 g dry weight) for RDA.

4. CONCLUSION

This study revealed that juicing increased Cu, Mn and Mo contents in two vegetables (*Manihot esculenta* and *Amaranthus sp*.) out of the six leafy vegetables investigated. Cu and Mn contents were also increased by juicing in *Launaea taraxacifolia*, *Piper guineense*, *Senecio biafrae* and *Ipomoea batatas*. Juicing effect seems less significant as it could not affect Zn content in the six leafy vegetable to yield the amount required as RDA in any of the vegetables investigated. Hence, juicing any of these vegetable as supplement for Zn may require more than 100 g dry weight to supply amount required as RDA of Zn. In addition, among the six leafy vegetables investigated, only juiced *Ipomoea batatas* yielded all the four micro-minerals below toxic level as it was observed that others yielded Cu at a level that is more than the upper limit of Cu RDA which may induce Cu toxicity – one the risk factors of ageing related diseases, atherosclerosis and Alzheimer's disease. Hence, caution be exercised when subject susceptible to Cu toxicity such as pregnant women, children and aged people are to consume juice of these (*M. esculenta, L. taraxacifolia, Amaranthus sp., Piper guineense, Senecio biafrae*) leafy vegetables. In addition, subjects using mineral supplements containing high amount of Cu should not take these juices of vegetables on prolonged time.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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