

Comparative Study of Mineral Composition of Selected Staple Green Leafy Vegetables in Nigeria

ABSTRACT

Six varieties of green leafy vegetables, namely, *Amaranthus hybridus*, *Corchorus olitorius*, *O. gratissimum*, *Talinum triangulare*, *Telfaria occidentalis* and *Vernonia amygdalina* were estimated in their raw, steamed and boiled forms for their mineral compositions. The effect of two different cooking methods (steaming i.e. water blanching and boiling) on fresh leafy vegetables were also evaluated. The mineral contents of fresh leaves of the vegetables were determined using Energy Dispersive X-ray Fluorescence techniques (EDXRF). The results from EDXRF analysis of mineral contents of the six leafy vegetables revealed that fresh, steamed and boiled *C. olitorius* had the highest quantity of K (133.5, 131.5 and 91.2 mg/100 g) and Zn (28.9, 28.4 and 21.4 mg/100 g) respectively. The fresh, steamed and boiled *T. occidentalis* had the highest quantity of Ca (124.2, 122.2 and 80.3 mg/100 g), Na (102.2, 100.6 and 71.1 mg/100 g) and Mg (324.5, 319.2 and 225.5 mg/100 g) respectively, while the fresh, steamed and boiled *A. hybridus* had the highest quantity of Fe (48.6, 47.7 and 29.1 mg/100 g) respectively. The results of percentage loss of mineral composition revealed that almost 40 % of minerals in boiled vegetables was lost, while the amount lost in steamed vegetables was < 2 %. The results obtained from evaluation of sodium/potassium (Na/K) ratio showed that all fresh and steamed leafy vegetables have the same values but the boiled leafy vegetables gave different values. The results have shown that vegetables could serve a better source of some important minerals that can contribute qualitatively to the nutritional need of humans. Based on the results from the processing of vegetables, we suggest that the daily nutritional needs can better be acquired by consuming the steamed processed vegetables rather than consuming the boiled processed vegetables, which would also probably reduce high blood pressure diseases.

Keywords: *Nutritional Composition, Heat processing, Staple Vegetables, EDXRF*

1. INTRODUCTION

The mineral composition of six raw, steamed and boiled staple leafy vegetables cultivated in South-Western Nigeria were investigated and compared. Furthermore, the effects of heat

processing were also emphasized. These six green leafy vegetables, namely, *A. hybridus*, *C. olitorius*, *O. gratissimum*, *T. triangulare*, *T. occidentalis* and *V. amygdalina* are regularly consumed by Nigerians to acquire the necessary minerals, primarily potassium, calcium, sodium, iron, magnesium and zinc that are important for human metabolism and protection of human body against diseases.

Leafy vegetables in general are the fresh and consumable portions of herbaceous plants. They are a great source of important minerals and vitamins [1]. Leafy vegetables contain important food ingredients which can be used as energy sources, body building, regulatory and protection. They also contain appreciable concentration of essential and toxic minerals, depending on the concentrations of those minerals in the soil in which the vegetable is planted [2]. Leafy vegetables have become regular ingredient in the daily diet of Nigerians in both urban and rural areas because they are readily available and affordable. Leafy vegetables can be consumed raw or cooked, their vitamins are mostly water-soluble and susceptible to heat. These water-soluble vitamins can extract out of vegetables when they are soaked in hot water [2].

In order to improve the palatability of leafy vegetables, they are usually subjected to various methods of processing and cooking [3]. They are perceived to be highly susceptible to loss of minerals during processing [4], hence most of these processing methods can alter the composition of minerals, particularly the thermolabile components, thereby reducing the quantity of the minerals required for daily body metabolism [5]. Therefore, this study evaluates and compares the mineral contents and their level of retention in six fresh and processed commonly consumed leafy vegetable species. Also emphasize on the significance of sodium/potassium ratio of the vegetables. The local names, botanical names, common names and parts of the plant used are listed in Table 1.

Table 1. Staple Leafy Vegetables, names and parts used

Local names	Botanical names	Common names	Part used
Tete	<i>Amaranthus hybridus</i>	African spinach	Leaves
Ewedu	<i>Corchorus olitorius</i>	Jute plant	Leaves

Efinrin-nla	<i>Ocimum gratissimum</i>	Basil	Leaves
Gbure	<i>Talinum triangulare</i>	Water lettuce	Leaves
Ugwu	<i>Telfaria occidentalis</i>	Fluted pumpkin	Leaves
Ewuro	<i>Vernonia amygdalina</i>	Bitter leaf	Leaves

2. MATERIALS AND METHODS

2.1 Reagents

All the chemicals and solvents used were of analytical reagent grade and used without further purification. Ethanol, and petroleum ether were purchased from BDH Chemicals, England, Trichloroacetic acid was obtained from E. Merck, Germany, Concentrated Sulphuric acid and hydrochloric acid were obtained from BDH Chemicals, England.

2.2 Collection of Plant Materials

Fresh leaves of the six vegetables were obtained from cultivated farmlands located at Okada, Edo State, Nigeria. The plant materials (leaves) were identified and authenticated at the Taxonomy section, Biological Sciences Department, Igbinedion University, Okada, Nigeria.

2.3 Plant Preparation

Each sample was thoroughly mixed, had their stalks removed, rinsed with de-ionized water and the residual moisture evaporated at room temperature before air-drying for few weeks on a clean paper with constant turning over to avert fungal growth. Each air-dried sample was ground into fine powder using pestle and mortar, and sieved through a 2.0 mm mesh sieve to obtain a dried powdered sample [6].

2.4 Heat Processing

Also, heat processing (steaming and boiling) was carried out on the fresh leaves of the samples following the procedure described by López-García et al. [7] with minor modification.

2.4.1 Steaming

5 g each of fresh vegetable leaves were cooked in a clean stainless-steel by the effect of saturated steam (produced with 200 mL of boiling water at atmospheric pressure) for 10 minutes. An electric cooker, fitted with temperature control, was used. After that, sample was placed in a water bath at 4°C for 30 seconds to stop cooking. Excess water was further removed with

desiccant paper. The steamed leaves were air-dried for few weeks on a clean paper with constant turning over to avert fungal growth. The air-dried sample was ground into fine powder using pestle and mortar, and sieved through a 2.0 mm mesh sieve to obtain a dried powdered sample and then stored in an air tight container for subsequent use.

2.4.2 Boiling

5 g each of fresh vegetable leaves were cooked with distilled water that was brought to boiling for 10 minutes. After boiling, the vegetables were sieved and allowed to cool at room temperature. The air-dried sample was ground into fine powder using pestle and mortar, and sieved through a 2.0 mm mesh sieve to obtain a dried powdered sample and then stored in an air tight container for subsequent use [7]. Both fresh air-dried, steamed air-dried and boiled air-dried samples were submitted for mineral analysis.

2.5 Mineral analysis

The Energy Dispersive X-ray Fluorescence (EDXRF) spectrometry at the Centre for Energy Research and Development (CERD), Obafemi Awolowo University, Ile-Ife, Nigeria was used to analyze the plant parts (leaves, stem bark and the root) using standard method of Preet *et al.* [8]. A powder pellet was prepared by mixing 2 g of plant powder with 0.2 g of pure wax (Hoechst Cridust wax) and homogenizing the mixture by using an agate mortar. The prepared sample was pressed at 20 ton for 120 sec. to obtain a cylindrical pellet 40 mm in diameter. The standard solution with known content of elements was deposited onto a pellet of pressed plants using micropipette. After drying at room temperature, this pellet was homogenized and again pelletized under the same conditions as for the analyzed samples. The quantitative analysis of samples was carried out using the XRF-FP Quantitative Analysis Software package which converts elemental peak intensities to elemental concentrations in weight percentage.

2.6 Statistical Analysis

Three analytical determinations were carried out on each independent replication for every parameter. Three independent replicates ($n = 3$) were obtained from each treatment and the results presented in tables and are reported as means \pm standard deviation (SD). Data were analyzed by ANOVA ($P < 0.05$).

3. RESULTS

3.1 Mineral composition of fresh and processed leafy vegetables

The EDXRF analyses of fresh and processed vegetables are summarized in Table 2a-2c. From the experimental results, potassium content ranges from (133.5 to 79.6) mg/100 g and (131.5 to 78.4) mg/100 g in *C. olerarius* to *A. hybridus* for both fresh and steamed leafy vegetables respectively, but ranges from 91.2 mg/100 g in *C. olerarius* to 54.6 mg/100 g in *V. amygdalina* for boiled leafy vegetables. Calcium content ranges from (124.2 – 61.5) mg/100 g and (122.2 to 60.5) mg/100 g in *T. occidentalis* to *O. gratissimum* for both fresh and steamed leafy vegetables respectively, but ranges from 80.3 mg/100 g in *C. olerarius* to 37.8 mg/100 g in *O. gratissimum* for boiled leafy vegetables. Sodium content ranges from (102.2 to 78.4) mg/100 g, (100.6 to 77.3) mg/100 g and (71.1 to 55.1) mg/100 g in *T. occidentalis* to *O. gratissimum* for fresh, steamed and boiled leafy vegetables respectively. Iron content ranges from (48.6 to 22.8) mg/100 g, (47.7 to 22.4) mg/100 g and (29.1 to 13.9) mg/100 g in *A. hybridus* to *T. triangulare* for fresh, steamed and boiled leafy vegetables respectively. Magnesium content ranges from (324.5 to 85.3) mg/100 g, (319.2 to 83.8) mg/100 g and (225.5 to 61.5) mg/100 g in *T. occidentalis* to *T. triangulare* for fresh, steamed and boiled leafy vegetables respectively. Zinc content ranges from (28.9 to 16.4) mg/100 g, (28.4 to 16.1) mg/100 g and (21.4 to 11.4) mg/100 g in *C. olerarius* to *V. amygdalina* for fresh, steamed and boiled leafy vegetables respectively.

Table 2a. Mineral composition of fresh leafy vegetables in mg/100g.

Vegetable	Mineral in mg/100g					
	K	Ca	Na	Fe	Mg	Zn
<i>Amaranthus hybridus</i>	79.6 ± 0.2	72.6 ± 0.2	82.4 ± 0.2	48.6 ± 0.2	148.2 ± 0.1	16.4 ± 0.2
<i>Corchorus olerarius</i>	133.5 ± 0.2	118.2 ± 0.1	92.1 ± 0.1	40.3 ± 0.1	126.8 ± 0.2	28.9 ± 0.2
<i>Ocimum gratissimum</i>	122.1 ± 0.2	61.5 ± 0.1	78.4 ± 0.1	34.3 ± 0.1	104.8 ± 0.1	19.3 ± 0.2
<i>Talinum triangulare</i>	84.5 ± 0.1	63.7 ± 0.1	80.2 ± 0.1	22.8 ± 0.2	85.3 ± 0.1	20.2 ± 0.2
<i>Telfaria occidentalis</i>	125.2 ± 0.1	124.2 ± 0.2	102.2 ± 0.2	42.6 ± 0.1	324.5 ± 0.1	23.3 ± 0.2
<i>Vernonia amygdalina</i>	85.6 ± 0.1	104.8 ± 0.1	93.1 ± 0.2	38.9 ± 0.2	126.4 ± 0.1	16.8 ± 0.2

Results are mean of triplicate determinations on a dry weight basis ± standard deviation.

Table 2b. Mineral composition of steamed leafy vegetables in mg/100g.

Vegetable	Mineral in mg/100g					
	K	Ca	Na	Fe	Mg	Zn
<i>Amaranthus hybridus</i>	78.4 ± 0.1	71.7 ± 0.1	81.2 ± 0.1	47.7 ± 0.1	145.5 ± 0.1	16.1 ± 0.1
<i>Corchorus olerarius</i>	131.5 ± 0.2	116.4 ± 0.1	90.8 ± 0.2	39.6 ± 0.1	124.6 ± 0.1	28.4 ± 0.2

<i>Ocimum gratissimum</i>	120.1 ± 0.1	60.5 ± 0.1	77.3 ± 0.1	33.7 ± 0.1	103.1 ± 0.1	19.0 ± 0.1
<i>Talinum triangulare</i>	83.2 ± 0.1	62.7 ± 0.1	78.8 ± 0.1	22.4 ± 0.1	83.8 ± 0.1	19.9 ± 0.2
<i>Telfaria occidentalis</i>	123.4 ± 0.2	122.2 ± 0.2	100.6 ± 0.2	41.9 ± 0.1	319.2 ± 0.2	22.9 ± 0.2
<i>Vernonia amygdalina</i>	84.2 ± 0.1	103.2 ± 0.2	91.6 ± 0.2	38.2 ± 0.2	124.3 ± 0.1	16.5 ± 0.2

Results are mean of triplicate determinations on a dry weight basis ± standard deviation.

Table 2c. Mineral composition of boiled leafy vegetables in mg/100g.

Vegetable	Mineral in mg/100g					
	K	Ca	Na	Fe	Mg	Zn
<i>Amaranthus hybridus</i>	55.6 ± 0.2	48.1 ± 0.1	58.6 ± 0.2	29.1 ± 0.1	108.9 ± 0.2	11.9 ± 0.2
<i>Corchorus olerius</i>	91.2 ± 0.1	80.3 ± 0.1	66.5 ± 0.1	24.3 ± 0.1	90.8 ± 0.2	21.4 ± 0.1
<i>Ocimum gratissimum</i>	80.2 ± 0.1	37.8 ± 0.2	55.1 ± 0.1	20.7 ± 0.2	73.1 ± 0.1	13.8 ± 0.2
<i>Talinum triangulare</i>	58.5 ± 0.1	39.9 ± 0.2	56.8 ± 0.2	13.9 ± 0.2	61.5 ± 0.1	13.8 ± 0.2
<i>Telfaria occidentalis</i>	77.1 ± 0.1	79.4 ± 0.1	71.1 ± 0.1	26.0 ± 0.1	225.5 ± 0.1	15.9 ± 0.2
<i>Vernonia amygdalina</i>	54.6 ± 0.2	71.7 ± 0.2	66.3 ± 0.1	23.2 ± 0.1	88.7 ± 0.2	11.4 ± 0.1

Results are mean of triplicate determinations on a dry weight basis ± standard deviation.

3.2 Percentage Loss of mineral composition of fresh and processed leafy vegetables

The evaluated percentage loss of mineral composition of fresh and processed vegetables is presented in Table 3a and 3b. The percentage loss of potassium ranges from (1.68 to 1.46 %) in *O. gratissimum* to *T. occidentalis* for steamed leafy vegetables and (38.4 to 30.2 %) in *T. occidentalis* to *A. hybridus* for boiled leafy vegetables. The percentage loss of calcium ranges from (1.62 to 1.31 %) in *T. triangulare* to *A. hybridus* for steamed leafy vegetables and (38.5 to 31.6 %) in *O. gratissimum* to *V. amygdalina* for boiled leafy vegetables. The percentage loss of sodium ranges from (1.70 to 1.42 %) in *T. triangulare* to *C. olerius* for steamed leafy vegetables and (30.1 to 27.8 %) in *T. occidentalis* to *C. olerius* for boiled leafy vegetables. The percentage loss of iron ranges from (1.96 to 1.67 %) in *A. hybridus* to *T. occidentalis* for steamed leafy vegetables and (40.4 to 38.9 %) in *V. amygdalina* to *T. occidentalis* for boiled leafy vegetables. The percentage loss of magnesium ranges from (1.82 to 1.62 %) in *A. hybridus* to *T. occidentalis* for steamed leafy vegetables and (30.5 to 26.5 %) in *T. occidentalis* to *A. hybridus* for boiled leafy vegetables. Percentage loss of zinc ranges from (1.81 to 64 %) in *C. olerius* to *V. amygdalina* for steamed leafy vegetables and (32.1 to 25.8 %) in *V. amygdalina* to *C. olerius* for boiled leafy vegetables.

Table 3a. Percentage loss of mineral composition of steamed leafy vegetables

Vegetable	% Loss of Minerals					
	K	Ca	Na	Fe	Mg	Zn
<i>Amaranthus hybridus</i>	1.52	1.31	1.48	1.96	1.82	1.75
<i>Corchorus olitorius</i>	1.49	1.52	1.42	1.82	1.72	1.81
<i>Ocimum gratissimum</i>	1.68	1.56	1.46	1.85	1.67	1.78
<i>Talinum triangulare</i>	1.54	1.62	1.70	1.88	1.78	1.70
<i>Telfaria occidentalis</i>	1.46	1.58	1.61	1.67	1.62	1.71
<i>Vernonia amygdalina</i>	1.61	1.52	1.60	1.70	1.68	1.64

Table 3b. Percentage loss of mineral composition of boiled leafy vegetables

Vegetable	% Loss of Minerals					
	K	Ca	Na	Fe	Mg	Zn
<i>Amaranthus hybridus</i>	30.2	33.7	28.9	40.1	26.5	27.4
<i>Corchorus olitorius</i>	31.7	32.1	27.8	39.6	28.4	25.8
<i>Ocimum gratissimum</i>	34.3	38.5	29.7	39.4	30.3	28.7
<i>Talinum triangulare</i>	30.8	37.3	29.2	39.2	27.9	31.6
<i>Telfaria occidentalis</i>	38.4	36.1	30.1	38.9	30.5	31.8
<i>Vernonia amygdalina</i>	36.2	31.6	28.8	40.4	29.8	32.1

3.3 Determination of sodium/potassium (Na/K) ratio of fresh, steamed and boiled leafy vegetables

Results of the Na/K ratio for six different fresh, steamed and boiled leafy vegetables are presented in Table 4. All the six fresh and steamed vegetables have similar sodium/potassium (Na/K) ratio, while the sodium/potassium (Na/K) ratio in boiled vegetables ranges from 0.53 in *Corchorus olitorius* to 0.84 in *Telfaria occidentalis*.

Table 4. Sodium/Potassium (Na/K) ratio of fresh, steamed and boiled leafy vegetables

Vegetable	Sodium/Potassium (Na/K) ratio		
	Fresh	Steamed	Boiled
<i>Amaranthus hybridus</i>	0.62	0.62	0.63
<i>Corchorus olitorius</i>	0.50	0.50	0.53

<i>Ocimum gratissimum</i>	0.50	0.50	0.54
<i>Talinum triangulare</i>	0.57	0.57	0.58
<i>Telfaria occidentalis</i>	0.74	0.74	0.84
<i>Vernonia amygdalina</i>	0.70	0.70	0.78

4. DISCUSSION

The mineral compositions of fresh and processed leafy vegetables were examined in the present research. The results have shown that the vegetables contain appreciable levels of essential minerals with fresh vegetables having higher concentrations, followed by the steamed vegetables and the least being the boiled vegetables. The difference observed in the concentrations the fresh leafy vegetables could be as a result of natural soil profiles, impacts of human activities and varied climatic factors [9], while the difference in the concentrations of the steamed and boiled leafy vegetables could be accredited to differences in analytical protocols, processing time and conditions.

Generally, green leafy vegetables are very rich in minerals that are essential for their physiological and biochemical growth [10-11]. The result for this study indicated that the fresh, steamed and boiled *T. occidentalis* had the highest quantity of calcium, sodium and magnesium, which is an indication that *T. occidentalis* can be consumed on regular basis, as calcium is a crucial nutrient that plays a vital role in neuromuscular function, blood clotting, gives rigidity to the skeleton by virtue of its phosphate salt and helps to maintain metabolism of the human body. The FAO/WHO has reported that the daily intake of calcium varies from 300 mg to 1200 mg depending on the age and physical health of the individual [8]. Sodium also plays a vital role in normal nerve, muscle function, controlling blood volume, maintaining fluid and sodium balance in older people [12]. Magnesium is also essential in stabilizing nucleic acids as a result of its interaction with phosphate. It was also reported that magnesium ions are required in our body by over 300 enzymes for their catalytic action [13]. *C. olerius* had the highest quantity of potassium and zinc, indicating that the existence of potassium in *C. olerius* can help in maintaining fluid and electrolyte balance in the bodies of humans and animals [14-15], as well as, the functioning of all living cells present in all plant and animal tissues [16]. The presence of zinc in *C. olerius* in substantial amount will play a vital role in the storage and secretion of insulin, which subsequently increases the uptake of glucose [17-18]. *A. hybridus* had the highest quantity of iron, indicating that appreciable amount of iron can be derived from the vegetable if

consumed. Reports have revealed that iron is a carrier of oxygen from the lungs to the tissues by red blood cells hemoglobin. It is also essential for the development of healthy brain and immune system [19-20].

The effect of heat processing on the mineral contents of the vegetables was also investigated and the results have shown that boiling had the most adverse effect on the mineral contents of the vegetables, accounting for almost 40 % loss while steaming accounted for almost 2 % loss. This agrees with the results of Onyeike *et. al.* [21]. We therefore suggest that steamed vegetables rather than boiled vegetables should be regularly consumed, in order to acquire the recommended daily intake of the aforementioned minerals. The sodium/potassium (Na/K) ratio of the fresh, steamed and boiled leafy vegetables was determined.

The sodium/potassium (Na/K) ratio (<1) is known to reduce the risk of cardiovascular health issues, helps in maintaining resting potential, affects transport and regulate cellular volume [22]. Therefore, the consumption of the selected leafy vegetables could be beneficial for populations in reducing the risk of cardiovascular events, due to the critical values (<1) of their sodium/potassium (Na/K) ratios, which indicates that they contain lower values of sodium and higher values of potassium.

5. CONCLUSION

The findings from this study have shown that the investigated green leafy vegetables are very rich in minerals that are essential for their physiological and biochemical growth. The results showed that among all the investigated vegetables, *T. occidentalis* had the highest quantity of calcium which plays a crucial role in neuromuscular function, blood clotting, gives rigidity to the skeleton by virtue of its phosphate salt and helps to maintain metabolism of the human body. Also, the results obtained from the effect of heat processing on the mineral contents of the vegetables have shown that boiling had the most adverse effect on the mineral contents of the vegetables, accounting for almost 40 % loss while steaming accounted for almost 2 % loss. Moreover, the sodium/potassium (Na/K) ratios of all the investigated vegetables have met the critical value of <1 indicating that they are all capable of reducing the risk of cardiovascular health issues.

COMPETING INTERESTS

No competing interests declared by Authors.

REFERENCES

1. Dhellot JR, Matouba E, Maloumbi MG, Nzikou JM, Safou-Ngoma DG, Linder M, Desobry S, Parmentier M. Extraction, chemical composition and nutritional characterization of vegetable oils: Case of *Amaranthus hybridus* (Var 1 and 2) of Congo Brazzaville. *African Journal of Biotechnology*. 2006; 5(11): 1095-1101.
2. Adeniyi SA, Ehiagbonare JE, Nwangwu SCO. Nutritional evaluation of some staple leafy vegetables in Southern Nigeria. *International Journal of Agriculture and Food Science*. 2012; 2(2): 37-43.
3. Oboh, G. Effect of some post-harvest treatments on the nutritional properties of *Cnidioscolusa contifolus* leaf. *Pakistan Journal of Nutrition*. 2005; 4(4): 226-230.
4. Favell DJ. A comparison of the Vitamin C content of fresh and frozen vegetables. *Food Chemistry*. 1998; 62: 59 – 64.
5. Davey MW, Montagu MV, Inze D, Sanmartin M, Kanellis A, Smimoff N, Benzie IFF, Strain JJ, Favell D, Fletcher J. Plant L ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*. 2000; 80:825-860.
6. Akubugwo IE, Obasi NA, Chinyere GC, Ugbogu AE. Nutritional and Chemical Value of *Amaranthus hybridus* L. Leaves from Afikpo. Nigeria. *African Journal of Biotechnology*. 2007; 6 (24): 2833-2839.
7. L'opez-Garc'ia G, L'opez-Mart'inez LX, Dubl'an-Garc'ia O, Baeza-Jim'enez R. Extraction and Characterization of the Fatty Acid Profile of Quintonil (*Amaranthus hybridus*). *Revista Mexicana de Ingenier'ia Qu'ımica*. 2017; 16 (3): 835-844.
8. Preet R, Gupta RC, Pradhan SK. Elemental Analysis and Biological Studies of *Physalis angulata* L. using Wavelength-Dispersive X-ray Fluorescence Technique, Wavelength Dispersion X-ray Fluorescence, from Rajasthan. *Asian Journal of Pharmaceutical and Clinical Research*. 2017; 10(8): 220-224.
9. Lamidi, WA, Murtadha, MA, Ojo, DO. Effects of Planting Locations on the Proximate Compositions of *Moringa oleifera* leaves. *Journal of Applied Science and Environmental Management*. 2017; 21(2): 331-338.

10. Anal JMH. Trace and essential elements analysis in *Cymbopogon citratus* samples by graphite furnace-atomic absorption spectroscopy and its health concern. *Journal of Toxicology*. 2014; Article ID 690758, 5 pages.
11. Kabata-Pendias A. Trace Elements in Soils and Plants, CRC Press, Boca Raton, Fla, USA. 2011.
12. Doello S, Burkhardt M, Forchhammer K. (). The essential role of sodium bioenergetics and ATP homeostasis in the developmental transitions of a cyanobacterium, *Current Biology*. 2021; 31(8): 1606-1615.e2.
13. Kass L, Weekes J, Carpenter L. "Effect of magnesium supplementation on blood pressure: a meta-analysis". *European Journal Clinical Nutrition*. 2012; 66 (4): 411–418.
14. Pohl HR, Wheeler JS, Murray HE. Chapter 2. Sodium and Potassium in Health and Disease. In Astrid Sigel, Helmut Sigel and Roland K. O. Sigel. *Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences*. 13. Springer. 2013; 29–47.
15. Clausen MJV, Poulsen H. Chapter 3 Sodium/Potassium Homeostasis in the Cell. In Banci Lucia (Ed.). *Metallomics and the Cell. Metal Ions in Life Sciences*, 12. Springer. 2013; .ISBN 978-94-007-5560-4.
16. Cogswell ME, Zhang Z, Carrquiry AL, Gunn JP, Kuklina EV, Saydah SH, Moshfegh AJ. Sodium and potassium intakes among U.S. adults: NHANES 2003-2008. *American Journal of Clinical Nutrition*. 2012; 96(3): 647-657.
17. Li YV. Zinc and insulin in pancreatic beta-cells, *Endocrine*. 2014; 45(2): 178–189.
18. Maret W. Zinc biochemistry: from a single zinc enzyme to a key element of life, *Advances in Nutrition*. 2013; 4(1): 82–91.
19. Ptyakowska K, Kita A, Janoska P, Polowniak M, Kozik V. Multi-element analysis of mineral and trace elements in medicinal herbs and their infusions. *Food Chemistry*. 2012; 135: 494-501.
20. Swaminathan S, Fonseca VA, Alam MG, Shah SV. “The role of iron in diabetes and its complications,” *Diabetes Care*. 2007; 30(7): 1926–1933.

21. Onyeike EN, Ihugba AC, George C. Influence of heat processing on the nutrient composition of vegetable leaves consumed in Nigeria. *Plant Foods for Human Nutrition*. 2003; 58: 1–11.
22. Averill MM, Young RL, Wood AC, Kurlak EO, Kramer H, Steffen L, McClelland RL, Delaney JA, Drewnowski A. Spot Urine Sodium-to-Potassium Ratio Is a Predictor of Stroke. *Stroke*. 2019; 50:321–327.