Available online at www.elixirpublishers.com (Elixir International Journal)

Food Science

Elixir Food Science 36 (2011) 3222-3225

ISSN: 2229-712X

Assessment of essential elements in *Moringa oleifera*

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ARTICLE INFO

Article history:

Received: 3 May 2011;

Received in revised form:

18 June 2011;

Accepted: 27 June 2011;

Keywords

Neutron Activation Analysis,

Atomic Absorption Spectrometer,

Risk.

ABSTRACT

Moringa is known to contain elements that are essential to life. Major roles played by elements include enzyme activation, nerve impulse conduction, oxygen transport, immune functions. The two major groups of elements are major and minor elements (trace elements). Major elements such as Na, Ca, K,P, Mg and trace elements such as Fe, V, Zn, Cr, Cu, Cd, Co , Mn, Pb, Al, Br were determined using both Neutron Activation Analysis(N.A.A) and Atomic Absorption Spectrometer(A.A.S). Further research is required to carry out the effectiveness of *Moringa* preparations in reducing the risks associated with diseases such as diabetes, hypertension.

Introduction

Elements are essential to life as living organisms use elements to activate enzymes and for production of hormones. Elements also function as constituents of bones and teeth, they form part of other organic molecules that participate in growth function and maintain life processes. Inorganic micronutrients play an important role in various metabolic processes and their deficiency or excesses may disturb the normal biochemical function of the body (Akhter et al., 2004). Elements that cannot be synthesized must be provided from plants, animals or mineral rich water sources (Prasad, 1998). However, some of the elements are known to be toxic to the human body, and even the known essential ones are also likely to exhibit toxic effects on the body when taken in excess.

The major route of entry for essential and toxic elements into living organisms is via the food chain. Due to the health hazards that minerals may pose when taken in excess, the World Health Organisation and other international bodies have set standards relating to daily allowances or tolerable intake of elements. Therefore all elements entering humans via foodstuffs need to be monitored and evaluated to be sure their amounts are within limits of standards set (Dermelj et al., 1996). The composition of elements in plants plays a crucial role in the medicinal values of plants and their therapeutic effects on health and diseases (Kaneez et al., 2001). As a result of increased awareness of the vital role of elements in human health, there has been a revival of interest in the use of plants as a source of conventional and complementary therapies. (Choudhary et al., 2002). A lot of nutritionally important elements and their presence in plants has been the subject of many studies (Sena et al., 1998). This has increased the need to study the elemental composition of many edible plants which could be used as important sources of elements. Ghana abounds in medicinally important plants. However, a few of them have been analysed for their mineral composition. One such important medicinal plant is *Moringa oleifera* which belongs to the family Moringaceae. It is grown and cultivated widely in Brong Ahafo, Ashanti, Northern and Eastern region to mention but a few places. It has become an all round important plant, traditionally important food commodity as the leaves, flowers, fruits and seeds of this tree are used as vegetables and for the treatment of various ailments in traditional pharmacology while the flowers are additionally considered to have anti-inflammatory properties. Moringa preparations have also been cited in scientific literature as having antibiotic, antiulcer, anti inflammatory properties. These properties have the ability of decreasing the severity of the following disorders hypertension, diabetes, anemia, skin infection and have a protective effect on the eye (Fuglie, 2000). Moringa contains several elements which are the basic building block of matter. Some of the elements in Moringa are calcium, chloride, magnesium, potassium, sodium, phosphorous (present as phosphates), nitrogen (present as nitrates) and the minor elements are iodine, iron, zinc, selenium, fluoride, chromium, copper, manganese and molybdenum. Essential elements are elements required for good health and are required for normal body functioning that either cannot be synthesized by the body at all, or cannot be synthesized in amounts adequate for good health and thus must be obtained from a food source. The body utilizes over eighty minerals for maximum function.

There are two categories of micronutrients, the organic compounds known as vitamins and the inorganic compounds, the minerals. These elements include iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc and molybdenum. The trace elements which may be essential includes Silicon, Vanadium, Arsenic, Boron, Nickel, Tin, Lead (Crews, 1998). According to Bowen (1979), a number of elements such as Al, Cd, Co, Cr (VI), Cu, Ti and Ni can be harmful to plants and humans even at quite low concentrations. However, many of these elements are also essential for regular growth.

An analytical technique with sufficient sensitivity is required for accurate determination of essential elements in plant samples. Major analytical techniques such as Atomic Absorption

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Spectrometery (AAS) and Neutron Activation Analysis (NAA) were employed in this study. Fundamentally, AAS is a matter of converting samples and standards in solutions, comparing the instrumental responses of standards and samples and using these comparative responses to establish accurate concentration values for the element of interest. Instrumental Neutron Activation Analysis (INAA) involves sample preparation, sample irradiation and counting as well as qualitative and quantitative analysis in the laboratory. It is often applied for the determination of elements in samples because of its multi-element analysis characteristics and precision. It has sensitivities in the order of parts per billion or better and it is often considered the "reference choice" when developing new procedures or when other methods do not agree (Glascock, 2004). The objectives of the study are to

• Assess the minor and trace elements present in *Moringa oleifera* studied

• To determine regional variations in the elements concentrations in Moringa.

Results obtained from this work will help create reliable nutritional information in addition to awareness creation on nutritional value of *Moringa oleifera.*

Materials and methods

Sample collection and preparation

Leaves of Moringa oleifera were collected from selected Moringa plants from two different areas into clean polythene containers and transported to the laboratory. Samples were then freed of dirt. Each sample was divided into three portions for air drying (room temperature ie.250C±40C). Each of the samples was milled after drying and placed in plastic materials. Special codes were given to each of the samples before storage. The time between sample collection, preparation and analysis was 72hours.

Preparation of samples for neutron activation analysis

About 0.2g of each of the samples was weighed into clean polythene foils, wrapped with forceps and the foils heat –sealed. Two (2) replicates of each of the samples were prepared. Two replicates of compositionally appropriate standard reference material of peach leaf 1547 from National Institute of Standards and Technology (NIST) were prepared and irradiated together with the samples. The reference materials were used as a comparator standard for gamma spectrum evaluation using the relative method of standardization for neutron activation analysis (NAA) and to check for the accuracy of the analytical method used.

Neutron activation analysis of medium-lived radioisotopes in samples

The reference material was 'sandwiched' between samples and stacked together as close as possible. This was to ensure that samples and standards were activated under the same conditions as possible since any variation can remarkably affect the precision. The stacked samples and standards were then placed in the same polyethylene irradiation vial and irradiated for 1hour with thermal neutrons at a neutron flux of 5x1011 neutrons cm-2s-1 at the inner irradiation sites of the Ghana research reactor-1 facility, situated at the Ghana Atomic Energy Commission (GAEC). The GHARR-1 is a 30kw tank-in-pool miniature neutron source reactor (China Institute of Atomic energy).It uses 90.2% enriched uranium(U-235)-Aluminium alloy as fuel. It is cooled and moderated with light water and beryllium acts as reflectors. (Akaho and Nyarko, 2002). At the end of the irradiation, samples and standards were allowed to cool to enable non-analyte radioisotopes induced together with radioisotopes of interest whose half- life are shorter than that of the radioisotopes of interest to decay. In addition to allow the activities induced in the samples to reach acceptable levels for handling to ensure safety of human health germanium(HPGe) N-type coaxial detector(model GR 2518-7500SL) with a resolution of (FWHM) of 1.8 keV relative to the 1332.5keV γ- energy line of 60Co ; a relative efficiency of 25% ;and a peak-to-Compton ratio of 55.Samples and standards were placed on the high purity germanium (HPGe) γ- ray detectors and γ-activity of the induced radioisotopes measured at the same position and distance from the detector. Samples were measured first followed by standards. A Plexiglas source support was mounted on the detector in order to ensure easy and reproducible source positioning.

Neutron activation analysis of short-lived radioisotopes in samples

Short-lived radioisotopes were induced in the samples and standards by 60s irradiation with thermal neutrons in the pneumatic sample transfer facility of GHARR-1 .Samples and standards were stacked together as close as possible and placed in the same polythene irradiation vials. Measurement of induced activity in the sample and standard followed immediately it was ejected from the reactor. Samples were measured first followed by standards.

γ- Activity concentration measurements in samples and standards

Analysis of induced radioisotopes of interest was performed on a PC-based gamma- ray spectroscopy system. The spectroscopy system consist of a Canberra high purity. Table 1 shows Nuclear data used to determine elemental concentrations.

Sample preparation for atomic absorption spectrometer

It involves sample weighing, addition of reagents and digestion in a microwave.0.5g of Moringa samples in three replicates were accurately weighed into TFM Teflon vessels of a microwave digester(Milestone ETHOS 900).6ml of HNO_3 and 1ml H_2O_2 were added to each vessel containing the sample .The vessels were swirled gently to mix well and fitted vertically into the microwave digester and digested for 20minutes.After digestion the solution containing the samples were cooled down in a water bath for 20 minutes to help reduce high temperature and pressure build-up within the vessels. The digestate was then transferred quantitatively into a volumetric flask and diluted to 10ml using deionised water. A blank was prepared in a similar fashion without the analyte. The sample was then analysed using VARIAN AA 240FS flame atomic absorption spectrophotometer in the inorganic laboratory, Department of Chemistry, Ghana Atomic Energy Commission.

To ensure the reliability of the analytical method during digestion and sample preparation blank samples were also digested along with each set of samples and subsequently analysed for appropriate elements through the same procedure. The analytical conditions including the elements, their wavelength, slit width, oxidant and fuel gas are shown in Table 2.

Results and discussion

The results for the analysis of essential elements in Moringa samples that were air dried and the ranges compared with studies from other areas are shown in Table 3.

Major elements

Major elements, also known as macro minerals, are those elements which are needed in the body in quantities greater than

100 milligrams per day. These elements make up more than 99% of the mass of human bodies. The macro minerals that are needed by the body to function and include Calcium, magnesium, phosphorus, sulphur and electrolytes elements (chlorine, potassium, and sodium).

Calcium: The concentration of calcium range between 13.60g/kg -37.10g/kg. A concentration of 20.950g/kg was recorded for Otiakrom and 35.390g/kg for Techiman. Calcium is for strong bone and teeth formation. Magnesium: The concentration of Magnesium range between 10.00 g/kg -98.200 g/kg. Techiman recorded a mean concentration of 60.99g/kg and 58.11g/kg for Otiakrom. Important in bone structure. Deficiency results in muscle spasms and can lead to a calcium deficiency. Potassium: The concentration of potassium range between 17.00g/kg -18.70g/kg. A mean concentration of 25.22g/kg was obtained for Techiman whereas that of Otiakrom was 16.950g/kg. The variation of K in the samples of leaves from different regions might be attributed to the variation in the agro climatic regions. It is a major electrolyte of blood and intracellular fluid. Required for the maintenance of pH and osmotic balance. Sodium: The concentration of sodium range between 65.74g/kg - 23.27g/kg. The mean concentration was 27.85g/kg for Otiakrom and that of Techiman was 33.42g/kg. It is a major electrolyte of blood and extracellular fluid. Required for the maintenance of pH and osmotic balance.

Phosphorous: The concentration of phosphorous range between 21.50g/kg -40.01g/kg. The mean concentrations range was 35.68g/kg for Techiman and 30.26g/kg for Otiakrom. Phosphorous is for strong bone and teeth formation. Required for ATP, the energy carrier in animals.

Trace elements

Trace elements are also known as micronutrients and are found only in minute quantities in the body - yet they are vitally important. The quantities in which they are found are so small, that they can only be detected by spectrographic methods or by using radioactive elements. Examples include Chromium, Cobalt, Copper, Iron, Manganese, Vanadium, Zinc.

Copper: A healthy adult body is supposed to have a copper content of 50mg-120mg (Sigel, 1981). A daily intake of 2mg is recommended considering potential losses through the skin. Copper concentration range between 3.22 mg/kg - 4.20 mg/kg. In the present studies mean concentration for Techiman was 3.88mg/kg and for Otiakrom the concentration was 3.34mg/kg. A structural element in the enzymes tyrosinase, cytochrome c oxidase, ascorbic acid oxidase, amine oxidases, and the antioxidant enzyme copper zinc superoxide dismutase. A copper deficiency can result in anemia from reduced ferroxidase function. Excess copper levels cause liver malfunction and are associated with genetic disorder Wilson's disease. Iron: The dietary recommended intake for iron is as follows; 8mg/day for men, 18mg for women (19years-50years) and 8mg/day for women above 51years.The concentration of iron range between 24.36 mg/kg -51.40 mg/kg. The mean concentration for Techiman in this study was 73.77mg/kg and 37.26mg/kg for Otiakrom. Contained in hemoglobin and myoglobin which are required for oxygen transport in the body. Anemia is the primary consequence of iron deficiency. Excess iron levels can enlarge the liver, may provoke diabetes and cardiac failure. Manganese: 10mg-20mg of this element is believed to be present in the adult human body. 2.5mg-5mg are recommended as the daily adult intake balancing intake with excretion. The concentration of manganese range between 0.17mg/kg-0.30mg/kg Manganese concentrations was found to be highly variable in this study with Otiakrom recording the highest mean concentrations of 0.33mg/kg. Techiman however recorded mean concentration level of 0.11mg/kg. The difference in the values of Manganese could be due to different factors like soil conditions ($_p$H, texture and drainage), climate and the drying method. The mechanism of action which manganese and magnesium exhibit in lipid metabolism may produce a synergistic effect in lowering cholesterol levels. Zinc: The recommended daily intake for zinc is 12g/day for adult women and 15mg/day for adult men. The daily intake is dependent on food, age, sex and general health status. For Techiman a mean concentration of 3.88mg/kg was recorded and 3.66mg/kg was recorded for Otiakrom.

Conclusion

Moringa oleifera leaves were analysed for elements of nutritional significance using both AAS and NAA. The results indicated that both major and minor elements were in higher concentrations in samples from Techiman compared to those from Otiakrom. Compared with studies from other cities the major elements (Ca, Mg, Na, P) studied in samples from Ghana had higher concentrations. However, the trace elements concentrations for samples from the other cities had higher concentrations levels than those from Ghana.

Recommendation

- Further research is required to carry out the effectiveness of Moringa preparations in reducing the risks associated with diseases such as diabetes, hypertension using volunteers who are willing to undertake clinical trials before recommending to humans.
- Localised studies are needed to test the leaves nutritional content and effects in different agro climatic areas since the Moringa plant has spread from its place of origin (India) to the tropical and sub-tropical world.

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Table 1 Nuclear data used to determine elemental concentrations (Filby et al., 1970)

Element	Reaction	Half life	Energy (Kev)
K	^{41}K (n, γ) ^{42}K	12.36h	1524.7
Na	^{23}Na (n, γ) ^{24}Na	15.02h	1368.6; 2754.1
Br	^{81}Br (n, γ) ^{82}Br	35.3h	554.3; 776.5
Mg	^{26}Mg (n, γ) ^{27}Mg	9.45min	1014.4
Mn	^{55}Mn (n, γ) ^{56}Mn	2.58h	846.7; 1810.7; 2112
Ca	^{48}Ca (n, γ) ^{49}Ca	8.7min	3084.4
Cl	^{37}Cl (n, γ) ^{38}Cl	3.7min	1642.4, 2167.5

Table 2 Analytical conditions for atomic absorption spectrometry

Element	Lamp current (mA)	Wavelength (nm)	Slit width (nm)	Fuel gas	Oxidant
Cd	4	228.8	0.5	Acetylene	Compressed air
Co	7	240.7	0.2	Acetylene	Compressed air
Cr	7	357.9	0.2	Acetylene	Compressed air
Fe	5	248.3	0.2	Acetylene	Compressed air
Pb	5	217.0	1.0	Acetylene	Compressed air

Table 3 Mean concentrations in this study compared with studies from other countries.

Elements Macroelements(g/kg)	This study(Techiman,Otiakrom)		Nicaragua	India	Niger	Pakistan (Bahawalnagar)	Pakistan (Sadigabad)
Calcium	35.39±25.71	20.95±5.90	17.50	26.40	13.90	22.93	18.95
Magnesium	60.99±67.59	58.11±34.71	0.11	0.11	0.11	0.10	0.10
Sodium	33.42±6.95	27.85±6.54	1.16	2.73	2.61	2.72	2.59
Potassium	25.22±7.91	16.95±0.125	19.10	21.7	18.4	20.98	19.73
Phosphorous	35.68±2.72	30.26±2.37	1.16	1.36	1.22	1.24	1.45
Microelements(mg/kg)							
Iron	73.77±16.73	37.26±9.71	582	175	347	205	397
Manganese	0.12±0.44	0.33±0.01	47.10	51.80	113.90	76.90	112.80
Zn	3.87±0.72	3.48±0.45	13.50	13.70	24.20	21.40	15.30
Copper	3.88±0.59	3.34±0.72	11.20	7.10	10.60	9.50	11.20