Available online www.jsaer.com

Journal of Scientific and Engineering Research, 2021, 8(2):194-202

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Heavy Metals and Mineral Contents of Some Commonly used Local Spices Sampled from Oil Mill Market, Port Harcourt, Nigeria

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Abstract The study was conducted to determine the concentrations of some heavy metals and mineral elements in four commonly used spices: Utasi (*Gongoronema latifolium*), Oziza seeds (*Piper guinenses*), Uyauak (*Tetrapleura tetraptera* and Uda (*Xylopia aethopica*). The spices were sampled from the popular Oil Mill market in Port Harcourt, digested and analysed using Inductively Coupled Plasma-Mass Spectroscopy and Optical Emission Spectroscopy (*ICP-MS* and *ICP-OES*) respectively. The levels of detectable concentrations ($\mu g/l$) for the heavy metals were in the order: Cr>Co>Se and As, while the concentrations (*mg/L*) of the mineral elements gave the order: *K>Ca>Mg>Na>Fe>*Zn. Selenium and Arsenic were below detection limits and the concentrations of both the heavy metals and the mineral elements were below FAO/WHO permissible limits judging from the available data on their permissible limits at the time of the study. Nevertheless, the results of both mineral elements and heavy metals showed significant variations ($p<0.05$) amongst the four spices, implying that the elemental burden on the spices could be sourced from anthropogenic activities of variable inputs. The study has also provided vital information on the concentrations of these minerals *vis-a-vis* their nutritive potentials which could be useful in evaluating dietary information on these spices and others in general.

Keywords Heavy metals, mineral elements, spices, icp-ms, icp-oes, oil mill market

Introduction

Plant materials form a major portion of the diet and their nutritive value is important in determining the growth and developments of all human beings. The story of spices is one of the most interesting chapters in the history of vegetable products [1]. The cravings for spices have been one of the great factors in human progress and have done much to change the course of history and geography and to promote international relations [2].

Spices refer to all of the edible parts of a plant including fruit, seed, root, rhizomes, barks, leaves, flowers and any other vegetative substances used in a very small quantity as food additives to colour, flavour or preserve food [3-4]. Spices have nutritional value and are often referred to as food accessories or adjuncts because of their ability to stimulate appetite and increase the flow of gastric juice.

Birt [3] also noted that, spices are a large group of such natural ingredients, and include dried seeds, fruits and roots. Spices add a glorious touch to food with their flavour and fragrance. In addition, spices add colour to food making it palatable, and also stimulates salivation and acid secretion of digestive enzymes like amylase [5]. They stimulate appetite by increasing the flow of gastric juice and are used in most homes and restaurants all over the world [6].

According to Kochhar [7], spices possess anti-inflammatory, antibacterial and antioxidant properties, and tend to reduce cholesterol levels which help to prevent heart diseases. Even though spices are not eaten as real functional foods but as supplements, their nutritional capacities should not be neglected. They are important sources of proteins, carbohydrates, vitamins, minerals, fats and oils, and therefore contribute to the nutritional enrichment of food in their own little way.

pices are used for preparing soups for mothers from the first day of delivery to prevent postpartum contraction and aid lactation. They are also used as vegetables for spicing meat, oil bean salad, and foods. Most of these spices have been associated with abundant bitter principle which is claimed to reduce blood sugar levels, and their liquor taken as a purge for colic, stomach pains, and worm infections. It is also believed that newborn babies grow rapidly when they are fed with food made of these spices [8]. There are also various claims about the usefulness of these spices, especially their use in fattening homes, and remarkable growth of new born babies whose mothers use these spices [9].

Spices may be contaminated with heavy metals during growth in the fields, processing and handling. In trace amounts, some heavy metals such as iron, chromium, copper, zinc, cobalt, manganese and nickel are essential for biological functions, though they may also be toxic if present in higher concentrations. Mercury, arsenic, lead, and cadmium are regarded as non-essential because even in trace amounts they can toxic, ad have the ability to bioaccumulate and disrupt the vital functions of human organs and glands such as brain, kidney and liver [10].

Plants may absorb heavy metals from the soil, water or air. In most plants, heavy metals content is observed to decrease in different parts of the plant in the following order –root< leaves< shoot< fruits and seeds. This is due to the fact that metals first face a root barrier from the soil to the plant that impairs penetration into the aerial parts of the plant [9]. Annan *et al* [11] also noted that the assimilation of heavy metals in plants is obvious because of the widespread presence of heavy metals in the soil due to geo climatic conditions.

Contamination of spices by hazardous heavy metals such as Cd, Cr, Cu, Ni and Zn generally, is a detrimental problem due to their non-biodegradable and cumulative nature; hence they are notoriously noted as persistent pollutants [12].

The beneficial health effects of spices have been documented [5]. These beneficial effects can be obtained through the utilization of the seeds, leaves or rhizomes of these spices which can be used as either fresh, dry or ground for medicinal purposes. Spices have been used for several purposes since ancient times. The specific uses of spices tend to vary considerably among cultures and countries: medicine, religious rituals, cosmetics, perfumery, and foods.

As food, they have also been shown to play an important role in health partially as sources of nutrients, Al-Bataina *et al* [13] observed that, irrespective of race, civilization and culture the use of plant product as spice is prominent. Spices are the agents which provide flavor to our food [14]. It is widely accepted that herbs and spices are significant nutritional sources of minerals.

The study therefore is an assessment of the heavy metals contents of some locally consumed spices as well as their dietary potentials with regard to some mineral elements.

Materials and Methods

The following spices: utasi leaves (*Gongoronema latifolium*), oziza seed (*Piper guinense*s), uyayak (*Tetrapleura tetraptera*) and uda seed (*Xylopia aethopica*) were bought at randomly selected in the popular Oil Mill market, Port Harcourt, as samples at regular intervals for a period of one month. Table 1 presents the classification of the spice samples

Table 1: Classifications of Spice Samples

Spice	English name	Local name	Scientific name	Part used
A	-	Utazi (Igbo)	*Gongoronema latifollium*	Leaves
B	Black Pepper	Oziza (Igbo)	*Piper guineense*	Seed
C	Galbanum	Uyayak (Ibibio) Arida (Yoruba)	*Tetrapleura tetraptera*	Fruit
D	Negro Pepper	Uda (Igbo)	*Xylopia aethopica.*	Fruit

Materials and Methods

Reagents and chemicals used for the preparation of solutions were of analytical grade. All the plastic and glassware were cleaned by soaking in dilute nitric acid and were rinsed with distilled water prior to use. Nitric acid (65%) and hydrogen peroxide (30%) were supplied by Merck (India).
Samples were digested using multiwave digestion method (Model: Anton Paar GmbH-multiwave 3000). Approximately 1.0 g was digested with 6 ml of HNO_3 and 2 ml of H_2O_2 in microwave digestion system. The samples and acid mixture were placed in suitably inert sealed polymeric microwave vessels and heated in the multiwave digestion system. The temperature program was as follows: 2 min for 400 w, 6 min for 400 w, 5 min for 400 w, 8 min for 800 w and 8 min for vent. The resulting clear solutions were cooled and diluted to 10 ml with distilled water and used for determination of metal contents.

Sample Analysis

The ICP-MS was used for As, Co, Cr, and Se determinations.The operating parameters were optimized. The ICP-MS was adjusted according to the specifications mentioned in Table 2, whileInductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Model: OPTIMA 2000DV, serial number: 080N3041701) afforded the mineral elements, Fe, K, Mg, Na and Zn and the ICP-OES operating conditions were optimized and the wavelengths, measurement parameters and standards for each element are given in Table 3.

Table 2: ICP-MS Operating Conditions and Measurement Parameters

Spectrometer	Perkins-Elmer Nexion300D
Spray chamber	Cyclonic
Nebulizer	Meinhard
RF Power (Kw)	1.35
Torch horizontal alignment	0.5-1.0
Scanning modes	Peak hopping
Torch vertical alignment	0.2-0.5
Ar gas flow rates (1/min	16.0
Dwell time (ms)	50
Sweep/reading	20
Lens voltage (v)	6.25
Rinse time (s)	10
Nebulizer flow rate (1/min)	1.0-1.07

Table 3: ICP-OES Operating Conditions and Measurement Parameters

Spectrometer	ICP – OES –thermo arcos
RF power (kw)	1.3
Nebulizer	Sea spray
RF generator	27.12mHz
Argon gas flow (L/min) plasma	16.0
Auxiliary flow (L/min)	1.5
Nebulizer flow	0.94
Spray chamber	Cyclonic
Plasma viewing	Axial
Data processing mode	Peak area
Road delay (s)	30
Rinse time	30

All samples were analyzed six times by the above two spectrometric methods.

Analysis of Certified Reference Material (CRM) of the Heavy Metals, Mineral Elements and Calibration

Aliquots of ICP multielement standard solution (0.1 – 10mg /L Merck) containing the mineral elements such as (Zn, Fe, Mg, Ca, Na, and K) and the heavy metals (Cr, Co, As and Se) were used in the preparation of calibration solutions. Working standard solutions were prepared by dilution of the stock standard solutions to the desired concentrations in 1% HNO_3. The ranges of the calibration curves (5 points) were selected to match the expected concentrations for all the elements

Calibration of Standards

For ICP-OES, calibration standards ranging from 0.1 -10mg/I were used to generate calibration curves. For ICP-MS, calibration standards ranging from 0.1 -10µg/I were used to generate calibration curve. The instrument responses were compared with the calibration curve and the concentration of the dilute samples obtained. The instrument result was multiplied by 10 to compensate for the dilution factor.

Statistical Analysis

On the platform of SPSS version 18, one way analysis of variance (ANOVA) was conducted to determine the differences in the concentrations of the elements in the four spices

Results and Discussions

The results of the heavy metals in the various spices are summarized in table 4.The Concentrations of the heavy metals Cr, Co, As and Se in the spices were compared with the maximum permissible limit (MPL) on the basis of the National Food Standards (MPL), applied to “other food and condiments”. The heavy metals were in the increasing order of Cr>Co>As>Se. Such ranking of metal concentrations in spices is good agreement with results obtained in Accra, Ghana [15] in their study on evaluation of trace metals contents of three local spices on Accra markets.

Table 4: Mean concentration (µg/l) of the heavy metals in the sample using ICP-MS (n=6)

Sample code	Cr (µg/l)	Co (µg/l)	As (µg/l)	Se (µg/l)
A	421.0±0.89	4.6±0.15	<1.0	<1.0
B	285.0±1.14	4.6±0.16	<1.0	<1.0
C	287.0±11.16	9.5±0.14	<1.0	<1.0
D	375.0±0.89	3.0±0.09	<1.0	<1.0
WHO/FAO Permissible Limit	3.5mg/kg (3500µg/l)	3.00mg/kg (3000µg/l)	1.0mg/kg (1000µg/l)	2.0mg/kg (2000µg/l)

± mean and standard deviation.

Chromium is an essential nutrient that potentiates insulin action and thus influences carbohydrates lipids and protein metabolism. The chromium contents in the spices under study showed the highest concentration in *Gongoronema latifolium* (421µg/l) and the lowest concentration in *Piper guineenses* (285µg/l). The FAO/WHO, [16] maximum permissible limit is 3.5mg/l. chromium regulates carbohydrate, nucleic acid and lipoprotein metabolism. It enhances the insulin’s action and thus plays a role in glucose metabolism [17]. Chromium deficiency leads to disturbance of glucose and lipids metabolism in humans and animals [18]. It is highly toxic, carcinogenic and is lethal at high dosage. It is required by the human body in very trace amount. Chronic exposure to chromium may results in liver, kidney and lung damage [19].

Cobalt was found higher in *Tetraplera tetraptera* (9.5µg/ll) and lower in *Xylopia aethiopica* (3.0µg/l). The FAO/WHO, (1984) permissible limit is (3.5mg/kg), (3500µg/l). Excess of cobalt causes cardiomyopathy, hyperglycemia, memory loss, allergic dermatitis [20]. Deficiency of cobalt causes pernicious anaemia, severe fatigue, and hyperthyroidism. Cobalt is a part of vitamin B12 which is essential for human health.

The concentration of both selenium and arsenic in the four spices were below (1.0µg/l) for all the four spice samples. These results are in line with Brima [21], who in his study of heavy metals in different medicinal plants using ICP-MS recorded that the concentrations of selenium and arsenic were the least amongst the concentrations of other metals analysed. Maximum permissible limits of selenium and arsenic are 2.0mg/kg (2000µg/l) and 1.0mg/kg (1000µg/l) respectively [16]. Selenium is an antioxidant and is involved in the biosynthesis of thyroid hormones.

Selenium plays an important role in the immune system and can help suppress the progression of HIV [22]. Intake of excess selenium is associated with Keshan disease, which causes an enlarged heart, cardiac arrhythmias, and congestive heart failure [23]. Selenium deficiency affects the function of seleno proteins and can cause damage to tissues and organs. In addition selenium deficiency can cause muscle weakness, skeletal myopathy, cardiomyopathy, and red blood cell macrocytosi [22].

High level of arsenic can cause harmful effect on the skin, lungs and bladder. Its effect can also cause nausea, vomiting or even damage to the blood vessels. From the results obtained generally, the concentration of minerals elements and heavy metals in the four local spices differs significantly ($p<0.05$). these variations in the concentrations of minerals in the spices so analysed in this study may be ascribed to the differences in the physical and chemical nature of the soil at various planting sites, absorption capacities of heavy metals by vegetables, atmospheric deposition of heavy metals which may have been influenced by innumerable environmental factors such as temperature, moisture and the nature of the spices i.e. leaves, roots, fruits and exposed surface area [24].

The levels of the mean concentrations of mineral elements are presented in Table 5. The Concentrations of the mineral elements Ca, Fe, K, Mg, Na and Zinc in the spices were compared with the maximum permissible limit (MPL) on the basis of the National Food Standards (MPL), applied to "other food and condiments". The statistical data revealed that potassium was the most predominant metal in the four spices among all the six mineral elements investigated, followed by calcium, magnesium and sodium. The mean concentration of mineral elements are in the increasing order of K>Ca>Mg>Na >Fe>Zn. This order is similar to those of Akwasi *et al* [15].

Table 5: Mean concentrations (mg/l) of the mineral elements in the samples using ICP-OES (n=6)

Sample code	Ca (mg/l)	Fe (mg/l)	K (mg/l)	Mg (mg/l)	Na (mg/l)	Zn (mg/l)
A	238.20±1.04	3.23±0.02	201.54±0.01	71.78±0.01	12.36±0.01	1.16±0.02
B	53.29±0.01	3.53±0.02	337.93±0.02	38.01±0.02	13.78±0.01	0.62±0.01
C	46.20±0.01	3.14±0.02	223.11±0.01	36.29±0.02	11.15±0.12	0.42±0.02
D	55.57±0.03	2.43±0.01	154.30±0.02	26.68±0.01	11.22±0.01	0.39±0.01
WHO/FAO Permissible Limit	-	300mg/kg (300mg/l)	-	200mg/kg (200mg/l)	-	100mg/kg (100mg/l)

± mean and standard deviation.

Potassium is the most abundant mineral in these spices under study. The highest mean concentration of potassium was also found in *Piper guinenses* (337.93 mg/l), while the lowest was in *Xylopia aethopica* (154.3 mg/l). This is in agreement with researchers who reported that potassium is the most abundant mineral in agricultural products [25]. The high values also agreed with the findings of Bouba, *et al* [4] and Uyoh, [26] in the same spices. Potassium helps in the proper functioning of the brain and nerves. It regulates the acid-base and water balance in the blood and tissues. It is required for bone formation and in the prevention of osteoporosis [27]. Potassium in the diet lowers blood pressures. High blood pressure is the major risk factor for stroke and heart diseases. The minimum daily intake of potassium is 3.5g /day [28].

Calcium amongst the four spices studied ranged from 53.29 to 238.2 mg/l. Calcium was found to be have its highest level in *Gongoronema latifolium* (238.2mg/l) and the lowest in *Piper guineenses* (53.29mg/l) which suggests that it is well fortified with calcium. It was opined that dry spices contain more calcium due to calcification and that happens during drying of seeds/leaves of spices than the fresh ones. These high values according to Akintola *et al* [29] may explain the reason these spices are often used in the diets of nursing mothers after delivery.

High concentration of calcium is important because of its role in bones, teeth, muscles, and heart functions. Calcium has a lot of benefits: it is good for blood clotting and also for good health and bone development. Calcium deficiency leads to hypochromic anemia, leucopenia and osteoporosis in children as corroborated by Dilek *et al* [30]. Ikem, and Egiebor [31], in their recent findings also posited that calcium is essential for good health but very high intake can cause adverse health problems such as liver and kidney damage. Calcium deficiency can lead to low bone mass, bone fractures, numbness, rickets and oesteoporosis [32].

Magnesium is the third most abundant mineral in the four spices studied. The maximum concentration was (71.78mg/l) in *Gongoronema latifollium* and the minimum (26.68mg/l) in *Xylopia aethopica*. According to British Nutrition Foundation (2005), magnesium has important interrelationships with Ca, K and Na. Edeoga and Gomina, [33] and Abii and Elegalam [34] noted that the high values of Mg content in the studied spices strengthen its usage in the treatment of heart diseases. Magnesium daily dietary intake ranges from 400-420mg/day [35]. The permissible level of 200mg/kg (200mg/l) is set by FAO/WHO [16]. Magnesium is important to all cells in humans. Magnesium is known to provide strength, aids enzyme function and helps nerve and heart function. According to Ladaniya, [36] magnesium deficiency leads to weakness, muscle pain, poor heart function, insomnia and nausea. Aremu, *et al* [25] stated that magnesium present in mitochondria and other enzymes is important in energy transfer processes

Sodium was found to be in the range of (11.22mg/l - 13.78mg/l). The maximum concentration (13.78mg/l) was recorded in *Piper guineenses* Like potassium, sodium is essential to the body. It is of great importance in the regulations of many systems in the body. The minimum daily intake of sodium is 24g/day [28]. Sodium, like potassium, is essential to all living organisms; it is also one of the major electrolytes in the blood. Without sodium the body cannot be hydrated, it would dry off. At the point when some vital processes are taking place, sodium is however not needed because too much of it can cause cells to break down [37]. Sodium deficiency has nevertheless been reported to cause loss of body weight and nerve disorder [38].

Iron was found to be in the range of (2.43mg/l -3.53mg/l). The highest concentration was found in *Piper* guineenses (3.53mg/l) and the lowest (2.43mg/l) in *Tetrapleura tetraptera*. The permissible limit as prescribed by WHO/FAO [16] is 300mg/kg (300mg/l). Therefore the concentrations of iron in the studied spices did not portend any health risk as at the time of the study. The concentrations of Fe in these spices also further strengthen its usage by lactating mother to regenerate their lost blood [34].

Iron plays an important role in oxygen and electron transfer processes in human body and is necessary for the synthesis of hemoglobin [39-40]. Iron is a component of numerous proteins and enzymes in the human body [41]. Iron deficiency results in anaemia, adverse pregnancy outcomes, developmental delays and impaired physical work performance, poor resistance to infection and general weakness.

Zinc held the highest uptake in *Gongoronema latifolium* (1.16mg/l) and the lowest in *Tetrapleura tetraptera* (0.42mg/l). This is in accordance with Ihedioha *et al* [42] who confirmed that zinc uptake is higher in leaves than in fruits. Ozturk *et al* [43] reported that zinc is one of the most important trace metals for normal growth and development of humans and helps with strong immune system. The concentrations recorded in this study are however, higher than those reported by Abii and Elegalam, [34] and Uyoh, [26] in the same spices. The difference may be attributed to the soil types and environmental factors associated with the different location of the studies. As important medicinal trace minerals in human body, zinc provides a natural protective mechanism against virus especially those causing respiratory tract infections [44].

Deficiency of zinc can result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism [45]. Zinc is an essential element in the nutrition of man and animal where it functions as an integral part of numerous enzymes or as a stabilizer of the molecular structure of sub-cellular constituents and membrane [41]. The permissible limit of zinc as prescribed by FAO/WHO [16] is 50mg/kg (50mg/l). The concentrations obtained in this study are below this permissible limit, implying that the concentrations of zinc in all the four spices under study are tolerable.

From the results obtained generally, the concentration of minerals elements in the four local spices differs significantly ($p<0.05$). These variations in the concentrations of minerals in the spices so analyzed in this study may be ascribed to the differences in the physical and chemical nature of the soil at various planting sites, absorption capacities by vegetables, atmospheric deposition which may have been influenced by innumerable environmental factors such as temperature, moisture and the nature of the spices i.e. leaves, roots, fruits and exposed surface area [24].

Conclusion

The mineral content analysis of four spices including Utasi (*Gongoronema latifolium*), Uyayak (*Tetrapleura tetraptera*), Uziza (*Piper guinenses*) and Uda seed *(Xylopia aethopica*) by Inductively Coupled Plasma–Optical

Emission spectroscopy has provided vital information on their concentrations as well as their nutritive potentials. The heavy metals in the four spices on the other hand, differ significantly ($p<0.05$). The results have also shown that these spices are safe for consumption, judging from the limits prescribed by the Food and Agricultural Organization and World Health Organization for the elements, except calcium, potassium and sodium for which such prescribed limits were not found.

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