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Trace metal concentrations in some tea leaves consumed in Ibadan, Nigeria

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This study was conducted to determine concentrations of seven trace metals and proximate analysis of some teas consumed in Ibadan, Southwestern Nigeria. Standard methods described by Association of Official Analytical Chemist (AOAC) were used for proximate analysis, while Flame Atomic Absorption Spectrometer (FAAS) was used for metal quantification. The mean of proximate analysis (%) were as follows: Nitrogen (3.01 ± 0.08), Protein (18.85 ± 1.87), Fat (4.63 ± 0.09), Ash (10.79 ± 1.42), and moisture content (8.40 ± 0.11). The ranges of the essential elements in percentage are as follows: Calcium (1.91 to 7.99), Potassium (0.90 to 2.91) and Magnesium (0.33 to 0.62), while the ranges for trace elements in µg/g are as follows: Copper (7.36 to 10.93), Iron (180.38 to 320.04), Manganese (104.78 to 117.85) and Zinc (21.17 to 40.00). Though the trace metals do not constitute any health risk at the present, regular monitoring of their levels in tea plant is necessary for future risk assessment.

Key words: Atomic absorption spectrometry, proximate analysis, tea, trace metals.

INTRODUCTION

After water, tea is the most widely consumed beverage in the world (Mcfarlane and Mcfarlane, 2004). Tea beverage is an infusion of the dried leaves of *Camellia sinensis*, a member of Theaceae family and tea is presently cultivated in at least 30 countries around the world (Sharma et al., 2007). Tea has been successfully grown in African countries, namely Kenya, Malawi, Zimbabwe, and South Africa (Greenop, 1997) as well as in Nigeria (Aroyeun et al., 2012). It is an evergreen shrub or tree that can grow to a height of 30 feet but is usually cut to a height of 2.5 feet in cultivation (Sharma et al., 2007). Depending on the manufacturing processes, teas are classified into 3 major types; non-fermented green tea (produced by drying and steaming the fresh leaves and thus no fermentation occurs); semi-fermented oolong tea (produced when the fresh leaves are subjected to partial fermentation before drying); and fermented black and red teas (which undergo a full fermentation stage before drying and steaming, although the fermentation of black tea is by oxidation and that of puerh is attained using microorganisms) (Zuo et al., 2002). Green tea is widely consumed in Japan and China. Western cultures favor black tea which is prepared through oxidation, curing process of maceration and exposure to atmospheric oxygen (Graham 1992). The consumption of oolong tea is most likely confined to China and Taiwan and roasted tea is consumed mostly in Japan (Sharma et al., 2007).

Tea contains all kinds of minerals such as Potassium (K); Calcium (Ca); Magnesium (Mg); Manganese (Mn); Phosphorus (P); Zinc (Zn) and Iron (Fe) (Han and Li, 2002). However, there is large variation in the elemental composition of tea (Costa et al., 2002) due to differences in climate and agricultural practices, including soil, water and fertilizers (Cabrera et al., 2003).

It is however worrisome that tea plant sometimes contains some of these beneficial elements in concentrations that are higher than would naturally occur. When this happens, the tea is said to be contaminated. These metals can get into the tea plant through anthropogenic sources such as metal-containing

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pesticides, metal-containing fertilizers, and irrigation water with high levels of these metals. These metal contaminants may also get to tea plants through atmospheric deposition. They eventually bioaccumulate higher up in the food chain and this makes humans to be at the receiving end. While there is a growing concern for adequate dietary availability of these elements, there has also been a growing awareness that excess exposure to nutritionally elements can be toxic (Goyer et al., 2004).

Consumption of tea is associated to a lower risk of diseases that cause functional disabilities, such as stroke, cognitive impairment, and osteoporosis in the elderly (Tomata et al., 2012). Tea consumed in Turkey has concentrations of Copper (Cu) in the range of 10.4 to 24.8 mg/g (Narin et al., 2004), while a concentration range for those consumed in the Czech Republic is given as 9.0 to 65.1 mg/g (Street et al., 2006). Xie et al. (1998) gave the range of 20 to 60 µg of Zn/g in different kinds and/or qualities produced in different regions of China. Narin et al. (2004) gave the concentration of Zn to range between 109.9 to 146.1 mg/g in all tested tea brands in Turkey.

Nearly all living things contain iron as an essential element. Iron is also found in tea leaves and concentrations of about 103 to 523 ppm have been determined (Street et al., 2006). A common symptom of Fe deficiency is anemia. Coma, metabolic acidosis, shock, liver failure, coagulopathy, long-term organ damage are some of the health effects of Fe contamination and sometimes, Fe contamination may result to death. Mg is regarded as essential for human nutrition because it is an activator and constituent of many enzymes present in humans (NAS/IOM, 2003). Some of the diseases caused by Mg deficiency include cardiac arrhythmias, coronary artery disease, diabetes milletus, fasciculation, and tremor (Maher, 2000). So far, no side effect is attributable to dietary intake of Mg. This study attempts to investigate the content of metals and proximate analysis in tea leaves that are sold in Ibadan.

MATERIALS AND METHODS

Apparatus and reagent used

All glassware, polypropylene bags and Teflon beakers used were first washed with detergents, rinsed with tap water and distilled water and then soaked in 10% HNO_3 for 48 h. They were rewashed with detergent and rinsed thoroughly with double distilled water. Afterward, the apparatus were oven dried for 12 h at a temperature of 80°C. Chemicals of analytical grade purity were used in the preparation of all reagents.

Sample collection and preparation

The samples for each tea were collected randomly from local markets in Ibadan, Oyo State, Nigeria. The composite dried tea samples were grounded using an electronic blender, sieved using 250 µm sieve and stored in a clean plastic bottle.

Digestion of sample for metal analysis

For metal analysis, 1 g of each tea sample was digested with a mixture of 1 ml 60% $HClO_4$, concentrated 5 ml HNO_3 and 0.5 ml H_2SO_4. This was followed by a boiling in a thermostated hot plate in a fume cupboard for about 45 min at 300°C. Replenishing of the acid was done as necessary to avoid dryness. The digested solution was cooled, filtered via an acid washed Whatman 40 filter paper into 100 ml volumetric flask and was made up to mark with double distilled water. Appropriate blanks were prepared to check for background contaminants. All samples were run in triplicates.

Quality assurance protocol

Blank determination

Blank experiments were run to check for background contaminants by the reagents and apparatus used. The values obtained from running blank experiments were subtracted from the analyte values as applicable.

Calibration of instrument

The calibration of the Flame Atomic Absorption Spectrometer (FAAS) used was done to evaluate the response of the analytical procedure with respect to known quantities of the standards of the heavy metals of interest so that the response to unknown quantities in the samples could be reliably estimated. For the FAAS 20, 18, 16, 14, 12, 10, 8, 6, 4, 2, and 0 µg/l concentrations of each metal solution were prepared by serial dilution for the determination of metals in soil samples. These solutions were run to obtain the working calibration graph. FAAS was used to estimate the levels of heavy metals in the samples by automatic interpolation with respect to the calibration graph (Table 3).

Recovery analysis

This was conducted to assess the error levels arising from contamination and also to ascertain the precision of the analytical procedures used in this study. A 1 g tea samples was put into a Teflon beaker in the presence of 6 M HNO_3 and later spiked with 25 ml of 50 µg/ml of the metals and digested as earlier described. The digested spiked sample was made up to the mark in a 25 ml volumetric flask with doubly distilled water. Also, a 25 ml each of 50 µg/ml of the standard heavy metal solution mixture was also prepared. The two samples were subjected to the same sample digestion procedures. The digested solution was quantitatively added to a 25 ml volumetric flask. The levels of each of these metals in the two samples were determined using the FAAS. The percentage recovery (% R) for each metal was calculated using the relationship:

$$\% R = \frac{A - B}{C} \times 100$$

where A = concentration of a metal in the spiked sample; B = concentration of a metal in the unspiked sample; and C = the amount of metal (ppm) used for spiking. The results of the recovery study are presented in Table 4.

Table 1. Composition of metals in tea leaves.

Metal	Green tea	Oolong tea	Black tea	Mean ± SD
Ca	7.99 ± 0.081	2.57 ± 0.0564	1.91 ± 0.023	4.16 ± 0.102
Mg	0.620 ± 0.0174	0.330 ± 0.014	0.440 ± 0.010	0.463 ± 0.024
K	2.91 ± 0.017	0.900 ± 0.017	1.20 ± 0.019	1.67 ± 0.031
Cu	9.16 ± 0.04	7.36 ± 0.03	10.93 ± 0.05	9.15 ± 0.07
Fe	180.38 ± 10.13	320.04 ± 11.40	258.76 ± 2.96	253.06 ± 15.54
Mn	114.64 ± 9.70	104.78 ± 2.83	117.85 ± 4.46	112.42 ± 11.05
Zn	21.17 ± 1.53	40.00 ± 3.13	30.66 ± 1.53	30.61 ± 3.81

Ca, Mg and K are expressed in %, while Cu, Fe, Mn and Zn are in µg/g.

Table 2. Proximate composition of Nigerian tea leaves (%).

Proximate	Green tea	Oolong tea	Black tea	Mean ± SD
Nitrogen	3.14 ± 0.05	3.05 ± 0.04	2.83 ± 0.05	3.01 ± 0.08
Crude protein	19.71 ± 1.38	19.06 ± 1.05	17.78 ± 0.71	18.85 ± 1.87
Crude fat	5.53 ± 0.05	5.12 ± 0.04	3.25 ± 0.06	4.63 ± 0.09
Ash	11.56 ± 0.97	11.22 ± 0.85	9.59 ± 0.60	10.79 ± 1.42
Moisture	8.70 ± 0.06	8.40 ± 0.08	8.10 ± 0.05	8.40 ± 0.11

Quantification process

The metal content of all the extracts in the digested solutions were determined using Buck Scientific Model 205 FAAS, East Norwalk, United States of America available at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Proximate analysis

The experimental samples were subjected to proximate analysis in accordance with Standard methods described by the Association of Official Analytical Chemist (AOAC, 2005).

Ash content was determined by subjecting the sample with known weight (2.3 g) to ignition in a muffle furnace set at 600°C for 6 h after which the samples were cooled in a desiccator and weighed. The percentage ash was calculated from the formula:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash x 100}}{\text{Original weight of sample}}$$

Moisture content was determined by subjecting the sample with known weight to drying in an oven at 100 to 102°C for 16 h. The loss in weight is reported as moisture content. The percentage dry matter content = 100 - % moisture content.

Crude protein was determined by the routine semi-micro Kjeldahl procedure. The percentage total nitrogen was calculated thus:

$$\text{\% Total Nitrogen} = \frac{\text{(S - B) x N x 140}}{\text{Wt x 1000}}$$

where N = Normality of the acid. The percentage crude protein was calculated by multiplying the total N by conversion factor of 6.25.

The crude fat content was determined quantitatively by extraction with a mixture of chloroform methanol (2:1). The mixture was allowed to stand overnight and lower lipid protein was transferred to a pre-treated and weighed flask and heated to dryness. The difference in the two weights of the wound joint flask gave the weight of the fat (Folch et al., 1957).

Statistical analysis of data

The mean standard deviation for the proximate composition and mineral composition of the experimental tea from replicate measurements were determined using the statistical package for social sciences (SPSS) software, 15.0 for window evaluation version.

RESULTS AND DISCUSSION

The concentration of metals and proximate composition of Nigerian tea leaves are presented in Tables 1 and 2, respectively. The results, based on plant's dry weight, are given as mean ± standard deviation. The trace metal concentration is in increasing order of Ca > K > Mg > Fe > Mn > Zn > Cu (Table 1).

Green tea was found to have the highest concentration of all the metals analyzed and the least concentration was found in black tea. There was no statistically significant difference ($P < 0.05$) in the mean total concentration of the entire elements determined for each type of tea. However, there was significant difference in the concentration of each metal in each tea. K has been reported to be the most abundant trace metal in tea (Soomro et al., 2008). In contrast, our result showed that, Ca was more abundant than K in all the brands of tea tested. Ca had the highest mean concentration of (4.16 ± 0.102%), while Cu had the lowest mean concentration (9.15 ± 0.07 µg/g).

Table 3. Operating conditions of FAAS.

Metal	Wavelength (Nm)	Slit width (Nm)	Detection limit (mg/l)	Detection range (mgl)
Ca	422.7	0.7	0.05	5.00
Mg	285.2	0.7	0.005	1.50
K	766.5	0.7	0.01	3.00
Cu	324.8	0.7	0.005	5.00
Fe	248.3	0.2	0.05	5.00
Mn	279.5	0.7	0.03	2.50
Zn	213.9	0.7	0.005	2.50

Table 4. Percentage recovery and R^2 value.

Metal	Percentage recovery	R^2 value
Zn	98.66 ± 3.06	0.9698
Cu	92.88 ± 4.53	0.9987
Mn	1 ± 5.31	0.9253
Fe	95.39 ± 5.60	0.9952
Ca	ND	ND
Mg	ND	ND
K	ND	ND

The concentration of Mn ranged from 104.78 ± 2.83 to 117.85 ± 4.46 µg/g. Oolong tea had the lowest concentration, while black tea had the highest concentration. These concentration ranges was lower than those reported for Mn by Street et al. (2006) and Narin et al. (2004) but were within the range reported by Aroyeun et al. (2012). The levels determined in this study were below the proposed upper limit of 2 to 3 mg/day (SCF, 1993; WHO, 1993).

Fe deficiency must be avoided as excess Mg affects the central nervous system and neurological effects have been observed in case of occupational exposure (Council of Europe, 2001). In this study, Fe was the fourth most abundant of the analyzed metals. The highest concentration was found in oolong tea, while the least concentration was found black tea. The concentration range of 180.38 to 320.04 µg/g revealed by our study is within the range of 103 to 523 µg/g reported by Street et al. (2006). The levels found in this study were below the recommended range of 10 to 15 mg/day (Normadic Council of Ministers, 1995). Cu was most abundant in black tea and least abundant in the oolong tea. The concentrations ranged from 7.36 ± 0.03 to 10.93 ± 0.05 µg/g. The results were within the same range with tea consumed in the Czech Republic (Street et al., 2006); slightly lower than those obtained from Mambilla highland in Nigeria (Aroyeun et al., 2012) but were generally lower to those consumed in Turkey (Narin et al., 2004). The levels of Cu in our investigation were lower than the upper level of average total dietary intake of 2.2 mg/day recommended by IPCS (1998).

Zn is an essential mineral, which is vital in biological and public health (Hambidge and Krebs, 2007). Zn deficiency resulted from inadequate dietary intake is of growing concern in the developing world (Korkmaz et al., 2010). The concentrations of Zn in our determination ranged from 21.17 ± 1.53 to 40.00 ± 3.13 µg/g, the highest being in oolong tea, while the lowest was found in green tea. These values were in agreement with those obtained from the Czech Republic (Street et al., 2006). According to SCF (1993), it would be unwise to exceed a daily intake of 30 mg in adults. WHO (1993) gave the average daily intake in the range of 15 to 20 mg. The levels found were below the upper limits. Mg was third most abundant of the metals determined. Given the health importance of Mg in related works (NAS/IOM, 2003; Maher, 2000), it is necessary that, adequate levels of this metal is present within the body to ensure a healthy living. The concentrations ranged from 0.330 ± 0.014% in oolong tea to 0.620 ± 0.017% in green tea. These values show that, tea is a good source of this essential element.

Proximate analysis

The proximate compositions of the various teas are shown in Table 2. The percentage N ranged from 2.83 ± 0.05 to3.14 ± 0.05% with a mean value of 3.01% and a standard deviation of 0.08. The percentage N content was in the order of green tea > oolong tea > black tea. This is probably due to gradual loss of N or organic

matter during transport, processing and storage. Also, variation may be caused by difference in organic matter contents of the samples as appreciable amount of N in tea samples occurs in organic form (Mohammed and Sulaiman, 2009). The values obtained are higher than the 0.88 to 1.75% range reported by Mohammed and Sulaiman (2009). Therefore, the percentage N protein content follows the same trend with percentage N content.

Ash content is an indication of the mineral content of a sample. The ash content ranged from 9.59 ± 0.60% in black tea to 11.56 ± 0.97% in green tea with a mean value of 10.79%. The high ash contents suggest that, the various teas can serve as good sources of minerals. The values were higher than the 4.90 to 7.20% range reported by Mohammed and Sulaiman (2009).

Moisture content can vary from one tea to the other, depending on the drying time and nature of the tea involved (Kurma et al., 2005). The moisture content ranged from 8.1 ± 0.05% in black tea to 8.7 ± 0.06% in green tea with a mean value of 8.40%. The range obtained in this study is higher than the 5.60 to 7.50% range reported by Mohammed and Sulaiman (2009). The moisture content is in the order of green tea > oolong tea > black tea. The crude fat content ranged from 3.25 ± 0.06 % in black tea to 5.53 ± 0.05% in black tea with a mean value of 4.63%.

Conclusion

In this study, the teas analyzed are high in N and ash contents, which suggest that, the teas can be rich sources of minerals. Trace elements in various plants can be a useful guide in the determining the safety of a particular plant for human consumption. The results of this study revealed that, the levels trace metals in various tea brands consumed in Ibadan are below the upper limits set by International Organizations. Therefore, trace metal concentrations in teas consumed in Ibadan, Southwestern Nigeria cannot be considered a critical issue for human health. However, regular monitoring of trace metal content in the various teas is necessary for future risk assessments as well as legislative actions when the metal reach critical levels.

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