The Contents of Fifteen Essential, Trace and Toxic Elements in Some Green Tea Samples and in Their Infusions

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Summary: The content of fifteen elements i.e. Ca, Mg, Na, K, Fe, Mn, Zn, Co, Cu, Cr, Ni, Pd, Cd, Ba and Al were determined for 30 sample from three types of green tea samples using flame atomic absorption spectroscopy. The samples were purchased from authentic tea dealer in Peshawar imported from India, China and Kenya. However, some samples were taken which were locally produced in Pakistan with branded packing and without package. The NBS tea leaves. The wet digestion and infusion procedure reference material was also analysed simultaneously with tea samples. The wet digestion and infusion procedures were employed for determination of total elements and aqueous extracted elements respectively. It was found that, considerable amount of essential and trace elements are present in total in tea infusion. The levels of toxic metals are low but level of aluminum is high in both forms. The results obtained form this analysis have shown good accuracy and reproducibility. The relative error and relative standard deviations were less than 10% for most of the elements analysed.

Introduction

Tea is taken in almost every country of the world in various forms, also being cheaper and easy to prepare and serve, it is the most popular drink of east and west. It has numerous medicinal benefits mainly due to its antibacterial and antioxidant properties [1].

The caffeine of tea keeps us awake. Green tea contains volatile oils, vitamins, minerals, and caffeine, but the active constituents are polyphenols, particularly the catechin called epigallocatechin gallate(EGCG). The polyphenols are believed to be responsible for most of its roles in promoting good health. Several animal and test tube studies have demonstrated an anticancer effect of polyphenols from green tea [2,3,4]. The polyphenol in green tea have also been associated with reduced risk of several types of cancer in humans [5,6,7]. In a double-blind study people with leukoplakia (a pre-cancerous oral condition) took 3 grams of mixed oral and topical green tea or placebo for six months. Those in the green tea group had significant decreases in the pre-cancerous condition compared to placebo [8].

Research demonstrates that green tea mildly guards against cardiovascular disease in many ways. Green tea lower total cholesterol levels and improves the cholesterol profile (the ratio of LDL 'good' cholesterol to HDL 'bad' cholesterol) reduces platelet aggregation and lowers blood pressure [9-15]. However, all studies dose note found that green tea intake lowers lipid levels [13]. Green tea is effective as an antioxidant in human beings [14].

In present studies green tea samples, marketed by different companies under different brand names as well as few unbranded samples available in the market have been collected and analyzed for essential, trace and toxic content by flame atomic absorption spectroscopy. Wet acid digestion procedure was employed for decomposing the organic matter in tea samples. The infusions was prepared by, boiling dry material in deionized water on electric hot plate. The infusion contains some water-soluble organic compounds and mostly inorganic materials.

Tea is one of the most popular beverages in the world, tea contains 4.0 – 9.0g/ 100g of inorganic matter. The plyphenolic compounds especially flavanoids are important for the binding of metals such as Al, Fe and Cu.

In green tea most of the toxic elements such as Al is bound with large and small organic compounds,

Table-1:Determination of Metals in *Camellia sinensis* Family Camelliaceae by Atomic Absorption Spectrophotometer mg/100g (Dried Basis)

Element	Indian tea 1	Indian tea 2	Indian tea 1	Indian tea 2
	597.0-883.19	538.5-829.8	439.6-686.7	556.7-699.7
Sodium	(387.6-465.66)	(491.6-642.5)	(356.3-427.9)	(245.8-343.3)
	622.6-1028.7	568.5-1028.7	406.1-541.4	3779.0-1109.9
Calcium	(257.2-473.7)	(216.6-311.3)	(216.6-176.0)	(270.7-338.4)
	916.2-970.2	931.7-1012.7	414.7-441.7	966.4-1722.6
Magnesium	(494.7-465.8)	(294.1-319.2)	(228.5-240.1)	(218.9-251.7)
	2702.0-3222.19	2962.12-3028.8	2968.7-3408.9	2714.86-3462.26
Potassium	(1614.43-1714)	(940.92-1027.61)	(994.27-1027.61)	(1364.-1737.8)
	14.0-15.9	23.1-24.7	12.2-14.4	9.6-19.9
Manganese	(2.2-2.8)	(4.4-4.7)	(1.9-2.2)	(4.5-5.6)
	0.269-0.299	0.167-0.233	0.173-0.227	0.209-0.233
Zinc	(0.072-0.090)	(0.090-0.119)	(0.179-0.376)	(0.131-0.167)
	0.890-1.2	1.1-1.6	0.482-0.727	1.2-1.7
Iron	(0.193-0.388)	(0.225-0.291)	(0.225-0.323)	(0.2253-0.3233)
Nickel	0.204-0.263	0.224-0.682	0.562-0.662	0.542-0.642
	(0.066-0.089)	(0.081-0.153)	(0.129-0.145)	(0.145-0.177)
	0.072-0.106	0.122-0.156	0.122-0.106	0.139-0.190
Cobalt	(0.036-0.049)	(0.062-0.083)	(0.036-0.056)	(0.036-0.049)
	0.122-0.175	0.149-0.201	0.122-0.175	0.149-0.228
Chromium	(0.049-0.070)	(0.049-0.059)	(0.049-0.070)	(0.049-0.081)
	0.264-0.385	0.385-0.565	0.264-0.445	0.264-0.445
Lead	(0.106-0.154)	(0.178-0.226)	(0.106-0.154)	(0.106-0.178)
	1.4-1.6	1.7-1.9	1.7-1.8	1.8-2.0
Copper	(0.069-0.098)	(0.156-0.214)	(0.156-0.200)	(0.142-0.171)
	0.152-0.198	0.152-0.183	0.261-0.308	0.214-0.245
Cadmium	(0.042-0.048)	(0.042-0.061)	(0.061-0.079)	(0.067-0.079)
	6.6-9.5	7.3-9.5	6.6-11.6	8.0-13.0
Aluminum	(2.9-3.5)	(2.3-2.9)	(3.2-4.3)	(2.3-2.9)
	9.5-10.7	10.5-12.4	1.8-2.2	3.0-3.6
Barium	(0.678-0.955)	(0.470-0.817)	(0.608-0.817)	(0.331-0.539)

Key: Values in ( ) for Water soluble metals or infusion values

and in the fermented form, the element species is more available.. Mineral contents in plants are affected not only by the genetic factor but also the environmental factor in the growing area, they are known to be different according to area of growth or variety. Thus fifty sample were analysed in present study by using different qualities of Camellia *sinensis* imported from India, China and Kenya.

Botanical Descriptions

It is a cultivated evergreen plant, usually trimmed to below six ft. in height. However, if left to grow wild, the bush can reach 30ft. Green, Oolong and black (normal) tea are all made from the leaves of the same plant species, camellia *sinensis*. Their chemical content and flavors are, however, very different due to their respective fermentation processes. Green tea leaves are allowed to wither in hot air then pan-fried at halt the oxidation (fermentation) processes. Green tea, unlike black and Oolong tea is not fermented, so the active constituents remain unaltered in the herb. The leaves of the plants are used both as a social and medicinal beverage.

Active Constituents

Tea leave contains many compounds, such as polysaccharides, volatile oils, vitamins, minerals, alkaloid (Caffeine) and polyphenols (catachins and flavonoids). The efficacy decrease in dark tea due to lower contents of antioxidant polyphenols. Tea contains chatechin including gallacatechin, epicate.

Pharmacological Action

Tea has antibacterial and antioxidizing activity. Flavonoids (Polyphenols) have medicinal properties include antioxidant, and antiviral effects. Green tea has also the ability to strengthen veins and decrease their permeability. It is a good source of vitamin C [20].

It has been used as an age-old home remedy for burn, wounds and it is known to be very rich in fluoride. The extract taken orally or applied to skin, inhibit skin tumor formation induced by chemical carcinogenic or ultraviolet radiation, due to polyphenol is constituents of tea and it is effective anticarcinogenic, the relative risk of cancer incidence decreased for those consuming over ten cups of green

Table-2:Determination of Metals in *Camellia sinensis,* family Camelliaceae by Atomic Absorption Spectrophotometer mg/100g (Dried Basis)

Element	China tea 1	China tea 2	Kenya tea 1	Kenya tea 2
Sodium	586.6-748.7 (255.8-389.3)	374.6-465.6 (213.3-336.8)	283.5-374.6 (148.2-278.3)	803.8-933.92 (460.5-545.0)
Calcium	1879.0-1999.9 (280.7-438.4)	379.0-703.8 (230.1-338.4)	270.7-758.0 (176.0-297.8)	351.9-487.3 (203.0-338.4)
Magnesium	988.4-1622.6 (278.9-281.8)	796.6-862.2 (259.4-286.6)	437.8-461.03 (278.7-407.99)	630.7-866.13 (259.4-390.63)
Potassium	2514.8-3262.2 (1364.1-1662.6)	2301.95-2906.7 (1487.7-1707.7)	1608.4-1888.5 (1027.6-1534.4)	2208.5-2942.1 (1104.2-1471.0)
Manganese	8.5-12.9 (2.5-3.6)	11.2-13.8 (4.5-5.6)	6.2-7.5 (4.4-5.3)	4.2-8.1 (2.2-2.8)
Zinc	0.229-0.243 (0.133-0.157)	0.185-0.239 (0.137-0.412)	0.072-0.108 (0.143-0.203)	0.161-0.221 (0.125-0.167)
Iron	1.3-1.5 (0.1653-0.2233)	1.0-1.2 (0.2580-0.3560)	0.645-0.890 (0.2580-0.3560)	2.3-2.5 (1.3-1.8)
Nickel	0.443-0.555 (0.139-0.199)	0.343-0.642 (0.097-0.129)	0.423-0.483 (0.081-0.169)	0.463-0.682 (0.153-0.177)
Cobalt	0.123-0.291 (0.038-0.052)	0.122-0.156 (0.042-0.062)	0.089-0.139 (0.042-0.049)	0.089-0.139 (0.036-0.049)
Chromium	0.159-0.258 (0.043-0.091)	0.122-0.201 (0.049-0.091)	0.122-0.175 (0.019-0.070)	0.090-0.122 (0.049-0.070)
Lead	0.244-0.343 (0.102-0.166)	0.264-0.385 (0.106-0.178)	0.325-0.505 (0.124-0.202)	0.264-0.445 (0.106-0.178)
Copper	1.4-2.8 (0.133-0.166)	0.391-2.2 (0.055-0.084)	1.4-1.6 (0.098-0.127)	1.0-1.6 (0.0554-0.084)
Cadmium	0.211-0.248 (0.087-0.099)	0.136-0.354 (0.036-0.048)	0.292-0.339 (0.042-0.061)	0.214-0.479 0.067-0.079)
Aluminum	8.4-12.0 (2.5-3.9)	9.5-14.4 (2.1-2.9)	5.4-6.6 (1.5-2.3)	8.0-23.7 (2.9-3.8)
Barium	3.5-4.5 (0.321-0.629)	1.6-2.2 (0.331-0.470)	2.2-3.0 (0.331-0.539)	2.0-10.8 (0.886-1.0)

tea. Green tea is effective in gastric problems the polyphenols have been shown to stimulate the production of several immune system cells, and have anti- bacteria properties even against the bacteria that cause dental plaque.

Possible Health Risks

Although all tea varieties posses less caffeine than both coffee and coke cola. It can induce insomnia and nervousness in sensitive and over indulgent individuals. It should also be noted that antioxidant action of (phenolic rich) tea extracts has been shown to reduce the ability of humans to utilize dietary iron. Thus people who are prone to anemia should avoid excessive intake of tea.

Results and Discussion

Essential, Trace and Toxic Content Found in Green Tea Samples

It was observed that the levels of essential and trace elements in all green tea samples are varying in great range. The level of toxic elements was very low in all tea samples and in infusions of tea samples, except aluminum. Mineral elements such as Na, K, Ca, Mg, Zn, Mn, Co, Cr, and Fe are present in all green tea samples and may be directly or indirectly helpful in the management of many diseases [16-18]

The level of essential elements such as potassium, calcium, magnesium and sodium found high as total and available form in infusion of all tea samples. Potassium is important for reducing blood pressure and also increasing blood circulation, as well as preventative aid on general heart health [19].

Calcium helps in the transport of long chain fatty acids which aid in prevention of heart diseases, high blood pressure and other cardiovascular diseases. Magnesium works with Ca to help transmitting nerve impulse in the brain, the both elements give relief in the patients having depression [21].

The level of Al is found to be high in most of the samples total as well as in aqueous extract. The uptake of Al is high in tea samples may be due to grown in soil having low pH. The presence of phosphate and organic compounds in green tea,

Table-3:Statistical Data for Standard of Elements

Elements	Conc. range ppm (x)	Absorption range (Y)	Statistical calculation y = mx +c		
			M	C	r
Sodium	0. 0 – 1. 0	0.0 – .313	0.3079	0.0032	0.999
Potassium	0. 0 –1. 0	0.0- 0.565	0.5654	0.0026	0.999
Calcium	0. 0 -1. 0	0.0– 0305	0.0304	0.0002	0.999
Magnesium	0. 0 –1. 0	0.0–0.883	0.886	0.0133	0.996
Iron	0. 0 – 4.0	0.0– .255	0.0641	0.001	0.999
Zinc	0. 0 – 1.0	0.0 -0.286	0.2861	0.0006	0.999
Manganese	0. 0 –1. 0	0.0 – 0.09	0.0886	0.0018	0.998
Chromium	0 .0 – 0.5	0.0 – 30*	60*	0.35	0.999
Cobalt	0 .0 –1. 0	0.0 – 72*	72*	0.4	0.999
Copper	0. 0 –1. 0	0.0 -0.097	0.0966	0.001	0.999
Aluminum	0. 0 – 10	0.0–0.031	0.0032	0.001	0.996
Nickel	0. 0 – 0.5	0.0 – 45*	89.6	0.4	0.999
Lead	0. 0 – 0.5	0.0 – 41*	82	0.175	0.999
Cadmium	0. 0 – 0.5	0.0 – 88*	176.8	0.35	0.999

* Chart divisions

which can form complexes with aluminum result in decrease of its toxicity. As the level of fluoride is high in all types of tea, the fluoride/aluminum association is of particular importance as it relates to Alzheimer's Disease. Aluminum by itself is not readily absorbed by the body. However, in the presence of fluoride ions, it combines with aluminum to form aluminum fluoride, which is absorbed by the body. In the body, the aluminum eventually combines with oxygen to form aluminum oxide or alumina (Alumina is the compound of aluminum that is found in the brains of Alzheimer's disease[22-23].

Experimental

Equipment Notes

A Hitachi 180-50 atomic Absorption Spectrophotometer equipped with standard hallow cathode lamps and air-acetylene flame was used for absorption measurements.

The nitric acid (65% w/v) and hydrogen peroxide (35% w/v) were supra pure reagents obtained from Merck. High-purity water (electrical resistivity $\rangle$ 10mΩ cm) was produced with a Milli-Q system Millipore, MA, USA). Reference sample of spinach was purchased from U.S. Dept. of Commerce, National Bureau of Standards.

Instrumental Parameters

Fifteen elements namely Na, K, Ca, Mg, Zn Fe,Mn, Co, Ni, Cu, Cd,Cr, Pb, Al and Ba were determined satisfactorily by maintaining different fuel oxidant ratios that is air-acetylene flame and acetylene nitrous oxide flame in absorption measurement mode instrumental conditions are summarized in table 4. The optimum flame region for measuring the cobalt and calcium absorption were higher than that observed for other elements for the determination of calcium the releasing agent lanthanide chloride was added in sample as well as in standard. Calibration was obtained with certified standards. The standards solutions were prepared by diluting a 1000mg/l multi element solution (ICP Multi element standard, Merck, Darmstadt, FRG) with the same acid mixture used for sample dissolution. The internal standard was also prepared by dissolving pure metal of analytical grade or metal compounds in 2N analytical-grade of nitric acid in deionized water. Glassware were cleaned by soaking with the contact over night in a 10% (w/v) nitric acid solution and then rinsed with deionized water.

Sampling

Five to ten samples of each quality were purchased from *Peshawar*, *Hyderabad* Authorized tea dealer and some samples were collected from National tea research station (NTRS) Pakistan.

Procedure

Wet Acid Digestion Procedure

All tea samples were dried at 105C° in oven. Replicate 0.5g samples of each batch weighed separately in 100ml conical flasks and treated with 5ml of nitric acid. 5ml of nitric acid was added empty conical flask serve as a blank. The flasks were covered with watch glasses, and their contents were heated to reflux gently on an electric plate. After refluxing for one hour the contents of flasks were treated with 5ml more of nitric, 2ml of 35 % hydrogen peroxide and the heating at gentle reflux

Table-4: Working Parameters: The Instrumental Conditions Used for the Analysis of Standards and Samples were as Follows

Elements	Wave length (nm)	Slit width (nm)	Lamp Current (mA)	Fuel flow (acetylene) (l/min)	Flow rate (Air) (l/min)	Burner height (mm)	Oxidant (Air) kg/cm^2	Fuel (Acetylene) kg/cm^2	Signal out put
Ca	423.0	2.6	7.0	2.61	9.41	12.5	1.60	0.4	100%
Mg	285.5	2.6	7.0	2.01	9.41	7.5	1.60	0.2	=
Na	590.2	0.4	7.5	2.21	9.41	7.5	1.60	0.25	=
K	266.8	2.6	7.5	2.31	9.41	7.5	1.60	0.3	=
Fe	248.5	0.2	7.5	2.30	9.41	7.5	1.60	0.3	=
Zn	214	1.3	7.0	2.01	9.41	7.5	1.60	0.2	=
Mn	279.8	0.4	=	=	=	=	=	=	=
Co	250.0	0.2	9.5	=	=	10.0	=	0.35	=
Cr	358.2	1.3	6.0	=	=	7.0	=	0.30	=
Cu	325.0	1.3	=	=	=	=	=	=	=
Ni	232.3	0.2	9.5	=	=	7.5	=	=	=
Pb	232.3	1.3	7.0	=	=	=	=	=	=
Cd	229.0	1.3	7.0	2.30	9.40	7.5	1.60	0.30	=
Al*	309.5	1.3	9.5	5.6	5.91(NO)	12.5	1.60 (NO)	5.61	=
Ba*	553.8	1.3	9.5	5.61	5.91(NO)	7.5	1.60	0.45	=

Key = * For these elements High temperature flame nitrous oxide- acetylene is used

was continued for another hour. The watch glasses were removed from the flasks, and the heating was continued until the volumes of their contents were reduced to 2-3ml. The contents of flask were cooled, diluted with high purity water, and filtered through whatman paper # 42 in to 25ml volumetric flasks. The contents of the flasks were brought to volume with high purity water and examined by atomic absorption spectrometry for their sodium, potassium, calcium, magnesium, iron, zinc, manganese, copper, nickel, lead, cobalt, cadmium and chromium levels. The results of these measurements are presented in table-3.

Calibration Standards for Elements and Efficacy

The calibration curves of fifteen elements were obtained for concentration Vs absorbance data were statistically analyzed using fitting of straight line by least square method. The statistical calculation for standard is given in table 3. Blank readings were also taken and necessary correction was made during the calculation of percentage concentration of various elements.

Percentage Recovery Test

The efficiency of extraction method was checked by standard addition method using reference sample of spinach NBS 1570. The matrix of standards, reference and sample solutions was same. The percentage recovery test for different elements by digestion method adopted was 98.5 - 99 % in range.

Preparation of Tea Infusions

Triplicate of each 2 g dried green tea samples were placed in conical flasks separately and 25 ml deionized water was added and solution was heated up to boiling point for ten minutes on electric hot plate. After cooling the content of flasks was filtered through whatman # 42 filter paper and total volume was completed to 50 ml in volumetric flasks. The blank experiment was carried out without tea samples. These samples were used for the determination of water extractable elements.

References

1. H.N.Graham,*Prev. Med.* **21**, 334 (1992).
2. G.D.Stoner, H. Mukhtar. *J. Cell Bioch.* **22,** 169 (1995).
3. K. Goto, S. kanaya, T.Ishigami and Y. Hara. *J. Nutr. Sci. vitaminol* **45,** 135 (1999).
4. C.S. Yang, M.J. Lee and G.Y. Yang, *Environ. Health Prospect,* **105** (Suppl.4), 971 (1997).
5. H. Mukhtar and N. Ahmad, *Toxicol Sci.* **52** (2 Suppl.): 111 (1996).
6. SK. Katiyar, H. Mukhtar, *World Rev. Nutr. Diet* **79**, 154 (1996) (review).
7. L.Kohlmeier, K.G.Weterings, S.Steck and F.J.Kok. *Nutr. Cancer,* **27**, 1 (1997).
8. N.Li Z.Sun, C.Han and J.Chen, *Proc. Soc. Exp. Biol.Med.,* **220**, 218 (1999).
9. S. Kono, K.Shinchi and N.Ikeda, Japan. Prev. Med., **21,** 526 (1992).
10. Y. Yamaguchi, H.Hayashi and H.Yamamzoe, *Nip.Yak. Zas*, **97**(6): 329 (1991).

11. Y.Sagesaka-Mitane, M.milwa and S.Okada, *Chem. Pharm.Bull,* **38** (3) 790 (1990).
12. I.Stensvold, A.Tverdal and K Solvoll, *Prev. Med.* **21,** 546 (1992).
13. Y.Tsubono, S.Tsugane, *Ann. Epidemiol* **7**, 280 (1997).
14. M. Serafini, A. Ghiselli, and A. Ferro-Luzzi, *Eur. J. Clin. Nutr.* **50**, 28 (1996).
15. K.H. Venhet, H. Deboer and SA. Viseman *Am. J. Clin. Nutr.* **66,** 1125 (1997).
16. F. Yasmeen, R. Aleem and J. Anwar *J. Chem. Soc. Pak.* **22**(2), (2000).
17. J.H. Salacinski and P.G. Riby *Anal. Chim. Acta*, 269, 1 (1992).
18. T.G. Kazi and S.A. Katz, *Spectroscopy letters,* **20** (7), 509 (1987).
19. T.G. Kazi, G.H. Kazi and T.P. Ansari, *J. ACGC,* **9,** 51 (1999).
20. D. Chopra.and S. David The chopra centre Herbal handbook. Three Rivers press, USA (2000).
21. P. Ody the Herb society's complete Medicinal Herbal. Dorling Kindersley ltd, London (1993).
22. P. Ody Home Herbal. Human and Rousseau (Pty) ltd, Cape Town. (1995).
23. B. Wyk, B.Oudtshoom and N.Gericke Medicinal Plants of South Africa. Briza Publications, Pretoria. (1997).