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Determination of Arsenic, Cadmium and Lead Concentration in Teas, Commercialized in Rio De Janeiro, Brazil, and Their Transfer to Tea Infusion

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Abstract: About 75% of the estimated 2.5 milliontons of dried tea harvested annually in the world is manufactured as black tea. Black tea (*Camellia sinensis*) is one of the most widely popular nonalcoholic beverages consumed by over two-thirds of the world's population, due to its medicinal, refreshing and mild stimulating effects; besides the positive effects, studies have also shown that drinking tea can have negative effects on human health. From tea leaves, different types of tea are produced: black, green and white. These teas mainly differ in their degree of processing and composition. In the present study, it has been hypothesized that tea leaves contain amounts of As, Cd and Pb, but these elements are leached not from tea leaves to infusion. To verify this hypothesis, concentrations of As, Cd and Pb were determined in tea leaves and infusion and results were compared. Five different brandswere collected in supermarkets and then analyzed. The samples were digested in a microwave digester. After digestion, the determination of As, Cd, Pb in tea leaves and infusions were done by inductively coupled plasma mass spectrometry (ICP-MS). The concentration of As, Cd and Pb in tea leaves were 0.02-0.4 mg kg^{-1}; 0.02-0.09 mg kg^{-1} and 0.1-3.6 mg kg^{-1}, and the concentration in tea infusions < 0.005-0.007mg L^{-1}; < 0.005-0.007 mg L^{-1} and 0.005-0.015 mg L^{-1}, so there was almost no transfer from tea leaves metal content to infusion. The concentration of Pb in white tea leaves (0.8-3.6 mg kg^{-1}) was higher than in green and black teas, and it was also higher than the limit established by Brazilian National Health Surveillance Agency (ANVISA) is of 0.6 mg kg^{-1}. The concentration of As and Cd in leaves and infusion was below ANVISA limits 0.4 mg kg^{-1}.

Keywords: Tea, arsenic, cadmium, lead and mass spectrometer.

1. Introduction

About 75% of the estimated 2.5 milliontons of dried tea harvested annually in the world is manufactured as black tea (Dambiec*et al*., 2013; Malik*et al*., 2013). Black tea (*Camellia sinensis*) is one of the most widely popular nonalcoholic beveragesconsumed by over two-thirds of the world's population, due to its medicinal, refreshing, mild stimulating effects and has an attractive aroma and good taste.

From tea leaves (*Camellia sinensis*), different types of tea are produced: fermented (black), unfermented (green) and semi fermented (white) (Chen*et al*., 2014). These teas mainly differ in their degree of processing and composition (Karak and Bhagat, 2010). The difference between green tea and black tea is through inactivationof

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leaf enzymes. The polyphenol composition of green tea is similar to that of fresh tea leaves, because steamingor roasting at the initial stage of green tea manufacture inactivates enzymes involving the oxidation and hydrolysis of chemical constituents of the leaves. In contrast, the enzymes play important roles in black tea manufacturing, producing the characteristic color and flavor (Karak and Bhagat, 2010; Tanaka and Kouno, 2013).

Various studies have proved that the presence of trace elements in tea may have both beneficial and adverse effects on human health (Malik *et al.,* 2003).

Tea is now considered a healthy drink with bioactive molecules and high antioxidant (Mandiwana, 2011). Owing to the importance of minerals and due toxicity of some trace elements present in tea many studies are carried out to determine their levels in tea leaves and their infusions.

During tea infusion, both essential mineral elements and toxic metals are extracted into the beverage and trace metals consumed in tea cause harmful effects on the human body.

Brazilian law by Brazilian NationalHealth Surveillance Agency–ANVISA- established quality parameters for the teas as: sensory, physico-chemical, microscopic and microbiological characteristics, as well as intentional additives and contaminants. (Vulcano *et al*.; 2008; Lima *et al*., 2009).

In the present study, it has been hypothesized that tea leaves contain amounts of arsenic (As), cadmium (Cd) and lead (Pb), but these elements are not leached from tea leaves to infusion. To verify this hypothesis, concentrations of As, Cd and Pb were determined in tea leaves and infusions and results were compared.

2. Materials and Methods

Instruments and reagents

An inductively coupled plasma mass spectrometer (ICP-MS), Perkin-Elmer SCIEX, model NexION 300D (Thornhill, Canada) was used. Argon gas with a minimum purity of 99.996% was obtained from White Martins (São Paulo, Brazil). The conventional sample introduction system equipped with a concentric nebulizer (MEINHARD®),and aglass cyclone spraychamber was also used. The used instrument conditions were: autolens mode on, peak hopping measurement mode, dwelltime of 15 ms, 50 sweeps by reading, 1 reading by replicate, 2 replicates.Nickel cone, skimmer and hyper-skimmer and a quartz ball joint injector 2.0 mm i.d. were used. A daily evaluation of the performance of the instrument using a standard solution containing 1 μg L^{-1} of Be, Ce, Fe, In, Li, Mg, Pb, and U in a 1% HNO_3medium was carried out and the results were compared with the parameters provided by the manufacturer. The samples were digested in a microwave digester model Speed Wave (BERGHOF, Germany).

All reagents were at least of analytical purity grade. Distilled and deionized water with resistivity of 18.2 mV Ωcm obtained by Milli-Q system from Millipore (Bedford, USA) was used. Concentrated nitric acid 65% (v/v) from Merck (Darmstadt, Germany) and hydrogen peroxide 30% (v/v) from Merck (Darmstadt, Germany). A standard solution containing 500 mg L^{-1} As was preparedfrom a Spex standard (New Jersey, USA), a 500 mg L^{-1} Cd solution was prepared from a Titrisol concentrate (Merck) and 500 mg L^{-1} Pb solution was prepared from a Titrisol concentrate (Merck). The calibration solutions were prepared daily through serial dilutionsof the stock solution with the addition of 1 % (v/v) nitric acid. The following rhodium (Rh) mono-element standard solution from Perkin-Elmer (Norwalk, USA) was employed as the internal standard.

Samples

Five different brands of green, black and white teas were collected in supermarkets and then analyzed. There were 6 green tea, 5 black tea, and 6 white tea samples, being a total of 17 samples. 0,5g of the sample was transferred to Teflon flask and after the addition of 3 mL of HNO_3, 2 mL of H_2O_2 and 2 mL of water, the flask was submitted to microwave digester using a five steps program. After digestion, the resulting solution was transferred to polypropylene tubes and the volume was completed to 25 ml. After that, an aliquot of 3 mL was diluted to 15 mL and Rh was added to a final concentration of 10 µg L^{-1} for its evaluation as internal standard. The infusions were prepared from an aliquot of 1 g from tea leaves and 50 mL boiled deionized water, leached for 15 min and filtered. After this time, the tea was filtered in sieve polyethylene, and mixed with sufficient amount of acid Merck Suprapur nitric ® to give a tea 1 % acid solution. Rh was added to a final concentration of 10 µg L^{-1} . All the samples were analyzed in triplicates.

ICP-MS procedure

The isotopes measured in this study were ^{75}As, ^{111}Cd, and ^{208} Pb.The radiofrequency and nebulizer gas flow rate optimizations wereaccomplished using an aqueous solution. Details of the instrumental operating conditions are depicted in Table 1. The external calibration was accomplished with aqueous solutions in the concentrations of 0.1; 0.5; 1.0; 5.0; and 10.0 µg L^{-1}. All calibration solutions were in 1% v/v nitric acid containing 10 µg L^{-1} of Rh. For the recovery test, three samples (white, green and black) were fortified in different concentrations: 0.5µg $L^{-1}$for Cd and 10µg $L^{-1}$for As and Pb. After the fortification, the samples were submitted to thesame procedure as the other analyzed samples.

Table 1. Experimental conditions used on ICP-MS equipment to determine As, Cd and Pb in tea leaves samples and their infusion.

ICP-MS	Experimental conditions
RF power	1400 W
Nebulizer flow rate	1.0 L min^{-1}
Auxiliary gas flow	1.1 L min^{-1}
Plasma gas flow	17.0 L min^{-1}
Dwell time	15 ms
Operation mode detector	Dual
Sweeps	50
Signal measurement	Peak Hopping
Readings by replicate	1
Auto lens mode	On

Table 2. Figures of merit the determination of trace metals by ICP-MS with Rh as internal standard.

Element	Isotope mass (u.m.a.)	Detection limit (ng L^{-1})	RSD (n=5)
As	75	5.5	**1.8%**
Cd	111	4.9	**2.0%**
Pb	208	43	**1.6%**

3. Results and Discussion

In this study on tea leaves samples, the most abundant element among the non–essential metal analyzed was As (Table 3) followed by Cd (Table 4) and Pb (Table 5).

Table 3. Concentration (mg kg^{-1}) of lead in tea leaves. Mean ± standard deviation; *n* = 5

Brands	White	Green	Black
C	0.36±0.01	XXXXXX*	0.04±0.002
D	0.11±0.001	0.1542±0.007	0.042±0.001
L	0.110±0.002	0.03±0.005	0.040±0.001
R	0.21±0.05	0.117±0.003	0.027±0.002
S	0.07±0.014	0.11±0.01	XXXXXXX*
T	0.140±0.009	0.06±0.002	0.120±0.003

*Green tea of C brand and lack tea of S brand were not analyzed.

Table 4. Concentration (mg kg^{-1}) of arsenic in tea leaves. Mean ± standard deviation; *n* = 5

Brands	White	Green	Black
C	0.092±0.001	*XXXXXX**	0.010±0.001
D	0.031±0.003	0.0547±0.003	0.022±0.001
L	0.040±0.007	0.010±0.005	0.022±0.001
R	0.043±0.003	0.022±0.001	0.0126±0.002
S	0.031±0.001	0.040±0.001	*XXXXXX**
T	0.052±0.001	0.021±0.001	0.021±0.001

*Green tea of C brand and lack tea of S brand were not analyzed.

Table 5. Concentration (mg kg^{-1}) of cadmium in tea leaves.Mean ± standard deviation; *n* = 5

Brands	White	Green	Black
C	3.61±0.06	*XXXXXX**	0.180±0.006
D	0.94±0.01	0.257±0.001	0.150±0.04
L	1.0±0.2	0.11±0.04	0.170±0.004
R	2.12±0.03	1.11±0.03	0.022±0.01
S	0.81±0.02	1.06±0.02	*XXXXXXX**
T	1.58±0.04	0.21±0.03	0.78±0.01

*Green tea of C brand and lack tea of S brand were not analyzed.

Arsenic

Arsenic is a mutagenic and carcinogenic element present in nature that can be toxic to human, animals and plants; however, its toxicity varies with their different chemical forms (Devesa *et al*., 2008, Medeiros *el al*.; 2012).InBrazilian legislation, the maximum total arsenic level established for tea leaves is 0.6 mg kg^{-1} (ANVISA, 2013).

The lowest and highest As levels observed in tea leaves were ≤ 0.04 mg $kg^{-1}$for black tea and 0.36 ± 0.01 mg kg^{-1} for white tea. Yuan *et al.,*(2007) reported As concentration ranging from below the detection limit to 4.81 mg kg^{-1} in Chinese tea leaves, and Madeja *et al*., (2012) mean As concentrations in tea available in different countries ranging from detection limit to 0.32 mg kg^{-1}.

The concentration of arsenic in tea infusions < 0.005to 0.007mg L^{-1} demonstrated there was almost no transfer from tea leaves metal content to infusion. Madeja *et al*., (2012) reported that arsenic concentrations in the infusion samples were low or even undetectable and that release of elements into tea infusions depends on whether they are strongly bound to the organic matrixor more soluble in the solution. In the analyzed samples showed that concentrations of arsenic higher than the detection limit were detected inorganic arsenic,the percentage of inorganic arsenic to total arsenic was from 29% to 88%.

Cadmium

Cadmium exerts toxic effects on the kidney, the skeletal system and the respiratory system and is classified as a human carcinogen. It is generally present in the environment at low levels; however, human activity has greatly increased those levels. The maximum Cd level for tea leaves established by the Brazilian legislation is 0.4 mg kg^{-1} (ANVISA, 2013).

The levels concentrations of Cd found in the present study were low or even undetectable. In different surveys the Cd concentration in tea leaves has not been frequently reported (Chen *et al*.,2009). Other studies showed Cd mean level ranges in tea leaves of 0.03 to 0.08 mg kg^{-1}.Thesewere in good agreement with the levels obtained in previous study reported by Karak *et al*., (2010).

In this study the concentration of cadmium in tea infusions < 0.005 to 0.007 mg L^{-1} showed there was almost no transfer from tea leaves metal content to infusion. Based on the existing literature, it was observed that Cd in tea infusion was generally low. Odegard and Lund (1997) reported that Cd in Lipton Yellow Label tea commonly used in Norway contents was 0.08 µg L^{-1}. Shen and Chen (2008) reported that only 52.8% of thetotal Cd was released from eighteen samples of oolong tea (0.005 µg per 100 mL), 40.3% from fifteen black tea samples (0.06 µg/100 mL), while no Cd was extracted from fifteen green tea samples in Taiwan (Karaka and Bhagat, 2010).

Lead

Lead is a toxic metal whose wide spread use has caused **.**The main exposure route of non-occupationally exposed individuals is by food consumption (Medeiros *et al*., 2012). Nowadays, Pb concentrations in commercial tea leaves have caused concern to both consumers and producers. The allowable limit of Pb for food and beverages in Europe and China is 5 mg kg^{-1}, in Japan it is 20 mg kg^{-1} while in Australia, Canada, and India it is 10 mg kg^{-1} (Karak and Bhagat, 2010). The guide line maximum value of Pb for tea leaves in the Brazilian legislation is 0.6 mg kg^{-1} (Anvisa, 2013). In this study the highest and the lowest Pb levels found were 3.61 ± 0.06 mg kg^{1} for white leaves tea and ≤ 0.02 mg kg^{-1} black tea. In comparison, Pb concentrations ranges were 0.2 to 97.9 mg kg^{-1} dryweight (DW) in 1225 samples from China (Han et al.,2006) and 0.04 and 1.36 mg kg in 100 tea sample collected from South India, Seenivasan et al. (2008). Chen et al. (2010) reported that theratio of Pb concentration in mature leaves to that in young leaves ranged from 1.7 to 6.5 where the minimum concentration of Pb in young leaf was 1 mg kg^{-1} and the maximum was 19.8 mg kg^{-1}.

Study on Pb concentration in tea infusion has not been frequently reported. In this, work the Pb concentration in tea infusions range from < 0.005 to 0.015 mg L^{-1}. However, soluble Pb concentration in some tea infusion could still exceed the 0.01 mg L^{-1} limit set for drinking water by Anvisa, 2011. Therefore, the Pb contamination in tea leaves remains a concern, and practices should be developed to avoid problems in the future.

4. Conclusion

This study was developed in order to provide information on metals concentrations in different tea brands and types. Metal concentrations showed a great variability even within the same tea types that is in accordance with the findings of other studies, nevertheless, all tea samples presented concentration of As and Cd in leaves and infusion values below the Maximum Tolerable Limits established by the Brazilian legislation.

The concentration of Pb in 100% of the white tea leaves were higher than in green and black teas, and the limit established by Brazilian National Health Surveillance Agency (0.6 mg kg^{-1}). 40% of the green tea leaves and17% of the black tea leaves had higher values than established by law.The concentration of Pb tea leaves is a public health issue and the authors suggest an extensive monitoring program in order to evaluate possible sources of contamination that can lead to decrease of this metal content in tea leaves.

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