LWT - Food Science and Technology 68 (2016) 491–498

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Trace elements in *Camellia sinensis* marketed in southeastern Brazil: Extraction from tea leaves to beverages and dietary exposure

Raquel F. Milani [a, b], Marcelo A. Morgano [a, *], Solange Cadore [b]

[a] Institute of Food Technology, Av. Brasil 2880, Jd. Chapadão, P.O. Box 139, 13070-178 Campinas, SP, Brazil
[b] Institute of Chemistry, University of Campinas, P.O. Box 6154, 13083-970 Campinas, SP, Brazil

A R T I C L E I N F O

Article history:
Received 24 April 2015
Received in revised form
3 December 2015
Accepted 18 December 2015
Available online 20 December 2015

Keywords:
Trace elements
Camellia sinensis
Dietary exposure
ICP-MS

A B S T R A C T

The presence of Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn in 30 samples of tea (*Camellia sinensis*) purchased from markets in southeastern Brazil were evaluated by ICP-MS. This study was performed for both tea leaves and their infusions. The results provide data that permits an evaluation of the exposure or dietary intake of these trace elements. For some samples, the levels in the tea leaves were above the standards established by the Brazilian and MERCOSUL regulatory bodies. For example, As was detected in 33% of the red oolong tea samples and Pb was detected in 67% and 100% of the white and red oolong samples, respectively. Although some of these tea leave samples possessed high toxic elements levels, the concentrations of the trace elements in the infusions were measured to be at safe levels. An evaluation of dietary intake demonstrated the remarkable importance of tea in achieving the daily intake of manganese. Indeed, a single cup of green tea may provide 16% of the daily value established by FDA. Principal component analysis classified the tea into three distinct groups on the basis of the inorganic constituents in the tea leaves.

1. Introduction

Tea is one of the most consumed non-alcoholic beverages in the world and is a traditional drink in some countries, for example, the United Kingdom and China. Its origin dates back 10,000 years to China, appearing in England only in the nineteenth century. Currently, China remains the only country where all types of tea are produced and consumed (Dattner, 2011).

Essentially, the types of tea consist of black, green, oolong and white, each with different sensorial characteristics. These characteristics are achieved by subjecting the leaves of *Camellia sinensis* to procedures that include fermentation (for black and oolong tea) or harvesting the leaves at different stages of their development; usually, young leaves are used to obtain green tea, whereas older leaves are used for black tea (Dattner, 2011).

The Food and Agriculture Organization (FAO) estimated that approximately three million tons of black tea were consumed worldwide in 2011, with almost 130 million tons of tea consumed in countries from North and South America: Brazil (5.8 mil tons), Argentina (5.5 mil tons), United States (103 mil tons) and Canada (12.5 mil tons) (FAO, 2012). Motivated by its unique nutritional composition, the consumption of tea has increased in recent years; indeed, tea is an excellent source of several minerals, vitamins, polyphenolic compounds and antioxidants, such as catechins and flavonoids (Karak & Bhagat, 2010; Kumar, Nair, Reddy, & Garg, 2005; Szymczycha-Madeja, Welna, & Pohl, 2012). Several health benefits are related to tea intake, including the prevention of diseases such as cancer, neurological disorders, myocardial infarction (Han, Shi, Ma, & Ruan, 2005; Jin et al., 2005), and reductions in the risk for type 2 diabetes and cholesterol blood levels (Pinto, 2013).

Nevertheless, the inorganic composition of a tea may not include only essential elements, such as Cu, Fe, Mn, and Zn, but also some non-essential minerals or contaminants, such as As, Cd, and Pb, (Malik et al., 2013). The main sources of contaminants in tea leaves are related to the environment or anthropic activities. Polluted air, soil and industrial equipment used for leaf processing are related to lead accumulation in the leaves (Yuan, Jiang, & He, 2005), whereas cadmium and arsenic may be found in additives, fertilizers, and wastewater sediments used in the growth process (Liu et al., 2013).

Accumulation of trace elements in tea leaves is attributable to the acidophilic nature of these plants because metal dissolution is greater in acidic soils than in neutral or alkaline soils (Karak,

* Corresponding author.
E-mail address: morgano@ital.sp.gov.br (M.A. Morgano).

http://dx.doi.org/10.1016/j.lwt.2015.12.041
0023-6438/

Abollino, Bhattacharyya, Das, & Paul, 2011). Aluminum absorption occurs via passive diffusion from the soil to the leaves during the plant's life (Hayacibara, Queiroz, Tabchoury, & Cury, 2004) and can have toxic effects in humans, particularly those with chronic renal diseases (Malik et al., 2013).

In this respect, some studies of trace elements have recently been conducted. Jin et al. (2005) studied several samples from Zhejiang Province (China) and reported Pb levels of 0.11–4.55 mg kg^{-1}, and Yuan et al. (2005) observed high concentrations of As in terrestrial plants (>90 mg kg^{-1} in rice). Mandiwana, Panichev, and Panicheva (2011) evaluated chromium levels in tea samples from South Africa and reported values up to 10.30 mg kg^{-1} for black tea. Finally, Seenivasan, Manikandan, Muraleedharan, and Selvasundaram (2008) verified high Cr values in their study, ranging from 1.1 to 21.2 mg kg^{-1}, in one hundred samples from India.

Despite these advancements, a comprehensive investigation of trace elements from tea leaves acquired from markets in southeastern Brazil and their extraction in infusion procedure is not available in the literature. In our previous work (Milani, Morgano, Saron, Silva, & Cadore, 2015), the applicability of the direct analysis method for assessment of trace elements in tea and herbal beverages by ICP-MS was evaluated. The method provided a reliable response, high sensitivity, low time of analysis and agreed with the "green chemistry" principles. The direct method was validated and applied to few samples of beverages (infusions and soft drinks) marketed in southeastern Brazil. Nevertheless, the analysis in the tea leaves, the evaluation of the extraction of elements from the leaves to the beverages prepared by infusion and the classification of the tea types through multivariate analysis was not performed.

For this reason, and with a view to inform all who may be exposed to these products, the aims of this study were to i) evaluate the extraction of Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn from tea leaves commercialized in Brazil and in beverages prepared by infusion; ii) estimate the dietary exposure and daily intake of these trace elements; and iii) classify the tea types using their trace elements composition through multivariate analysis. To provide these data, thirty tea samples of black, green, white and red oolong tea were acquired in southeastern Brazil and the trace elements levels were determined using inductively coupled plasma-mass spectrometry (ICP-MS) both in the leaves and the beverages.

2. Materials and methods

2.1. Reagents and solutions

Nitric acid (sub-boiling distilled acid, Berghof, Eningen, Germany) and reverse osmosis-purified water (18.2 MΩ cm) were used for the analyses. Analytical curves (0.1–100 μg L^{-1} for As, Cd, Cr, Cu, Ni, Pb and Se and 10–2000 μg L^{-1} for Al, Ba, Fe, Mn and Zn) and internal standard solutions were obtained by successive dilutions of 1.000 mg L^{-1} (Merck, Darmstadt, Germany) and 100 mg L^{-1} standard solutions (Specsol, São Paulo, Brazil) in 2.5% (v/v) isopropyl alcohol for As and in 0.2% (v/v) nitric acid for the other elements.

2.2. Instrumentation

Trace elements were determined using ICP-MS (7700×, Agilent Technologies, Tokyo, Japan). Optimized operational conditions were used: RF power, 1500 W; Ar and auxiliary gas flow rate, 15 and 0.9 L min^{-1}, respectively; He gas flow, 5 and 10 L min^{-1}; double pass spray chamber and micro-mist nebulizer gas flow rate, 1.1 L min^{-1} and 3 replicates with 0.3–1.0 s for integration time. The internal standards (^{45}Sc, ^{72}Ge, ^{89}Y) and the isotopes monitored (^{27}Al, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{80}Se, ^{111}Cd, ^{138}Ba, ^{206}Pb) were used in conditions that minimize isobaric and polyatomic interferences.

2.3. Samples and analytical procedure

Thirty samples of the four most commonly consumed types of tea leaves (black, n = 9; green, n = 9; red oolong, n = 3 and white, n = 9) were purchased from markets in southeastern Brazil. Data concerning the origin or age (i.e., young or old) of the tea leaves were unavailable. Beverages were prepared by infusion of the tea leaves: the proportion of 1 bag (approximately 1.5 g) for a 200-mL cup was used, and the leaves were kept in contact with boiling reverse osmosis-purified water for 3 min. Beverages were filtrated through a 250 μm polymeric membrane and acidified to obtain a 0.2% (v/v) acid concentration.

2.3.1. Determination of trace elements in tea leaves

Sample digestion was performed in a closed microwave digestion system (Start D, Milestone, Sorisole, Italy). Approximately 200 mg of sample (composed for 3–4 bags of each package and homogenized in a stainless steel mill (M-20, IKA, Staufen, Germany) was transferred to a PTFE digestion vessel, and 3 mL of water and 5 mL of concentrated HNO_3 were added. Sample decomposition was performed at a maximum temperature of 170 °C for 32 min. The final solutions were transferred to 25 mL volumetric flasks using purified water.

2.3.2. Determination of trace elements in beverages

Previously acidified infusions were directly analyzed using a discrete sampling introduction system (ISIS-DS, Agilent Technologies, Tokyo, Japan). A sample loop of 150 μL, an uptake time and acquisition delay of 20 s and a rinse during data acquisition was applied for trace elements determination (Milani et al., 2015).

2.4. Quality control

Analysis was performed in triplicate, and the experiments with the analytical blanks followed the same procedure used for the samples. Analytical methods were validated according to INMETRO (2011) using certified reference materials SRM 1547 *Peach leaves* (NIST, Maryland, USA) and INCT-TL-1 *Tea leaves* (Instytut Chemii i Techniki Jądrowej, Warszawa, Poland) and spiked samples. Limits of detection and quantification were calculated as 3 and 10 times the standard deviation of 10 blank experiments.

2.5. Statistical analysis

Mean concentrations of the trace elements were evaluated for significance using Tukey's test and one-way analysis of variance (ANOVA) using the XLSTAT software (Addinsoft, Paris, France). PCA was performed using the Pirouette software (Infometrix, Woodinville, WA, USA).

3. Results and discussion

3.1. Trace element levels in tea leaves

The results obtained for the method validation are shown in Table 1.

Low values of limit of quantification were observed, often below 0.20 mg L^{-1}, except for Al, Fe and Zn. Recoveries between 77 and 118% were obtained for all analytes in the certified references materials. The results of the trace element analysis in tea leaves are presented in Table 2.

According to the ANOVA and Tukey's test results, there was low

Table 1
Results of the method validation for tea leaves analysis.

Element	LOD (mg L^{-1})	LOQ (mg L^{-1})	CRM *Peach leaves*				CRM *Tea leaves*			
			Certified value (mg kg^{-1})	Measured value (mg kg^{-1})	Recovery (%)	CV (%)	Certified value (mg kg^{-1})	Measured value (mg kg^{-1})	Recovery (%)	CV (%)
Al	2.0	6.7	249 ± 8	268 ± 27	108 ± 11	10%	2290 ± 28	2196 ± 62	96 ± 3	3%
As	0.005	0.018	0.060 ± 0.018	0.071 ± 0.002	118 ± 3	3%	0.106 ± 0.021	0.125 ± 0.013	118 ± 13	10%
Ba	0.042	0.14	124 ± 4	127 ± 5	102 ± 4	4%	43.2 ± 3.9	44.8 ± 1.6	104 ± 4	4%
Cd	0.001	0.004	0.026 ± 0.003	0.025 ± 0.001	97 ± 5	4%	0.0302 ± 0.0040	0.0242 ± 0.0011	80 ± 4	4%
Cr	0.031	0.10	1[a]	0.84 ± 0.09	84 ± 9	11%	1.94 ± 0.22	1.48 ± 0.04	77 ± 2	3%
Cu	0.051	0.17	3.7 ± 0.4	3.6 ± 0.2	98 ± 5	5%	20.4 ± 1.5	21.9 ± 1.4	107 ± 7	6%
Fe	1.1	3.7	218 ± 4	191 ± 5	88 ± 2	3%	432[a]	418 ± 6	97 ± 1	1%
Pb	0.013	0.042	0.87 ± 0.03	0.89 ± 0.03	102 ± 4	3%	1.78 ± 0.24	1.42 ± 0.15	80 ± 8	11%
Mn	0.032	0.11	98 ± 3	82 ± 4	83 ± 4	5%	1570 ± 11	1482 ± 57	94 ± 4	4%
Ni	0.019	0.064	0.69 ± 0.09	0.63 ± 0.03	92 ± 4	5%	6.12 ± 0.52	4.87 ± 0.16	80 ± 3	3%
Se	0.006	0.021	0.120 ± 0.009	0.112 ± 0.007	93 ± 6	6%	0.076[d]	0.087 ± 0.006	114 ± 8	7%
Zn	1.4	4.8	17.9 ± 0.4	15.3 ± 1.4	86 ± 8	9%	34.7 ± 2.7	27.3 ± 1.8	79 ± 5	7%

[a] Reference value. **LOD** = Limit of detection; **LOQ** = Limit of quantification; **CRM** = Certified Reference Material; **CV** = Coefficient of variation.

Table 2
Determination of elements (mean, standard deviation and range) in tea leaves acquired from markets in southeastern Brazil.

Element	Tea leaves levels (mg kg^{-1})			
	Black tea (n = 9)	Green tea (n = 9)	Oolong red tea (n = 3)	White tea (n = 9)
Al	1872 ± 1079[a] (750–3937)	2219 ± 572[a] (1613–3468)	2045 ± 78[a] (1997–2135)	1702 ± 251[a] (1305–2063)
As	0.021 ± 0.019[a] (<0.018–0.048)	0.042 ± 0.014[a] (0.029–0.063)	0.56 ± 0.09[b] (0.50–0.67)	0.15 ± 0.07[c] (0.09–0.27)
Ba	57 ± 23[a] (29–87)	68 ± 18[a] (54–101)	35 ± 5[b] (32–41)	38 ± 6[b] (25–49)
Cd	0.013 ± 0.003[a] (0.010–0.020)	0.013 ± 0.006[a] (<0.004–0.019)	0.066 ± 0.008[b] (0.061–0.075)	0.054 ± 0.028[b] (0.020–0.089)
Cr	1.46 ± 1.20[a] (0.16–3.38)	1.67 ± 0.47[a] (1.02–2.33)	5.61 ± 2.27[b] (4.20–8.23)	1.19 ± 0.77[a] (0.35–2.92)
Cu	12 ± 1[a] (11–13)	12 ± 2[a] (11–16)	24 ± 2[b] (22–27)	19 ± 6[b] (14–34)
Fe	94 ± 23[a] (72–136)	151 ± 72[ab] (61–252)	867 ± 182[c] (657–987)	231 ± 103[b] (134–426)
Mn	1123 ± 784[a] (108–1960)	1323 ± 174[a] (1118–1642)	932 ± 120[a] (800–1036)	1169 ± 74[a] (1007–1264)
Ni	3.53 ± 2.07[a] (0.88–6.04)	4.64 ± 1.72[a] (3.09–8.02)	5.30 ± 0.45[a] (4.81–5.69)	4.22 ± 0.38[a] (3.65–4.72)
Pb	0.16 ± 0.02[a] (0.13–0.20)	0.20 ± 0.12[a] (0.05–0.37)	2.05 ± 0.45[b] (1.54–2.41)	1.45 ± 0.94[ab] (0.29–2.85)
Se	0.075 ± 0.031[a] (0.046–0.14)	0.069 ± 0.020[a] (0.051–0.11)	0.10 ± 0.02[a] (0.074–0.11)	0.10 ± 0.05[a] (0.040–0.18)
Zn	21 ± 2[a] (17–25)	20 ± 2[a] (17–23)	44 ± 3[b] (41–46)	30 ± 2[c] (25–34)

[a, b, c] Mean values between different columns with the same letter are not significantly different at $p > 0.05$, according to Tukey's test.

variability between the trace elements present in the black and green tea. However, a significant statistical variation was observed for some tea leaves: Ba levels in the green and white teas; high Cr, Cu and Pb levels in the red oolong tea; and high levels of Zn in both the white and red oolong teas.

The concentration of Al ranged from 750 to 3937 mg kg^{-1} in the tea samples analyzed, which is similar to the findings of the study by McKenzie, Jurado, and de Pablos (2010). The highest levels of Al were found in green (3468 mg kg^{-1}) and black tea (3937 mg kg^{-1}), and these levels were not statistically significantly different ($p < 0.05$). Al has been the subject of numerous studies because of its potential contribution to the development of Alzheimer's disease (WHO, 2011). Cao, Qiao, Zhang, and Chen (2010) studied Puerh tea samples from China and reported Al levels between 390 and 980 mg kg^{-1}. In their study of *C. sinensis*, Mehra and Baker (2007) also found low levels of Al in teas from India (458–605 mg kg^{-1}), Sri Lanka (712–1092 mg kg^{-1}) and China (1053–1307 mg kg^{-1}).

The nickel levels from this study were comparable to those in samples from the Czech Republic obtained by Malik, Szakova, Drabek, Balik, and Kokoska (2008). For iron and manganese, however, high levels were observed in samples from Brazil, ranging from 61 to 987 mg kg^{-1} and 108–1960 mg kg^{-1}, respectively.

The barium levels were slightly higher than those found by Nookabkaew, Rangkadilok, and Satayavivad (2006), with mean values ranging from 35.3 to 68.0 mg kg^{-1}. However, the Se levels determined in this study were lower (ranging from 0.040 to 0.18 mg kg^{-1}) than the values reported by the same group.

For the Cu and Zn levels, the samples acquired in Brazil showed the following trend: black ~ green < white < oolong. This trend is unlikely to be associated with the tea production. That is, green tea that is not fermented has a similar composition to a fermented black tea (Szymczycha-Madeja et al., 2012), which indicates that these tea leaves may come from different regions or countries. Notably, McKenzie et al. (2010) observed a similar behavior for green and black teas.

The observed differences in the trace element levels for samples purchased from Brazil and the other countries may be due to the age of the leaves when collected (young or old), soil nutrients, rainfall incidence or altitude (Malik et al., 2008). Unfortunately, the origin of most tea leaves is often unknown because commercial samples consist of a pool of tea leaves from different locations.

Regarding the toxic elements (contaminants) levels, Brazilian and MERCOSUL regulations have established maximum limits for some elements, including As (0.6 mg kg^{-1}), Cd (0.4 mg kg^{-1}), and Pb (0.6 mg kg^{-1}) in tea, yerba mate, and other infused vegetables (Brasil, 2013). As shown in Table 2, the concentration levels of some elements were above these maximum limits for some samples in this study.

High levels of lead were detected in red oolong tea (100% of the samples) and white tea (67%). The results obtained by Nookabkaew et al. (2006) for *C. sinensis* samples from Thailand, China, Japan, Sri Lanka, India and Indonesia found Pb levels up to 53.89 mg kg^{-1}. The levels determined in this study are similar to the levels reported by Seenivasan et al. (2008) for black tea samples from India (i.e., 0.04–1.36 mg kg^{-1}).

For arsenic, only 33% of the red oolong tea possessed levels above the maximum allowed by Brazilian and MERCOSUL regulations (Brasil, 2013). The As levels determined in this study (with a maximum of 0.238 mg kg^{-1} in *C. sinensis* teas) were higher than those found by Nookabkaew et al. (2006).

Cadmium was not detected in high levels in the samples we investigated (0.002–0.100 mg kg^{-1}), which is lower than the results obtained by Seenivasan et al. (2008), who reported black tea samples with Cd levels of 0.69 mg kg^{-1}.

According to Canadian regulations, the maximum limit for Cr is 2 mg kg^{-1}. In this study, high levels of Cr in black and green (18%), white (11%) and red oolong (100%) tea were observed. In some countries, including Canada, China, Malaysia, Singapore, and Thailand, the limits for As (2–5 mg kg^{-1}), Cd (0.3–1 mg kg^{-1}), and Pb (10–20 mg kg^{-1}) in herbal medicines and products are often higher than the limits established by Brazilian and European regulations (WHO, 2007). Mandiwana et al. (2011) found low Cr levels in green and white tea, with maxima of 0.95 and 0.61 mg kg^{-1}, respectively. For black tea, however, Cr levels up to 10.30 mg kg^{-1} were reported.

3.2. Trace element levels in tea infusions

Trace element analysis was performed in the thirty tea infusions prepared using the tea leaves (item 3.1) and analyses were performed in analytical triplicates. In our previous study, the direct analysis method was validated according to INMETRO, 2011 recommendations (Milani et al., 2015). Briefly, limits of quantification obtained were below 2.2 μg L^{-1}, except for Al and Zn, and recoveries between 82 and 120% were obtained for all analytes in spiked experiments. The results of the trace element analysis in the beverages are presented in Table 3.

In general, the results obtained in this study are in agreement with analyses prepared with samples from the Czech Republic (Malik et al., 2008) and Spain (Fernández, Pablos, Martín, & González, 2002). However, an exception was found for Cu and Zn. In these cases, the levels reported from the samples from the Czech Republic were higher than those found in this study by a factor of approximately 10. The present study also detected higher values than those reported for black tea samples from Turkey (Görür, Keser, Akçay, Dizman, & Okumuşoğlu, 2011), with values up to 368.57 and 9.17 μg kg^{-1} for Mn and Cu, respectively.

These differences are possibly the result of different local tea cultivation practices or because of different processing habits for the beverages (i.e., brew or infusion and the ratio and contact time between tea leaves and boiling water). This study considered the infusion procedure according to the recommendation by Brazilian government agencies and manufacturers, which consists of exposing tea leaves to boiling water for 3 min (Brasil, 2010). The adopted procedure is consistent with the findings of Szymczycha-Madeja et al. (2012), who determined that the highest extraction of trace elements occurs in the first five minutes of contact between boiling water and tea leaves.

High levels of manganese and aluminum were observed for all types of tea, especially for the black, green and white teas. The values ranged between 2061 and 6222 μg L^{-1} for Al and 173–3111 μg L^{-1} for Mn. As seen in Table 4, these elements had one of the highest percentages of extractions, except for Ni in green and white tea, which exhibited a mean extraction of 95 and 92%, respectively. Szymczycha-Madeja et al. (2012) verified better leaching of some elements (such as nickel and zinc) in green tea than in black and oolong teas. The results obtained in this study support this observation.

For copper and iron, low extraction percentages were observed, especially for red oolong tea. The use of boiling water is usually associated with low extractions of these elements from the leaves because phenolic compounds containing Fe and Cu are insoluble at this temperature (Szymczycha-Madeja et al. 2012). Values obtained in this study are in agreement with those obtained by Nookabkaew et al. (2006), who reported extraction percentages of 2.39% and 12.96% for Fe and Cu, respectively.

The extraction of aluminum was similar to that found by Mehra and Baker (2007), who reported 29.7% in the first tea infusion. Moderate extractions (of 20–55%) were reported by Szymczycha-Madeja et al. (2012) for Al, Cr, Cu, Mn and Zn. This group also noted that tannins strongly bind with Al, Cr, Cu and Fe. We observed low extraction values for these elements in red oolong tea, possibly because of a high content of tannins in this type of tea. Additionally, poor extraction of As, Cd and Pb was found in this study; indeed, these elements were often found at low levels or even below quantification limits.

Regarding the contaminant levels, Brazilian and MERCOSUL regulations established maximum limits for As (0.05 mg kg^{-1}), Cd (0.02 mg kg^{-1}), and Pb (0.05 mg kg^{-1}) in non-alcoholic beverages, excluding juices (Brasil, 2013). From the data in Table 3, it is possible to see that none of the investigated samples possessed concentrations above these limits.

3.3. Dietary intake and an evaluation of exposure

Presently, the estimate for dietary intake and exposure considered a daily consumption of a cup of tea (200 mL) by a 70 kg (bw = body weight) adult. The data are presented in Tables 5 and 6. This assumption is consistent with the study performed by Camargo and Toledo (2002), who determined an average daily

Table 3
Determination of elements (mean, standard deviation and range) in tea beverages prepared using tea leaves acquired from markets in southeastern Brazil.

Element	Tea beverages levels (μg L^{-1})			
	Black tea (n = 9)	Green tea (n = 9)	Oolong red tea (n = 3)	White tea (n = 9)
Al	5085 ± 2739[a] (2061–9647)	5103 ± 794[a] (3823–6222)	954 ± 76[b] (882–1033)	3216 ± 829[ab] (2168–4468)
As	<0.46[a]	<0.46[a]	1.17 ± 1.01[b] (<0.46–1.79)	<0.46[a]
Ba	36 ± 22[a] (8–62)	32 ± 11[a] (20–54)	15 ± 4[b] (13–20)	17 ± 5[b] (10–24)
Cd	<0.053[a]	<0.053[a]	<0.053[a]	<0.053[a]
Cr	4.0 ± 3.5[a] (<0.3–10.6)	3.9 ± 1.6[a] (2.0–6.7)	0.7 ± 0.1[b] (0.6–0.7)	1.1 ± 0.9[b] (0.4–2.5)
Cu	22 ± 4[a] (17–28)	17 ± 5[ab] (10–27)	6 ± 3[b] (4–10)	32 ± 12[c] (19–48)
Fe	8.1 ± 1.3[a] (6.1–10.0)	22.6 ± 8.1[b] (11.0–34.0)	64.6 ± 16.9[c] (47.2–80.9)	35.8 ± 10.7[d] (21.8–54.6)
Mn	1651 ± 1150[ab] (173–3111)	2409 ± 609[a] (1612–3223)	1020 ± 97[b] (951–1131)	1828 ± 378[ab] (1433–2464)
Ni	18 ± 13[a] (2–42)	33 ± 14[b] (20–57)	18 ± 3[ac] (16–22)	29 ± 3[bc] (24–35)
Pb	0.35 ± 0.28[ab] (<0.39–0.70)	<0.39[a]	0.78 ± 0.69[b] (<0.39–1.27)	0.61 ± 0.60[b] (<0.39–1.50)
Se	<0.50[a]	<0.50[a]	<0.50[a]	<0.50[a]
Zn	22 ± 26[a] (<35–50)	45 ± 19[ab] (<35–62)	30 ± 26[ab] (<35–45)	70 ± 7[b] (61–79)

[a, b, c, d] Mean values between different columns with the same letter are not significantly different at p > 0.05, according to Tukey's test.

Table 4
Percentage of extraction (mean, standard deviation and range) of Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn during the preparation of tea beverages.

Element	Extraction (%)			
	Black tea (n = 9)	Green tea (n = 9)	Oolong red tea (n = 3)	White tea (n = 9)
Al	37 ± 6[a] (24–45)	32 ± 9[ab] (20–51)	6.2 ± 0.6[c] (5.9–6.9)	23 ± 10[bc] (0–37)
As	0[a]	0[a]	27 ± 24[b] (0–45)	0[a]
Ba	7.7 ± 3.1[a] (3.7–13)	6.3 ± 1.0[a] (4.3–7.1)	5.9 ± 2.0[a] (4.2–8.0)	5.9 ± 1.4[a] (3.8–8.2)
Cd	0[a]	0[a]	0[a]	0[a]
Cr	27 ± 22[a] (0–55)	31 ± 6[a] (24–38)	1.7 ± 0.4[b] (1.2–2.1)	17 ± 14[ab] (2.2–41)
Cu	24 ± 5[a] (18–34)	18 ± 4[b] (11–23)	3.6 ± 1.8[c] (2.3–5.7)	22 ± 6[ab] (15–30)
Fe	1.2 ± 0.3[a] (0.8–1.9)	2.2 ± 0.8[b] (1.2–3.4)	1.0 ± 0.1[a] (0.9–1.1)	2.3 ± 0.9[b] (1.1–4.0)
Mn	21 ± 4[ab] (14–26)	24 ± 6[a] (16–33)	15 ± 1[b] (14–16)	21 ± 4[ab] (17–27)
Ni	61 ± 19[a] (38–93)	95 ± 17[b] (65–111)	47 ± 12[a] (37–60)	92 ± 9[b] (77–105)
Pb	31 ± 24[a] (0–53)	0[b]	5.9 ± 5.5[ab] (0–11)	3.8 ± 3.2[b] (0–8.7)
Se	0[a]	0[a]	0[a]	0[a]
Zn	14 ± 17[a] (0–35)	30 ± 12[a] (0–43)	9.2 ± 8.0[a] (0–14)	31 ± 2[a] (28–34)

[a, b, c] Mean values between different columns with the same letter are not significantly different at $p > 0.05$, according to Tukey's test.

Table 5
An evaluation of trace element exposure for the daily consumption of one cup of tea (200 mL) by an adult (body weight, bw, 70 kg) (WHO, 2005, 2014).

Element		Black tea	Green tea	Oolong red tea	White tea
Monthly estimated intake					
Cd	Mean (range), μg kg^{-1} bw	<0.023	<0.023	<0.023	<0.023
	% PTMI	<0.09	<0.09	<0.09	<0.09
Weekly estimated intake					
Al	Mean (range), μg kg^{-1}	509 (206–965)	510 (382–622)	95.4 (88.2–103)	322 (217–447)
	% PTWI	25 (10–48)	26 (19–31)	4.8 (4.4–5.2)	16 (11–22)
Ba	Mean (range), μg kg^{-1}	3.6 (0.8–6.2)	3.3 (2.0–5.4)	1.5 (1.3–2.0)	1.7 (1.0–2.4)
	% PTWI	–	–	–	–
Pb	Mean (range), μg kg^{-1}	0.035 (<0.039–0.070)	<0.039	0.078 (<0.039–0.13)	0.061 (<0.039–0.15)
	% PTWI	–	–	–	–
Daily estimated intake					
As	Mean (range), μg kg^{-1}	<0.007	<0.007	0.011 (<0.007–0.026)	0.001 (<0.007–0.007)
	% BMDL 0.5	<0.22	<0.22	0.36 (<0.22–0.85)	0.03 (<0.22–0.24)
Ni	Mean (range), μg kg^{-1}	0.26 (0.04–0.60)	0.47 (0.28–0.82)	0.26 (0.23–0.31)	0.41 (0.35–0.49)
	% TDI	2.2 (0.3–5.0)	3.9 (2.4–6.8)	2.2 (1.9–2.6)	3.4 (2.9–4.1)
Cu	Mean (range), μg kg^{-1}	0.31 (0.24–0.40)	0.24 (0.14–0.39)	0.09 (0.06–0.14)	0.45 (0.27–0.68)
	% PMTDI	0.06 (0.05–0.08)	0.05 (0.03–0.08)	0.02 (0.01–0.03)	0.09 (0.05–0.14)
Fe	Mean (range), μg kg^{-1}	0.12 (0.09–0.14)	0.32 (0.16–0.49)	0.92 (0.67–1.16)	0.51 (0.31–0.78)
	% PMTDI	0.01 (0.01–0.02)	0.04 (0.02–0.06)	0.12 (0.08–0.14)	0.06 (0.04–0.10)
Zn	Mean (range), μg kg^{-1}	0.31 (<0.50–0.71)	0.64 (<0.50–0.89)	043 (<0.50–0.64)	1.00 (0.87–1.13)
	% PMTDI	0.03 (<0.05–0.07)	0.06 (<0.05–0.09)	0.04 (<0.05–0.06)	0.10 (0.09–0.11)

PTMI (Provisional Tolerable Monthly Intake): Cd = 25 μg kg^{-1} bw; **PTWI** (Provisional Tolerable Weekly Intake): Al = 2 mg kg^{-1} bw; Pb = withdrawn in 2010; Ba: not established; **PTMDI** (Provisional Maximum Tolerable Daily Intake): Cu = 0.5 mg kg^{-1} bw; Fe = 0.8 mg kg^{-1} bw; Zn = 1.0 mg kg^{-1} bw; **BMDL0.5** (Benchmark Dose Lower Limit): As = 3.0 μg kg^{-1} bw (for inorganic arsenic); **TDI** (Tolerable Daily Intake): Ni = 12 μg kg^{-1} bw.

Table 6
Estimated intake of trace elements for the daily consumption of one cup of tea (200 mL) based on a caloric intake of 2000 calories*.

Element		Black tea	Green tea	Oolong red tea	White tea
Cr	Mean (range), μg kg^{-1}	4.0 (0.3–10.6)	3.9 (2.0–6.7)	0.66 (0.58–0.74)	1.11 (0.40–2.49)
	% DRI	1.15 (0.08–3.03)	1.13 (0.57–1.90)	0.19 (0.17–0.21)	0.32 (0.11–0.71)
	% DV	0.33 (0.02–0.88)	0.33 (0.17–0.55)	0.06 (0.05–0.06)	0.09 (0.03–0.21)
Cu	Mean (range), μg kg^{-1}	21.5 (16.8–28.3)	16.8 (9.5–27.3)	6.3 (4.5–9.7)	31.8 (18.8–47.7)
	% DRI	0.24 (0.19–0.31)	0.19 (0.11–0.30)	0.07 (0.05–0.11)	0.35 (0.21–0.53)
	% DV	0.11 (0.08–0.14)	0.08 (0.05–0.14)	0.03 (0.02–0.05)	0.16 (0.09–0.24)
Fe	Mean (range), μg kg^{-1}	8.1 (6.1–10.0)	22.6 (11.0–34.0)	64.6 (47.2–80.9)	35.8 (21.8–54.6)
	% DRI	0.006 (0.004–0.007)	0.02 (0.01–0.02)	0.05 (0.03–0.06)	0.03 (0.02–0.04)
	% DV	0.005 (0.003–0.006)	0.01 (0.01–0.02)	0.04 (0.03–0.04)	0.02 (0.01–0.03)
Mn	Mean (range), μg kg^{-1}	1651 (173–3111)	2409 (1612–3223)	1020 (951–1131)	1828 (1433–2464)
	% DRI	7.2 (0.8–13.5)	10.5 (7.0–14.0)	4.4 (4.1–4.9)	7.9 (6.2–10.7)
	% DV	8.3 (0.9–15.6)	12.0 (8.1–16.1)	5.1 (4.8–5.7)	9.1 (7.2–12.3)
Se	Mean (range), μg kg^{-1}	<0.50	<0.50	<0.50	<0.50
	% DRI	<0.15	<0.15	<0.15	<0.15
	% DV	<0.07	<0.07	<0.07	<0.07
Zn	Mean (range), μg kg^{-1}	22.0 (<35.0–50.0)	45.0 (<35.0–62.0)	30.0 (<35.0–45.0)	70.0 (61.0–79.0)
	% DRI	0.03 (<0.05–0.07)	0.06 (<0.05–0.09)	0.04 (<0.05–0.06)	0.10 (0.09–0.11)
	% DV	0.01 (<0.02–0.03)	0.03 (<0.02–0.04)	0.02 (<0.02–0.03)	0.05 (0.04–0.05)

* **DRI** = Daily recommend intake (Brasil, 2003): Cr = 35 μg/100 g; Cu = 900 μg/100 g; Fe = 14 mg/100 g; Mn = 2.3 mg/100 g; Se = 34 μg/100 g and Zn = 7 mg/100 g.
* **DV** = Daily value (FDA, 2013): Cr = 120μg/100 g; Cu = 2 mg/100 g; Fe = 18 mg/100 g; Mn = 2 mg/100 g; Se = 70 μg/100 g and Zn = 15 mg/100 g.

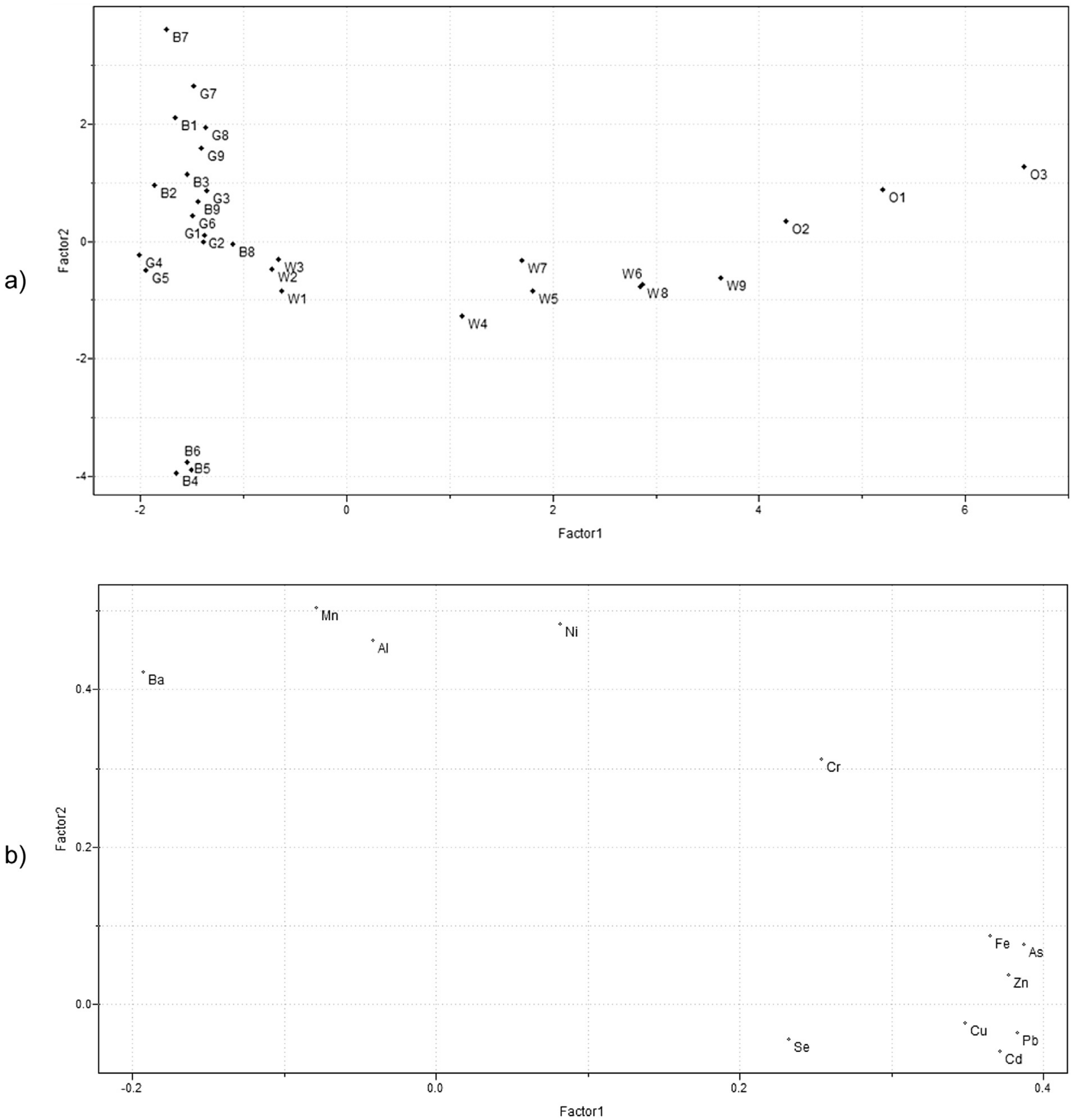

Fig. 1. Score (a) and loading (b) plots for tea samples marketed in southeastern Brazil. Different letters indicate tea variety (B = black, G = green, W = white, O = red oolong) and different numbers indicate brands (1–3 = brand “A”, 4–5 = brand “B”, 7–9 = brand “C”).

consumption of 263.34 mL of tea by the population of Campinas, SP, Brazil.

An evaluation of the exposure to this amount of tea is presented in Table 5. The results were compared to the threshold values established by the WHO (2014). Overall, most of the trace elements were within safe levels. For aluminum, our evaluation suggested that the consumption of a single cup of black tea per day may contribute up to 48% of the provisional weekly intake (PTWI) of aluminum. Although this value represents almost half of the Al PTWI, Villa, Peixoto, and Cadore (2014) noted that an exposure analysis should also consider the bioaccessibility and bioavailability of a contaminant. A recent study of lettuce and cole samples from Brazil by Silva et al. (2015) reported that the bioaccessibility of Al is less than 11% of the total Al content in these vegetables.

Regarding the nickel content in the tea infusions, its contribution to the tolerable daily intake (TDI) ranged between 0.3 and 6.8%. The highest contribution of nickel was found in green tea (mean value = 3.9%). Inorganic arsenic levels were used as the benchmark dose lower limit (BMDL = 0.5) to evaluate the total arsenic levels, which is consistent with the results described by Yuan, Gao, He, and Jiang (2007), who verified that inorganic As species were predominant in both tea leaves and beverages. Although copper, iron and zinc are micronutrients, FAO/WHO have defined limits for these elements to prevent high dosage. The measured levels of these species were not relevant. For example, a maximum contribution of 0.14% to the provisional maximum tolerable daily intake (PMTDI) was measured for Fe in red oolong tea.

Table 6 contains information regarding the intake estimates for the daily consumption of a cup of tea based on a caloric intake of

2000 calories. For Fe, Se and Zn, low contributions (<0.12%) for the daily intake of these nutrients was observed relative to both of the Brazilian and Food and Drug Administration (FDA) recommended values (Brasil, 2003; FDA, 2013).

For Cr and Cu, contributions ranging from 0.02 to 0.88% of the daily value (DV) and 0.08–3.03% of the daily recommended intake (DRI) were measured in the tea infusions. The beneficial influence of tea consumption is verified for manganese, a well-known essential micronutrient also present in some cereals, grains and nuts. Values of 16.1% for the DV of Mn and 14.0% of its DRI can be reached by the daily consumption of one cup of green tea.

3.4. Multivariate analysis

Principal component analysis (PCA) was applied to interpret and to classify the samples on the basis of their trace elements composition. Fig. 1 presents the score and loading plots for the thirty samples of tea leaves investigated in this study. The three brands were identified by numbers: 1 to 3 correspond to brand "A", 4 to 6 correspond to brand "B" and 7 to 9 correspond to brand "C". The tea varieties were identified by their initial name: "B" for black, "G" for green, "W" for white and "O" for red oolong tea.

Auto escalated pre-processing was chosen to provide the same weight for the loadings (i.e., trace elements) and no transformations of the data were applied. PCA was applied to the data matrix (30×12) such that each line and column corresponds to the mean value of the tea samples and trace elements, respectively. No samples were considered outliers.

Two principal components can explain 74.14% of the variance (PC1 = 49.10% and PC2 = 25.04%) and, for this reason, were chosen for further analysis. The first principal component is related to the trace elements As (0.3925), Cd (0.3631), Cu (0.3439), Fe (0.3719), Pb (0.3769) and Zn (0.3790), whereas the second principal component is related to Al (0.4650), Ba (0.4405), Mn (0.5092) and Ni (0.4722). The values in the parentheses correspond to the loading values.

Three groups could be recognized from this analysis:

- Group 1 constituted by the red oolong tea samples, associated with the levels of As, Cd, Cu, Fe, Pb and Zn (trace elements with high loading values in PC1);
- Group 2 consisting of black and green tea samples that presented low variability in their composition (see Table 2). This group is characteristic because of its content of Al, Ba and Mn (trace elements with low loading values in PC1);
- Group 3 constituted only by white tea samples. Because these samples were located near the origin of PC1 and PC2, this group cannot be assigned on the basis of a predominance of select trace elements.

Although the tea leaves acquired in markets in southeastern Brazil may be a mixture of tea leaves derived from different countries, PCA successfully classified them into three groups based on similarities in their trace elements composition.

4. Conclusion

In this study, twelve trace elements were determine in tea samples and their beverages prepared by an infusion procedure. Some samples of tea leaves exhibited trace element levels above the maximum limits allowed by Brazilian and MERCOSUL regulations: As (33% in red oolong tea) and Pb (67% and 100% in white and red oolong, respectively). However, safe levels were found in the infusions. Moreover, the alleged benefits of a daily consumption of a single cup of tea were supported for the intake of manganese, with values of up to 16% for the DV established by the FDA.

Although a lack of information regarding the age, origin or use of a pool of tea leaves (i.e., mixture of tea leaves from different countries), multivariate analysis presented reasonable results. Principal component analysis allowed classifying the tea leaves into three groups based on only similarities of their trace elements composition.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors gratefully acknowledge the FAPESP (Process 2012/19142-7), the CNPq and INCTAA (CNPq process 573894/2008-6 and FAPESP process 2008/57808-1) for financial support. Fabio F. Silva from Agilent Technologies Brazil, Letícia K. Silvestre and Leandro I. Peron are also gratefully acknowledged for analytical support.

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