Assessment of Human Exposures: Bioaccessibility studies of Some Toxic Metals in Tea (*Camellia sinensis* L.) Samples

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Abstract

In this study, As, Cd, Cr, and Pb were determined from black, green, and earl grey tea samples after microwave digestion and in-vitro methods that simulate stomach and intestinal digestive conditions. Leachabilities were assessed after brewing of tea samples. Inductively coupled plasma-mass spectrometry (ICP-MS) was used for all measurements. The bioaccessibility from tea samples were found to be varied between 3 to 100% of the leachable levels.

Keywords: Tea *(Camellia sinensis* L.), bioaccessibility, toxic elements, inductively coupled plasma-mass spectrometry (ICP-MS)

1. Introduction

Tea (*Camellia sinensis* L.) is one of the oldest and most popular non-alcoholic beverages consumed next to water globally (Szymczycha-Madeja *et al*., 2012; Yemane *et al*., 2008). While the tea plant is grown in more than 45 countries (Karak and Bhagat, 2010), various kinds of teas including black, green and herbal ones are consumed at high levels (Soylak *et al*., 2007). The tea plant can be cultivated in many regions having with humidity, adequate rainfall, fair temperature, good drainage, and acidic soils (Dufresne and Farnworth, 2001; Karak and Bhagat, 2010) and leaves are rich in trace elements at different levels (Kumar *et al*., 2005; Szymczycha-Madeja *et al*., 2012; Yemane *et al*., 2008) mainly originating form growth media, insecticides, herbicides, fertilizers, etc. (Soylak *et al*., 2007) as well as climate conditions. Moreover, the regular consumption of tea can contribute to the daily dietary requirements of some of the elements. Thus, determining their levels is important in order to evaluate their nutritional value and health risks (Karak and Bhagat, 2010). Nevertheless, determination of available levels for absorption will be more important rather than total elemental levels associated with the consumption (Intawongse and Dean, 2006). The aim of this study was to investigate the bioaccessibility of some toxic elements (As, Cd, Cr, and Pb) from commonly consumed black, green, and Earl Grey tea samples in Turkey and to evaluate the effects of different infusion times on bioaccessibility as well as consumption habits.

2. Material and Methods

2.1 Chemicals and reagents

A multi-element standard solution at concentrations of 10 $\mu g\ mL^{-1}$ of Cd, Cr, Pb and 100 $\mu g\ mL^{-1}$ of As as well as a single-element standard solution of Ca at concentration of 1000 $\mu g\ mL^{-1}$ were purchased from Merck (Merck 110580) and PerkinElmer, respectively. A certified reference material (NCS ZC73014 tea leaves) was used for method validation. All of the reagents used were obtained from local suppliers of Merck KGaA. Apart from these, enzymes, pepsin, pancreatin, and bile extract were purchased from Sigma-Aldrich. Sodium bicarbonate was a Carlo Erba reagent. Ultrapure water was supplied from Zeener Power I purification system. Hydrophilic PVDF (polyvinylidene fluoride) syringe filters were obtained from Millipore Corp. and used for all filtration purposes.

2.2 Sample preparation

Nine different commercially available brands of black, Earl grey (black tea flavored with bergamot), and green tea samples were purchased from local markets in Bursa-Turkey. The nine final samples prepared from each tea type in teabags were used for experimental parts. Total elemental levels were determined after digestion of the samples with 6 mL of HNO_3 and 1 mL of H_2O_2 using microwave digestion system (Erdemir and Gucer, 2014). Tea brews were prepared by 25 mL of boiled water and 0.5 g of leaves at different infusion times of 2, 5, and 10 min. Prepared infusions were filtered and analyzed directly by ICP-MS after dilution. 5 mL of the obtained effluents at each extraction period were used according to the method described by Erdemir and Gucer (Erdemir and Gucer, 2014) for enzymatic digestion with the modification of reagents volume and sample amount derived from the central composite design. Enzyme residues were filtered through PVDF filters and analyzed by ICP-MS. All the sampling procedures were repeated in triplicate as well as blank samples that were prepared in the same manner as the samples. Finally, 5 mL of 2 min infused black teas were used after lemon juice, table sugar, skimmed milk, calcium, citric and tannic acids were added separately to the samples at three addition levels. (0.5, 1.0, or 2.0 mL of lemon juice or milk, 0.4, 0.8, or 1.2 g of table sugar; 7, 14, or 28 $\mu g\ L^{-1}$ calcium or citric acid, 5, 10, or 20 $mg\ kg^{-1}$ of tannic acid.

2.3 Apparatus and Instrumentation

Samples were digested using Microwave Labstation MLS 1200 Mega, for total element analysis. The microwave

Table 1. Total contents, leachability and bioaccessibility of the studied tea samples (*n=3*)

Tea type and code		*Total element* Mean, *mg kg*$^{-1}$	SD[a]	*Leachability* Mean, *mg kg*$^{-1}$	*Bioaccessibility* Mean, *mg kg*$^{-1}$	SD	%[b]	%[c]
Black-1	Pb	0.456	0.090	0.056	0.020	0.015	4	36
	Cr	2.477	0.458	0.396	0.146	0.082	6	37
	Cd	0.060	0.010	<LOD[d,e]	<LOD	-	-	-
	As	0.019	0.004	0.103	0.006	0.011	29	5
Black-2	Pb	0.507	0.040	0.043	0.043	0.002	8	99
	Cr	3.012	0.191	0.470	0.464	0.581	15	99
	Cd	0.089	0.014	0.006	<LOD	0.002	-	-
	As	0.042	0.005	0.094	0.042	0.010	101	45
Black-3	Pb	0.466	0.017	0.070	0.039	0.008	8	56
	Cr	3.314	0.572	0.591	0.123	0.055	4	21
	Cd	0.012	0.019	0.005	<LOD	0.001	-	-
	As	0.031	0.001	0.094	0.006	0.007	19	6
Earl Grey-1	Pb	0.389	0.062	0.044	0.040	0.005	10	91
	Cr	2.119	0.174	0.720	0.719	0.055	34	100
	Cd	0.044	0.035	<LOD	<LOD	0.001	-	-
	As	0.036	0.009	0.134	0.024	0.005	67	18
Earl Grey-2	Pb	0.405	0.062	0.039	0.039	0.003	10	100
	Cr	2.430	0.427	0.839	0.778	0.038	32	93
	Cd	0.042	0.046	0.006	0.006	0.000	14	100
	As	0.024	0.002	0.176	0.025	0.002	103	14
Earl Grey-3	Pb	0.500	0.024	0.039	0.021	0.012	4	53
	Cr	4.170	1.018	0.802	0.728	0.052	17	91
	Cd	0.030	0.023	0.007	<LOD	0.000	-	-
	As	0.043	0.006	0.167	0.024	0.005	57	15
Green-1	Pb	0.327	0.007	0.027	0.019	0.011	6	70
	Cr	0.857	0.205	0.602	0.601	0.062	70	100
	Cd	0.018	0.013	<LOD	<LOD	0.001	-	-
	As	0.021	0.003	0.261	0.009	0.029	44	4
Green-2	Pb	1.595	0.083	0.269	0.269	0.017	17	100
	Cr	1.343	0.039	0.444	0.418	0.039	31	94
	Cd	0.039	0.022	0.015	<LOD	0.001	-	-
	As	0.086	0.006	0.136	0.009	0.001	11	7
Green-3	Pb	0.186	0.018	0.042	0.027	0.014	15	65
	Cr	1.059	0.173	0.604	0.347	0.041	33	57
	Cd	0.036	0.031	0.006	0.004	0.000	12	79
	As	0.010	0.001	0.305	0.010	0.008	98	3

[a] Standard Deviation
[b] Percent bioaccessibility depending on total elemental levels
[c] Percent bioaccessibility depending on leachable elemental levels
[d] *<LOD*= under detection limit
[e] Dilution factor = 20 for leachability studies. For the total and bioaccessibility studies no dilution was applied before analysis.

operating conditions were detailed elsewhere (Erdemir and Gucer 2014) A pH meter (Minilab IQ125 Isfet pH tester) an unstirred thermostatic water bath (Clifton NE1-22), and leaves, infusions, and enzyme digestions by an Elan 9000 ICP-MS. The optimized instrumental conditions of ICP-MS were as follows: RF (radio-frequency) power, 1000 W; plasma argon flow rate, 17.0 L min^{-1}; nebulizer gas flow rate, 0.85 L min^{-1}; sample uptake rate, 1.5 mL min^{-1}; dwell time, 50 ms; scanning mode, peak hopping; and detector mode, dual.

3. esults and discussion

Table 1 represents the total elemental contents, leachability and bioaccessibility of the studied tea samples. These levels were calculated by instrumental signal, dilution factor, and sample mass. The analytical accuracy was ascertained using certified reference standard (NCS ZC73014 tea leaves). t-test was used to evaluate any statistical differences between measured and the certified levels together with their standard deviations. There was good agreement at the 95% confidence level according to paired t-test between measured and certified values of the elements (three independent replicates (*n=3*) were prepared and analyzed for each group). Measured concentrations of 1.45 ± 0.10, 0.52 ± 0.01, 0.053 ± 0.005, 0.070 ± 0.010 μg g^{-1} for P, Cr, Cd, and As, respectively were consistent with the certified values of 1.5 ± 0.2, 0.45 ± 0.10, 0.062, 0.004, 0.09 ± 0.01 μg g^{-1}, respectively, for those elements in tea leaves. Thus, measured values of elements were consistent with the certified values. Limits of detection (LOD) values of the elements were calculated as being three times the standard deviation of blank samples while limits of quantification values were calculated as 10 times ($n = 3$). These parameters were converted to μg g^{-1} levels using dilution factor, and sample mass. LOQ values of Pb, Cr, Cd, and As were 0.023, 0.115, 0.021, and 0.012 μg g^{-1} respectively. Intra-day precisions were in the ranges from 1% to 10% respectively for the determined elements. Leachable levels of studied elements were still comparable nowadays with the published work of Gurses and Artik (1982) regarding Turkish tea infusions. Bioaccessible levels of the elements were also highlighted in Table 1. Elemental levels in the infusions were used rather than the total levels in the leaves to simulate bioaccessibility from tea as it is consumed. For Cd, under detection levels were screened for some of the samples in comparison to the rest of the elements. Bioaccessibilities were found in the range from 3 to 100% of the leachable levels for the other elements. Elemental contents were also determined in tea infusions obtained after 5, and 10 min. Although small changes were observed, 2 min values were outlined as standard infusion time recommended by manufacturers. As diluted infusions were analyzed without digestion to simulate metabolic processes for *in vitro* bioaccessibilities, potential interferences that may be caused primarily from the sample matrix should be taken into account. So, some interferences were eliminated in this study to reach accurate results by ICP-MS. The effects of some consumption habits, and dietary factors on bioaccessible levels were also studied after addition of lemon juice, citric acid, milk, calcium, sugar, and tannic acid and found to have increasing or decreasing effects. Further studies were conducted for data treatment and numerical calculations as a MSE Mistral 2000 centrifuge were used for in-vitro studies. Levels of toxic elements were determined in tea

well as to evaluate the health risk of toxic elements depending on daily allowable values.

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