Biochemistry & Physiology: Open Access

Han, et al., Biochem Physiol 2014, 3:2
DOI: 10.4172/2168-9652.1000132

Research Article Open Access

Multi-Element Detection in Green, Black, Oolong, and Pu-Erh Teas by ICP-MS

Qiang Han*, Shozo Mihara, and Tatsuya Fujino

Department of Chemistry, Tokyo Metropolitan University, Hachioji-Shi, Tokyo

***Corresponding author:** Qiang Han, Department of Chemistry, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji-Shi, Tokyo 192-0397, Tel: +81 042 677 3135; Fax: +81 042 677 3135; Email: han-qiang@ed.tmu.ac.jp

Rec date: May 08, 2014, **Acc date:** May 21, 2014, **Pub date:** May 28, 2014

Abstract

The contents of various elements in green, black, oolong, and pu-erh teas were measured by ICP-MS. The dependence of the dissolution rate of each element on the extraction time and the number of infusion was determined. By calculating the estimated daily dietary intake as a result of consuming 15 g of tea leaves a day, it was revealed that Cr and Mn exceeded the adequate intake for one day.

Keywords: Tea; ICP-MS; Minerals elements; Infusions

Introduction

Tea is one of the most popular beverages because of its aroma, taste, variety, and health-promoting effects. However, it contains not only organic compounds, such as caffeine, but also minerals and trace elements regardless of their beneficial or toxic effects. Therefore, it is important to know the contents of elements in tea leaves and the influence of daily tea consumption on human health. In general, tea leaves are classified by the degree of fermentation: unfermented green tea (GT), fully fermented black tea (BT), and partially fermented oolong tea (OT) are the most popular and frequently consumed [1]. Post-fermented pu-erh tea (PT) has seen a boom in consumption because of its beneficial health effects [2].

Due to its high sensitivity and multi-element capabilities, inductively coupled plasma mass spectrometry (ICP-MS) is well used in food analysis laboratories to determine elements at trace or ultra-trace levels [3]. Therefore, ICP-MS is considered one of the most effective tools for elemental detection in tea.

In this study, the infusions from those four types of teas leaves were subjected to ICP-MS for elemental detection. Although there are reports about the elemental contents in tea infusions [4,5], the dependence on the extraction time and the number of infusions is reported here for the first time. In addition, the influence of tea consumption on human health was investigated by comparing the estimated daily dietary intake of the elements with their respective upper tolerable intake and adequate intake.

Materials and Methods

Tea samples

Four types of teas: GT, BT, OT, and PT harvested in 2011 were purchased from Hangzhou, China. Each sample was wrapped individually, freeze-dried, and stored at room temperature (293 K) before being pulverized prior to measurement.

ICP-MS performance

ICP-MS (Spectro Analytical Instruments, Kleve, Germany) precision was confirmed by measuring a mixed internal standard solution (XSTC-331, SPEX, USA) that had a concentration of 1000 μg L^{-1}. To calculate the amount of each element that could be ingested, the intrinsic amount of each element in the four types of tea leaves (bulk amount) was first determined. For this purpose, NIES CRM No. 23 tea leaves II provided by the National Institute for Environmental Studies (NIES, Japan) was used as the reference standard. A spiking recovery which used with analysis of spiked samples could reveal mistakes made in the sample preparation. The spiked recoveries were evaluated with adding a known amount (100 μg L^{-1}) of standard multi-element solution into the tea samples during the experiment process.

Infusion preparation

Each of the four types of tea leaves was accurately weighed with 20 ± 1 mg and soaked in 1 mL of Milli-Q water which was pre-heated to 368 K, then put into water bath pot (temperature accuracy ± 1 K) with the specified length of time. During the process, the vessel was shocked 5 seconds with the vortex (G-560, Scientific Industries, Inc. Bohemia, New York, USA) every 5 minutes. The efficiency of the dissolution rate of each element on different extraction time (0.08, 0.17, 0.5,1, 3, 5, 10, 15, 20, 25, 30, 45, 60 min) and the number of infusion (3 times) was determined. The dissolution rate (extracted amount/bulk amount) for each element at various extraction times was calculated. Each sample was carried out in triplicate.

Digestion procedures

Bulk tea and infusions were digested prior to analysis with ICP-MS. The digestion was carried out in PTFE beakers on a hot plate. Approximately 10 mg of bulk tea sample was weighed into each beaker and dissolved in 3 mL of 69% HNO_3 (AnalR, sub-boiling quartz still redistilled, Wako, Asaka, Japan). The beakers were covered with PTFE plates and placed on a hot plate set at 393 K for two hours. The solution was quantified in pre-cleaned 50 mL volumetric flasks, and then transferred into PTFE bottles for ICP-MS measurement.

Results and discussion

ICP-MS precision

Our measured values for the eight elements (Mg, K, Ca, Mn, Ni, Cu, Sr, and Al) found in the tea leaf standard showed good agreement with the certified values. The recovery rates ranged from 96.5 ± 4.9% to 112.2 ± 27.3%; 100% of those elements were extracted within experimental error, indicating complete digestion. Then, the above digestion method was used to estimate the bulk amounts of those eight metals in four types of tea leaves (GT, BT, OT, and PT).

The calibration curves were linear in the range of 0 to 1000 μg L^{-1}. The different concentrations of stand solutions (0, 10, 25, 50, 100, 250, 500, 1000 μg L^{-1}) were used for making working curve. The correlation coefficients for all the calibration curves were at least 0.9994 and thus good linear relationships were attained throughout the concentration range studied. The minimum and maximum LODs values for all the trace elements with being 0.000795 μg L^{-1} for Cu and 0.153 μg L^{-1} for Mg, respectively. The spiking recoveries were in the acceptable range between 94.1% (Cu) and 101% (Sr). The results of recoveries indicated that no significant losses occurred during the ICP-MS detection.

Extraction time and the number of infusion

The extraction time dependence of the dissolution rates of Mg, Mn, Ni, and Cu from GT were shown in Figure 1(a). Although the dissolution rates varied, similar time dependences were observed except that for Cu. The dissolution rate for each element showed an almost constant value when the extraction time exceeded 25 min.

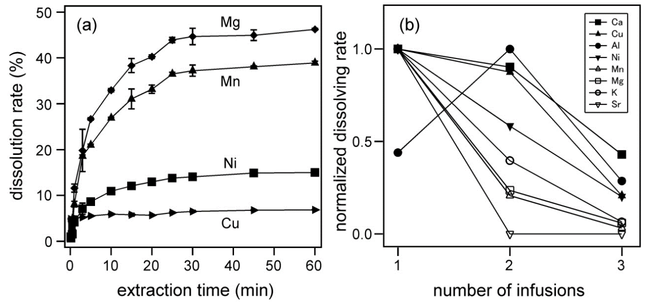

Figure 1: Figure 1(a) shows the normalized dissolution rate plotted against the number of infusions. Figure 1(b) shows that Mn, Mg, K, and Sr could be extracted almost completely extracted in the 1st infusion.

As the same batch of tea leaves may be used repeatedly to prepare tea infusions, we also determined the dissolution rates for the 2nd and 3rd 25-min infusions, and the results are summarized in Table 1.

Figure 1(b) shows the normalized dissolution rate plotted against the number of infusions. Here, the highest dissolution rate for each extraction was set to 1.

Elements	1st time dissolution rate (%)	2nd time dissolution rate (%)	3rd time dissolution rate (%)
Sr	3.94 ± 1.06	ND[a]	ND
Ca	2.77 ± 0.31	2.50 ± 1.19	1.19 ± 0.30
Cu	6.25 ± 0.16	5.61 ± 0.47	1.33 ± 0.46
Al	3.86 ± 1.23	8.77 ± 1.85	2.51 ± 1.58
Ni	13.8 ± 0.7	7.82 ± 2.74	2.70 ± 0.23
Mn	36.5 ± 0.4	7.33 ± 0.32	1.12 ± 0.08
Mg	43.9 ± 0.6	10.4 ± 0.4	2.65 ± 0.04
K	50.2 ± 0.4	19.9 ± 4.1	3.25 ± 0.29

Table 1: Bulk amounts and dissolution rates of elements in green tea. [a]ND=not detected because the value is below the detection limit

It was understood from Figure 1(b) that Mn, Mg, K, and Sr could be extracted easily; they were almost completely extracted in the 1st infusion. In contrast, Ca, Cu, Al, and Ni were difficult to extract regardless of their bulk amounts. The total dissolution rate, which is the sum of the dissolution rates for the 1st, 2nd, and 3rd 25-min extractions of the other types of tea leaves (BT, OT, and PT) was evaluated and the results are summarized in Table 2.

Elements in tea and its infusion

K was abundant in all the four types of tea leaves and was the most easily extractable of the eight elements. It is common knowledge that K is the most important element in fertilizers as it functions as a coenzyme to promote various reactions, such as glycolysis, in plants [6]. Therefore, it was easily understood that the amount of K is the largest among the eight elements examined.

As for alkaline earth metals, Mg and Ca were also abundant in tea leaves. However, Mg was easily extractable and Ca was not. Although Sr is also an alkaline earth metal, it was excluded from consideration because its bulk amount was quite small and its dissolution rates for the 2nd and 3rd extractions could not be determined. The difference in extraction efficiency between Mg and Ca could be explained by considering the localization of those elements in tea leaves. Ca reacts with pectin to produce a gel that binds cells tightly in tea leaves [7]. There are many calcium-binding sites in the cell wall, so Ca is mainly distributed in the middle layer of the cell wall and plasma membrane. Therefore, it can be assumed that the extraction of Ca is somewhat difficult even though there is a considerable amount of Ca. On the other hand, Most of the Mg combine with inorganic anion or an organic anion such as malate, citrate exist in the cytoplasm of the plant. Belong to movable diffusion portion, so can easily be extracted by water solution. Meanwhile, Mg is important for photosynthesis and Mg^{2+} existing at the center of a chlorophyll molecule. Therefore, it is considered that Mg^{2+} can be extracted with hot water when making tea infusion.

Al is a toxic to plants and many plants do not accumulate Al from soil. However, the tea plant is unique as it accumulates Al in leaves [8]. Table 2 shows that Al content in OT was the highest. It has been reported that Al tends to accumulate in old leaves as old tea plants have higher Al tolerance than young tea plants. In the case of OT, it is known that old tea leaves are commonly used for production [9].

Elements	Green tea	Rate (%)	Oolong tea	Rate (%)	Black tea	Rate (%)	Pu-erh tea	Rate (%)
	(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})		(mg kg^{-1})	
Sr	17.3 ± 0.5	3.94 ± 1.06	13. 9 ± 0.3	2.07 ± 0.88	6.24 ± 0.28	6.36 ± 2.36	27.1 ± 1.2	ND[a]
Ca	4.17×10^3 ± 386	6.46 ± 1.80	3.62×10^3 ± 151	13.2 ± 1.3	2.67×10^3 ± 150	11.3 ± 2.6	4.56×10^3 ± 323	1.89 ± 1.85
Cu	11.4 ± 0.1	13.4 ± 1.1	5.80 ± 0.22	15.0 ± 2.1	22.1 ± 0.1	14.4 ± 1.2	16.1 ± 1.1	15.8 ± 1.5
Al	368 ± 2	15.1 ± 4.6	973 ± 28	39.8 ± 8.3	580 ± 9	31.1 ± 3.1	858 ± 35	19.3 ± 3.9
Ni	8.82 ± 0.06	23.9 ± 3.7	2.95 ± 0.02	29.9 ± 2.3	6.76 ± 0.06	57.5 ± 5.6	10.2 ± 0.2	38.9 ± 5.3
Mn	810 ± 39	43.8 ± 0.8	915 ± 11	31.1 ± 0.3	767 ± 6	36.5 ± 0.7	1.02×10^3 ± 2	35.5 ± 0.8
Mg	1.46×10^3 ± 45	57.1 ± 1.0	1.81×10^3 ± 65	39.7 ± 0.6	1.87×10^3 ± 26	55.0 ± 1.8	2.03×10^3 ± 24	44.5 ± 1.4
K	1.89×10^4 ± 734	73.4 ± 4.8	1.76×10^4 ± 67	87.4 ± 1.9	2.27×10^4 ± 253	76.2 ± 4.2	2.67×10^4 ± 160	66.7 ± 3.1

Table 2: Contents and dissolution rates of elements in oolong, black, and pu-erh teas. [a]ND=not detected because the value is below the detection limit

Considering that the Al extraction efficiency from old and young tea leaves is almost the same, the higher Al content in OT than in the other tea leaves could be due to the difference in age of the tea leaves used for production. Transition metals (Mn, Ni, and Cu) are also essential for growing tea leaves. In the case of Cu, however, it has been pointed out that contamination during harvesting and processing should be taken into account [10]. The fact that the extraction time dependence of the dissolution rate of Cu in Figure 1(a) was quite different from those of the other elements indicates that the extracted Cu does not originate from the tea leaves.

Estimated daily dietary intake of tea

To examine the influence of tea consumption on human health, we adopted the estimated daily dietary intake (EDDI) reported previously [11], and calculated the EDDI values for the eight metals by assuming the consumption of 15 g tea leaves in a day. In addition, the EDDI values for Cr, Fe, and Zn were calculated (Table 3).

All metals had EDDI values that were lower than their respective upper tolerable intake (UI) [12] or adequate intake (AI) [13], except Cr and Mn. The toxicity of Cr is dependent on its oxidation state: Cr (III) is essential for human health but Cr(VI) is harmful. Although it is not possible to identify the oxidation state of Cr in tea by ICP-MS, further experiments are necessary because of the abundance of Cr in tea. In contrast, it has been reported that Mn is beneficial for hypertensive patients. Therefore, tea could be an important source of Mn.

Conclusion

It was observed that the elemental concentrations in the four type's teas and their infusions showed wide variability. It was demonstrated that 25 min was appropriate for tea extraction, and the elements of Mn, Mg, K, and Sr were extracted easier then Ca, Cu, Al, and Ni in the first time infusion. Generally, Tea could be an important source of Mn and the large amount of K could be beneficial for hypertensive patients.

Elements	Max value	EDDI	AI	UI
	(mg kg^{-1})[a]	(μg d^{-1})	(μg d^{-1})	(μg d^{-1})
Sr	0.681	10.2	-[b]	-
Ca	476	7.14×10^3	1.00×10^6-1.20×10^6	2.50×10^6
Cu	3.5	52.5	900	1.00×10^4
Al	973	5.81×10^3	-	-
Ni	3.88	58.2	ND[c]	1.00 ×10^3
Mn	354	5.31×10^3	2.30×10^3 [d] (1.80×10^3)[e]	1.10×10^4
Mg	1.03 ×10^3	1.54×10^4	4.20×10^5 (3.20×10^5)	3.50×10^5
K	1.78 ×10^4	2.68×10^5	-	-
Cr	3.23	48.5	30.0-35.0	ND
Fe	120	1.80×10^3	8.00×10^3 -1.80×10^4	4.50×10^4
Zn	90.7	1.36×10^3	1.10×10^4 (8.00×10^3)	4.00×10^4

Table 3: EDDI values of elements from consumption of 15 g of tea leaves a day, compared with respective AI and UI values. [a]The highest content of element among the four types tea infusion. [b]AI and UI values not found through the Institute of Medicine (IOM). [c]Not determined due to lack of adverse effects data in this age group (19-70 years old). [d]AI for males. [e]AI for females.

Acknowledgement

Qiang Han, et al. acknowledges the Asian Human Resources Fund from the Tokyo Metropolitan Government.

References

1. Welna M, Szymczycha-Madeja A, Pohl P (2013) A comparison of samples preparation strategies in the multi-elemental analysis of tea by spectrometric methods. Food Research International 53: 922-930.
2. Cao H, Qiao L, Zhang H, Chen J (2010) Exposure and risk assessment for aluminium and heavy metals in Puerh tea. Sci Total Environ 408: 2777-2784.
3. Walczyk T (2001) The potential of inorganic mass spectrometry in mineral and trace element nutrition research. Fresenius J Anal Chem 370: 444-453.
4. Salahinejad M, Aflaki F (2010) Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. Biol Trace Elem Res 134: 109-117.
5. Mehra A, Baker CL (2007) Leaching and bioavailability of aluminium, copper and manganese from tea (Camellia sinensis). Food Chemistry 100: 1456-1463.
6. Pytlakowska K, Kita A, Janoska P, PoÅ‚owniak M, Kozik V (2012) Multi-element analysis of mineral and trace elements in medicinal herbs and their infusions. Food Chem 135: 494-501.
7. Mossion A, Potin-Gautier M, Delerue S, Le Hécho I, Behra P (2008) Effect of water composition on aluminium, calcium and organic carbon extraction in tea infusions. Food Chemistry 106: 1467-1475.
8. Street R, Drabek O, Szakova J, Mladkova L (2007) Total content and speciation of aluminium in tea leaves and tea infusions. Food Chemistry 104: 1662-1669.
9. Chan EWC, Lim YY, Chew YL (2007) Antioxidant activity of Camellia sinensis leaves and tea from a lowland plantation in Malaysia. Food Chemistry 102: 1214-1222.
10. Karak T, Bhagat RM (2010) Trace elements in tea leaves, made tea and tea infusion: A review. Food Research International 43: 2234-2252.
11. Salahinejad M, Aflaki F (2010) Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. Biol Trace Elem Res 134: 109-117.
12. Lv H-P, Lin Z, Tan J-F, Guo L (2013) Contents of fluoride, lead, copper, chromium, arsenic and cadmium in Chinese Pu-erh tea. Food Research International 53: 938-944.
13. Sofuoglu SC, Kavcar P (2008) An exposure and risk assessment for fluoride and trace metals in black tea. J Hazard Mater 158: 392-400.