Integrative Food, Nutrition and Metabolism

Research Article ISSN: 2056-8339

Leaching and *in vitro* bio-accessibility of aluminium from different teas

Tingting Lin and Xiaoping Yang*

Key Laboratory of Horticultural Plant Biology (Huazhong Agricultural University), Ministry of Education, Wuhan 430070, P.R. China

Abstract

To assess human exposure to aluminum (Al) from tea, the total Al contents in different teas (green tea, black tea and oolong tea processed with same fresh leaves) and the leaching of Al from these teas were analyzed, and the bio-accessibility of Al from these teas and their infusions was evaluated under simulated gastrointestinal condition. The results showed that green tea contained a high level of Al, but the leaching rate of Al from it was low, and the brewing condition significantly affected the leaching rate. It was also found that the leaf age significantly affected the content of Al in tea, but no significant differences were observed in the Al content among different teas and in the Al leaching from these teas. Results also showed that the bio-accessibility of Al from green tea infusion was very low after simulated gastrointestinal digestion, and there were no significant differences in the bio-accessibility of Al from different tea infusions. It is concluded that drinking tea is relatively safe and it is no cause for concern about Al toxicity in healthy individuals.

Introduction

Aluminum (Al) is toxic to humans. It has been reported that a high level of brain Al damages not only nervous system but also cardiovascular system, hepatobiliary system, respiratory system, endocrine system, urinary system, and so on [1]. Epidemiological studies also showed that excessive Al in the body might lead to Alzheimer's disease (AD), osteomalacia, dialysis encephalopathy, microcytic anemia, and so on [2]. Therefore, a reduced intake of dietary Al is of crucial importance to prevent the toxicity of Al.

Tea is one of the most popular drinks around the world, and hundreds of millions of people drink tea daily for health benefits such as antioxidant activity, preventing cardiovascular disease, and weight management [3]. However, tea plant is an Al accumulator [4], and Al is mainly accumulated in leaves [5]. Therefore, tea drinking may be a potentially important source of dietary Al, and more and more people have been becoming interested in whether drinking tea increases the risk of some diseases such as AD.

The intake of Al from tea drinking depends on the following aspects: total Al content in tea, Al leaching into tea infusion, and the bioavailability of Al from tea infusion in the body [6]. The total Al content in tea varies with many factors, such as soil characteristics [7], tea plant variety, especially leaf age [5]. It has been reported that old leaves contain average 5600 $mg.kg^{-1}$ Al and young leaves average 997 $mg.kg^{-1}$ [8]. The leaching of Al into tea infusion depends on the solubility of Al and brewing conditions besides the amount present in the tea. It has been reported that the solubility of Al is low for Al in tea is bound by polyphenols, fluoride, and so on [9-11]. The bioavailability of Al from tea infusion seemed also be low in the body. Powell *et al.* [12] reported that only a small proportion of Al in tea infusion was available for absorption in the gastrointestinal tract. Yokel and Florence [13] reported that oral Al bioavailability from tea infusion was only 0.37%. From these findings, drinking tea might not contribute significantly to the total body burden of Al.

To accurately estimate the daily intake of Al from tea consumption and the risk of Al, it is important to evaluate total Al content in tea, the leaching of Al from the tea, and the bio-accessibility of Al from its infusion after digestion at the same time. It is also necessary to compare the difference of total Al contents in green tea, black tea, and oolong tea, of the Al leaching from these teas, and of the bio-accessibility of Al from their infusions because green tea, black tea, and oolong tea were processed with different manufacturing processed, and different manufacturing processed might lead to the different effects on the total Al content in tea, thus affecting the leaching of Al from them and the bio-accessibility of Al from their infusion. The present study aims to determinate total Al contents in green tea, black tea, and oolong tea processed with same fresh leaves and the leaching of Al from these teas, and to evaluate the bio-accessibility of Al from their infusions under simulated gastrointestinal conditions.

Materials and methods

Materials

Porcine pepsin was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Porcine pancreatin (CAS. 8049-47-6) and porcine stomach mucin were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (China). Porcine pancreatic lipase (CAS. 9001-62-1) was purchased from Shanghai Aladdin Industrial Inc. (China). All reagents and chemicals used were of analytical grade.

Processing of tea

Fresh tea leaves of *Camellia sinensis* L. were harvested from tea

Correspondence to: Xiaoping Yang, College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, P.R. China, Tel: 862787286948; Fax: 862787282010; E-mail: yangxp@mail.hazu.edu.cn

Key words: *tea, aluminium, bio-accessibility, leaching*

Received: October 20, 2016; **Accepted:** November 07, 2016; **Published:** November 11, 2016

plantation at Huazhong Agricultural University (HZAU), Wuhan, China. These leaves were respectively manufactured into green tea, black tea, and oolong tea at the tea processing factory on HZAU campus. The teas were ground, sieved through a 40-mesh stainless steel sieve (0.42 mm), and stored at -20℃ for further use.

Total Al content in tea

1.0 g of oven-dried tea sample was dry-ashed according to the method of Akinyele and Shokunbi [14]. Total Al content in the tea was analyzed by atomic absorption spectrometry (AAs) as described in Bertsch and Bloom [15].

Leaching of Al from tea

2.0 g of milled green tea, black tea, and oolong tea was soaked in 100 mL deionized water at 90℃ for 20 minutes, respectively. The mixtures were centrifuged at 4846×g for 10 min to get tea infusions. 10 mL of tea infusion was transferred into porcelain crucibles. The porcelain crucibles were heat overnight to vaporize water according to the method of Moreno *et al.* [16]. The residues in porcelain crucibles were dry-ashed and analyzed for Al content as described above. The leaching rate of Al was calculated as the percentage between the amount of Al in tea infusion and in tea.

Effect of brewing condition on Al leaching from tea

Batch experiments, including tea to water ratio (tea/water), leaching temperature, leaching time and leaching times, were carried out by adding the above milled green tea into 250 mL glass conical flasks containing deionized water in thermostatic water bath for some time. Then, the mixtures were centrifuged at 4846×g for 10 min and the supernatants were collected for the determination of Al content as described above. The effect of tea/water (1:20, 1:40, 1:60, 1:80, 1:100 and 1:150 g.mL^{-1}) was conducted by adding the milled tea (5.0, 2.5, 1.67, 1.25, 1.0 and 0.67 g) to 100 mL deionized water at 90℃ for 20 minutes. The effect of leaching temperature was done by adding 2.0 g of the milled tea into 100 mL deionized water at different temperature (60, 70, 80, 90 and 100℃) for 20 minutes. The effect of leaching time was conducted by adding 2.0 g of the milled tea into 100 mL deionized water at 90℃ for different time (3, 5, 10, 20, 40 and 60 min). The effect of leaching times was done according to the following description: 2.0 g of the milled tea was added to 100 mL deionized water at 90℃ for 20 minutes, the mixture was centrifuged and the supernatant was collected as the first infusion; the same tea sample was used for making a second and then a third infusion, using 100 mL of deionized water at 90℃ each time for 20 minutes, respectively.

Determination of *in vitro* bio-accessibility of Al from tea infusion and tea

The preparation of simulated gastric, duodenal, and bile juices and the *in vitro* gastrointestinal digestion of tea infusion were done according to the method of Flores *et al.* [17]. Briefly, simulated gastric juice was composed of 2.5 g pepsin, 3.0 g mucin, 2.752 g NaCl, 0.824 g KCl, 0.266 g NaH_2PO_4, 0.399 g $CaCl_2.2H_2O$, 0.306 g NH_4Cl, 0.085 g urea, 6.5 ml concentrated HCl in 500 ml H_2O. Simulated duodenal juice was composed of 9.0 g pancreatin, 1.5 g lipase, 7.012 g NaCl, 0.564 g KCl, 3.388 g $NaHCO_3$, 80.0 mg KH_2PO_4, 50.0 mg $MgCl_2$, 0.1 g urea, 0.180 ml concentrated HCl in 500 ml H_2O. Simulated bile juice was composed of 30.0 g bile salts, 5.259 g NaCl, 0.376 g KCl, 5.785 g $NaHCO_3$, 0.25 g urea, 0.150 ml concentrated HCl in 500 ml H_2O. The pH of the simulated gastric, duodenal and bile juices was adjusted to 1.30 ± 0.02, 8.1 ± 0.2, 8.2 ± 0.2, respectively with 1 M HCl or 1 M NaOH.

10 mL of tea infusion (3.0 g of milled teas was extracted with 100 mL deionized water at 90℃ for 10 min.) was digested sequentially as follows: stomach - addition of 12 mL of gastric juice and mixing for 2 h; and intestine-addition of 12 mL of duodenal and 6 mL of bile juices and mixing for 2 h. Aliquots were collected at 0.5 h intervals for a total of 4 h, and centrifuged at 4846×g for 10 min. The supernatants were stored at -20℃ prior to further analyses for Al content. The amount of Al in the supernatant was considered bioaccessible and available for the absorption into the systematic circulation from the gut. The bioaccessibility of Al was calculated as the percentage between the concentration after digestion and before digestion. The *in vitro* bioaccessibility of Al from teas was calculated after 0.5 g of milled tea was digested as described above.

Statistical analysis

All data were expressed as the mean ± SD of three replications of the experiment. Statistical analysis was performed by one-way analysis of variance followed by LSD test. Significant difference was considered at $p < 0.05$.

Results

Total Al contents in tea

The total Al contents in green tea with different leaf ages are shown in Table 1. We found that the content significantly increased with the age of leaf, and it in four leaves and a bud green tea was 6.9 times as high as that in a bud green tea. The total Al contents in black tea and oolong tea showed similar changing trends to that in green tea with the increase of leaf age (data not shown). This was in agreement with the report of Xie *et al.* [7], who showed that the content of Al in old leaves was 1.1- 3.3 times higher than that in mature leaves and 5.2-17.8 times higher than that in young leaves. Hence, consumers can reduce Al intake through drinking tea processed with young leaves.

The total Al contents in different types of teas processed with same fresh leaves (bud with two leaves) are shown in Table 2. No significant differences were observed in the total Al contents of green tea, black tea, and oolong tea. This was not in agreement with several previous reports that tea types significantly affected total Al contents in teas. For example, Zhou *et al.* [18] found that Al contents in teas were oolong tea (1943 mg.kg^{-1})>black tea (1565 mg.kg^{-1})>green tea (699 mg.kg $^{-1}$), and Wong *et al.* [8] also reported that Al contents were oolong tea (1010 mg.kg $^{-1}$)>black tea (782 mg.kg^{-1})>green tea (675 mg.kg $^{-1}$). The disagreement might be mainly attributed to different fresh leaves for processing teas. The teas in the study of Zhou *et al.* [18] were purchased

Table 1. The content and leaching of Al from green tea[a].

Tender	Total Al (mg.kg^{-1})	Leached Al (mg.L^{-1})	Leaching rate (%)
Bud	250.51 ± 22.67^{e}	1.42 ± 0.14^{e}	29.25 ± 0.39^{b}
Bud with a leaf	646.21 ± 23.04^{d}	3.57 ± 0.17^{d}	28.91 ± 1.36^{b}
Bud with two leaves	1137.09 ± 17.59^{c}	6.40 ± 0.11^{c}	29.41 ± 0.51^{ab}
Bud with three leaves	1434.06 ± 26.91^{b}	8.42 ± 0.18^{b}	30.67 ± 0.64^{a}
Bud with four leaves	1717.33 ± 33.41^{a}	9.39 ± 0.17^{a}	29.13 ± 0.57^{b}

[a]Means followed by different letters in the same column indicate significant differences at p<0.05 according to LSD test.

Table 2. The content and leaching of Al from different types of teas (bud with two leaves).

Type of tea	Total Al (mg.kg^{-1})	Leached Al (mg.L^{-1})	Leaching rate (%)
Green tea	1183.31 ± 23.61	6.79 ± 0.22	30.44 ± 0.96
Black tea	1148.60 ± 29.44	7.04 ± 0.11	30.65 ± 0.50
Oolong tea	1203.15 ± 31.62	6.97 ± 0.03	30.19 ± 0.14

from supermarket, and the teas in the study of Wong *et al.* [8] were processed with fresh leaves from different plantations. It was difficult to ensure that the quality of fresh leaves was same, thus affecting the total Al contents in teas processed with these fresh leaves, for the total Al contents in fresh leaves were affected by soil characteristics, plant variety, especially leaf age. It was noted that green tea was generally made from young and tender leaves, and black tea, especially oolong tea, were made from more mature leaves than green tea. Wong *et al.* [8] had also showed that the green tea was processed with buds and two leaves while old leaves were used for black tea and oolong tea in their study. Therefore, they showed that tea types significantly affected total Al contents in teas. In our study, green tea, black tea and oolong tea were processed with same fresh leaves through different manufacturing processes, and the effect of tea types on the Al content was only from different manufacturing processes. We did not observe significant differences in total Al contents of these teas, which suggested that different manufacturing processes did not significantly affect the Al contents in teas and the Al contents in teas might be mainly related to the quality of fresh leaves, especially leave ages.

Leaching of Al from tea

As shown in Tables 1 and 2, the leaching of Al from green tea, black tea and oolong tea was low, and the leaching rate was only about 30%, although the total contents in them were considerably high. This was consistent with the report of Wróbel *et al.* [19], who showed that the leaching rate of Al from green tea was 28.7%, and of Moghaddam *et al.* [20], who showed that the leaching rates from different teas were from 23.9%-34.6%. The low leaching rate might be due to that Al in teas was bound by organic compounds such as polyphenols [10,11], and it also formed complexes with fluoride (F) [9], which hindered the leaching of Al from teas. No significant differences were observed in the leaching of Al from green tea, black tea and oolong tea.

With the increase of leaf age, the total Al content in green tea increased significantly, the leached Al in its infusion also increased significantly (Table 1). However, the leaching rate of Al did not show the similar trend. The leaching rate from four leaves and a bud green tea was significantly lower than that from three leaves and a bud green tea. This might be due to that the F content in tea also increased with leaf age, and Al formed more complexes with F in old leave [5,21], thus decreasing the leaching rate. The leaching of Al from black tea and oolong tea also showed a similar trend to that of green tea with the increase of leaf age.

Effect of brewing condition on Al leaching from tea

The leaching of Al into tea infusion depends on not only the Al amount present in the tea, its solubility but also the brewing condition. As shown in Figure 1, tea/water, brewing temperature, times and time significantly affected the leaching of Al from green tea. The Al content in

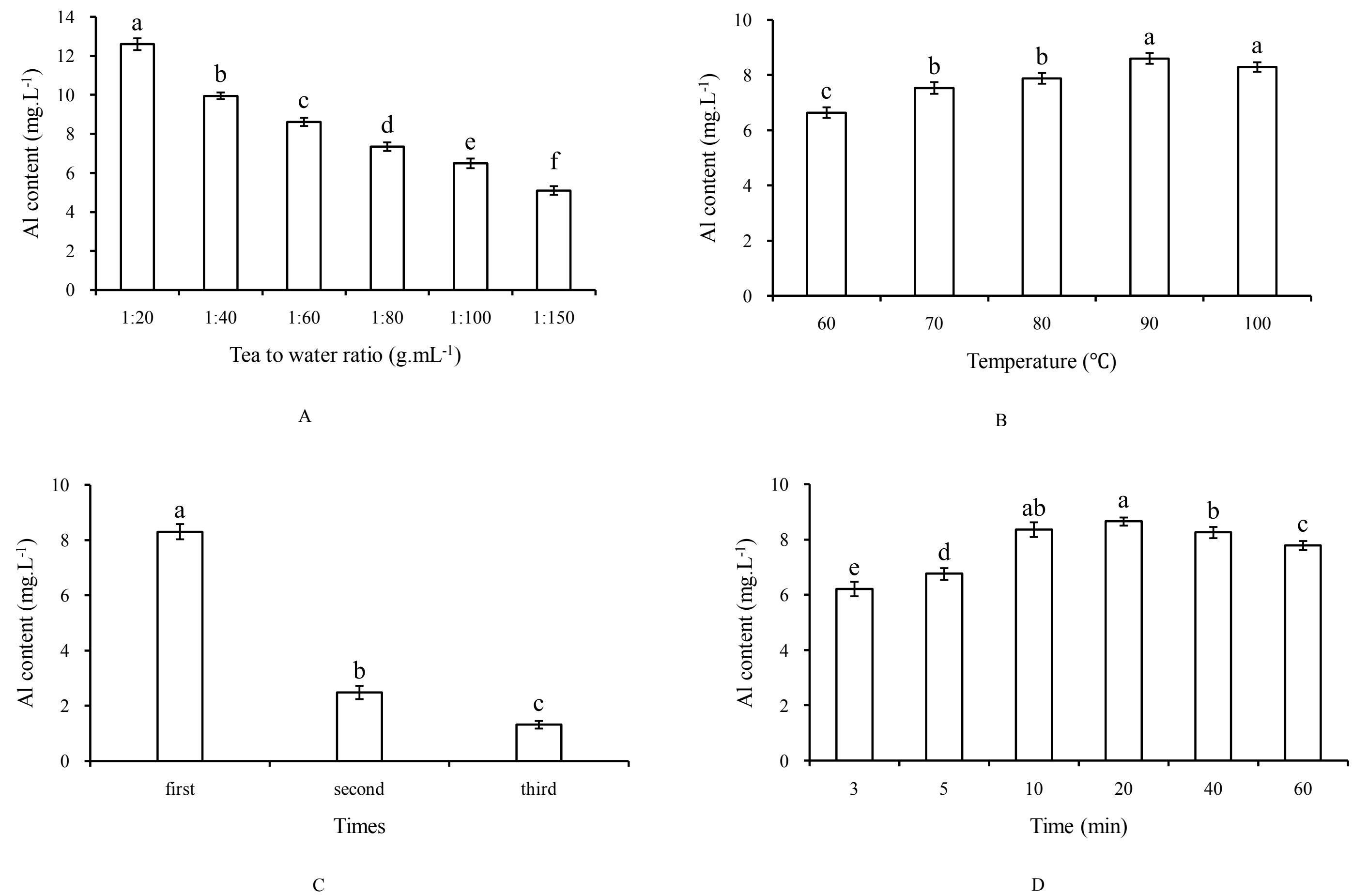

Figure 1. Effect of tea to water ratio (A), temperature (B), times (C), and time (D) on Al leaching from tea [The milled green tea was added into 250 mL glass conical flasks containing 100 mL deionized water in thermostatic water bath for some time. Then, the mixtures were centrifuged at 4846×g for 10 min and the supernatants were collected for the determination of Al content. Means followed by different letters indicate significant differences at p<0.05 according to LSD test].

tea infusion decreased significantly with the decrease of tea/water, and increased significantly with the increase of brewing temperature when the brewing temperature was not higher than 90℃. It decreased when the brewing temperature was over 90℃, but no significant difference was observed between 90℃ and 100℃. We also observed that the Al content in the first infusion was significantly higher than that in the second infusion, and 68.6% of soluble Al was leached into the first infusion. It in the second infusion was significantly higher than that in the third infusion, and 20.5% of soluble Al was leached into the second infusion. A similar finding has been reported by Mehra and Baker [22]. Finally, the Al content in tea infusion significantly increased with increasing infusion time in the first 10 min of infusing, and significantly decreased after 20 min of infusing. The effect of brewing condition on Al leaching from black tea and oolong tea showed a similar trend to that of green tea. These suggest that it is conductive to reduce Al intake through increasing water consumption, decreasing water temperature and brewing time when making tea.

In vitro bio-accessibility of Al from tea infusion and tea

Table 3 shows the bio-accessibility of Al from the infusions of green tea, black tea and oolong tea after simulated gastrointestinal digestion. The results revealed that the bio-accessibility of Al from tea infusions was very high and over 94% after 2 h of gastric digestion, while it was very low and only about 5.1% after another 2 h of duodenal digestion. Similarly, the bio-accessibility of Al from teas was relatively high and about 47% after 2 h of gastric digestion, while it was very low and only about 5.5% after another 2 h of duodenal digestion (Table 4). These showed that the bio-accessibility of Al from both teas and their infusions was very low in the intestine, which was in agreement with several previous studies. Although teas contained high concentrations of Al, only a small proportion was available for absorption in the gastrointestinal tract [12]. Mehra and Baker [22] also reported that the availability of Al from teas was only 4.96% for absorption in the intestine. The poor bio-accessibility of Al might be due to its poor solubility in the intestine. It has been reported that Al was relatively insoluble at pH 6.0-8.0 [23]. These suggested that tea consumption impossibly provided an appreciable proportion of the daily dietary intake of Al because Al absorption mainly occurred in the intestine [24]. The bio-accessibility of Al from teas was significantly lower than that from their infusions after 2 h of gastric digestion, which might be due to the action of polyphenols in teas, which avidly bound trivalent metals (Al^{3+}) [12], and of F in them, which formed complexes with Al [9].

Table 3. The bio-accessibility (%) of Al from tea infusions after *in vitro* gastrointestinal digestion[a].

Time (h)	Type of tea	0.5	1.0	1.5	2.0
Gastric digestion	Green tea	74.72 ± 3.01^{cde}	91.23 ± 1.89^{bd}	97.00 ± 1.81^{a}	97.84 ± 2.07^{a}
	Black tea	71.45 ± 3.09^{ce}	88.11 ± 2.72^{bde}	94.02 ± 1.99^{a}	94.93 ± 1.63^{a}
	Oolong tea	76.91 ± 1.58^{cd}	86.51 ± 2.38^{be}	94.94 ± 2.03^{a}	96.41 ± 2.22^{a}
Duodenal digestion	Green tea	4.73 ± 0.95	5.31 ± 0.99	5.19 ± 0.92	5.13 ± 0.87
	Black tea	4.91 ± 0.83	5.75 ± 0.97	5.26 ± 0.84	5.08 ± 0.96
	Oolong tea	4.82 ± 0.97	5.84 ± 0.83	5.52 ± 0.79	5.43 ± 1.01

[a]Means followed by different letters in the same row (a,b,c) and in the same column of gastric digestion (d,e) indicate significant differences at $p<0.05$ according to LSD test.

Table 4. The bio-accessibility (%) of Al from teas after *in vitro* gastrointestinal digestion[a].

Time (h)	Type of tea	0.5	1.0	1.5	2.0
Gastric digestion	Green tea	28.28 ± 0.86^{cde}	35.33 ± 0.93^{b}	46.12 ± 1.09^{a}	46.78 ± 0.99^{a}
	Black tea	29.81 ± 0.53^{cd}	36.67 ± 1.30^{b}	46.13 ± 0.64^{a}	46.93 ± 1.28^{a}
	Oolong tea	27.85 ± 0.96^{ce}	36.12 ± 0.76^{b}	46.49 ± 1.05^{a}	47.13 ± 1.12^{a}
Duodenal digestion	Green tea	5.85 ± 0.93^{c}	11.88 ± 1.29^{a}	8.03 ± 1.15^{b}	5.46 ± 1.05^{c}
	Black tea	4.32 ± 0.75^{c}	12.28 ± 1.10^{a}	6.32 ± 1.13^{b}	5.52 ± 1.20^{bc}
	Oolong tea	4.23 ± 1.01^{c}	10.30 ± 1.20^{a}	6.45 ± 1.01^{b}	5.72 ± 0.92^{bc}

[a]Means followed by different letters in the same row (a,b,c) and in the same column of gastric digestion (d,e) indicate significant differences at $p<0.05$ according to LSD test.

We observed that digestion time affected the bio-accessibility of Al from the teas and their infusions under simulated gastrointestinal conditions. The bio-accessibilities of Al from both teas and their infusions significantly increased with the prolonged gastric digestion time and reached equilibrium after 1.5 h of digestion. However, Al bio-accessibilities from teas first increased significantly with the prolonged intestinal digestion time, and then significantly decreased after 1.0 h of digestion. Al bio-accessibilities from tea infusions showed a similar trend of change with the prolonged intestinal digestion time, but no significant differences were observed.

In the comparison of the bio-accessibilities of Al from green tea, black tea and oolong tea after simulated gastrointestinal digestion, no significant differences were observed between them except that the bio-accessibility from black tea was significantly higher than that from oolong tea at 0.5 h of gastric digestion. There were also no significant differences between the bio-accessibility of Al from different tea infusions after simulated gastrointestinal digestion, except that the bio-accessibility from oolong tea infusion was significantly higher than that from black tea infusion at 0.5 h of gastric digestion, and it from green tea infusion was significantly higher than that from oolong tea infusion at 1.0 h of gastric digestion. These were similar to the report of Fujii *et al.* [25], who showed that there were no significant differences in the availability of Al from green tea infusion, black tea infusion and oolong tea infusion in the gastrointestinal tract.

We assume that an adult drinks 10 g of tea daily, then 5.1 mg of Al can be ingested from drinking tea infusion according to 1700 $mg.kg^{-1}$ of average total Al content and 30% of leaching rate, and about 0.28 mg of Al (5.5% of the bio-accessibility) is accessible in the intestine. Then, the maximum amount of daily Al intake from tea into the body should be 0.28 mg because Al may not be completely absorbed in the intestines [26], and daily consumption of tea is often less than 10 g. The amount is markedly below the standard of the United Nations food and agriculture organization/the world health organization (FAO/WHO) that the acceptable daily intake (ADI) of Al is 1.0 $mg.kg^{-1}$ body weight/day [27]. The evidences suggest that tea consumption impossibly contributes an appreciable daily dietary intake of Al, thus leading to markedly negative health effect. Contrarily, it has potentially positive effect on human health for its rich antioxidant polyphenols [28].

In general, green tea, black tea, and oolong tea contained high contents of Al, and the total Al contents in them significantly increased with the leaf age. However, the leaching of Al into tea infusions was low, and brewing condition significantly affected the leaching. Moreover, the bio-accessibility of Al from the teas and their infusions were very low after simulated gastrointestinal digestion. No significant differences were observed in the total Al contents in green tea, black tea, and oolong tea processed with same fresh leaves, and there were also no significant differences in the leaching of Al from these teas, and in the bio-accessibility of Al from these teas and their infusions after simulated gastrointestinal digestion. From our findings, we suggest that drinking tea is no cause for concern about Al toxicity in healthy individuals.

Acknowledgments

This work was financially supported by the Fundamental Research

Funds for the Central Universities, China (grant no. 2013PY091).

References

1. Nayak P (2002) Aluminum: impacts and disease. *Environ Res* 89: 101-115.[crossref]
2. Soni MG, White SM, Flamm WG, Burdock GA (2001) Safety evaluation of dietary aluminum. *Regul Toxicol Pharmacol* 33: 66-79.[crossref]
3. Pinto MS (2013) Tea: A new perspective on health benefits. *Food Res Int* 53: 558-567.
4. Fung KF, Zhang ZQ, Wong JW, Wong MH (2003) Aluminium and fluoride concentrations of three tea varieties growing at Lantau Island, Hong Kong. *Environ Geochem Health* 25: 219-232.[crossref]
5. Shu WS, Zhang ZQ, Lan CY, Wong MH (2003) Fluoride and aluminium concentrations of tea plants and tea products from Sichuan Province, PR China. *Chemosphere* 52: 1475-1482.[crossref]
6. Karak T, Bhagat RM (2010) Trace elements in tea leaves, made tea and tea infusion: A review. *Food Res Int* 43: 2234-2252.
7. Xie ZL, Dong DM, Bao GZ, Wang ST, Du YG et al. (2001) Aluminum content of tea leaves and factors affecting the uptake of aluminum from soil into tea leaves. *Chinese Geogr Sci* 11: 87-91.
8. Wong MH, Zhang ZQ, Wong JWC, Lan CY (1998) Trace metal contents (Al, Cu and Zn) of tea: tea and soil from two tea plantations, and tea products from different provinces of China. *Environ Geochem Health* 20: 87-94.
9. Nagata T, Hayatsu M, Kosuge N (1993) Aluminum kinetics in the tea plant using 27 Al and 19 F NMR. *Phytochemistry* 32: 771-775.
10. Flaten AK, Lund W (1997) Speciation of aluminium in tea infusions studied by size exclusion chromatography with detection by post-column reaction. *Sci Total Environ* 207: 21-28.[crossref]
11. Tang DS, Shen SR, Chen X, Zhang YY, Xu CY (2004) Interaction of catechins with aluminum in vitro. *J Zhejiang Univ Sci* 5: 668-675.[crossref]
12. Powell JJ, Greenfield SM, Parkes HG, Nicholson JK, Thompson RP (1993) Gastro-intestinal availability of aluminium from tea. *Food Chem Toxicol* 31: 449-454.[crossref]
13. Yokel RA, Florence RL (2008) Aluminum bioavailability from tea infusion. *Food ChemToxicol* 46: 3659-3663.[crossref]
14. Akinyele IO, Shokunbi OS (2015) Comparative analysis of dry ashing and wet digestion methods for the determination of trace and heavy metals in food samples. *Food Chem* 173: 682-684.[crossref]
15. Bertsch PM, Bloom PR (1996) Aluminum, in Methods of Soil Analysis, Part 3, Chemical Methods, ed. by Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, et al. Soil Science Society of America, Inc., Madison, Wisconsin, pp.517-550.
16. Moreno IM, González-Weller D, Gutierrez V, Marino M, Cameán AM, et al. (2008) Determination of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr and Zn in red wine samples by inductively coupled plasma optical emission spectroscopy: Evaluation of preliminary sample treatments. *Microchem J* 88: 56-61.
17. Flores FP, Singh RK, Kerr WL, Pegg RB, Kong FB (2014) Total phenolics content and antioxidant capacities of microencapsulated blueberry anthocyanins during in vitro digestion. *Food Chem* 153: 272-278.
18. Zhou CY, Wu J, Chi H, Wong MK, Koh LL, et al. (1996) The behaviour of leached aluminium in tea infusions. *Sci Total Environ* 177: 9-16.
19. Wróbel K, Wróbel K, Urbina EMC (2000) Determination of total aluminum, chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of blacktea, green tea, hibiscus sabdariffa, and ilex paraguariensis (Mate) by ETA-AAS. *Biol Trace Elem Res* 78: 271-280.
20. Moghaddam MA, Mahvi AH, Asgari AR, Yonesian M, JahedGh, et al. (2008) Determination of aluminum and zinc in Iranian consumed tea. *Environ Monit Assess* 144: 23-30.[crossref]
21. Xie ZL, Chen Z, Sun WT, Guo XJ, Yin B, et al. (2007) Distribution of aluminum and fluoride in tea plant and soil of tea garden in Central and Southwest China. *Chinese Geogr Sci* 17, 376-382.
22. Mehra A, Baker CL (2007) Leaching and bioavailability of aluminium, copper and manganese from tea (Camellia sinensis). *Food Chem* 100: 1456-1463.
23. Driscoll CT, Postek KM (1996) The chemistry of aluminum in surface waters, in The Environmental Chemistry of Aluminum (2nd ed.), ed. by Sposito G. Lewis Publishers, New York, pp. 363-418.
24. Berthon G (2002) Aluminum speciation in relation to aluminum bioavailability, metabolism and toxicity. *Coord Chem Rev* 228: 319-341.
25. Fujii W, Kusumoto A, Nakada T, Suwa Y (2002) Gastrointestinal absorption of aluminum from teas in rats. *J Food Sci* 67: 2552-2554.
26. Ganrot PO (1986) Metabolism and possible health effects of aluminium. *Environ Health Perspect* 65: 363-441.[crossref]
27. JECFA (Joint FAO/WHO Expert Committee on Food Additives) (1999) Summary of evaluations performed by the Joint FAO/WHO expert committee on food additives, JECFA 1956-2003. ILSI Press, International Life Sciences Institute, Washington, DC, USA.
28. Pękal A, Dróżdż P, Biesaga M, Pyrzynska K (2012) Screening of the antioxidant properties and polyphenol composition of aromatised green tea infusions. *J Sci Food Agric* 92: 2244-2249.[crossref]