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

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**Abstract**

To assess human exposure to aluminium (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 Biotechnology 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...
planted 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°C 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 Shokumbi [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°C 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 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°C 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°C) for 20 minutes. The effect of leaching time was done by adding 2.0 g of the milled tea into 100 mL deionized water at 90°C 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°C 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°C 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 in 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 NaH₂PO₄, 0.399 g CaCl₂·2H₂O, 0.306 g NH₄Cl, 0.085 g urea, 6.5 ml concentrated HCl in 500 ml H₂O. 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 NaHCO₃, 80.0 mg KH₂PO₄, 50.0 mg MgCl₂·6H₂O, 0.150 ml concentrated HCl in 500 ml H₂O. Simulated bile juice was composed of 30.0 g bile salts, 5.259 g NaCl, 0.376 g KCl, 5.785 g NaHCO₃, 0.25 g urea, 0.150 ml concentrated HCl in 500 ml H₂O. The pH of the simulated gastric, duodenal and bile juices were 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 tea was extracted with 100 mL deionized water at 90°C 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°C 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 at p<0.05 according to LSD test. Significant difference was considered at p < 0.05.

**Table 1. The content and leaching of Al from green tea**

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

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   | 6.70 ± 0.02        | 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     |

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

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**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. The temperature difference was not higher than 90°C. It decreased when the brewing temperature was over 90°C, but no significant difference was observed between 90°C and 100°C. 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 tea infusions 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.

| Time (h) | Type of tea | 0.5 | 1.0 | 1.5 | 2.0 |
|----------|-------------|-----|-----|-----|-----|
| **Gastric digestion** | Green tea | 74.72 ± 3.01c | 91.23 ± 1.80d | 97.00 ± 1.81a | 97.84 ± 2.07a |
| | Black tea | 71.45 ± 3.09c | 88.11 ± 2.72bc | 94.02 ± 1.99a | 94.93 ± 1.63a |
| | Oolong tea | 76.91 ± 1.58b | 86.51 ± 2.38bc | 94.94 ± 2.03a | 96.41 ± 2.22a |
| **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 |

*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.

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