

Total content and speciation of aluminium in tea leaves and tea infusions

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Abstract

A total of 29 tea samples of different origin, 13 green tea samples, 13 black tea samples, two semi-fermented and one white tea, imported to the Czech Republic, were collected and analysed for total content of aluminium (Al) in tea leaves and tea infusions, as well as for Al compounds in these infusions. The total content of metals in tea leaves differs according to the type of tea (green or black) and is probably influenced by many factors, e.g., soil properties. The HPLC/IC speciation of Al in tea infusions was performed for all samples. The addition of Al^{3+} to the tea infusion proved that Al^{3+} , Al(Y)^{2+} and Al(X)^{1+} species can be determined in tea infusions. Increased extraction time did not show any affect on Al speciation, neither did the addition of sugar. After the addition of lemon juice, the speciation changed in one sample of black tea and five samples of green tea. These findings suggest that lemon juice as an additive can significantly influence Al speciation in tea infusions.

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1. Introduction

Aluminium (Al) in most of its forms presents no harm to living organisms. However, under certain conditions such as low pH, Al tends to form species that are potentially toxic to all living organisms, including humans (McLachlan, 1995). Recent studies show that Al is involved in Alzheimer's disease, Parkinson's disease and dialysis encephalopathy (Exley & Korchazhkina, 2001). The toxic effect of certain Al species on different plants has been well documented (Lukaszewski & Blevins, 1996; Takabatake & Shimmen, 1997).

The chemistry of Al in soil solution is complex, and many factors, such as pH and soil organic matter content and quality, influence Al speciation. Aluminium has a minimum solubility in the pH range 5.5–6.0; concentrations of total dissolved Al increase at higher and lower pH values

(ISO, 1997). Hydroxyls, fluoride, sulphate, phosphate, silica and organic matter are the most important relevant ligands with respect to terrestrial and aquatic ecosystems. In addition, Al can form complexes with various organic compounds (e.g., humic and fulvic acids, and low mass organic compounds) and inorganic ligands (e.g., fluoride, chloride, sulfate and phosphate), most but not all of which are soluble (CCME, 1988).

The relative distribution of Al between its various organic and inorganic complexes influences its mobility in the environment, bioavailability, and toxicity (Adams & Hathcock, 1984; Hue, Cradock, & Adams, 1986). This toxicity to plants qualitatively decreases in the order: polymer $\text{AlO}_4\text{Al}_{12}(\text{OH})_{24}(\text{H}_2\text{O})_{12}^{7+}$, Al^{3+} , $\text{Al}(\text{OH})^{2+}$, $\text{Al}(\text{OH})_2^+$, $\text{Al}(\text{OH})_4^-$ and AlSO_4^+ (the toxicity of AlSO_4^+ is not always accepted). Aluminium bound in fluorides or organic complexes, phosphate or silicate Al polymers and $\text{Al}(\text{OH})_3$ is said to be non-toxic (Boudot, Becquer, Merlot, & Rouiller, 1993).

Polymer $\text{AlO}_4\text{Al}_{12}(\text{OH})_{24}(\text{H}_2\text{O})_{12}^{7+}$ is commonly referred to as Al_{13} . Its toxicity is often considerably higher than

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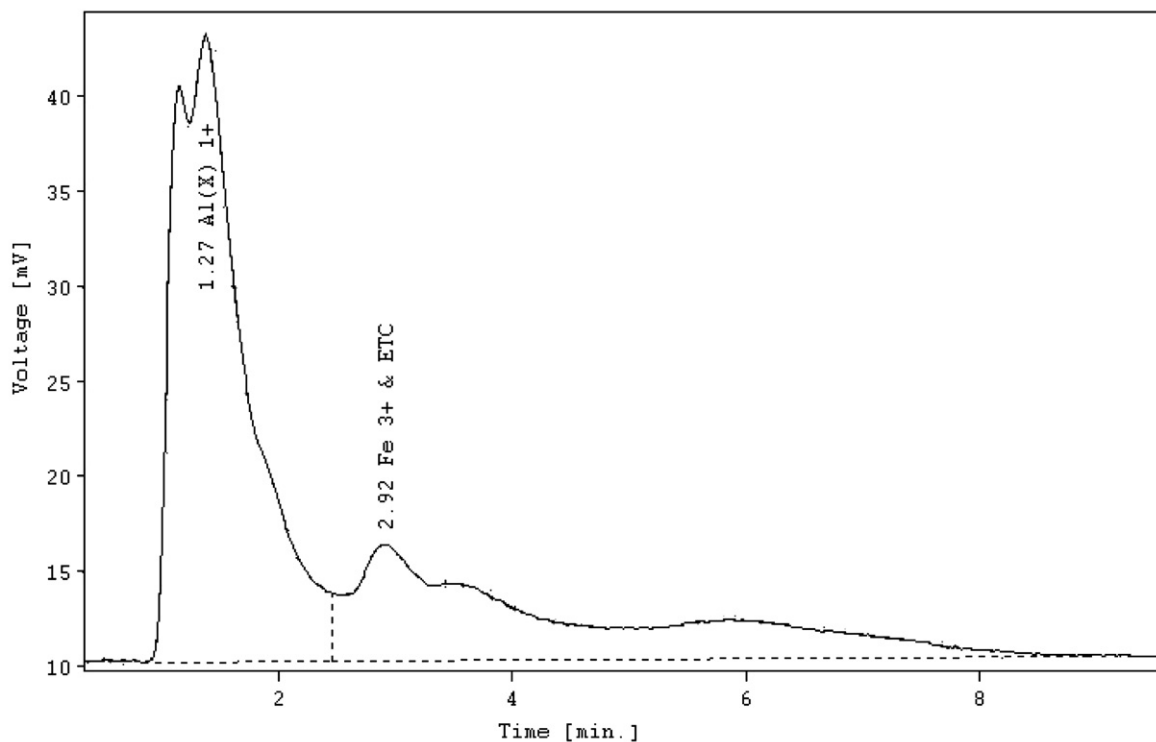


Fig. 1. Typical chromatogram of tea infusion sample showing the splitting of the first peak and the influence of metallic interference.

that of Al^{3+} (Parker & Bertsch, 1992). About 1–11.5 times less Al as Al_{13} than as Al^{3+} (i.e., about 13–150 times on a molar basis) was needed to obtain an inhibition of 20% of both soybean and wheat root elongation. The Al_{13} polymer has been recently reported to occur in soils (Hunter & Ross, 1991), under an adsorbed state in podzol humus. As chelating organic matter is regarded as an inhibitor of Al_{13} formation, this presence is very surprising. Additionally, the occurrence of $\text{Al}(\text{OH})_4^-$ is believed to be a prerequisite to the formation of Al_{13} (Bertsch, 1987) and it is not easily conceivable that podzol humus offers favourable conditions to its formation. Clay surfaces, however, would be highly favourable to Al hydrolysis and polymerisation, even in unsaturated solutions (Boudot et al., 1993).

A range of plant species has evolved mechanisms that enable them to grow on acid soils, where toxic concentrations of Al^{3+} can limit plant growth. Organic acids play a central role in these Al tolerance mechanisms. Some plants detoxify Al in the rhizosphere by releasing organic acids that chelate Al (Ryan & Delhaize, 2001). There are approximately 25 plant families with members that accumulate Al at concentrations exceeding 1000 mg kg^{-1} in their leaves (Jansen, Watanabe, Dessein, Smets, & Robbrecht, 2003). Among them, tea plants (*Camellia sinensis* L.) are among the most important; old leaves of tea may contain up to $30 \text{ mg Al per gram dry weight}$ (Matsumoto, Hirasawa, Morimura, & Takahashi, 1976). Tea plants not only tolerate high Al but also their growth is strongly enhanced by Al supply (Konishi, Miyamoto, & Taki, 1985).

A number of studies have reported the total concentration of Al in tea infusions (Flaten, 2002; Powell, Burden, & Thompson, 1998; Fung, Zhang, Wong, & Wong, 2002). The total metal components in tea plants depend on many factors, primarily the age of the tea leaves, but also the soil conditions, rainfall, altitude, genetic make up of the plant, etc. This affects the metal concentration in an infusion. The preparation method (infusion time, temperature, tea–water ratio) also has a large influence (Marcus, Fisher, Ree, & Hill, 1996). Despite the variation in infusion conditions, the reported Al concentrations in tea infusions are remarkably consistent. With few exceptions, the Al values are in the range of $1\text{--}6 \text{ mg l}^{-1}$.

Tea is a major dietary source of Al, and tea drinking can more than double an individual's intake of Al (Flaten, 2002). It is suggested that Al in tea infusions is fully or partly bound with relatively large organic molecules, which may be polyphenolic compounds. Tea polyphenols have a high affinity for metals and also for biological macromolecules, such as alkaloids, carbohydrates and proteins (Alberti, Biesuz, Profumo, & Pesavento, 2003). Yang, Sivagutu, Horst, and Matsumoto (2000) found the pH of readily available tea (commercial products) to be lower than that of the infusions, and stated it was probably due to the presence of citric acid and other products generally added for the preservation of commercial drinks. Citric acid is known to markedly enhance Al absorption. Tea contains a large number of compounds that are able to complex Al. It has often been assumed that the polyphenols, which make up 40% of the dry matter present in

tea, are the most important Al-complexing compounds in tea due to the many phenolic hydroxyl groups that provide a large number of potential complexation sites. Infusions of green and black tea differ chemically, mainly within the polyphenolic fraction. The difference results from the oxidation of simple polyphenols to more complexed and condensed ones. A large fraction of black tea polyphenols has not been chemically identified, and these compounds are often referred to as thearubigens (Flaten, 2002).

In this study, the total Al content in a number of tea plants, and the amount of Al available in the tea extracts, depending on the type of tea and on the method of preparation, was evaluated as well as HPLC/IC Al speciation in these extracts. Additionally, the effect of sugar and lemon addition into the tea infusions on Al speciation was investigated.

2. Materials and methods

A total of 29 tea samples of different origin imported to the Czech Republic were collected and analysed. There were 13 green tea samples, 13 black tea samples, two semi-fermented and one white tea (Table 1). Only two soil samples were analysed, as soil samples from tea plantations are very difficult to obtain. One sample was the substrate used for growing black tea (pH 3.74), and the second sample was the substrate used for growing green tea (pH 4.12). Where the locality was given for the remaining tea samples,

the prevailing soil unit and characteristics were approximated from The Handbook of Soil Science (Sumner, 2000).

For the determination of the accumulation of total Al in tea leaves, aliquots (0.5 g) of the dried and powdered biomass were heated in 50 ml quartz–glass beakers up to 500 °C for 16 h on a hot plate and in a muffle furnace, with a stepwise increase of the ashing temperature (Mader, Száková, & Miholová, 1998). The ash was then heated for 5–10 min at 100 °C in 3 ml of *Aqua Regia* and then transferred quantitatively to test tubes and filled up to 20 ml with deionised water. Tea infusions were prepared, to test the solubility of the Al, after (i) 5 min, (ii) 60 min, and (iii) 24 h, as follows: 1 g of tea was carefully weighed out into standardised glass beakers. Boiled distilled water (50 ml) was poured into the glass beakers, after which they were covered with watch glasses. After the given time, the extracted solution (tea infusion) was filtered through filter paper (blue label) into test tubes, and immediately measured. When the influence of tea additives was tested, 5.5 g of sugar and/or 0.5 ml commercial lemon juice was added to 50 ml of filtered tea infusion and carefully stirred.

For the extraction of mobile forms of Al in soils, the following procedures were applied: (i) exchangeable forms: 0.5 M KCl (adjusted to pH 5.8) in ratio 1:10 w/v, shaking for 24 h, (ii) weakly organically bound forms: 0.3 M CuCl₂ solution in ratio 1:10 w/v, shaking for 2 h, (iii) total organically bound forms: 0.05 M Na₄P₂O₇ solution in ratio 1:20 w/v, shaking for 24 h. Extracts were separated from suspen-

Table 1
Tea samples: type of tea, name, location, prevailing soil unit of the region

Sample no.	Type of tea	Name of tea	Location	Soil unit
1	Black	Golden Nepal	Darjeeling	Acrisols
2	Black	Gruzie OP	Caucasian	Arenosols
3	Black	Kenya GFOP – 1 Milima	Kenya	Alisols
4	Black	Ceylon OP Dimbula Uduwela	Sri Lanka	Acrisols
5	Black	Pu – Erh	Yunnan, China	Alisols
6	Black	Assam OP blend	Assam, India	Cambisols
7	Black	Ceylon OP decaffeinated Superior	Ceylon	Acrisols
8	Black	Gunpowder black	Unknown	Unknown
9	Black	Nilgiri	South India	Acrisols
10	Black	Turkey BOP	Turkey	Alisols
11	Black	Darjeeling FF FTGFOP1 Lucky Hill Inbetween	Darjeeling	Acrisols
12	Black	Golden Yunnan FOP	Yunnan, China	Alisols
13	Green	Formosa Gunpowder	Unknown	Acrisols
14	Green	Jade Arrow	Unknown	Alisols
15	Green	Bancha	Unknown	Alisols
16	Green	Java OP green	Indonesian Island	Acrisols
17	Green	Japan Kokaicha	Japan	Luvisols
18	Green	Lung Ching	Zhejiang Province, China	Alisols
19	Green	China Sencha	China	Alisols
20	Green	Darjeeling SFTGFOP Moondakotee	Darjeeling	Acrisols
21	Green	Chun Mee	Chun Mee, China	Fluvisols
22	Green	Vietnam Ché ngon So	Vietnam	Alisols
23	Green	Bancha	Yunnan, China	Alisols
24	Green	Yunnan green	Yunnan, China	Alisols
26	Semi-fermented	Ti Kuan Yin	Unknown	Acrisols
27	Semi-fermented	Formosa Fine Oolong	Taiwan	Acrisols
28	White	Pai Mu Tan	Fujian, China	Alisols
29	Green	Unspecified	China	Alisols
30	Black	Unspecified	China	Alisols

sion by centrifugation (20 min, 13,500 rpm), and, when necessary, further purified by filtration. Content of weakly organically bound Al was calculated as the difference between Al amount extracted by CuCl_2 and by KCl. Content of strongly organically bound Al was calculated as the difference between Al amount extracted by $\text{Na}_4\text{P}_2\text{O}_7$ and by CuCl_2 (Drábek, Borůvka, Mládková, & Kočárek, 2003).

The content of Al in the plant digests, tea infusions, and soil extracts were determined by inductively coupled plasma optical emission spectrometry with axial plasma configuration (ICP-AES – Varian VistaPro, Australia, equipped with autosampler SPS-5), at spectral line $\lambda = 308.2$ nm. Aliquots of certified reference material, CRM CTA-OTL-1 tobacco leaves, were mineralised under the same conditions, for quality assurance of the analytical data of the total Al content in tea samples. Statgraphics Plus 4.0 for Windows (Manugistics, 1997) was used to perform analysis of variance of the data.

Speciation of Al forms in 0.5 M KCl and water extracts were determined by means of HPLC/IC, according to Drábek et al. (2003). The principle of the method consists in the separation of Al forms according to the value of their positive charge. Final spectroscopy detection in the UV range is then enabled by post-column derivatization. The HPLC instrument consisted of a Consta Metric 4100 pump (LDC Analytical Company) with additional pulse suppressor, automatic sampler Triatlon (Spark), cation column Alltech Cation/R SN IC1 818 (Alltech), pneumatic sampler RDU 010 (Watrex), T-like conjunction reactor with mixing loop, and UVD 200 detector (DeltaChrom). The signal from the detector was processed and stored by chromatographic software CSW (DataApex). Sodium sulfate solution (0.1 M Na_2SO_4 in 7.5 mM H_2SO_4 , pH ~ 2.4) was used as the mobile phase. The flow rate was 0.8 ml/min. The flow rate of the derivatization agent (6×10^{-4} M Tiron solution in 1 M ammonium acetate solution) was adjusted correspondingly to the flow rate of mobile phase (~ 1.6 ml/min through the detector). Detection of derivatized Al forms was performed online in the UV region (310 nm). Standard solutions were prepared from certified Al solution (Analytika, Czech Republic) with an initial concentration of 1000 mg l^{-1} , with respect to extracting agent. The analyses were run under stable laboratory conditions (temperature around 22°C). The HPLC/IC method enables to separate Al forms into three different groups, according to their charge: $\text{Al}(\text{X})^{1+}$, $\text{Al}(\text{OH})_2^+$, $\text{Al}(\text{SO}_4)^+$, AlF_2^+ , $\text{Al}(\text{oxalate})^+$, $\text{Al}(\text{H-citrate})^+$, etc.); $\text{Al}(\text{Y})^{2+}$ ($\text{Al}(\text{OH})^{2+}$, AlF^{2+} , etc.) and Al^{3+} (Al^{3+} and transformed hydroxyl-Al polymers).

3. Results and discussion

3.1. Content of mobile Al forms in soil

The contents of weakly organically bound Al in the soils were 464 mg kg^{-1} , and 340 mg kg^{-1} , and of strongly organically bound Al in the soils were 148 mg kg^{-1} and

271 mg kg^{-1} , for the black and green tea soil samples, respectively. The KCl-exchangeable Al in the samples was 367 mg kg^{-1} for the black tea soil sample and 79 mg kg^{-1} for the green tea soil sample. These findings suggest that there is a relatively high pool of mobile Al forms in soils. Unfortunately, we could not study Al content and speciation in soil solution. However, we can hypothesise that under such conditions, some more sensitive plants would suffer Al stress. Nevertheless, these conditions do not affect tea growing in such localities, as was orally confirmed by the provider of the soil samples.

3.2. Total content of Al in tea leaves and tea infusions

The contents of total Al in tea leaves and Al concentrations in tea infusions are summarised in Table 2. Average Al content in black tea was 1070 mg kg^{-1} whereas in green tea it was 1340 mg kg^{-1} ; this corresponds to the results reported by Matsuura, Hokura, Katsuki, Itoh, and Haraguchi (2001) Wrobel, Wrobel, and Urbina (2000) that Al content in green tea is higher than in black tea. The difference in the total content could be influenced by many aspects, primarily the age of the tea leaves, but also the genetic make up of the plant soil conditions, rainfall and

Table 2
Total Al contents found in tea (mg kg^{-1}), and concentration of Al in tea infusions after 5 min, 60 min and 24 h infusions (mg l^{-1})

Sample no.	Total	Infusions		
		5 min	60 min	24 h
1	883	2.19	3.32	3.12
2	1620	3.53	3.89	4.86
3	1250	4.91	2.36	4.53
4	1140	1.74	2.86	4.12
5	1280	0.669	2.22	3.12
6	1020	1.14	1.81	1.83
7	825	2.71	2.27	3.61
8	1290	2.59	3.14	2.50
9	888	3.03	2.57	5.49
10	1770	2.79	3.87	7.60
11	869	2.10	2.67	3.10
12	659	1.84	2.22	2.62
13	1480	2.20	3.68	5.48
14	293	0.284	0.398	0.246
15	3260	7.83	15.0	20.3
16	1560	3.04	6.05	7.57
17	1070	2.60	1.62	3.64
18	1230	2.75	4.37	3.79
19	2410	7.00	9.53	14.4
20	966	1.66	1.19	3.28
21	852	1.35	2.17	3.36
22	542	1.80	2.22	2.28
23	2660	9.38	14.2	20.5
24	828	1.64	2.36	3.36
26	1650	1.71	3.07	5.99
27	1010	2.15	2.86	4.68
28	805	0.951	1.17	1.17
29	272	0.279	0.304	1.01
30	908	0.712	0.814	0.373

altitude. However, we did not find any statistically significant differences in total Al contents between different prevailing soil type units. Marcus et al. (1996) stated that metal contents in tea leaves differ according to the type of tea (green or black) and geological conditions. A study done by Fung et al. (2002) found that Al concentrations in individual parts of the tea plant (young leaves, old leaves, branches) were different at different locations.

The results for the Al content of 5 min tea infusions agreed with the results compiled by Flaten (2002), who found the values rarely fell outside the range of 1–6 mg l⁻¹. In our samples, the average content of Al for the 5 min tea infusion was 2.75 mg l⁻¹ (2.3 mg l⁻¹, and 3.2 mg l⁻¹ for black and green tea infusions, respectively). These values represented 11.4% of total Al content for black, and 11.1% for green tea, respectively. There is a small, non-significant difference between the 5 min and 60 min tea infusions, but a statistical significant difference between the 5 min and 24 h infusions, in which it is clear that the Al content in the tea infusions increases over time. Comparisons were made between black and green tea for 5 min, 60 min and 24 h infusions. In each case the green tea infusions contained a higher amount of Al but not at a statistically significant level.

No health-based criteria have been proposed for Al levels in drinking water by the WHO (1998). At present there is a WHO guideline for the maximum level of Al in drinking water of 0.2 mg total Al per litre; therefore the Al con-

centration in tea infusion is 10–100 times more than that in drinking water. This does not mean that tea infusion is 10–100 times more toxic because toxicity is dependant on Al speciation. Aluminium is generally very poorly absorbed in the gastrointestinal tract; roughly in the order of 0.1% of the dietary intake is absorbed, depending on the chemical species of Al (Priest et al., 1996). Thus, if the species of Al present in tea are more bioavailable than the species present in other dietary sources, tea could make a larger contribution to human uptake of Al than is indicated by the total concentration present. However, the possible dietary risk of Al contents in tea lead to the proposal that Al in soils of tea plantations should be at least partly immobilised. This could be achieved by, for example, increasing the organic matter content. The introduced organic matter should contain humic substances which are generally known for their high Al-binding capacity (Sposito, 1996).

3.3. Aluminium speciation in tea infusions

The pH levels of the tea infusions were in the range of 4.04–5.08 (average: 4.42). The black tea was found to be more acidic (pH 4.3) than green tea (pH 4.5), but the semi-fermented tea had the highest average acidity of all (pH 4.21). The white tea had a pH of 4.88. The pH of the tea infusions after adding sugar was in the range of 4.22–5.08 (mean average: 4.57), causing it to be only slightly higher than the pH of the simple tea infusion. Citric

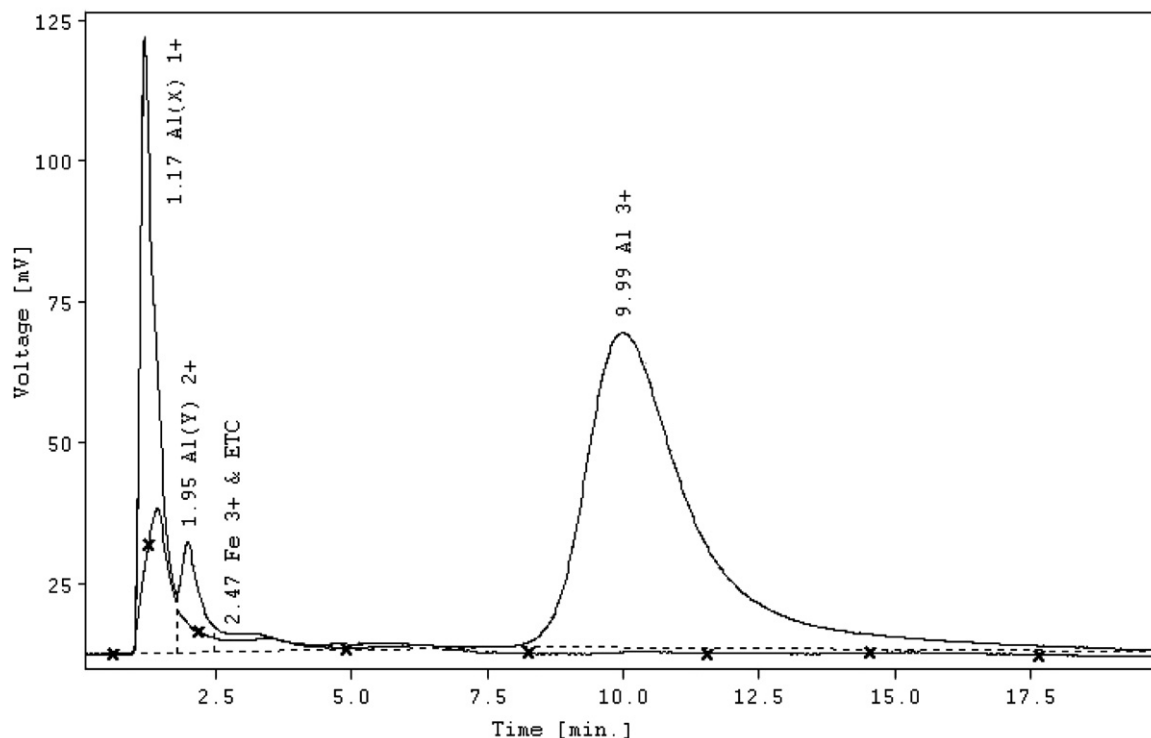


Fig. 2. Al³⁺ added to tea infusion. The crossed line represents black tea (No. 8) without additives and the normal line represents black tea (No. 8) with the addition of Al³⁺.

acid (e.g., lemon) is a common tea additive, and is known to markedly enhance Al absorption in humans (Flaten, 2002). Adding lemon juice to the tea infusions with sugar

resulted in lowering the pH, with the range being 2.38–2.78 (average: 2.52). The mean pH value for the black tea was then 2.5 while the green tea was more acidic (pH

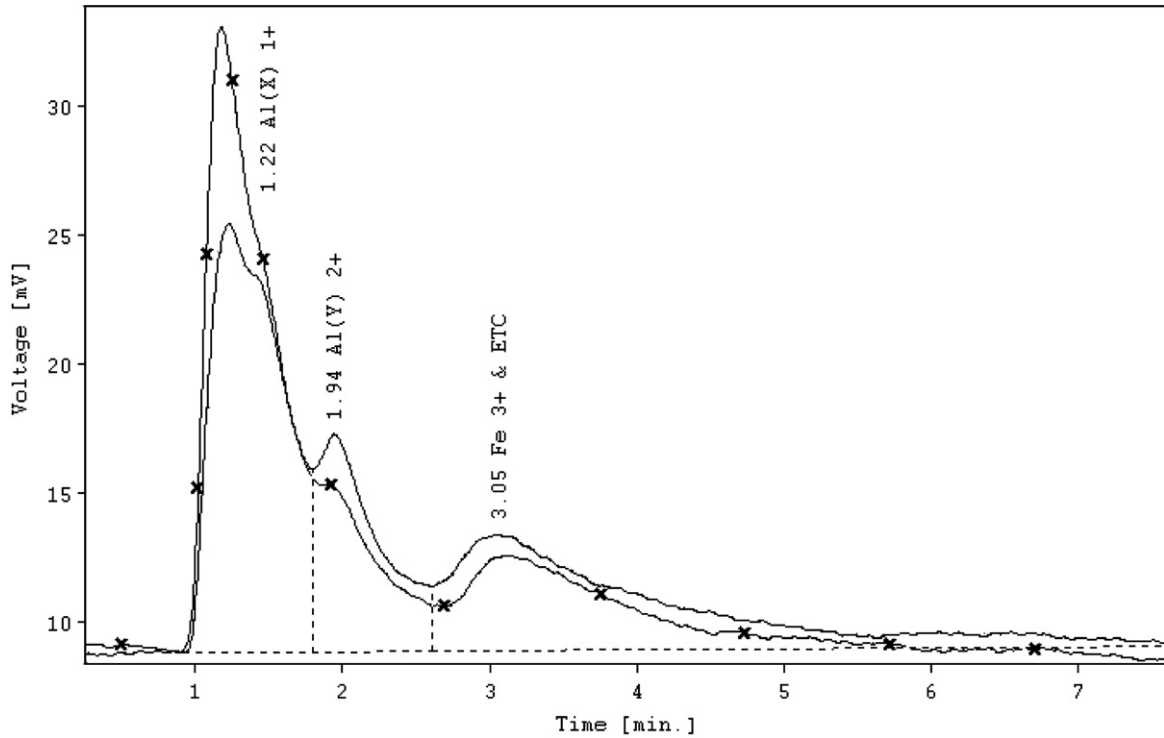


Fig. 3a. Changes in speciation caused by the addition of lemon juice. The crossed line represents green tea (No. 18) without additives and the normal line represents green tea (No. 18) after the addition of lemon juice.

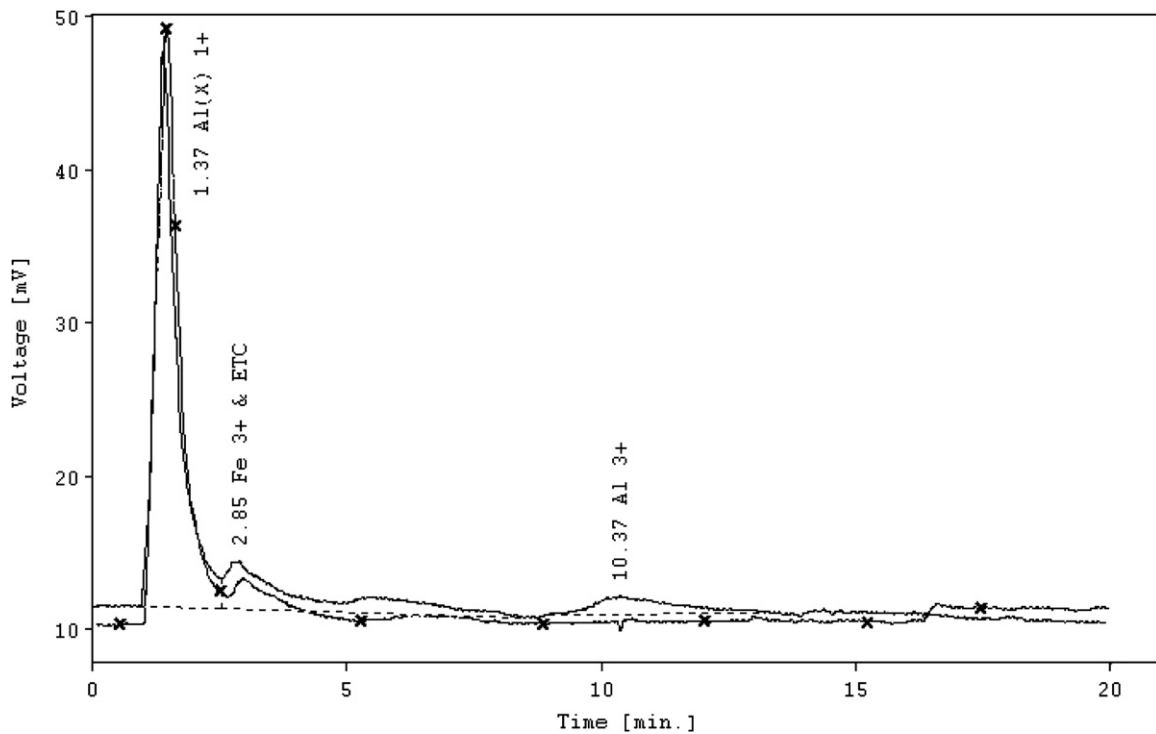


Fig. 3b. Changes in speciation after the addition of lemon juice. The crossed line represents green tea (No. 20) without additives and the normal line represents green tea (No. 20) after the addition of lemon juice.

2.4). This suggests that the buffering capacity of green tea is less than that of black tea. The semi-fermented tea (after sugar and lemon juice addition) had the lowest pH of all (2.3). Matsuura et al. (2001) found that after brewing black tea, the pH of the tea infusion was 5.8, and suggested that the elements tested in the black tea may only be sparingly soluble at pH 5.8. However, the brewing method used in that study (boiling the tea leaves for 5 min in water) is not a typical standard method for preparation of tea infusion. A standard way to prepare tea infusion used by many would be to leave the tea to lixiviate for 3–5 min. The results of our pH experiments are in agreement with those of other workers who showed that the pH of readily available tea is lower than that of tea infusions (Alberti et al., 2003; Behrendt, Oberste, & Wetzels, 2002). The readily available teas in most cases contain lemon juice or citric acid as an additive, and their pH ranges from 4.04 to 2.63.

All simple tea infusions (with no additives) showed similar Al speciation, with no general differences between black and green tea. The Al^{3+} species, were not detected in any sample. This observation confirms the fact that Al present in tea infusions does not exist in “free” forms of hexahydrates or in the form of hydroxypolymers. The second peak containing $\text{Al}(\text{Y})^{2+}$ species was clearly determined only in three green tea samples, numbered 15, 18 and 23. The rest of Al was determined as $\text{Al}(\text{X})^{1+}$ species. Composition of tea infusion matrix and latest findings of Kralj, Bukovec, Krizaj, Slejko, and Milacic (2005) suggest that some of these species carry negative or zero charge. This is in agreement with the fact that the $\text{Al}(\text{X})^{1+}$ peak, was broad with an irregular shape and in some cases even split (Fig. 1). However, the presence of some $\text{Al}(\text{Y})^{2+}$ in the broad right shoulder of this peak is expected. Moreover, interferences of other metals present in tea infusions were also apparent and significantly influenced the shape of the chromatograms. These are the reasons why speciation is described mainly qualitatively. Increased time of extraction (24 h) did not show any effect on Al speciation.

In one specific case, Al^{3+} from certified stock solution (Al in 5% HCl) was added to create final a Al concentration of 55 mg l^{-1} . The quite high Al concentration and lower pH ensured that some part of the Al remains in original Al^{3+} form. This solution equilibrated for 1 h and then the speciation was performed. The distribution of Al species into three regular peaks was observed (Fig. 2). This observation proved that mainly Al^{3+} and $\text{Al}(\text{Y})^{2+}$ but also $\text{Al}(\text{X})^{1+}$ species are possible to determine in tea infusion.

The influence of milk as a common tea additive was not studied because of possible chromatography column damage. The pH of mobile phase is quite low (2.8) so presumably milk would precipitate during the chromatography run and probably irreversibly plug the column. Adding sugar to the tea infusion did not show any effect on Al speciation. This fact was expected as sugar addition did not change the pH of the tea infusions. Moreover, addition of sugar increased the noise of the chromatography signal

and so when lemon juice was added to tea infusions, the addition of sugar was omitted.

Interesting results appeared when artificial lemon juice as a tea additive was chosen. After the addition of lemon juice, samples were equilibrated for at least 1 h and then the HPLC/IC speciation of Al was done. The pH of all samples significantly decreased and in six samples different Al speciation was observed. The speciation changed in only one sample of black tea (No. 10) but in five samples of green tea. This confirms the lower buffering capacity of green tea. In the original sample 10, only $\text{Al}(\text{X})^{1+}$ species were observed but after lemon juice amendment an additional 30% of Al present was transformed into $\text{Al}(\text{Y})^{2+}$ form. Samples 18 and 26 showed similar trends 40 and 38% of original $\text{Al}(\text{X})^{1+}$ species transformed into $\text{Al}(\text{Y})^{2+}$ form, respectively. In samples 19 and 20 there were 8 and 17% of original $\text{Al}(\text{X})^{1+}$ species transformed, even into Al^{3+} species (Figs. 3a and 3b). The $\text{Al}(\text{Y})^{2+}$ species were not detected in this case. These findings suggest that lemon juice as an additive can in some, rare cases, significantly influence Al speciation in tea infusion.

4. Conclusions

Tea plantations have a high Al pool and a low pH in their soil, which would be considered too acidic for normal agricultural practices. Organic substances, especially humic acids, should be added to the soil, which would partly immobilise Al, as they have a high Al-binding capacity. This action should reduce Al accumulation in tea plants. More research needs to be done regarding Al uptake from soil to plants. The total content of aluminium in tea leaves differs according to the type of tea (green or black) and is probably influenced by many other factors, e.g., soil properties.

The addition of sugar and lemon juice changed the pH of the tea infusions. The addition of lemon juice lowered the pH of the infusion and caused the green tea, which originally had a higher pH than black tea, to have a lower pH than the black. This suggests that the buffering capacity of green tea is low compared to that of black tea.

These findings together with the work of Kralj et al. (2005) lead to the conclusion that HPLC/IC is suitable for studying Al speciation in tea infusions but it cannot be recommended as a general speciation tool for, organically rich matrices. Use of an anionic column which would enable researchers to further separate the first peak according to negative charges, or the use of gel permeation chromatography, separating Al species according to size, is strongly recommended for Al speciation in tea infusions.

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