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Analytical Methods Determination of certain micro and macroelements in plant stimulants and their infusions

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ABSTRACT

The quantitative analysis of Al, B, Cu, Fe, Mn, P and Zn by inductively coupled plasma optical emission spectrometry (ICP-OES) and Ca, K and Mg by atomic absorption spectrometry (AAS) has been carried out in both the raw material and infusions from 31 samples of traditional plant stimulants (tea and coffee) and mate, rooibos, honeybush and chamomile. The results were discussed with respect to differences to the beverage quality and their role in the human diet. The levels of elements not significantly differ between tea types (black, green, oolong, white), and between Arabica and Robusta coffee. In comparison with tea, coffee was found to be a poor source of elements with the exception of Ca and Fe. High levels of B, Ca, Cu, Mn, Mg and Zn were found in mate (mainly green type) and of B, Ca, Cu, Fe and P in chamomile, whereas the amounts of all elements in rooibos and honeybush infusions were low (except of Ca). Apart from tea, other stimulants appeared to not represent important sources of potentially harmful amounts of Al for the human diet.

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1. Introduction

During the past few decades, extensive research on nutrient elements has been carried out to define their role in the human diet. Heavy metals are the most often controlled negative elements of food or any other product due to their ability to accumulate in the food chain. Their maximal levels have therefore become worldwide quality standards. Essential and non-essential elements are also recognised and their required or recommended contents in the human diet have been established (e.g. Co, Cr, Fe, Mn, Mo, Ni, Se, Sn, V and Zn are essential whereas As, Cd, Pb, Hg are ranged among unambiguously toxic elements). In spite of this reality, elevated levels of both essential and non-essential elements can also cause morphological abnormalities, reduced growth, increased mortality, and mutagenic effects in humans. This leads to the result that consumption of a balanced diet of all nutrients in amounts to maintain desirable body weight is necessary ([Mertz, 1998\)](#page-5-0).

Tea is the most widely consumed drink after water, due to its refreshing and mildly stimulant effects ([Harbowy & Balentine,](#page-5-0) [1997\)](#page-5-0), and plays a major role in the intake of a number of nutritional and toxic trace elements in humans [\(Nkono & Asubiojo,](#page-5-0) [1997\)](#page-5-0). Many plants have been collected or cultivated for stimulating properties, and tea is fundamental to the modern period expansion of similar used plants or herbs to the global market ([van der](#page-5-0) [Vossen & Wessel, 2000](#page-5-0)). The history of tea, a hot water infusion from the dried leaves of Camellia sinensis (L.) Kuntze (Theaceae), dates more than 5 000 years back to China. Tea types, based on processing modifications or harvested leaf development level are: black or red (fermented), oolong (semi-fermented), green (non-fermented), white (harvested leave buds with white trichomes, nonfermented or semi-fermented; [Valter, 2001](#page-5-0)).

Besides essential macro- and microelements, tea plants strongly accumulate Al at concentrations exceeding 1000 mg kg^{-1} in their leaves ([Jansen, Watanabe, Dessein, Smets, & Robbrecht, 2003\)](#page-5-0), such that old leaves of tea may contain up to 30 mg Al per gram dry weight ([Matsumoto, Hirasawa, Morimura, & Takahashi, 1976\)](#page-5-0). Tea plants not only tolerate high Al, but also their growth is strongly enhanced by Al supply [\(Konishi, Miyammoto, & Taki,](#page-5-0) [1985\)](#page-5-0). 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 $1-6$ mg l^{-1} . Therefore, tea is a major dietary source of Al, and tea drinking can increase than double an individual's intake of Al [\(Flaten, 2002\)](#page-5-0). Recent studies show that Al is involved in some important human

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disease pathologies, such as Alzheimer's disease, Parkinson's disease and dialysis encephalopathy [\(Exley & Korchazhkina, 2001](#page-5-0)).

The second most widely known stimulant used for longer than 3 000 years for preparing beverages is coffee, roasted and melted beans of Coffea arabica L., C. liberica Bull ex Hiern or C. canephora Pierre ex Froehn. belonging to the family Rubiaceae.

In addition to traditional beverages of tea and coffee, there are some another plant stimulants, prepared as a hot water infusion, which have been used only for a very short period in history, no longer than several hundred years. The beverage with the biggest potential to compete with the popularity of tea and coffee is mate, which is the infusion from dried leaves of Ilex paraguariensis A.St.- Hil. in Aquifoliaceae family. It has been commercially used since the end of 17th century and currently exceeds tea in popularity in South America. The dried leaves (green mate), dried thin branches and leaves (yerba mate) or roasted dried leaves (roasted or red mate) are used [\(van der Vossen & Wessel, 2000\)](#page-5-0). The first records of next two ''newcomers" are not older than 300 years and their commercial usage starts at first half of the past century. The rooibos (Aspalathus linearis (Burm. f.) Dahlgren) and honeybush (Cyclopia intermedia Meyer) both belong to the family Fabaceae, and are very similar, 1 m high, woody, perennial shrubs, whose leaves are dried (green) or fermented and dried (red) and consumed also as hot water infusions. They are often recommended for people hypersensitive to caffeine, because they are naturally caffeine free.

Also the dried flower heads of Matricaria recutita L. (Synonym Chamomilla r. L., Asteraceae), commonly called (German) chamomile, are used for preparation with hot water as a non-caffeine stimulant beverage. The principal components of the essential oil extracted from the flowers are the terpenoids alpha-bisabolol and its oxides and azulenes, including chamazulene. Chamomile has moderate antioxidant and antimicrobial activities, and significant antiplatelet activity in vitro. Animal model studies indicate potent anti-inflammatory action, some antimutagenic and cholesterol-lowering activities, as well as antispasmodic and anxiolytic effects [\(McKay & Blumberg, 2006\)](#page-5-0). Although it has been used for medicinal purposes, it is increasing in daily usage as a soft calming ''herbal tea" and gentle sleep aid agent or as supplement to tea mixtures to increase the flavour and bulk [\(van der Vossen & Wes](#page-5-0)[sel, 2000\)](#page-5-0). In this paper it is included especially because, thanks to low soil demands and high tolerance to toxic elements, chamomile has the ability to accumulate minerals, especially cadmium ([Kovacik, Tomko, Backor, & Repcak, 2006\)](#page-5-0).

As a result of national standards, only a few ''most important" elements are monitored (as quality markers or health risks) and no others. Moreover, only the commonly used foods are researched for their influence in the human diet. According to these realities we decided to compare the certain elements contained in traditional plant stimulants (tea and coffee) with several lesser-known species (mate, rooibos, honeybush and chamomile) in their raw material and infusions. In addition, aluminium contents in individual species were determined to evaluate their potential toxicity in contrast to expected high levels of this element in tea.

2. Material and methods

A total of 31 samples of plant stimulants (tea, coffee, mate, rooibos, honeybush and chamomile) available in the market in Czech Republic were collected and analysed. There were 15 types of pure mixture of tea leaves: four black teas (one decaffeinated and one with the addition of tea flowers), four green teas (one decaffeinated), four oolong teas (semi-fermented) and six white teas (one semi-fermented). Followed by three arabica coffees (one decaffeinated), one arabica and robusta mixture and one pure robusta, three sorts of mate, two samples of both rooibos and honeybush and one of chamomile flowers (Table 1).

Table 1

Table 2

Results for certified reference material (mg kg^{-1} of dry matter)

Element	Certified value	Obtained value
B	15 ± 4	15 ± 1
Ca	4300 ± 400	3856 ± 8
Cu	17.3 ± 1.8	18.7 ± 0.1
Fe	264 ± 15	213 ± 36
K	16600 ± 1200	16846 ± 1113
Mg	1700 ± 200	1526 ± 111
Mn	1240 ± 70	1071 ± 144
Ni	4.6 ± 0.5	4.7 ± 0.5
P	2840 ± 90	2544 ± 113
Zn.	26.3 ± 2.0	25.4 ± 0.5

For the determination of the accumulation of total elements in raw material, aliquots (1 g) of the dried and powdered biomass were decomposed in 50 ml quartz-glass beakers at 500 \degree C for 16 h on a hot plate and in a muffle furnace with a stepwise increase of the ashing temperature ([Mader, Szakova, & Miholova, 1998\)](#page-5-0). The ash was then heated for 5–10 min at 100 °C in 3 ml of Aqua regia and than transferred quantitatively to test tubes and made up to 20 ml with deionised water [\(Street, Szakova, Drabek, & Mladkova, 2006](#page-5-0)).

Tea infusions were prepared as follows: 1 g of tea was carefully weighed out into standardised glass beakers. Fifty millilitres of boiled distilled water was poured into the glass beakers after which they were covered by watch glasses to extract the components of tea leaves. After 15 min the extracted solution (tea infusion) was filtered through filter paper (blue label) into test tubes, and immediately determined. All the samples were analysed in triplicate and blanks represented 10% of the total number of samples.

The determination of Al, B, Cu, Fe, Mn, Ni, P and Zn in plant digests and infusions was carried out by inductively coupled plasma

optical emission spectrometry with axial plasma configuration (ICP-OES – Varian VistaPro, Australia), equipped with an autosampler SPS-5, at spectral lines λ = 308.2 nm for Al, λ = 249.7 nm for B, λ = 327.4 nm for Cu, λ = 238.3 nm for Fe, λ = 257.6 nm for Mn, λ = 231.6 nm for Ni, λ = 214.9 nm for P and λ = 206.2 nm for Zn.

Flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-300) was used for determination of Ca, K and Mg in plant digests and infusions. Aliquots of the certified reference material DC73351 tea (China National Analysis Center for Iron and Steel, Beijing, China) were mineralised under the same conditions for quality assurance of the analytical data of the total selected elements content in raw materials (Table 2).

3. Results and discussion

The results given in Tables 3 and 4 show that the contents of elements analysed in our study vary between plant species (raw materials) as well as between their hot water infusions. Enhanced levels of analysed elements were found in infusions of mate and chamomile that exceeded by several times the current levels found in the traditionally used tea and coffee species. For example in mate, there were in comparison to tea or coffee always determined contents more than five times higher of all elements. Over the broad differences between the species from the group of non-traditional plant stimulants, we can generally say, that in comparison with traditionally used species, the elements of the less known plants researched in this study are present in similar or higher amounts. Interestingly from the point of view of human consumption, if the elements from the group of commonly used plant stimulants are considered as rather positive (Mn, B), they are in the other group represented in similar or higher (up to very high) levels, whereas the rather negative elements (Al, Ni) are contained in lower levels.

It is well documented, that plant species commonly used to prepare stimulant beverages (tea and coffee) contain certain microand macroelements; however, their concentrations vary with many factors: the age of the plant when harvested, the genetics of the plant, soil conditions, rainfall, altitude, and, in case of infusions, preparation conditions (amount of material relative to water, infusion time and temperature, etc.; [Flaten, 2002\)](#page-5-0). Additionally, it has been observed that coffee and tea differ in contents of microand macroelements between their raw materials and infusions ([Jaganyi & Madlala, 2000; Street et al., 2006\)](#page-5-0). Our results are in agreement with these findings. Moreover, we detected differences between traditional and non-traditional (chamomile, honeybush, mate, rooibos) plant stimulants analysed in this study. The variances were found between the particular species as well as between their raw materials and infusions. The results confirmed relatively poor conversion of Fe and Ca, almost quantitative conversion of K, and the conversion varying between 30% and 50% for Cu, Mn, P, Zn in most of cases between raw materials and infusions as discussed also by [Gallaher, Gallaher, Marshall, and](#page-5-0) [Marshall \(2006\)](#page-5-0).

The elements contents were generally similar for all tea infusions and raw materials where the total contents fitted to the same order of magnitude as observed by other authors (e.g. [Kumar, Nair,](#page-5-0) [Reddy, & Garg, 2005\)](#page-5-0). Among tea infusions, significantly higher were the contents of Ni and Mn in decaffeinated black Japan Sencha tea and the amount of Al in both Assam teas (green and black). Our results are in agreement with the previous study of [Fernandez,](#page-5-0) [Pablos, Martin, and Gonzales \(2002\)](#page-5-0) in which no significant differences between infusions of green and black teas, except of manganese in black tea have been reported. The average Mn and Cu portions in released to the infusions represented 31% and 26% of total element content in raw material, respectively, and were comparable to results of [Mehra and Baker \(2007\)](#page-5-0). In contrast, average Al content in tea infusions, which representing 10% of total tea aluminium content was lower compared to the results of these authors (30%) even in the case of similar sources of tea samples (Assam, Darjeeling).

A considerable number of studies have reported the total concentration of Al in tea infusions for its toxicity. Because the plant is a natural aluminium accumulator, tea beverages are a major source of dietary Al exposure, and heavy tea drinking may more than double an individual's intake of Al [\(Flaten, 2002; Nagata,](#page-5-0) [Hayatsu, & Kosuge, 1993\)](#page-5-0). In correspondence with previously described wide ranges of Al content in tea infusions (0.7–3.5 mg l^{-1}) by [Wong, Zhang, Wong, and Lan \(1998\)](#page-5-0), we have detected a broad spectrum for this element ranging from 0.055 to 1.734 mg l^{-1} in our samples.

The Al contents in other ''non-tea" stimulants, analysed in this paper are without significant differences and are close to WHO's drinking water limit, which is set at 0.2 mg Al l^{-1} . The maximum normal daily intake of Al (5 mg day $^{-1}$; [WHO, 2004\)](#page-5-0) may not be exceeded by drinking these infusions.

By comparison of all species analysed in this study, the highest content of Al in raw material has not been observed for tea, but for coffee (Indonesian arabica, near to five times higher). Because of poor Al leaching from melted coffee beans into water, the levels in infusions reached only to half of the average tea content (ca 0.25 mg 1^{-1}). Actually the raw material of the coffee contained even more than 10 times higher amounts of Fe than tea but in the infusion we found similar results to those previously detected by [Oni](#page-5-0)[anwa, Adetola, Iwegbue, Ojo, and Tella \(1999\)](#page-5-0) that approached only to the average rates of iron in tea.

In contrast to Al and Fe contents, the amounts of Ca in raw materials of coffees were lower than those in teas (two times). Moreover, we have detected over 13.1 mg l^{-1} of Ca in the solutions of decaffeinated arabica, which were close to four times more than in tea samples. [Bundt, Kretschmar, Zech, and Wilcke \(1997\)](#page-5-0) observed enhanced uptake of Mn by coffee plants growing in Costa Rica as dependent on season and manganese mobility in soil. In our case, low levels of manganese and zinc were detected in all coffee samples analysed, suggesting the effect of different growing areas. In correspondence with results of [Martin, Pablos, and Gonzales](#page-5-0) [\(1999\),](#page-5-0) the significant differences between robusta's and arabica's elemental contents have not been detected during our study.

In comparison to the traditional species used for preparing an infusion, all mate samples analysed in our study were generally characterised by the high contents of B, Ca, Cu, Mg, Mn and Zn, whereas the obtained concentrations were greatest in mate green, followed by roasted mate and halved for yerba mate (mixture of leaves and twigs). The conversion of elements to the infusions was comparable to the results obtained by [Giulian et al., 2007.](#page-5-0) Interestingly, the contents of boron are up to seven times higher (green mate), than in tea or coffee. This level (1.55 mg l $^{-1}$) is much more than recommended daily allowance (RDA 2.0 μ g day $^{-1}$), but the maximal level is not stipulated. Since coffee and milk are considered as the two top contributors of boron in the human diet, representing up 12% of its total intake ([Rainey et al., 1999\)](#page-5-0), mate also seems to be, according to our results, important as a potential source of B in the human diet.

The level of Ca determined in green mate was 39.3 mg l⁻¹, which was the highest amount within all infusions and was 10 times that found in tea. In addition, in green mate as well as in chamomile the maximum levels of copper were detected. Our findings suggest that one litre of this beverage containing 0.340 mg may provide 10-20% RDA (1.5-3 mg day $^{-1}$) of copper [\(National Re](#page-5-0)[search Council, 1987\)](#page-5-0). Extremely high values of Mg and Mn in comparison to the commonly used plant species have been observed in beverages made from green and roasted mate.

Magnesium is in all species between 10 and 20 mg I^{-1} , with exception of coffee which contains up to 30 mg l^{-1} , and green mate which has an average level of 106.4 mg l $^{-1}$. The level of magnesium in green mate equates to approximately one-third to half of the magnesium RDA (210–320 mg day^{–1}; [National Research Council,](#page-5-0) [1987\)](#page-5-0). The manganese contents are very low in all non-tea samples except green and roasted forms of mate. According to the literature data, Mn is the only element with a significant dietary amount in tea, especially in black tea ([Powell, Burden, & Thompson, 1998;](#page-5-0) [Street et al., 2006\)](#page-5-0). However, the levels determined in raw tea material (samples 8, 9, 10, 11) were almost similar to mate (23, 26). The content of mate infusions were three times higher than tea infusions, suggesting that intake of one litre of mate with 15.5 mg Mn could provide the entire RDA of Mn $(2-5 \text{ mg day}^{-1})$; [National Research Council, 1987](#page-5-0)). Also average zinc contents in raw material of teas as well as in all types of mate are similar and higher than in all other species. As far as the content of Zn in all types of infusions is considered, green and yerba mate are two forms with its highest content (0.603 mg l^{-1}), whereas, surprisingly, one of the lowest amounts has been observed in roasted mate (0.124 mg l $^{-1}$).

Despite some promotional claims, made by the commercial distributors that beverages prepared from leaves of rooibos and honeybush are valuable sources of several nutrient elements, in the majority of samples analysed during our study we found lower amounts of minerals in comparison with the traditionally used plant stimulants. This suggested that the beneficial nutritional effect of the infusions made from both these species is disputable. We did not detect significant differences in element quantities contained in infusions between rooibos and honeybush species. In general, the infusions of red forms of these plant materials contained lower amounts of elements than green forms containing higher or same amounts of all 11 and eight (except Fe, Al, K) elements determined in our analyses, respectively. Calcium content in infusions of both green and red forms was three or four times higher than in the raw materials, reaching only half of the amounts in tea. Additionally, Ca levels in both rooibos and honeybush green forms were significantly higher in comparison to tea or coffee levels. The similar results were also found in the case of iron, with the slight exception of red rooibos characterised by 0.165 mg l^{-1} .

For chamomile, total amounts of all elements analysed in this study were determined as the second highest after green mate. The unusually high content of Al in the raw material, which is more than five times higher than in tea, is not reflected in the final infusion (1.5% of the total content). In this case, possible secondary contamination of the sample during harvest and/or technological processes must be taken into account, and high heterogeneity of Al content in the sample as well as low conversion of this element to the infusion seem to support this theory. However, more samples will have to be analysed in further research to verify it. The Al contents determined by [Ba](#page-5-0)şgel and Erdemoğlu (2006) in chamomile (356 mg kg^{-1}) indicated relatively high ability of chamomile plant to accumulate this element with its low conversion to the infusion as compared to other medicinal plants such as Tilia vulgaris, Foeniculum vulgare, Rosa caninae, or Salvia officinalis. In comparison to commonly used plants for preparing stimulant infusions, high levels have also been measured in the cases of boron, calcium and phosphorus (five, eight and two times more than tea, respectively). Whereas, the content of microelements in the raw material is similar as in other samples, the chamomile infusion is the most abundant source of copper $(0.348 \text{ mg l}^{-1})$ that represents about 20% of RDA $(1.5-3 \text{ mg day}^{-1})$. In comparison with other materials analysed, the level of Fe is relatively high in both the raw material and infusions; however, the contribution of chamomile to human iron intake seems to be quite poor due to its low percentage toward the RDA (less than 2%) and decreased absorption of non-haem Fe by the human body ([Breet, Kruger, Jer](#page-5-0)[ling, & Oosthuizen, 2005\)](#page-5-0). Only the level of Ni (0.04 mg l^{-1}) is lower in comparison to tea or coffee, and is near to the drinking water limit $(0.02 \text{ mg l}^{-1}/60 \text{ kg of human body weight})$ set by [WHO](#page-5-0) [\(2004\).](#page-5-0)

4. Conclusion

In summary, our results show that in contrast to both traditional plant stimulants (coffee and tea), the contents of certain selected micro and macroelements in less commonly used species (chamomile, honeybush, mate, rooibos) are significantly different. The great amounts of B, Ca, Cu, Mn, Mg and Zn were determined in mate (mainly green type) and of B, Ca, Cu, Fe and P in chamomile; whereas, the analysed volumes of Al and Ni in rooibos and honeybush infusions were significantly lower than in tea or coffee.

In the case of traditional plant stimulants, our results demonstrate that the levels of elements analysed in this study did not significantly differ by tea type (black, green, oolong, white). Similarly, we have not detected variations between Arabica and Robusta coffee; however, in comparison with tea, coffee is a poor source of elements with exception of Ca and Fe.

Overall, the study also shows that the infusions of some plant materials of non-traditional species used to make stimulant beverages could be a valuable source of nutrient elements in the human diet. The results suggest that chamomile and mate could be an interesting source of B, Ca (mate and chamomile) and Mg, Mn (mate), because their contents approximate to the respective RDAs.

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