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ANALYTICAL METHODOLOGY

Content of trace elements and chromium speciation in Neem powder and tea infusions

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ABSTRACT

Total concentrations of selected trace elements in Neem powder and in Neem tea were determined by inductively coupled plasma mass spectrometry (ICP-MS). The data revealed that despite high total concentrations of the potentially toxic elements Al and Ni in Neem powder, their amounts dissolved in Neem tea were low. Total concentrations of the other toxic elements Pb, As and Cd were also very low and do not represent a health hazard. In contrast, total concentrations of the essential elements Fe, Cu, Zn, Se Mo and Cr in Neem powder were high and also considerable in Neem tea. Consuming one cup of Neem tea (2 g per 200 mL of water) covers the recommended daily intakes for Cr and Se and represents an important source of Mo and Cu.

Speciation analysis of Cr by high performance liquid chromatography (HPLC) coupled to ICP-MS with the use of enriched Cr isotopic tracers to follow species interconversions during the analytical procedure demonstrated that toxic Cr(VI) was not present either in Neem powder or in Neem tea. Its concentrations were below the limits of detection of the HPLC–ICP-MS procedure applied. The speciation analysis data confirmed that even Cr(VI) was added, it was rapidly reduced by the presence of antioxidants in Neem leaves. By the use of enriched Cr isotopic spike solutions it was also demonstrated that for obtaining reliable analytical data it is essential to apply the extraction procedures which prevent Cr species interconversions, or to correct for species transformation.

Introduction

Neem (*Azadirachta indica* A. Juss) is an evergreen tree in the mahogany family Meliaceae. The leaves, fruit and bark of thee Neem tree, which all possess beneficial medicinal properties, have been used traditionally in Ayurvedic medicine in India. More than 135 chemical compounds have been isolated, but only a few have been tested for their medicinal properties [1]. Extracts from different parts of the Neem plant were found to have antifungal [2], acaricidal [3], antibacterial, antisecretorial, antihaemorrhagic [4], antiviral [5], larvicidal [6], insecticidal and antifeedant [7] activities. Due to its beneficial properties, Neem is widely used in the personal hygiene and cosmetic industry, and in insecticide and pesticide production. Moreover, it is plant useful in combating deforestation and desertification [8].

Since some trace elements are essential for normal functioning of the human organism and several have health promoting properties, Neem extracts have been subjected to numerous trace element analyses, most commonly by atomic absorption spectroscopy [9–11] and neutron activation analysis [12–14]. Among the essential and toxic elements Zn, Cu, Fe, Co, Ni, Al and Cr were frequently determined in different Neem extracts.

Oxidation state is the most important factor determining the toxicity of Cr and its essentiality towards living organisms. In general, trivalent Cr (Cr(III)) is considered to be essential, while hexavalent Cr (Cr(VI)) is toxic [15,16]. In several studies, the content of Cr has been determined in digested Neem leaf samples [9,11] and in aqueous extracts of Neem leaves [11]. Determination of total Cr concentrations in Neem leaves provides data which is important for estimating its contribution to daily intake, but to evaluate the biological effects of Cr on humans, or its potential toxicity, speciation analysis is mandatory.

Regarding Cr(VI), consumption of foodstuffs is considered to be safe, since Cr(VI) is rapidly reduced in these matrices by organic matter [15]. Despite this well-known fact, several articles have been published reporting the presence of Cr(VI) in plants [17] tea [18,19] and bread samples [20]. The authors used alkaline

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Table 1
ICP-MS operating parameters for determination of element concentrations.

Parameter	Type/value	He mode (4.5 mL He min^{-1})	No gas mode
Sample introduction			
Nebuliser	Miramist		
Spray chamber	Scott		
Skimmer and sampler	Ni		
Plasma conditions			
Forward power	1550 W		
Plasma gas flow	15.0 L min^{-1}		
Carrier gas flow		1.05 L min^{-1}	0.75 L min^{-1}
Dilution gas flow		0.10 L min^{-1}	0.45 L min^{-1}
QP bias		−15.0 V	−3.6 V
Oct bias		−18.0 V	−8.0 V
Cell entrance	−40.0 V		
Cell exit		−60 V	−50 V
Deflect		−2.2 V	13.4 V
Plate bias		−60 V	−40 V
Sample uptake rate	0.3 mL min^{-1}		
Data acquisition parameters			
Isotopes monitored		^{27}Al, ^{51}V, ^{52}Cr, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{95}Mo	^{111}Cd, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{201}Hg, ^{208}Pb
Isotopes of internal standards		^{45}Sc, ^{72}Ge, ^{103}Rh	^{45}Sc, ^{72}Ge, ^{103}Rh

extraction procedures similar to that reported by James and co-workers [21] for the extraction of Cr(VI) from solid samples, or hot water to prepare Neem tea infusions. The content of Cr in the extracts determined by electrothermal atomic absorption spectrometry (ETAAS) was ascribed to Cr(VI), without the use of any speciation analysis for the identification of Cr(VI) [17,18,20]. Chen et al. [19] performed speciation analysis of Cr in tea leaves infusions by separation of Cr(III) from Cr(VI) using a pre-concentration of positively charged Cr(III) species on the negatively charged surface of titanium dioxide nanotubes, while negatively charged Cr(VI) remained in solution. The fraction of Cr in the tea infusion which was not adsorbed on the nanotube sorbent was ascribed as Cr(VI). The authors did not consider that in tea infusion Cr(III) is complexed by available organic ligands, forming negatively charged and neutral complexes, which are not retained by the negatively charged nanotube surface. Assigning the Cr content that was not adsorbed as Cr(VI) may lead to erroneous interpretation of the data. To check whether data reporting the existence of Cr(VI) in tea infusions and bread was obtained as an artefact of inappropriate methodology, Novotnik and co-workers [22] repeated the experiments of Mandiwana et al. [18] and Soares et al. [20]. By applying speciation analysis using high performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP-MS) and enriched stable Cr isotopes to follow species interconversions during the extraction procedures, the authors proved that in the presence of antioxidants and organic matter, Cr(VI) cannot exist in tea infusions and bread samples. The importance of the use of adequate analytical tools in Cr speciation was emphasised also in a review article by Ščančar and Milačič [23].

Since Neem leaf powder is rich in essential trace elements and can also contain elevated Cr concentrations, the aim of the present work was to determine the total concentrations of selected trace elements in Neem leaf powder and tea infusions by ICP-MS. Further, in Neem tea infusions, speciation of Cr(VI) was performed by HPLC–ICP-MS using enriched stable isotopic tracer solutions of ^{50}Cr(VI) and ^{53}Cr(III) to follow species interconversions during the extraction procedures.

Experimental

Instrumentation

Total elemental concentrations were determined by an inductively coupled plasma mass spectrometer (ICP-MS), model 7700x from Agilent Technologies (Tokyo, Japan). ICP-MS operating parameters are presented in Table 1. For HPLC separations an Agilent (Tokyo, Japan) series 1200 quaternary pump equipped with a Rheodyne model 7725i (Cotati, CA, USA) sample injection valve fitted with a 0.05 mL injection loop was used. An anion-exchange FPLC column of Mono Q 5/5 GL (GE Healthcare, Bio-Sciences AB, Uppsala, Sweden) (column dimensions 5 mm × 50 mm, matrix polystyrene/divenyl benzene, pH stability 2–12, particle size 10 μm) was applied for separation of Cr species. After the chromatographic separation, Cr was detected by ICP-MS. The outlet of the chromatographic column was directly connected to the nebuliser of the ICP-MS instrument. ICP-MS operating conditions for Cr speciation are presented in Table 2. Data were treated with Agilent MassHunter software. Data processing was based on peak area. Experimental working conditions for ICP-MS were optimised for plasma robustness and sensitivity using a High Matrix Introduction (HMI) system. To eliminate the polyatomic interferences of chlorine and carbon on m/z 52 and 53, the High Energy Collision Mode (HECM) was applied [24].

Table 2
ICP-MS operating parameters for chromium speciation.

Parameter	Type/value
Sample introduction	
Nebuliser	Miramist
Spray chamber	Scott
Skimmer and sampler	Ni
Plasma conditions	
Forward power	1550 W
Plasma gas flow	15.0 L min^{-1}
Carrier gas flow	0.64 L min^{-1}
Dilution gas flow	0.53 L min^{-1}
Total carrier gas flow	1.17 L min^{-1}
HECM	10 mL He min^{-1}
QP bias	−97 V
Oct bias	−100 V
Cell entrance	−130 V
Cell exit	−150 V
Deflect	−80 V
Plate bias	−150 V
Sample uptake rate	1.5 mL min^{-1}
Data acquisition parameters	
Isotopes monitored in Cr speciation	^{50}Cr, ^{52}Cr,^{53}Cr
Isotopes of internal standards	^{45}Sc, ^{72}Ge
Total acquisition time	599 s