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Copper complexing properties and physico-chemical characterisation of the organic matter in Greek herbal infusions

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ABSTRACT

Complex formation is among the mechanisms affecting metals' bioaccessibility. Evaluating the extent of interactions between trace elements and several constituents of food items is of great interest. This paper examines the release of copper-complexing ligands in herbal infusions of 13 aromatic plants commonly used in Greece. The concentration of ligands (L_T) and the copper-binding strength ($logK_{app}$) of herbal infusions were determined with Differential Pulse Anodic Stripping Voltammetry (DPASV).

All herbal infusions were found to release Cu complexing ligands, at concentrations ranging from 8.8 to 112.5 μM in rosemary and marjoram, respectively. In all infusions the total copper concentrations were lower than the corresponding L_T values, indicating that Cu is fully complexed.

Aiming to partially characterise the physico-chemical properties of the released organic material, the surface active substances (SAS), reduced sulphur species (RS) and catalytically active compounds (CAC) were measured, for the first time, in herbal infusions by sensitive electrochemical techniques.

1. Introduction

Herbs are widely used for pharmaceutical and culinary purposes and are consumed in the form of infusions worldwide, due to their flavour, mild features and low side effects. Herbal infusions are usually prepared by soaking the dried plants into hot water, resulting in the extraction of various classes of organic compounds, including carbohydrates, proteins, amino acids, aroma-forming substances, vitamins, volatile oils and several phytochemicals. In addition, brewing results in the differential extraction of certain amounts of trace elements, making the prepared beverage a source of major, minor and trace elements, including metals, which could have certain health implications (Jin et al., 2005; Mehra & Baker, 2007).

The amount of metals which can actually be retained by the human body from the consumption of herbal infusions is primarily related to the metal content of herb leaves, to the fraction of metal content eluted to the infusion and to the bioavailability of metal species present in the infusion (Wrobel, Wrobel, & Urbina, 2000).

Metal chelation comprises, together with free radical scavening and reducing capacity, the modes of antioxidant action exerted by plant phenolics (Mira et al., 2002; Rice-Evans, Miller, & Paganga, 1996). Redox active metals like Fe, Cu, Cr, Co can undergo redox cycling reactions in biological systems and produce reactive oxygen species (ROS) or reactive nitrogen species (RNS), the most representative examples of which are the superoxide anion radical and nitric oxide, respectively. Recently, another group of active species with stressor properties similar to the ones found in ROS, namely reactive sulphur species (RSS), has emerged in the literature (Giles, Tasker, & Jacob, 2001; Gruhlke & Slusarenko, 2012). RSS include thiyl radicals, disulphides, sulfenic acids and disulphide S-oxides (Giles & Jacob, 2005). The accumulation of ROS, RNS and RSS is linked to oxidative stress, which results to chronic inflammation; the latter seems to be implicated in the pathogenesis of several degenerative diseases involving cancer, diabetes, cardiovascular diseases, atherosclerosis and neurodegenerative diseases (Jomova & Valko, 2011; Chassaing et al., 2013). Chelation of redox-active metals maintains them in a stable oxidation state and prevents their participation in redox reactions, thus avoiding the subsequent oxidative damage (Jomova, Baros, & Valko, 2012). Recently Hyung et al. (2013) reported that green tea flavonoids interfere in the formation of Cu, Fe and Zn containing metalloproteins, which are

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related to Alzheimer disease. Additionally, stimulation of phenolic metabolism in response to the toxicity provoked by several metals was found in the cases of chamomile (*Matricaria chamomilla* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (Diaz, Bernal, Pomar, & Merino, 2001; Kovacik & Backor, 2007; Winkel-Shirley, 2002).

Since aromatic plants contain significant amounts of phytochemicals, several classes of which are known to exert metal chelating capacities, their simultaneous consumption with other food items is expected to affect the overall bioavailability of redox-active and toxic metals. Indeed, recent research revealed that the co-consumption of phytochemicals-rich foods like tea, coffee or fruits, may reduce the bioaccessibility of toxic metals from fish and seafood (He & Wang, 2011; Ouédraogo & Amyot, 2011; Passos et al., 2007). Since such studies are limited, the study of metal chelating capacity of herbal infusions is of interest.

In the present study we investigated the release of copper-complexing ligands in the infusions of 13 aromatic plants widely consumed by Greeks. Specifically, the apparent complexing capacity of Cu (L_T) and the apparent conditional stability constant ($logK_{app}$) were determined in herbal infusions with Differential Pulse Anodic Stripping Voltammetry (DPASV). The partial physico-chemical characterisation of the organic material released in the infusions, regarding its functional groups and type of compounds, was performed with recently developed chronopotentiometric stripping analysis with constant current (Strmečki, Plavšić, & Ćosović, 2010; Strmečki & Plavšić, 2012). Surface active substances (SAS) were measured by an electrochemical technique as well. The potential correlation of total polyphenols, classes of simple polyphenols, antioxidant capacity and terpenic acids with Cu complexation was also examined.

2. Materials and methods

2.1. Plant material

The herb species studied were: chamomile, Cretan dittany, Cretan marjoram, lemon balm, marjoram, mountain tea, oregano, pennyroyal, pink savory, rosemary, sage, St John's wort and thyme (Table 1). The majority of herbs originated from the island of Crete (southern Aegean Sea), St. John's wort was purchased from Ano Poroia (central Macedonia, northern Greece) and lemon balm was obtained from an organic farm in Aitoloakarnania (western Greece). All samples were provided dried, wrapped in paper-cellophane bags and were stored in a cool, dark place.

Table 1
Herb species the infusions of which were tested.

	Common name	Species	Family	Distribution
1	Chamomile	*Matricaria chamomilla* L.	Asteraceae	Europe, temperate Asia
2	Cretan dittany	*Origanum dictamnus* L.	Lamiaceae	Endemic, Crete island
3	Cretan marjoram	*Origanum microphyllum* (Benth.) Vogel	Lamiaceae	Endemic, Crete island
4	Lemon balm	*Melissa officinalis* L.	Lamiaceae	Southern Europe, worldwide
5	Marjoram	*Origanum majorana* L.	Lamiaceae	Indigenous, Mediterranean
6	Mountain tea	*Sideritis syriaca* L.	Lamiaceae	Mediterranean, Central Europe
7	Oregano	*Origanum vulgare* ssp. hirtus L.	Lamiaceae	Mediterranean and SW Eurasia
8	Pennyroyal	*Mentha pulegium* L.	Lamiaceae	Europe
9	Pink savory	*Satureja thymbra* L.	Lamiaceae	Native, Mediterranean
10	Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Native, Mediterranean
11	Sage	*Salvia officinalis* L.	Lamiaceae	Native, Mediterranean
12	St. John's wort	*Hypericum perforatum* L.	Clusiaceae	Worldwide
13	Thyme	*Thymus vulgaris* L.	Lamiaceae	South Europe

2.2. Preparation of infusions

Infusions were prepared by infusing 3 g of each dried herb into 200 mL of boiling water (equivalent to a tea cup) on a preheated hot plate for 3 min. To conform with the traditional practice, only the leaves and flowers of the herbs were infused, while in the case of mountain tea all the aerial parts were used. Infusions were left to cool to room temperature for 10 min and were filtered through filter paper. Next, infusions were dehydrated by lyophilisation (Heto Lyolab 3000, Heto-Holten, Allerod, Denmark) and the dry residues were weighed and stored at −40 °C for further analysis.

2.3. Electrochemical measurements

2.3.1. Sample preparation and equipment

For the determination of [L_T], solutions of 150 mg L^{-1} of aqueous herbal extracts were prepared by dissolving 15 mg of lyophilised infusion in 100 mL of Milli-Q water 18.2 MΩ cm (Millipore, Bedford, MA, USA). Following the addition of 10 drops of 3 M NaCl, samples were immediately subjected to copper complexing capacity (L_T), apparent stability constant (K_{app}) and organic carbon (OC) determinations.

For surface active substances (SAS) and reduced sulphur species (RS) measurements solutions of 10 mg L^{-1} of the aqueous herbal extracts were prepared by dissolution of lyophilised infusions in Milli-Q water. For the measurements of catalytically active compounds (CAC) 10 mg L^{-1} concentrations of each extract were prepared in 0.55 M NaCl (with the addition of acetate buffer for pH 5.1 and organic free (UV-irradiated) seawater for pH 8.2). Samples were measured immediately after preparation.

Electrochemical measurements were carried out using a μAutolab type III (Eco-Chemie, The Netherlands) instrument connected to a three electrode cell (663 VA Stand, Metrohm, Switzerland) with a static mercury drop electrode (SMDE) as the working electrode. The reference electrode was an Ag/AgCl (3 M KCl). A carbon rod electrode served as the auxiliary electrode.

2.3.2. Copper complexing capacity (L_T)

Differential Pulse Anodic Stripping Voltammetry (DPASV) was used for complexing capacity determinations (Plavšić, Krznarić, & Branica, 1982). The values of complexing capacity as well as of corresponding stability constants (K_{app}) were calculated by applying the linear transformation plot, through linearly transforming titration data assuming 1:1 metal to ligand complexation (Ružić, 1982; van den Berg, 1982). The equation used for calculation is: [Cu]/[CuL] = [Cu]/L_T + 1/K L_T, where Cu is the copper ion detected by anodic stripping voltammetry, CuL is the copper ion bound in a complex with ligand L, L_T is the total concentration of binding ligands (complexing capacity) and K is the apparent stability constant. The plot of [Cu]/[L_T] vs. [Cu] yields a straight line with a slope of 1/L_T and an intercept of 1/KL_T. The repeatability calculated (n = 5) was lower than 10%.

2.3.3. Surface active substances (SAS), reduced sulphur species (RS)

SAS were determined by phase selective alternating current voltammetry (PSACV) (Ćosović, 1985). The deposition potential of −0.6 V vs. Ag/AgCl Ref. electrode and deposition time of 60 s controlled by stirring were applied. Surfactant activity was expressed