Journal of Food Composition and Analysis 40 (2015) 70–77

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

# Determination of antioxidant activity, rutin, quercetin, phenolic acids and trace elements in tea infusions: Influence of citric acid addition on extraction of metals

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ARTICLE INFO

*Article history:*
Received 28 May 2014
Received in revised form 10 December 2014
Accepted 17 December 2014
Available online 12 February 2015

*Keywords:*
Antioxidant activity
Tea
Red Lapacho
HPLC/MS/MS
HR-CS GFAAS
Trace elements
Rutin
Phenolic acids
Food analysis
Food composition

ABSTRACT

Antioxidant activity of tea, content of bioactive compounds (rutin, quercetin, phenolic acids including: gallic, chlorogenic, protocatechuic, *p*-coumaric, caffeic, ferrulic, syringic and sinapic as well as other selected organic acids) and trace elements (Mn, selected as a metal playing a role in oxidative metabolism; Al; Cd; and Pb – the so-called heavy or noxious metals) were studied as these parameters greatly influence the quality of tea infusions. A number of different samples were tested including white, green, black and lemon-flavored teas, as well as the Red Lapacho tea. Antioxidant activity was measured using DPPH and ABTS tests, and a strong correlation between obtained results was observed. Infusions made from lemon teas packed in bags showed increased levels of rutin, quercetin and phenolic acids. Nevertheless, the positive effect of the increased level of phenolic compounds extracted is also connected with higher level of extracted noxious elements. Citric acid added to tea and herbal Red Lapacho infusions significantly increased Al, Cd, and Pb trace elements concentrations. Nevertheless, the infusions prepared from the lemon tea bags contained even the 10–70-fold higher content of these elements. On the other hand, addition of citric acid to tea infusions increased amount of extracted Mn only in black teas and Red Lapacho.



## 1. Introduction

Tea (*Camellia sinensis* L., Family: *Theaceae*) is a common beverage that is important source of bioactive compounds which influence human health, especially acts as antioxidant in cancer diseases, cardiovascular diseases or in diabetes (Sharangi, 2009). Tea leave production is widespread in over 36 countries which export tea to consumers throughout the world.

There are six main categories of tea: white, green, oolong, black, compressed and flavored teas differing in the quality and quantity of compounds responsible for the unique aroma, taste and bioactive functions (The United Kingdom Tea Council Ltd, 2014: Vinson et al., 2004). Especially green tea leaves and its infusions contain numerous bioactive compounds, among which catechins or flavan-3-ols are the most thoroughly investigated. Primary compounds belonging to these groups are: catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (Friedman et al., 2005; Horzic et al., 2009; Naldi et al., 2014; Poon, 1998; Rusak et al., 2008; Zimmermann and Gleichenhagen, 2011). Black tea leaves and infusions include flavonoids as oligomeric theaflavins and thearubigins formed in the oxidation process (converted from monomeric catechins or flavan-3-ols). White tea is produced from unopened buds (categorized as silver needle) or from buds and immature leaves. All types of tea infusions also contain glycosides of flavonols i.e. quercetin, such as rutin and other quercetin derivatives, phenolic acids and organic acids (Lin et al., 1996, 1998, 2008; Ding et al., 1997; Horie et al., 1998). The composition of tea also varies with variety, season, age of leaves, climate, and horticultural practices (Kim et al., 2011; Lin et al., 2003).

Red Lapacho herbal tea made from the bark of plant *Tabebuia impetiginosa* contains such bioactive compounds as: iridoid glycosides, lignan glycosides, isocoumarin glycosides, phenylethanoid glycosides and phenolic glycosides; aroma compounds such as lapachol and beta-lapachone (Steinert et al., 1996; Warashina et al., 2004).

Nevertheless, not only the phenolic compounds are responsible for total antioxidant activity of tea infusions but also some trace elements. Tea is a rich source of Mn, which is a part of endogenic

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http://dx.doi.org/10.1016/j.jfca.2014.12.015
0889-1575/

antioxidant in the fight with reactive oxygen species (ROS) and then oxidative stress in organism (Szymczycha-Madeja et al., 2012; Yanai et al., 2008). Antioxidant activity is defined "as an inhibition of the oxidation of lipids, proteins, DNA or other molecules that occurs by blocking the propagation step in oxidative chain reactions" and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction (Huang et al., 2005).

Drinking several cups of tea daily is a tradition of Chinese people and is also popular in northern Europe countries. Therefore for consumers' health it is very important to know the quantity of harmful contaminants in tea infusions. Some of metals, especially Cd and Pb, are toxic and accumulate in the tea bush and also during tea processing and storage. These elements, especially Cd, are potentially carcinogenic, teratogenic or even immunotoxic. Aluminum is also accumulated in the tea bush. This element is associated with neurological dysfunction such as Alzheimer's disease due to its bioavailability (Yokel and Florence, 2008). Latest research showed that Al content of ferritin seems to be related to different disease stages of Alzheimer's disease (De Sole et al., 2013). Phenolic substances form complex with Al to promote the absorption of Al and its transporting to each part of the tea. The Al in tea plant is positively correlated with catechins and total phenolics content (Chen et al., 2011).

Therefore it is interesting to analyze these compounds and elements in three different types of tea (white, green and black) available on the market. The aims of the study were: (1) measurement of the antioxidant activity using ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays, (2) determination of the content of total phenolics, two most important flavonols in a diet: rutin and quercetin and the most common phenolic acids and organic acids in white, green and black tea infusions to compare them with Red Lapacho herbal infusion, (3) determination of trace elements (Al, Mn, Cd, and Pb) in tea leaves and in tea infusions with or without addition of citric acid. Possible correlations between content of particular compounds and antioxidant activity are also in the scope of this study.

## 2. Materials and methods

### 2.1. Chemicals and gases

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-hydroxyethyl cellulose (HEC), β-alanine, malic acid, gallic acid chlorogenic acid, succinic acid, quercetin, caffeic acid, *p*-coumaric acid, protocatechuic acid, salicylic acid, ferulic acid, syringic acid, sinapic acid, tartaric acid, quinic acid, glutamic acid, aspartic acid and Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). MS-grade acetonitrile and methanol were from POCH (Gliwice, Poland) and MS-grade formic acid was from Sigma–Aldrich. Citric acid and hydrochloric acid were also purchased from POCH. Acetic acid and chemical modifier solutions: magnesium modifier stock solution, $10.0 \pm 0.2\ g\ L^{-1}$ $Mg(NO_3)_2$; palladium/magnesium modifier stock solution, $10.0 \pm 0.2\ g\ L^{-1}$ Pd + $Mg(NO_3)_2$; and phosphate modifier stock solution, $10.0 \pm 0.2\ g\ L^{-1}$ $NH_4H_2PO_4$ were purchased from Merck (Darmstadt, Germany). Compressed high-purity argon obtained from Air Products (Warsaw, Poland) was used as a carrier gas.

Standard solutions of Al, Cd, Mn, and Pb were prepared from a 1000 mg $L^{-1}$ atomic absorption standard solutions (Merck, Darmstadt, Germany). Mineral acids (65% (v/v) $HNO_3$ and 40% (v/v) HF) and hydrogen peroxide 30% (v/v) of the highest quality (Suprapur, Merck, Darmstadt, Germany) were used. High-purity water – deionized (DEMIWA 5 ROSA, Watek, Czech Republic) and doubly distilled (quartz apparatus, Bi18, Heraeus, Hanau, Germany) was used throughout the research. The resistivity of the water was 18 MΩ cm.

### 2.2. Material and extraction process

Fourteen teas (*C. sinensis*) including one herbal tea: two white teas (loose tea), three green teas (loose tea), three black teas (loose tea), three lemon green teas (bag tea), two black lemon teas (bag tea) and Red Lapacho herbal tea (bark) were purchased from a local market. 2 g of leaves/piece of bark was extracted by 100 mL of distilled water (94 °C) for 15 min. This extraction process was previously optimized (Jeszka-Skowron and Zgoła-Grześkowiak, 2014). Then the solution was decanted, cooled to room temperature and filtered through 0.45 μm polytetrafluoroethylene syringe filter from Agilent Technologies (Santa Clara, CA, USA) and finally diluted to proper volume with distilled water. The tea solution was prepared directly before analysis.

To prepare infusion with citric acid (as a substitute of lemon juice), 2 g of leaves/piece of bark was extracted by 100 mL of distilled water (94 °C). After 7.5 min 1 mL of citric acid solution ($0.066\ g\ mL^{-1}$) was added and extraction was continued up to 15 min. The amount of citric acid to be added was established previously on a basis of an average quantity of citric acid content in lemons which was determined in a sample of five lemons purchased from a local market (isotachophoretic separation).

### 2.3. ABTS scavenging activity

Total antioxidant capacity was determined using ABTS radical cation as described previously by Re et al. (1999). Antioxidant activity was calculated as a percentage of ABTS change in absorbance. The absorbance of samples was measured at 730 nm using Beckman UV-VIS Spectrophotometer 7500DU (Brea, CA, USA). Antioxidant activity was expressed as percentage ABTS scavenging relative to control using the following equation:

$$\text{ABTS scavenging activity}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### 2.4. DPPH scavenging activity

The ability of tea infusions to scavenge DPPH radicals was determined according to the method of Blois with a slight modification (Blois, 1958). Briefly, 1.0 mL of a 0.5 mM methanolic solution of DPPH was mixed with 3 mL of extract diluted in methanol. The mixture was then mixed and left for 30 min at room temperature in the dark. The absorbance of samples was measured at 516 nm using Beckman UV-VIS Spectrophotometer 7500DU (Brea, CA, USA). Antioxidant activity was expressed as percentage DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### 2.5. Total phenolic content

Total phenolic content in infusion of leaves/bark was analyzed using Folin–Ciocalteu's reagent (Singleton and Rossi, 1965). Gallic acid as an external standard was used ($r^2 = 0.999$). The absorbance of samples was measured at 754 nm using Beckman UV-VIS Spectrophotometer 7500DU (Brea, CA, USA). The results were