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Spectrophotometric determination of total iron content in black tea

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Abstract: The aim of this work was the assessment of total iron (Fe) content in some black tea brands using mineral digestion and spectrophotometric method. Four samples of black tea from different manufactures in three parallels were prepared by digestion and oxidation with a mixture of sulphuric and nitric acid. The total Fe content in analyzed black tea varies from 21.3 mgFe/kg to 37.6 mg Fe/kg. The used spectrophotometric method is simple and sensitive method that can be applied for the determination of total Fe content in plant material.

INTRODUCTION

Tea is one of the most popular beverages consumed worldwide. Tea infusion is made from the processed leaves of the plants *Camellia sinensis L., familia Thecea* and the three most popular types of tea (green, oolong, and black) are distinguished on the basis of degree of fermentation. The leaves of green tea are dried and roasted but not fermented, whereas black tea leaves are additionally fermented. The chemical composition of tea leaves consists of tanninic substances, flavonols, alkaloids, proteins and amino-acids, enzymes, aroma-forming substances, vitamins, minerals, and trace elements (Jha et al**.,** 1996).

Among the minerals and essential trace elements that are essential to human health, Ca, Na, K, Mg, and Mn are present in tea leaves at g/kg level, while Cr, Fe, Co, Ni, Cu, Zn are present at mg/kg level (Cao et al., 1998; Fernandez-Caceresetal., 2001). Plants obtain these trace elements from growth media such as nutrient solutions and soils. The extent to which they take up metals depends on the extent to which trace elements are bound to soil constituents and the other sources include pesticides and fertilizers.

Habitual drinking of tea infusions may significantly contribute to daily dietary requirements for specific elements. The total contents of metals in tea leaves differ according to the type of tea (green or black) and are probably influenced by many other factors, e.g. soil properties. Iron, one of the most abundant metals on Earth, is essential to most life forms, and to normal human physiology (Dobrinas et al., 2011; Jha et al., 1996; Mose et al., 2014). Iron is an integral part of many proteins and enzymes that maintain good health. In human metabolism, iron is an essential component of proteins involved in oxygen transport. It is also essential for the regulation of cell growth and differentiation. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity. On the other hand, excess amounts of iron can result in toxicity and even death (Moroydor Derun et al., 2012). Many studies have concluded that tea has numerous beneficial effects on health, including the prevention of many diseases such as skin cancer,

diabetes, Parkinson's disease, myocardial infarction, and coronary artery disease (Schwalfenberg et al. 2013). The knowledge of both micronutrients and toxic elements content in beverages is important, taking into account nutrition requirement and intoxication risk related with their consumption (Afsana et al., 2004; Powell et al. 1998; Nelson M. and Poulter J., 2004; Temme E.H.M. and Van Hoydonck P.G.A., 2002).
The aim of this work was the assessment of total iron (Fe) content in some black tea brands using mineral digestion and spectrophotometric method.

EXPERIMENTAL

Biological material

Plant samples (black tea leaves) - Ceylon Tea (Turkey), Hazir Harman Çay (Turkey), Indian black tea (Franck Zagreb, Croatia) and Ceylon Tea (Emona Brand extra quality, Kosovë).
Four plant samples were prepared by wet digestion (open system) where weighed mass of 1.0000 g was heated with mixture of concentrated nitric acid and sulphuric acid for 4 hours at temperatures 110-130^{0}C. Solid phase separated by filtration (blue filtered paper), dissolved in mixture of nitric and hydrochloric acids (concentration of 0.05mol/L, 1:1 v/v), transferred in volumetric flask (100mL) and diluted to mark with same mixture of acids. Digestion of each samples was done in triplicate.

Spectrophotometric method

Spectrophotometric method was performed with a Genesys 2 UV- VIS Spectrometer, Model TM2. Solutions of prepared twenty samples were yellow to pale red color. Before spectrophotometric analysis, intensity of color was increased by addition of potassium thiocyanate (Sigma-Aldrich Co. LLC) for complexation of iron ions and formation of red complex with different composition from $[FeSCN(H_2O)_5]^{2+}$ to $[Fe(SCN)_6]^{3-}$ (Itodo et al., 2012; Paul E. Adams, 1995). Standard stock solutions of iron(III) ions was prepared by dissolving 20 mg of iron (III) chloride (Sigma-Aldrich Co. LLC) in 100 ml deionized water in a volumetric flask (100 ml). The calibration solutions were prepared by pipetting volumes of 0.05, 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 2.50 ml, respectively of the stock standard solution into volumetric flasks (10 ml). Next, of volumes of 1.00 ml of nitric acid and 1.20 ml of potassium thiocyanate (concentration of both solution was 5 M) were added to each volumetric flasks to obtain a concentration range from 1.40 to 69.81 µg/ml Fe. The absorbance of each solution (working and analyzed solutions) was measured at absorption maximum of 481.0 nm using 10 mm quartz cuvette (Figure 1).

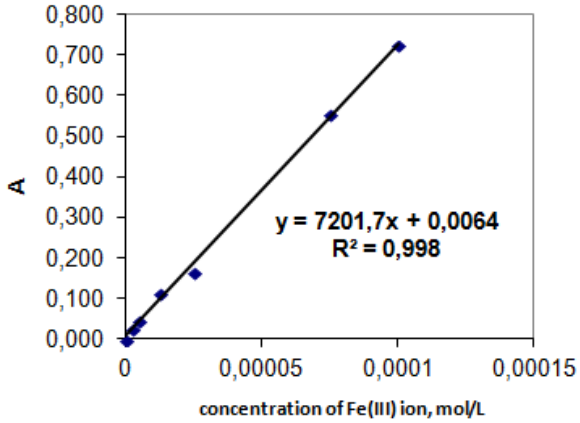

Figure 1. Standard calibration curve

RESULTS AND DISCUSSION

The total Fe content after mineral digestion determined by using an spectrophotometric method in different brands of black tea is shown in Table 1.

Table 1. Total content of iron in analyzed black teas (**mean±S.E.M**)

Sample	Black tea	Total Fe content (mg/kg)
I	Ceylon Tea	37.6±1,13
II	Hazir Harman Çay	21.3±0,88
III	Indian black tea	29.01±0,37
IV	Ceylon Tea-extra quality	27.4±0,98

The result shows that these four brands of black tea product contained Fe concentration ranging from 21.3 to 37.6 mg/kg with a mean value of 28.8 mg/kg. The lowest concentration (21.3 mg/kg) was observed in Hazir Harman Çay tea brand and the highest concentration (37.6mg/kg) was observed in Ceylon Tea brand.
A number and different instrumental techniques, such as induced coupled plasma-optical emission spectroscopy (ICP-OES), induced coupled plasma-mass spectrometry (ICP-MS), flame atomic absorption spectrophotometry (AAS), etc. (Moroydor Derun et al., 2012; Marbaniang et al., 2011;Achudume and Owoeye, 2010; Moseti et al., 2013; Gebretsadik and Chandravanshi, 2010),are used for determination of minerals and essential trace elements in plant and soil. The reason for such a number of methods is that no single method fulfills all the conditions, such as precision, accuracy, selectivity, speed, etc. In all methods the sample must be degraded by wet or dry digestion. In order to quantify the use of any of the above mentioned methods, iron must be converted into the appropriate form (to be oxidized to iron (III) or iron (II)). Conversion of iron in ferri and ferro ions and decomposition of the sample is carried out with a mixture of concentrated acids H_2SO_4/HNO_3. Problem with digestion is that, when sample is heated in such acidic conditions, Fe may evaporate in the form of its volatile compounds, so it is

very important to control heat to avoid evaporation of Fe (Moroydor Derun et al., 2012;Achudume and Owoeye, 2010; Street et al., 2006). Also, some quantity of iron precipitated and is lost during degradation and oxidation of biological material. Determination of level of iron in plant material is influenced by many factors.. The preparation method (time duration of digestion, temperature, etc.) has also a great influence (Marcus 1996). Tea polyphenols have a high affinity for metals and also for biological macromolecules. Many factors may be contributing to the metals accumulation in the tea leaves, such as soil composition, its organic matter contents, manufacturing process and environmental pollutions. It was confirmed that the content of metals might be an adequate discriminator of tea varieties and their geographical origin. The main source of trace elements in plants including tea is their growth media (pH soils), use of fertilizers (e.g. nitrogenous), insecticides and herbicides. Industrialization have also been shown to influence the element content in plants (Fernandez-Caceres et al., 2001; Dobrinas et al., 2011).

A previous report showed that the Fe concentrations in imported teas in Czech Republic varied from 0.037 to 0.142 mg/mL (Street et al., 2006), which are consistent with our results (0.021 mg/mL to 0.376 mg/mL). Our results indicated that Fe contents in our samples were lower than some black teas from the markets of India (Marbaniang et al., 2011) and Nigeria (Achudume and Owoeye, 2010), which were reported to have Fe contents from 0.439 mg/mL and 0.99 to 2.39 mg/mL, respectively. Also, MoroydorDerun and others (2012) found the highest Fe of 2.396±0.040 mg/L in black teas from the market from Turkey.

Many elements present in food at major, minor and trace level are reported to be essential to human's health. Human body requires both metallic and nonmetallic elements for healthy growth, development and the proper functioning of the body. The determination of these elements in beverages and plant is of the most importance and is currently the subject of studies by various researchers (Dobrinas et al., 2011; Mose et al., 2014). Analysis of content of the metals in tea is very important especially determination of their concentrations that need to be presented in properly recommended values. Tea has a recognized therapeutic value. It is important in the prevention and treatment of many diseases (Fernandez-Caceres et al. 2001). The study of trace elements in tea has been taken up as trace elements play an important role in the complex metabolic pathways in human system and their deficiency or excess may cause disease. Identification of metal containing components of tea is not simple due to the analytical difficulties associated with both the separation of such components and their quantitative measurement.

CONCLUSION

These data demonstrate the tea plant's ability to accumulate the studied element (iron) as nutrient, further underlining tea consumption as a potential dietary source of the nutritionally essential inorganic nutrients necessary for various biological processes. The used spectrophotometric method is simple and sensitive method that can be applied for the determination of total Fe content in plant material.

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Summary/Sažetak

Cilj ovog rada je procjena ukupnog sadržaja željeza (Fe) u nekim brendiranim crnim čajevima primjenom mineralne digestije i spektrofotometrijske metode. 4 uzorka crnog čaja različitih proizvođača i u 3 paralelke pripremljeni su digestijom i oksidacijom sa smjesom sumporne i nitratne kisleine. Sadržaj ukupnog željeza u uzorcima crnog čaja varirao je od 21 mg Fe/kg do 37,6mg Fe/kg. Primjenjena metoda spektrofotometrije je jednostavna i osjetljiva i može biti primjenjena za određivanje ukupnog sadržaja željeza u biljnom materijalu.