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Željka J. MILOVANOVIĆ[1], *Slađana S. POPOVIĆ*[1], *Ana S. PANTELIĆ*[2*], *Jelena R. MILINKOV*[3], *Dragana L. MILOŠEVIĆ*[1], *Vladimir M. PETROVIĆ*[1], *Milka M. VIDOVIĆ*[1]

[1] University of Belgrade, SI Institute of Chemistry, Technology and Metallurgy
Department of Ecology and Technoeconomics
Njegoševa 4, Belgrade 11001, Serbia
[2] University of Belgrade, SI Institute of Chemistry, Technology and Metallurgy
Department of Chemistry
Njegoševa 4, Belgrade 11001, Serbia
[3] Hemofarm A.D.
Beogradski put bb, Vršac 26300, Serbia

DETERMINATION OF INORGANIC ANIONS IN HERBAL TEA INFUSIONS USING ION CHROMATOGRAPHY

ABSTRACT: The ionic content was examined in nine aqueous tea extracts in which time of boiling, acidification of the medium using lemon juice and way of preparation were observed as factors. Ion chromatography was used for determination of inorganic anion content, and data were processed using CANOCO program for multivariate analysis. The variations in ionic content were observed among different tea samples. The highest concentrations of chloride, nitrate, phosphate, and sulphate ions were found in nettle, while the highest concentrations of fluorides were detected in elderflower tea infusion. The effect of boiling time (5, 10, and 20 min), acidification of the medium and different preparation procedure (boiling and cooling at room temperature) were statistically presented using principal component analysis. The examined factors did not have a significant effect on the ionic concentration in tea infusions.

KEYWORDS: herbal tea, inorganic anions, ion chromatography, principal component analysis, tea infusion

INTRODUCTION

Nowadays, traditional methods of treatment that replace synthetic pharmaceuticals are increasingly used. One of such traditional treatment methods includes phytotherapy, which involves the use of herbal teas, more precisely,

* Corresponding author. E-mail: annapantelic@gmail.com

herbal tea infusions (Pohl et al., 2016; Pytlakowska et al., 2012; Martín-Domingoa et al., 2017). Herbal tea infusion represents a combination of boiling water and different parts of a plant, such as leaves, flowers, berries, seeds, roots, separately or combined (Altintig et al., 2013; Emekli et al., 2009; Kara, 2009; Pohl et al., 2016). These infusions are often consumed as an alternative or non-conventional medicaments for their physical or medicinal effects, especially for their stimulant, relaxant or sedative properties, helping thus in the treatment of different diseases (Altintig et al., 2013; Atoui et al., 2005; Kara, 2009; Malinowska et al., 2008; Martín-Domingoa et al., 2017; Pohl et al., 2016; Pytlakowska et al., 2012). It is known that herbal teas can positively influence human health due to the presence of different useful compounds such as amino acids, polysaccharides, vitamins, antioxidants, minerals and ions (Das et al., 2017; Pohl et al., 2016). The concentrations of these compounds in plant depend on many factors like plant species, the origin of the plant, climatic conditions, the characteristics of the substratum on which certain plant grows, the capability of plant to accumulate certain substances, and industrial processes in tea production and preparation (Martín-Domingoa et al., 2017; Pohl et al., 2016; Pytlakowska et al., 2012). Inorganic anions frequently released into water solution during tea preparation include fluorides, chlorides, nitrates, sulphates, and phosphates. Michalski (2006) and Mincă et al. (2013) recorded that inorganic anions and carboxylic acid contribute to the acidity of the medium influencing the flavor and taste of tea itself. For most of them, important biological role, as well as beneficial effects on the function of the human organism has been recorded. Chlorides are the basic constituent ions of extracellular fluid responsible for the proper muscle functioning (Mincă et al., 2013). Phosphates are responsible for the growth and tissue regeneration, and also for proper cell metabolism functioning (Mincă et al., 2013), while sulphates are the main source of sulfur that is necessary for the synthesis of amino acids cysteine and methionine (Balcerzak and Janiszewska, 2015). Fluorides, regarded as the most prominent anions, are very beneficial in small concentrations, enabling proper bones and teeth functioning (Chan et al., 2013; Das et al., 2017; Giljanović et al., 2012). However, many substances that have the positive effect on human health can be harmful if used in higher concentrations than allowed: for example, fluorides in higher concentrations can cause dental and skeletal fluorosis (Emekli et al., 2009; Koblar et al., 2012; Chan et al., 2013; Das et al., 2017; Giljanović et al., 2012) and nitrates and nitrites paralysis of vasomotor center (Mincă et al., 2013).

The aim of this paper was to analyze the inorganic anions in herbal tea infusions commonly consumed in this geographical area using the ion chromatography. The effect of different extraction time at the same temperature, as well as the way of the tea preparation were also taken into consideration, as in Popović et al. (2017) where the influence of these factors on metals release was observed.

MATERIALS AND METHODS

Analytical procedure

The content of inorganic anions was determined by ion chromatography using ICS 2020i Dionex ion chromatographic system consisting of an isocratic eluent delivery pump with the flow of 0.8 mL/min, sample injection port, guard and separation column (AG22 and AS22 Dionix IonPac), suppressor (ASRS 300 Dionix), and conductivity detector. As an eluent, a mixture containing 3.2 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ was used. Calibration solutions of analyzed inorganic anions were prepared by diluting the stock standard solution (1000 mg/L fluorides (F^-), chlorides (Cl^-), nitrates (NO_3^-), phosphates (PO_4^{3-}), and sulphates (SO_4^{2-})) to the required concentration. Ultra-pure water with the electrical conductivity of <0.05 µS/cm was used during the experiment. The solution was injected into the chromatographic column after filtration through 0.45 µm membrane filter.

Sample preparation

As described in Popović et al. (2017), eight different commercial herbal tea samples and one green tea sample of the same brand were used for the analyses: hawthorn (*Crataegus monogyna* Jacq.), St John's wort (*Hypericum perforatum* L.), nettle (*Urtica dioica* L.), elderflower (*Sambucus nigra* L.), green tea (*Camellia sinensis* (L.) Kuntze), bearberry (*Arctostaphylos uva-ursi* (L.) Spreng.), thyme (*Thymus serpyllum* L.), yarrow (*Achillea millefolium* L.), and mint tea (*Mentha piperita* L.). Some of them were associated to "leaf" or "flower" teas depending on which part of the plant was mostly used for the preparation of tea when packed in tea bags. Bags were opened and content was mixed and homogenized prior to drying at 105 °C to constant weight. After homogenization of each sample, five different sets of aqueous extractions were prepared as described in Popović et al. (2017). Each set was prepared by weighing approximately 2 g of each tea sample and soaking it in 100 mL of boiling ultra-pure water: the first set was boiled for five minutes, the second for ten and the third for twenty minutes; for the fourth set, boiling water was added to the tea sample, then it was covered and left at room temperature for five minutes; the fifth set included the addition of lemon juice (5 mL), then it was covered and left for five minutes at room temperature. Conductivity and pH value were also measured in each tea sample water extract using WTW LF 191 conductivity meter and pH meter WTW inoLab pH 730. For each set of samples, a blank was prepared following the same procedure.

Limits of detection (LOD) and limits of quantification (LOQ) of the instrument were calculated as three times and ten times of the residual standard deviation of the low concentration standard (Table 1).

Table 1. Limit of detection and limit of quantification for all anions

Anion	F^-	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
LOD, mg/L	0.29	0.18	0.30	0.51	0.29
LOQ, mg/L	0.99	0.60	1.00	1.70	0.99

Data analysis

Statistical analysis was done using CANOCO for Windows, Version 5.0 (Ter Braak and Smilauer 2012). Two principal component analyses (PCA) were performed. The first PCA was used to evaluate the relationship between anion concentration released in water after boiling for 5, 10, and 20 min with following supplementary variables included: tea samples, time of boiling, part of the plant included in the making of the tea, pH, and conductivity. The second PCA was used to demonstrate the relationship between the same supplementary variables and anions released in water solution from samples that were boiled for 5 min, that were covered and left at room temperature for 5 min, and samples that were treated with lemon juice covered and left at room temperature for 5 min.

RESULTS AND DISCUSSION

The average contents of the determined anions in the examined tea samples are given in Table 2. The highest variation among different tea samples is observed in nitrate ion values. According to the results, in most tea samples phosphates had the highest concentrations, while the concentrations of other anions were significantly lower. The highest values of phosphates, as well as nitrates, were found in nettle and elder-flower water extracts.

Few data exist for nitrates, chlorides, phosphates, and sulphates. The analysis of these anions was done in few tea samples, mainly in green and mint tea. According to Ozcan et al. (2007) the accumulation of nitrates is possible in leaves, as well as in roots of vegetables, and the concentration itself depends on the plant type, fertilization form, and amount and harvest time, used together with organic fertilizer or industrial fertilizer. So, the concentration of nitrates can be very variable in different plants, but also in the same plant of different geographical origin. Michalski (2006) in his study reported higher

Table 2. The average concentrations of the determined anions in tea infusions

Type of tea	Concentration (mg/g)±SD				
	Fluorides	Chlorides	Nitrates	Phosphates	Sulphates
Hawthorn	2.13±0.08	0.20±0.02	0.66±0.06	3.83±0.10	1.61±0.07
Yarrow	0.39±0.02	2.56±0.01	0.36±0.08	3.12±0.05	1.51±0.03
St John's wort	0.79±0.03	0.99±0.04	1.04±0.06	3.58±0.01	1.38±0.02
Elderflower	2.37±0.10	0.40±0.07	7.34±0.03	6.60±0.04	2.56±0.04
Nettle	0.97±0.05	2.84±0.01	8.27±0.02	7.00±0.03	4.70±0.02
Thyme	0.51±0.04	1.93±0.05	1.29±0.07	3.01±0.07	2.01±0.02
Mint tea	1.49±0.02	1.52±0.01	1.25±0.08	4.58±0.02	2.97±0.08
Bearberry	0.97±0.09	0.16±0.20	0.22±0.07	1.86±0.05	0.73±0.08
Green tea	1.47±0.07	0.37±0.02	0.18±0.02	1.92±0.04	1.28±0.03

nitrates content than in this research and lower content was reported in the study done by Mincă et al. (2013), Balcerzak et al. (2015), and Ozcan et al. (2007), with similar values of this anion for green tea. Considering chlorides, we obtained lower values in green tea than Mincă et al. (2013) and Balcerzak et al. (2015), and also in mint tea reported by Michalski (2006). For phosphates, Balcerzak et al. (2015) reported similar results for green tea, while the content of sulphates was slightly higher. The concentrations of phosphates and sulphates were higher for mint tea (Michalski 2006) and for green tea in study by Mincă et al. (2013). When speaking about fluorides, the data are much more diverse compared to other mentioned anions. Lower concentration of fluorides than in this study was reported by Chan et al. (2013), Emekli et al. (2009), Karak and Bhagat (2010), Kjellevold et al. (2006), Michalski (2006), and Yuwono (2005). According to Das et al. (2017) the content of fluorides in some herbal teas was lower, but similar results for green tea were documented. Similar values of fluorides in tea infusions were also reported by Chan et al. (2013) (0–30 mg/L). On the other hand, Michalski (2006) and Mincă et al. (2013) reported higher values of this anion in their examined tea infusions. According to Malinowska et al. (2008) and Yi and Cao (2008) the content of fluorides can be used as a tool for the estimation of the tea quality. Furthermore, the content of fluorides in green tea, for example, depends on the age of plant burgeon, where young burgeons (with two leaves) release significantly lower concentration of fluorides, compared to older ones where the fluoride concentration can be 2–4 times higher, and according to Chan et al. (2013) even to 10 times higher. Fluoride concentration can also be dependent on the land type, for example, higher concentrations of fluorides in plants are observed when the plant is growing on acidified substratum (Emekli et al. 2009; Chan et al. 2013).

Figure 1 shows the relationship between ion concentrations in infusions, tea samples, and time of tea boiling. PCA explained a total of 81.2% of variations in our data (PCA axis 1 and PCA axis 2 together explained 73.7% of the variation). It can be seen that the highest content of nitrates and sulphates are in nettle and elderflower extracts, while chlorides have high concentrations in nettle, but also in thyme and yarrow. The highest concentrations of fluorides and phosphates were found in elderflower tea infusion. Supplementary variables that refer to 5, 10, and 20 min of boiling are placed near the center of the ordination diagram. As it was the case with extracted metals from the same tea samples (Popović et al. 2017), their position indicates that significant differences were not observed in the concentrations of ions from the tea samples with changes in boiling time. Nominal variables "flower" and "leaf" were also placed in the center of the ordination diagram, meaning that ions were equally extracted from these plant parts. Conductivity vector and pH are oriented toward the right side of the ordination diagram, and their highest values were recorded in nettle tea infusion.

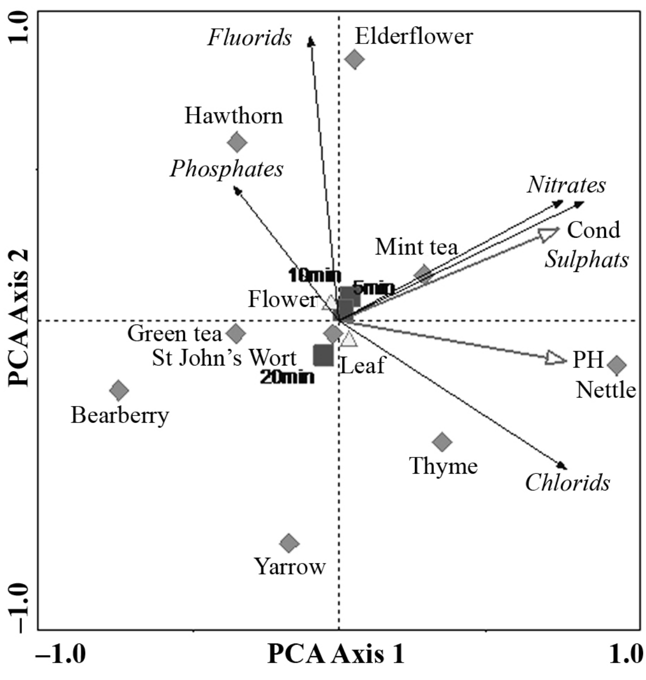

Figure 1. Principal component analysis (PCA) of ions from water extract after boiling for 5, 10, and 20 min. The supplementary variables include tea as blue diamonds (hawthorn, St John's wort, nettle, elderflower, green tea, bearberry, thyme, yarrow, and mint tea); red squares refer to boiling time (5, 10, and 20 min); and white triangles represent the part of the plant from which the tea is primarily produced (leaves, flowers)

Fluorides are the most examined ions when speaking about tea infusions and time as a factor. Giljanović et al. (2012) examined how brewing time affected fluoride concentration for tested tea samples, and concluded that in most cases fluoride concentration reached maximum in 10 to 20 min, which especially referred to mint tea. However, different tea samples acted completely different: the increase in fluoride concentrations with time was observed in mint and pomegranate samples, while in green tea a positive correlation was documented (Giljanović et al., 2012). Similar findings were given by Yuwono et al. (2005), where the increase was observed only in green and black tea samples. Emekli et al. (2009) demonstrated that there was no significant difference in fluoride content in different herbal teas if time of boiling was higher than five minutes. According to Chan et al. (2013) the highest concentration of anions was released after two minutes of boiling, and the prolonged time of boiling did not influence the changes in the concentration of anions – the concentration did not increase with time. On the other side, Chan et al. (2013), Karakand Bhagat (2010), and Malinowska et al. (2008) documented the increase in fluoride content with time of boiling. Giljanović et al. (2012) also recorded that the content of released fluorides in the tea water solution depended on the way of tea packing procedure, and also highlighted that the extraction was better from teas that consisted of smaller parts of plants, than from those consisting of larger parts. Kjellevold et al. (2006) pointed out that in some cases tea plant acted as an adsorption agent when concentrations of fluorides were higher in tea infusions, and emphasized the fact that the concentration of total fluorides in tea leaf did not affect its adsorption capacity. In this study, higher concentrations of fluorides were obtained when compared to a large number of papers dealing with this topic. It is possible that in this case some form of balance between the release and absorption exists, which is responsible for similar results obtained after 5, 10, and 20 min of tea boiling.

Since no major differences were observed after 5, 10, and 20 min of tea boiling, we decided to analyze samples obtained after 5 minutes of tea preparation (tea samples boiled for five minutes, tea samples in which boiling water was added, then covered and left at room temperature for five minutes; tea samples in which lemon juice (5 mL) was added, after which they were covered and left for five minutes at room temperature). According to Das et al. (2017) and Yuwono et al. (2005) five minutes represent the ideal length for the tea preparation since during five minutes the best taste and low levels of tannins are guaranteed.

The analysis of differently prepared tea extracts for 5 minutes time showed similar results (Figure 2). PCA explained a total of 91.3% of variations in our data (PCA axis 1 and PCA axis 2 together explained 74.0% of the variation). Unlike metals (Popović et al. 2017) ions behaved differently when these three different ways of preparation for 5 minutes were observed. All supplementary variables referring to 5 minutes tea preparation are placed in the center of the ordination diagram, showing that different ways of preparation do not influence the ion concentrations in tea infusions. So, unlike metals, higher concentrations of ions have not been observed in acidified tea infusions. The relationship between ion concentrations in infusions and tea samples is exactly the same as in the previous figure, leading to the conclusion that ion release after all five different ways of preparation is the same, or that a different way of preparation available in this study does not influence the changes in the ion concentrations in tea infusion.

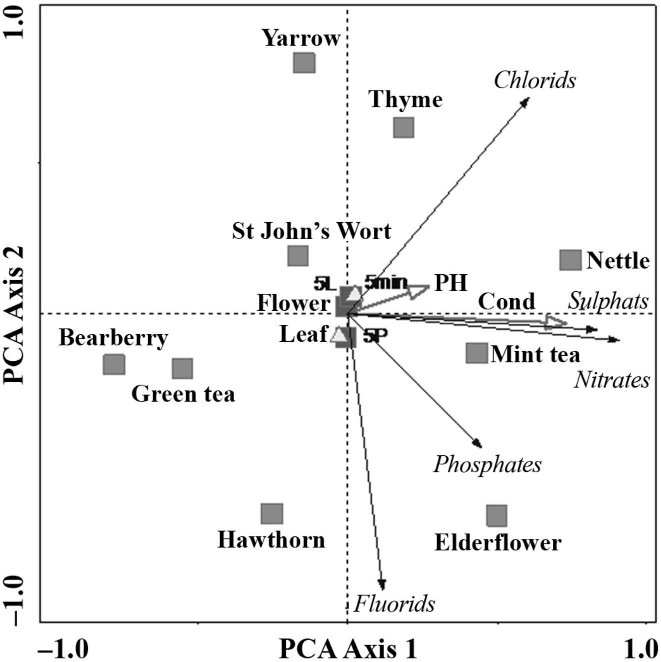

Figure 2. Principal component analysis (PCA) of ions from water extract after boiling for 5 min (5 min), after samples were covered and left at room temperature for 5 min (5P), and after samples were covered and left at room temperature for 5 min with added lemon juice (5L). The supplementary variables include tea as blue diamonds (hawthorn, St John's wort, nettle, elderflower, green tea, bearberry, thyme, yarrow, and mint tea); red squares refer to boiling time; and white triangles represent the part of the plant from which the tea is primarily produced (leaves, flowers)

In the majority of tea samples, pH value showed slightly lower values – the samples were slightly acidic. The same finding was observed by Mincă et al. (2013) where infusions of green, white, and black tea were examined. The values of pH ranged from 4.71 to 5.91 in all tea samples except in nettle tea infusion, where pH of 7.74 was measured. The conductivity ranged from 474 µS/cm (bearberry) to 1995 µS/cm (nettle).

Acidification of samples with lemon juice does not significantly influence the release of anions in tea infusions. However, in nettle infusions some differences not seen in the ordination diagram were observed in acidified samples. More precisely, nettle infusion is the only infusion having pH values above 7. After acidification, a slight increase in chlorides and nitrates was observed, while sulphate content was decreased. On the other hand, the content of phosphates was significantly higher in acidified sample. This suggested that pH had an indirect impact on the anion concentration in samples.

It is worth mentioning that the results of the anion analysis were hard to compare with the results from other studies. It is probably due to the different methods used, the origin of herbs itself, different examined herbal teas, type of the land on which the herb is grown and its acidity, or content of fluorides in land.

CONCLUSION

Results indicated that all investigated anions showed variations in their concentrations among different tea samples. In almost all tea samples phosphate ion had the highest concentrations and its highest release was observed in elderflower and nettle tea infusions. Beside phosphate content, nettle tea infusion also had the highest chloride, nitrate, and sulphate content. According to the performed principal component analysis, no significant difference in ion concentration after boiling for 5, 10, and 20 minutes, as well as after acidification of the medium was observed. In the aqueous solution of herbal tea, pH of the solution did not have an effect on ion concentration, but slight variations were observed in nettle, where the highest pH of the tea infusion was measured. The only difference was found in nettle infusions, where acidification showed higher ion concentrations, except for fluoride ions. A presumption could be made that there is some indirect influence on the concentration change rather than on pH value. The obtained results can contribute to the general knowledge of inorganic anions in herbal tea infusions that can be beneficial in human diet, as well as to better understanding of the effect of different factors on their release.

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ОДРЕЂИВАЊЕ НЕОРГАНСКИХ ЈОНА У РАСТВОРУ БИЉНИХ ЧАЈЕВА ЈОНСКОМ ХРОМАТОГРАФИЈОМ

Жељка Ј. МИЛОВАНОВИЋ[1], Слађана С. ПОПОВИЋ[1], Ана С. ПАНТЕЛИЋ[2], Јелена Р. МИЛИНКОВ[3], Драгана Л. МИЛОШЕВИЋ[1], Владимир М. ПЕТРОВИЋ[1], Милка М. ВИДОВИЋ[1]

[1] Универзитет у Београду, НУ Институт за хемију, технологију и металургију
Центар за екологију и техноекономику
Његошева 12, Београд 11001, Србија
[2] Универзитет у Београду, НУ Институт за хемију, технологију и металургију
Центар за хемију
Његошева 12, Београд 11001, Србија
[3] Хемофарм а.д.
Београдски пут бб, Вршац 26300, Србија

САЖЕТАК: Јонски садржај испитиван је код девет водених раствора чаја, при чему је на екстракцију јона посматран утицај дужине кувања, киселости средине (која је постигнута додатком лимуновог сока) и начина припреме. Јонска хроматографија коришћена је за одређивање садржаја неорганских анјона, а подаци су обрађени помоћу *Canoco* програма за мултиваријациону анализу. Јонски садржај варира код различитих узорака чаја. Највеће концентрације хлоридних, нитратних, фосфатних и сулфатних јона одређене су у коприви, док су највеће концентрације флуорида одређене у инфузији кантариона. Утицај времена кључања (5, 10 и 20 мин.), киселост медијума и различити начини припреме (кључање и хлађење на собној температури) статистички су приказани помоћу анализе главних компоненти. Испитивани фактори нису имали значајан утицај на јонску концентрацију у чајним инфузијама.

КЉУЧНЕ РЕЧИ: анализа главних компоненти, биљни чајеви, чајне инфузије, јонска хроматографија, неоргански анјони