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Article

Multi-Element Analysis, Bioavailability and Fractionation of Herbal Tea Products

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Chás de ervas (*Mentha piperitae folium* e mistura de *Marticaria chamomilla flos* com *Lavandula officinalis flos*) foram comparados considerando o conteúdo total de microelementos (Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Ti, V) e macroelementos (C, H, N, S, Ca, Mg, P), biodisponibilidade e fracionamento. Diferentes métodos (espectrometria de emissão óptica com plasma indutivamente acoplado (ICP OES), espectroscopia no infravermelho com transformada de Fourier (FTIR) e análise elementar (CHNS)) foram aplicados. O procedimento de digestão assistida por micro-ondas mostrou-se mais efetivo do que placa de aquecimento para a digestão ácida por via úmida do chá. A aplicação do procedimento de extração sequencial BCR (Community Bureau of Reference) modificado apresentou diferenças nas concentrações de metal ligado com frações redutíveis e oxidáveis. A precisão do método foi verificada pela análise do material de referência certificado INCT-TL-1 Tea Leaves. A ingestão diária de todos os elementos da infusão de chá de ervas analisados não excedeu os níveis máximos permitidos e não constitui um risco para a saúde.

Herbal teas (*Mentha piperitae folium* and mixture *Marticaria chamomilla flos* with *Lavandula officinalis flos*) were compared considering the total contents of micro (Al, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr, Ti, V) and macro (C, H, N, S, Ca, Mg, P) elements, bioavailability and fractionation. Different methods (inductively coupled plasma optical emission spectrometry (ICP OES), Fourier transform infrared spectroscopy (FTIR) and CHNS elemental analysis) were applied. The microwave-assisted digestion procedure was found to be more effective than the hot-plate heating for the wet acid digestion of tea. The application of the modified BCR (Community Bureau of Reference) sequential extraction procedure exhibited differences in the concentrations of metal bound to reducible and oxidizable fractions. The accuracy of method was verified by analysis of certified reference material INCT-TL-1 Tea Leaves. The daily intake of all elements from the analyzed herbal tea infusion did not exceed the maximum permissible levels and does not constitute health risk.

Keywords: herbal tea, infusion, sequential extraction, FTIR spectroscopy, CHNS analysis

Introduction

The last few decades have witnessed a rapid development in the diet studies concerning the determination of trace elements, which reflect their role in human health and nutrition. Deficiency, excess or imbalance of trace element intake into human body may result in various diseases. Water, food and commonly used beverages are the different sources of trace elements in the diet, however, food and beverages are considered as the major ones. The knowledge of both micronutrients and toxic element contents in the beverages is important taking into account nutrition requirement and/or intoxication risk related with their consumption. The determination of trace elements in plants, used traditionally for preparing teas, was done either to assess the exposition to heavy metals or to verify the possibility of utilizing them as nutritional supplements owing to their role in many biochemical and physiological processes.

Plants constitute important connection with the transfer of heavy metal pollutants from soil into human body. The uptake of metals by plants is dependent on various factors, including kind of soil, climate, pollutions and the ability of the plants to selectively accumulate some of these elements.[1,2]

Several works have been devoted to determine the macro and microelement contents of different teas, herbal teas and medicinal plants, from many parts of the

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world.[3-12] A number of studies has been focused on the total concentration of metals in tea infusions.[10,13-15] In general, the determination of the element contents in different teas and medicinal plants has been done in previous publications with the use of various methods, such as flame atomic absorption spectrometry (F AAS),[2,5,7,10,12,16,17] graphite furnace atomic absorption spectrometry (GF AAS),[18] electrothermal atomic absorption spectrometry (ET AAS),[2,4] inductively coupled plasma optical emission spectrometry (ICP OES)[3,8,16] and inductively coupled plasma mass spectrometry (ICP MS).[13,19]

Fourier transform infrared spectroscopy (FTIR) has been widely used in the food industry for the identification of various components such free fatty acids in palm olein[20] and aflatoxin B_1 in red chili powder.[21] Until now, several attempts have been made to apply FTIR spectroscopy to the analysis of tea samples.[22-24]

Peppermint *(Mentha piperita)*, chamomile (*Matricaria chamomilla*) and lavender (*Lavandula officinalis*) are the most popular herbs, also consumed for medicinal purposes and for maintaining good health. Peppermint tea is traditionally used as a digestive aid for occasional indigestion and is particularly helpful with flatulence and a sensation of fullness. The combination of chamomile and lavender is useful for nervous stomach and restlessness associated with upset stomach.

The present study undertook to determine macro and microelements in herbal teas (peppermint, mixed chamomile and lavender) and in their infusions, as well as to obtain more complex information about the tea products by application of IR spectroscopy and extraction. The main purpose was to establish the levels of sixteen elements (Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, Sr, Ti, V and Zn) in the teas and infusions. Organic matter was investigated with the aid of FTIR spectroscopy, and CHNS analysis was also performed.

Experimental

Apparatus

A Jobin Yvon ICP sequential spectrometer JY38S was used to measure element concentrations. The plasma operating conditions are listed in Table 1. The high-pressure microwave sample decomposition method was performed using a Milestone digestion system (MLS-1200 Mega) equipped with a rotor (MDR 300/10). The ultrasonic extraction of the samples was performed using an ultrasonic bath (UltrasonsH Selecta). A MPW-350 centrifuge (Med. Instruments) was used to separate the supernatant from the solid residue. The FTIR spectrum in the region 4000-400 cm^{-1} was acquired on a Perkin-Elmer FTIR-2000 spectrophotometer using KBr pellets. Elemental analysis (C, H, N, S) was carried out by using a VarioEL analyzer.

Table 1. ICP operational parameters

Generator / MHz	40.68
Rf power / W	1000
Injector i.d. / mm	2.5
Spray chamber	Cyclonic
Nebulizer	Burgener (Mira Mist)
Monochromator	1 m with 4320/2400 grooves *per* mm grating
Observation zone	12 mm above load coil
Air flow rate / (L min^{-1})	plasma gas: 13 sheath gas: 0.2 carrier gas: 0.3
Solution uptake / (mL min^{-1})	0.75
Line (wavelength / nm)	Al I (396.1), Ba II (233.5), Ca I (317.9), Cd II (228.8), Cr II (267.7), Cu I (324.7), Fe II (259.9), Mg II (280.3), Mg I (285.2), Mn II (259.4), Ni II (221.6), P I (214.9), Pb II (220.3), Sr II (407.8), Ti I (334.9), V II (292.4), Zn I (213.8)

I: atomic line; II: ionic line.

Reagent and standard solutions

All reagents used in this study were of analytical grade. For sample digestion, concentrated HNO_3 (Merck KGaA, Germany, p.a.) and 30% (m/v) H_2O_2 (POCh S.A., Poland, p.a.) were used. The extractant solutions were prepared using 80% acetic acid (POCh), ammonium acetate (Fluka GmBh, Switzerland) and hydroxylamine hydrochloride (POCh). Working standard solutions were prepared by dilution of the concentrated (1000 μg mL^{-1}) ICP multi-element standard (Merck). All dissolutions and dilutions were performed with deionized water (18.3 MΩ cm^{-1}) obtained from EASYpure™ (Baenstead, Thermolyne Corporation, USA). Glassware and plastic bottles were cleaned with 10% nitric acid in an ultrasonic bath and rinsed several times with deionized water.

Samples

Four herbal teas commercially available as tea bags, Organic Peppermint from Traditional Medicinals (USA), Peppermint from Herbapol Lublin Ltd (Poland), Organic Chamomile with Lavender from Traditional Medicinals (USA) and Organic Chamomile & Lavender from Clipper (UK), were examined. The raw herbal material from all tea bags were placed in one box and put in polyethylene

containers, mixed, ground and stored at room temperature. Reliability of the digestion procedure and the accuracy of the determination method were confirmed by the analysis of the plant standard reference material (INCT-TL-1 Tea Leaves).

With each set of digestions and extractions, a blank sample was simultaneously prepared through the complete procedure, analyzed and then used for correction of the analytical signals.

Digestion procedures

Two different procedures were applied in order to digest the herbal tea samples.[25-27] For each type of analyzed sample, five replicated samples were prepared and measured.

Microwave-assisted digestion procedure (MW)

0.5 g of herbal tea were accurately weighed into a Teflon digestion vessel, and 6 mL of concentrated nitric acid and 1 mL of hydrogen peroxide (30%) were added. The samples were subjected to microwave heating with maximum power of 600 W for 45 min. After the cooling, the colorless solution was quantitatively transferred into a 25 mL volumetric flask and made up to the volume with deionized water. The above procedure was also applied for the decomposition of the certified reference materials.

Hot-plate heating procedure (HP)

1 g of each sample was accurately weighed and mineralized in a mixture of concentrated HNO_3 and 30% (m/v) H_2O_2 on hot-plate at 85 ºC for about 3 h, i.e., until brown fumes stopped evaporating and the digest became clear. Then, the mineralized solution was evaporated to a small volume. After cooling, the solution was quantitatively transferred to a 25 mL volumetric flask and brought up to volume with deionized water.

Infusions of tea samples

The tea infusions were prepared according to the usual method of tea preparation. Deionized water was used for this purpose. One bag of each sample was placed in a glass beaker, 100 mL of boiling deionized water were added, covered and left at room temperature for 5 min. The infusions were filtered before measurements.

BCR sequential extraction procedure

The four-step sequential extraction procedure was applied to compare the mobility of elements. 0.5 g tea samples and 20 mL of reagents were used for the extraction, maintaining the same weight-to-volume ratio as in the original BCR (Community Bureau of Reference) protocol. The extraction (assisted with ultrasound to shorten the process time) was carried out for 15 min at 25 ºC in each step. The extraction was carried out in triplicate. Extracts were centrifuged at 14000 g for 15 min and filtered through a hard filter paper into a polyethylene container.

Step 1: (water-soluble fraction) 20 mL of deionized water were added to the 0.5 g of tea sample.

Step 2: (acid-soluble fraction): 20 mL of 0.11 mol L^{-1} acetic acid were added to the residue from step 1.

Step 3: (reducible fraction): 20 mL of 0.1 mol L^{-1} freshly prepared hydroxylamine hydrochloride were adjusted with nitric acid to pH 2 and added to the residue from step 2.

Step 4: (oxidizable fraction) The residue from step 3 was heated with 10 mL 30% hydrogen peroxide with ultrasounds for 15 min at 25 °C and then evaporated to near-dryness in a thermostatic bath at 85 °C for 1 h. A second aliquot of 10 mL of 30% hydrogen peroxide was added and the mixture was heated at the same temperature for 1 h. After cooling, 20 mL of 1.0 mol L^{-1} ammonium acetate (adjusted to pH 2 with nitric acid) was added.

Between each extraction step the residue was washed with 10 mL of deionized water, manually shaken for 1 min and centrifuged for 15 min at 9700 g. The supernatant was decanted and discarded carefully to avoid the loss of any of the solid residue.

Digestion of the residue is not specified in BCR protocol. The residual fraction was calculated as the difference between the total element concentration and the sum of all previous steps.

Results and Discussion

Total metal concentrations

Analysis of standard reference material (INCT-TL-1 Tea Leaves) was performed for validation of the applied analytical procedure. The concentrations of the elements were measured in the samples after microwave mineralization with the use of the mixture of nitric acid and hydrogen peroxide. The *t*-test was used to find out whether statistically significant differences occurred between the found and certified values at 95% confidence level. The measured and certified results with the significance level notation (S or NS) are given in Table 2. A good agreement between the obtained data and the certified values was observed. In case of Al, Ba, Ca, Cr, Cu, Mg, Mn, Ni, Sr, V and Zn, the differences in concentrations (measured and certified) were found to be within the limits of standard deviation uncertainties. For Fe, P and Ti, only information values were given. The measured concentrations

Table 2. Verification of the digestion procedure and measurement accuracy, analysis of standard reference material (INCT-TL-1 Tea Leaves)

Element	Concentration[a] / (μg g^{-1})		Value of *t*-test	Significance[b]
	Certified value	Experimental data, MW		
Al[d]	0.229 ± 0.028	0.203 ± 0.015	3.00	NS
Ba	43.2 ± 3.9	38.7 ± 1.9	4.08	NS
Ca[c]	0.582 ± 0.052	0.624 ± 0.019	3.83	NS
Cr	1.91 ± 0.22	1.71 ± 0.19	1.82	NS
Cu	20.4 ± 1.5	20.5 ± 1.6	0.11	NS
Fe	432[c]	466 ± 4		
Mg[d]	0.22 ± 0.017	0.234 ± 0.007	2.47	NS
Mn[d]	0.157 ± 0.011	0.178 ± 0.012	3.03	NS
Ni	6.12 ± 0.52	5.72 ± 0.55	1.26	NS
P	1810[c]	2438 ± 30		
Sr	20.8 ± 1.7	18.2 ± 1.1	4.09	NS
Ti	30[c]	24.1 ± 2.0		
V	1.97 ± 0.37	1.77 ± 0.19	1.82	NS
Zn	34.7 ± 2.7	32.4 ± 1.6	2.49	NS

[a]Mean value ± standard deviation; [b]significance of *t*-test (n = 3) at 95% confidence level; [c]information value; [e]in wt.%. MW: microwave-assisted digestion procedure; NS: not significant.

of these elements were different than the informed data. For Fe and P, the obtained values were higher (Fe 8%, P 35%), but lower in case of Ti (about 20%).

The total concentrations of the elements determined in the analyzed herbal teas expressed as arithmetic mean and their standard deviation uncertainties are given in Table 3. On the basis of the results shown in this table, microwave digestion was found to be the most effective herbal tea digestion method. This technique provided a better solubilization of the matrix, increasing the reproducibility between different replicates and satisfactory precision.

The precision of the concentration measurements (expressed as relative standard deviation (RSD)) varied between types of tea. For Organic Chamomile & Lavender tea (UK), the RSD values remain at the level of a small percentage, varying from 0.9% (Sr) to 4.2% (Mg). Only for Cr and Ni, the RSD values exceeded 10%. In the case of Peppermint tea (Poland), except for Cr, for most elements, the RSD values were below 5%. For Organic Peppermint tea and Organic Chamomile with Lavender tea (USA), only for Cr, Mg, Ni and V, low precisions (RSD > 5%) were obtained.

The analyzed herbal teas are rich in macronutrient elements such as Ca, Mg and P. The concentrations of Ca, Mg and P were in the range of 6630-19020, 2380-6880 and 2370-5480 μg g^{-1}, respectively, with the lowest levels for Organic Chamomile & Lavender (UK). These results were in agreement with other studies,[14,19,28] which indicated that these three elements were the most abundant elements in many medicinal plants, herbal medicines and teas. The obtained tea Ca concentrations in this study were higher than those found in medicinal plants,[5,26,27] but similar to those measured by other authors[2,6,14,25] In the case of Mg and P, our results agreed with those described in the literature,[2,3,5] but were also much higher (2-3 times) than those reported by Başgel *et al.*,[14] Kara[25] and Özcan *et al.* [26,27]

Fe was generally the most abundant metal in all of the tea samples. The highest (597 μg g^{-1}) and the lowest (333 μg g^{-1}) levels of Fe were found in Organic Chamomile with Lavender (USA) and Peppermint teas (Poland), respectively. These values were close to the results obtained by Özcan,[3] Başgel *et al.*[14] and Kara[25] and were appreciably higher than those reported by Łozak *et al.*,[2] Ražić *et al.*[6] and Özcan *et al.*,[27] in similar herbs and herbal tea products.

The content of Al varied between 206 and 585 μg g^{-1}. According to the literature, most of the investigated foodstuffs (meat, vegetables and dairy products) contained less than 5 μg g^{-1} of Al and high concentrations were found in spices (145 μg g^{-1}) and black tea leaves (899 μg g^{-1}).[29] The concentrations of Al were lower than those reported for black tea, but were similar to the obtained value for different herbal medicines, herbal and green tea products and herbs.[14,28,30]

The concentration of Mn differed markedly between the tea brands. The highest Mn content (72.5 μg g^{-1}) was in Peppermint tea (Poland) and the lowest value (38.6 μg g^{-1}) was determined in Organic Chamomile & Lavender tea (UK). Close results were also obtained for the Organic Peppermint tea and Organic Chamomile with Lavender tea

Table 3. Metal contents in the analyzed teas (n = 5)

Element	Concentration / (μg g^{-1})			Concentration / (μg g^{-1})		
	Organic Peppermint (USA)		Peppermint (Poland)	Organic Chamomile with Lavender (USA)		Organic Chamomile & Lavender (UK)
	HP[a]	MW[b]	MW[b]	HP[a]	MW[b]	MW[b]
Al	219 ± 20	206 ± 3	325 ± 17	527 ± 14	585 ± 23	500 ± 20
Ba	18.6 ± 0.7	17.3 ± 0.4	22.3 ± 0.3	22.9 ± 0.9	22.2 ± 0.3	9.00 ± 0.24
Ca	13150 ± 300	13417 ± 220	19016 ± 627	11906 ± 466	13431 ± 262	6628 ± 254
Cd	< LOD[c]	< LOD[c]	< LOD[c]	< LOD[c]	< LOD[c]	< LOD[c]
Cr	0.53 ± 0.04	0.65 ± 0.08	0.56 ± 0.07	2.95 ± 0.35	2.74 ± 0.30	1.05 ± 0.24
Cu	19.7 ± 1.1	16.7 ± 0.6	10.3 ± 0.8	10.2 ± 0.3	12.4 ± 0.5	9.35 ± 0.30
Fe	489 ± 62	471 ± 9	333 ± 10	583 ± 7	597 ± 14	529 ± 11
Mg	6524 ± 205	6880 ± 400	6340 ± 420	4122 ± 176	4418 ± 360	2375 ± 100
Mn	46.3 ± 2.2	45.9 ± 0.3	72.5 ± 2.3	57.1 ± 1.2	56.1 ± 0.8	38.6 ± 0.9
Ni	1.62 ± 0.72	7.75 ± 0.55	3.08 ± 0.22	3.51 ± 0.19	8.84 ± 0.71	5.08 ± 0.42
P	5005 ± 130	5482 ± 126	4408 ± 125	3364 ± 175	3596 ± 137	2373 ± 67
Pb	< LOD[c]	< LOD[c]	< LOD[c]	< LOD[c]	< LOD[c]	< LOD[c]
Sr	28.6 ± 0.8	31.6 ± 0.8	33.0 ± 0.5	45.0 ± 1.5	37.5 ± 4.0	55.4 ± 0.5
Ti	17.1 ± 0.7	38.9 ± 0.6	12.4 ± 1.0	27.7 ± 0.3	18.1 ± 1.1	26.8 ± 0.6
V	0.96 ± 0.10	1.20 ± 0.11	0.84 ± 0.06	1.43 ± 0.14	1.12 ± 0.16	1.36 ± 0.04
Zn	35.1 ± 0.5	34.0 ± 0.3	33.3 ± 0.7	32.3 ± 0.9	39.9 ± 1.3	19.3 ± 0.7

[a]Hot-plate heating procedure; [b]microwave digestion; [c]below the limit of detection.

(USA). The amount of Mn was generally similar to results previously reported.[3,14,25,26]

The total content of Sr for both Peppermint tea (USA, Poland) was in the range of 31.6-33.0 μg g^{-1}, and 45.0-55.4 μg g^{-1} for Organic Chamomile with Lavender teas (USA, UK).

The Ba concentration was the lowest (9.00 μg g^{-1}) in Organic Chamomile & Lavender tea (UK), in the others it varied from 17.3 to 22.3 μg g^{-1}. Similar results for Peppermint, Chamomile and Lavender tea were reported by Kara[25] and Özcan *et al.*[26]

The Cu and Zn concentrations in the herbal teas were in the range of 9.40-16.7 and 19.3-32.3 μg g^{-1}, respectively. The highest contents of the both metals were found in Organic Chamomile & Lavender tea (UK), whereas the lowest values were found in Organic Peppermint tea (USA). The obtained results are in agreement with those reported for other plants.[5,15,25]

The highest Ti (38.9 μg g^{-1}) concentration was found in Organic Peppermint tea (USA) and the lowest (12.4 μg g^{-1}) value was found in Peppermint tea (Poland). Our results were much higher than those previously reported[2,28] in herbal teas and medicines.

The V, Cr and Ni concentrations were low if compared with the results for other metals. The highest V, Cr and Ni concentrations (1.43, 2.74 and 8.84 μg g^{-1}, respectively) were found in Organic Chamomile with Lavender tea (USA), whereas the lowest levels of all the elements (0.84, 0.56 and 3.08 μg g^{-1}, respectively) were determined in Peppermint tea (Poland).

In all of the examined teas, the toxic Cd and Pb were not detected.

Infusions of tea samples

The element concentrations in herbal tea infusions were determined to assess an exposure to these elements during drinking of the tea. The concentrations of 14 elements determined in four types of herbal teas are presented in Table 4. On the basis of a comparison of metal concentrations and in the infusions, the percentage of release (calculated as a ratio between infusion extractable metal and total content in the raw material) of each element was calculated (Table 5).

The most abundant elements present in all tea infusions were Mg and P. The concentrations of Mg and P were in the range of 981-3268 and 1292-2827 μg g^{-1}, respectively. The lowest levels for these elements were found in Organic Chamomile & Lavender (UK) and the highest contents were determined in Organic Peppermint tea infusions (USA).

Table 4. Mineral contents in the analyzed herbal tea infusions (n = 3) in comparison with recommended and tolerable dietary values (Organic Peppermint, USA (tea 1), Peppermint, Poland (tea 2), Organic Chamomile with Lavender, USA (tea 3), and Organic Chamomile & Lavender, UK (tea 4))

Element	Concentration / (μg g^{-1})				ADDI[a] / (μg day^{-1}), this study[b]	Dietary reference intake / (mg day^{-1})[32]	
	Tea 1	Tea 2	Tea 3	Tea 4		Recommended allowance/ Adequate intake	Upper tolerable level
Al	4.31 ± 0.23	5.40 ± 0.97	10.4 ± 0.5	11.3 ± 0.6	12.9-33.9		7000[c]
Ba	3.20 ± 0.04	4.05 ± 0.33	5.59 ± 0.21	2.36 ± 0.10	7.08-16.8		
Ca	3742 ± 61	4457 ± 51	3574 ± 190	1532 ± 101	4596-13371	1000-1300	2500
Cu	5.48 ± 0.31	3.53 ± 0.25	5.62 ± 0.12	4.38 ± 0.17	10.6-16.9	0.7-0.9	5-10
Fe	8.20 ± 0.08	6.23 ± 0.40	10.8 ± 0.7	9.10 ± 0.52	18.7-32.4	8-18	40-45
Mg	3268 ± 65	2671 ± 79	1908 ± 28	981 ± 11	2943-9804	240-420	350
Mn	14.2 ± 0.21	22.2 ± 0.1	14.1 ± 0.9	8.64 ± 0.33	25.9-66.6	1.8-2.3	6-11
Ni	1.16 ± 0.06	2.69 ± 0.28	2.37 ± 0.12	1.73 ± 0.09	3.48-8.07		0.6-1.0
P	2827 ± 71	2296 ± 117	2114 ± 77	1292 ± 6	3876-8481	700-1250	3000-4000
Sr	15.1 ± 0.5	14.4 ± 0.7	23.7 ± 0.9	16.4 ± 1.3	43.2-71.1		
Ti	0.07 ± 0.02	0.03 ± 0.01	< LOD[d]	0.68 ± 0.08	0.09-2.04		
V	< LOD[d]	< LOD[d]	0.15 ± 0.02	0.15 ± 0.09	0-0.45		1.8
Zn	12.0 ± 0.5	8.80 ± 1.05	11.0 ± 1.4	7.57 ± 0.10	22.7-36.0	8-11	23-40

[a]ADDI: average daily dietary intake (min.-max.); [b]3 cups *per* day (one cup of infusions was prepared from one bag of tea in 100 mL of hot deionized water); [c]expressed (mg kg^{-1} body weight *per* week), on assumption of provisional permissible weekly intakes, Joint FAO/WHO;[33] [d]below the limit of detection (LOD).

The percentage of P release was high and varied between 51.6-58.8%. In the case of Mg, this value was slightly lower and achieved 41.3-47.5%. Our results are similar to Łozak *et al.*[2] and Lemberkovics *et al.*[31]

In spite of very high Ca content in the teas, the percentage of Ca in infusion was relatively low, and amounted from 23.2 to 27.9% for Organic Chamomile & Lavender tea (UK), Peppermint tea (Poland), Organic Chamomile with Lavender tea and Organic Peppermint tea (USA), respectively. Ca concentration in all herbal tea infusions ranged from 1532 μg g^{-1} (Organic Chamomile & Lavender tea, UK) to 4457 μg g^{-1} (Peppermint tea, Poland). Compatible results for percentage of release were also obtained by Lemberkovics *et al.*,[31] but our results were higher than those reported by Łozak *et al.*[2] and Başgel *et al.*[14]

The Sr content in tea infusions was between 14.4-23.7 μg g^{-1}. For Organic Chamomile & Lavender (UK), only 29.7% of Sr were released, whereas 43.6, 47.7 and 52.7% of Sr were leached from both peppermint tea (Poland, USA) infusions and from organic chamomile with lavender tea (Traditional Medicinals, USA), respectively. Our results were lower than those reported by Łozak *et al.*,[2] however in accordance with the obtained by Başgel *et al.*[14] and Nookabkaew *et al.*[19]

The Ba concentration in herbal tea infusions was in the range of 2.36-5.59 μg g^{-1}. For both peppermint tea infusions (Poland, USA), 18.2-18.5% of Ba were released, whereas 25.2-26.2% of Ba were extracted from the organic chamomile with lavender tea infusions (USA, UK). The results of both peppermint teas were similar to the obtained by Başgel *et al.*[14] and Kalny *et al.*[18]

The Cu content in tea infusions varied from 3.53 to 5.62 μg g^{-1}. The percentage of Cu release was very similar

Table 5. Release of metal extraction from raw samples to infusions

Element	Release[a] / %			
	Organic Peppermint (USA)	Peppermint (Poland)	Organic Chamomile with Lavender (USA)	Organic Chamomile & Lavender (UK)
Al	2.10	1.66	1.79	2.26
Ba	18.5	18.2	25.2	26.2
Ca	27.9	23.4	26.6	23.1
Cu	32.8	34.2	45.3	46.9
Fe	1.74	1.87	1.80	1.72
Mg	47.5	42.1	43.2	41.3
Mn	31.0	30.7	25.2	22.4
Ni	15.0	87.3	26.8	34.0
P	51.6	52.1	58.8	54.4
Sr	47.7	43.6	52.7	29.7
Ti	0.17	0.22	–	2.53
V	–	–	10.3	10.8
Zn	35.3	26.4	34.0	39.2

[a]Calculated as a ratio between infusion extractable metal and total content.

for the both peppermint tea infusions (32.8-34.2%) and for both organic chamomile with lavender tea infusions (45.3-46.9%).

The level of Zn determined in tea infusions was in the range of 7.57-12.0 μg g^{-1}. The percentage of release for Peppermint tea (Poland) was 26.4, but for other teas these values were higher and varied from 34.0 to 39.2%. Comparing with values described in the literature, our results were similar to those reported by Başgel *et al.*,[14] Nookabkaew *et al.* [19] and Lemberkovics *et al.*[31]

Mn was released at the highest amount (22.2 μg g^{-1}) in Peppermint tea infusions (Poland), and the lowest concentration (8.64 μg g^{-1}) was in Organic Chamomile & Lavender tea (UK). The percentage of release in all teas was found in the range of 22.4-31.0%. According to Powell *et al.*[34] 40% of the manganese is potentially bioavailable.

The highest percentage of Ni release (87.3%) was achieved in Peppermint tea infusions (Poland), whereas only 15.0% in Organic Peppermint (USA) infusion. The obtained value for Peppermint Tea (Poland) was similar to those reported by Łozak *et al.*,[2] Soomro *et al.*[17] and Kalny *et al.*[18]

The percentage of Fe release into the infusions of the herbs was very low (1.72-1.87%). The Al content in infusions was in the range 4.31-11.3 μg g^{-1}, which indicates only 1.66-2.26% of the release. Similarly to Fe, Al has also very low bioavailability due to the actions of polyphenols, which avidly bind to trivalent metal ions (Fe^{3+} and Al^{3+}), preventing their intestinal absorption.

The small percentage of release was observed also in the case of Ti (0.17-2.53%). Its concentration ranged from 0.03 μg g^{-1} (Peppermint tea, Poland) to 0.68 μg g^{-1} (Organic Chamomile & Lavender tea, UK). In the case of Organic Chamomile with Lavender tea (USA), Ti was not detected.

V was detected only in organic chamomile with lavender tea infusions. The V content was 0.15 μg g^{-1} and the release was about 10%.

The release of elements into tea infusions depends on whether they are strongly bound to the organic matrix of tea or are more soluble in the solution. Accordingly, considering the extraction efficiencies in tea infusions, the analyzed elements can be classified into two groups: i.e., moderately (20-60%, Ca, Cu, Mg, Mn, P, Sr and Zn) and poorly (< 20%, Al, Fe, Ti and V) extractable. Some elements, such as Ti and V, were even undetectable in the infusions of particular teas. The highest discrepancies were found for Ni. Its extractability varied from poor to high and the effect was involved with the type of tea.

Dietary reference values for elements (i.e. recommended allowances/adequate daily intakes, upper tolerable intake levels for individuals in the 9-70 life stage group and maximum level of daily intake without detriment to health) cited in Table 4 can serve for the evaluation of the nutrition characteristics of the examined herbal teas. The concentrations of all the metals for daily intake were below the safety levels for human consumption and can be considered as a supplement in the diet. Generally, the monitoring of commercially distributed herbal tea products is an important task if these products are very widely used in our daily consumption.

BCR sequential extraction procedure

Sequential extraction methods were employed in this study to evaluate the mobility of metals present in tea samples. The extraction efficiency (calculated as a ratio between elements in extraction step and total content in the raw material) of each element was used to estimate reducible and oxidizable fractions. The results, including the mobility of some elements in each extraction step except for the residual fractions, are shown in Figures 1 and 2. As can be seen, the extraction efficiencies depended on the extract solution, element and differed markedly between the type of tea. Generally, the distribution of most metals in extracts was similar and characteristic of the investigated type of tea.

Very small concentrations of Al, Cr, Fe and Ti were observed in the first, second and third extraction steps for all analyzed herbal teas. These elements behaved similarly and were essentially bound to the residual fraction. It suggested relatively low mobility of the metals.

Water leached the smallest amounts (< 10%) of Ba and V from all analyzed herbal teas and Ni from both peppermint teas (Poland, USA). Cu, Mn and Zn were relatively easily extracted by deionized water. These values were close to the results obtained by us for infusion tea samples.

The acid-soluble fraction (metal bound to carbonate) and reducible fraction (metal bound to iron and manganese oxides) generally contained small percentage (below 10%) of the total metal concentrations in all teas. Only percentages above 10% Ba, Mn, Sr and Zn were found to be associated with carbonates. The extraction efficiency in this fraction was considerably higher (about 8, 15, 10 and 6%, respectively) for both peppermint teas (Poland, USA) than for both organic chamomile with lavender teas (USA, UK). Among the studied metals, the highest reducible fraction content was also observed for Ba, Mn, Sr and Zn, and the extraction effectiveness achieved about 20% for all cases.

Oxidizable fraction shows the amount of metals bound to organic matter and sulfide. Based on the results, Ba, Cu, Sr and Zn were easily extracted under oxidizing condition. Ba was extracted at the highest amount (57%) in Organic

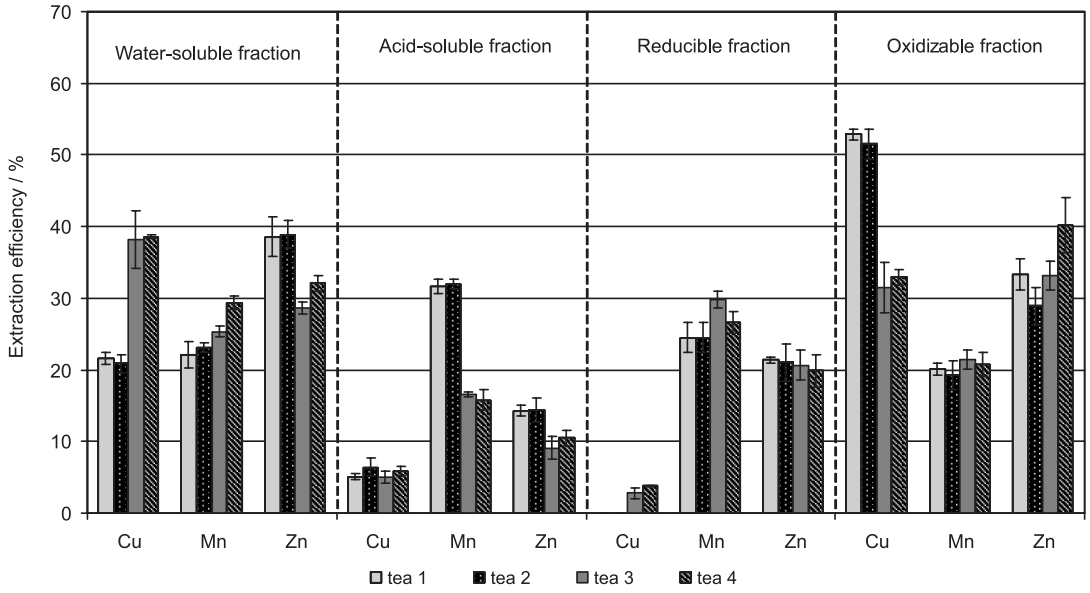

Figure 1. Cu, Mn and Zn distributions in different fractions (%) in: Organic Peppermint, USA (tea 1), Peppermint, Poland (tea 2), Organic Chamomile with Lavender, USA (tea 3), and Organic Chamomile & Lavender, UK (tea 4).

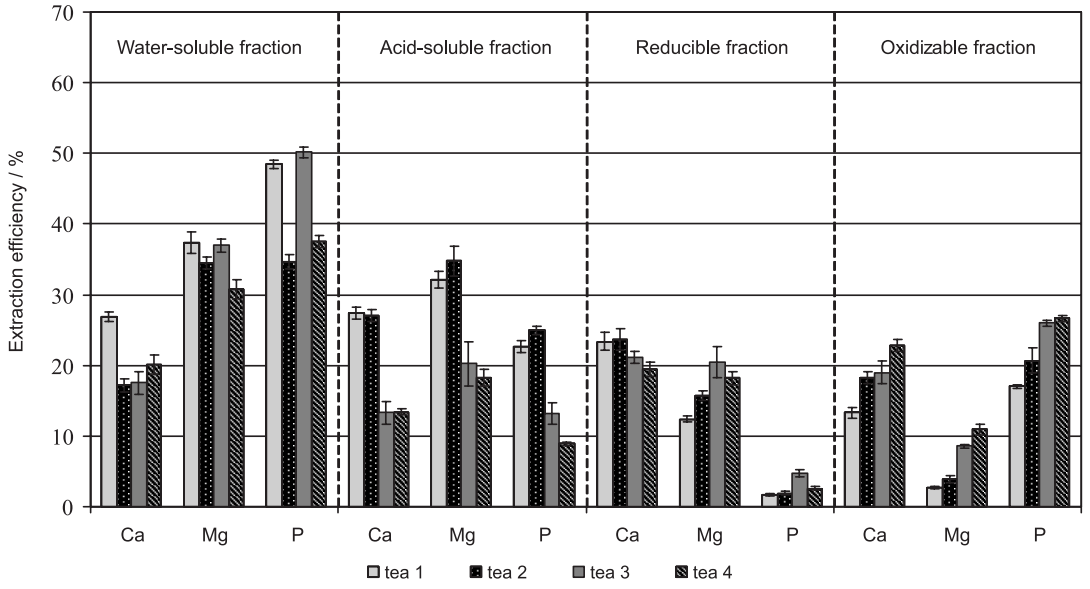

Figure 2. Ca, Mg and P distributions in different fractions (%) in tea 1: Organic Peppermint, USA, tea 2: Peppermint, Poland, tea 3: Organic Chamomile with Lavender, USA, and tea 4: Organic Chamomile & Lavender, UK.

Peppermint tea (USA), and the lowest extraction efficiency (44%) was in Organic Chamomile & Lavender tea (UK). From both organic chamomile with lavender teas (USA, UK), only 31% of Cu were extracted, whereas about 50% of Cu were leached from both peppermint teas (Poland, USA). In the case of Sr and Zn, these values were approximately 35% for all teas. For other elements, the leaching efficiency varied in the range of 10-20%. The extraction efficiency for Al, Fe and Ti in both organic chamomile with lavender teas (USA, UK) did not exceed 10% of their total concentrations.

The quantity of major elements such as Ca, Mg and P (Figure 2) in the water-soluble fraction was relatively high and the extraction effectiveness was in the range of 17.2-26.9, 30.7-37.4 and 34.6-50.2%, respectively. The elements in the water-soluble fraction decreased in order: P > Mg > Ca. In the fraction associated with carbonates, the contents of Ca, Mg and P were lower than in the first fractions and differed markedly between the type of tea. The highest extraction efficiency of Ca, Mg and P (about 27, 33 and 23%, respectively) was observed in both peppermint teas (Poland, USA) and the lowest value (13, 20 and 10%, respectively) were found in organic chamomile with lavender teas (USA, UK). The mobility of these elements in the second fraction decreased in the order Mg > Ca > P.

The reducible and oxidizable fractions (third and fourth steps) contained a considerable amount of Ca (about 20%) in all examined teas. The quantity of Mg in the third step was similar to Ca, while in the fourth step, this value was less than half. The extraction efficiency of P in reducible fraction did not exceed 5% of its total concentrations. This value was much higher for oxidizable fraction and achieved approximately 22%. The relation of these elements followed the order: Ca > Mg > P and P > Ca > Mg, in reducible and oxidizable fractions, respectively.

FTIR and CHNS analysis

Similar absorption bands in FTIR spectra were identified in all the analyzed teas, but their intensities were different (Figure 3a). The broad band centered between 3410-3371 cm^{-1} corresponded to O–H stretching of hydroxyl groups (alcohols, phenols and carboxylic acids) and also of N–H stretching in amines I, II and amides. The peaks between 2920 and 2850 cm^{-1} were due to C–H vibration (stretch) of aliphatic groups. The band at 1750-1620 cm^{-1} was assigned to the C=O vibration of bonded conjugated ketones, aldehydes, quinines and esters. The strong band at about 1640 cm^{-1} was attributed to the C=C vibration of aromatic structures, C=O stretching of amide I and carboxylic acids. Aromatic skeletal vibration of the lignocelluloses absorbed around 1520 cm^{-1}. Another broad band was noted between 1440-1410 cm^{-1} arising from the O–H in plane bend of carboxylic acids, and C–O stretch vibration of carbonates. A peak around 1320 cm^{-1} was attributed to the C–N stretch vibration of aromatic primary and secondary amines. The band around 1250 cm^{-1} was generally linked to C–H stretch and O–H deformation of carboxyl groups and to the N–H of amide II. Another broad band centered around 1050 cm^{-1} was attributed to Si–O–Si silicates, to aromatic ethers and polysaccharides (C–O–C stretch). The bands at 900-600 cm^{-1} correspond to primary and secondary amines and amides.

The infrared spectra of raw teas and the teas after infusion exhibited the same band pattern, indicating that no noticeable qualitative changes occurred during the infusions (Figure 3b).

Table 6 contains the results of CHNS analysis of all investigated teas. The nitrogen content for peppermint teas (Poland, USA) was higher than for organic chamomile with lavender teas (USA, UK). As a result, the elemental ratio of carbon to nitrogen was significantly lower in both peppermint teas. The carbon and hydrogen contents in all teas were comparable. Elemental analysis showed that significant amounts of sulfur were released to infusions. The ratio of H/C in the teas after infusion is higher

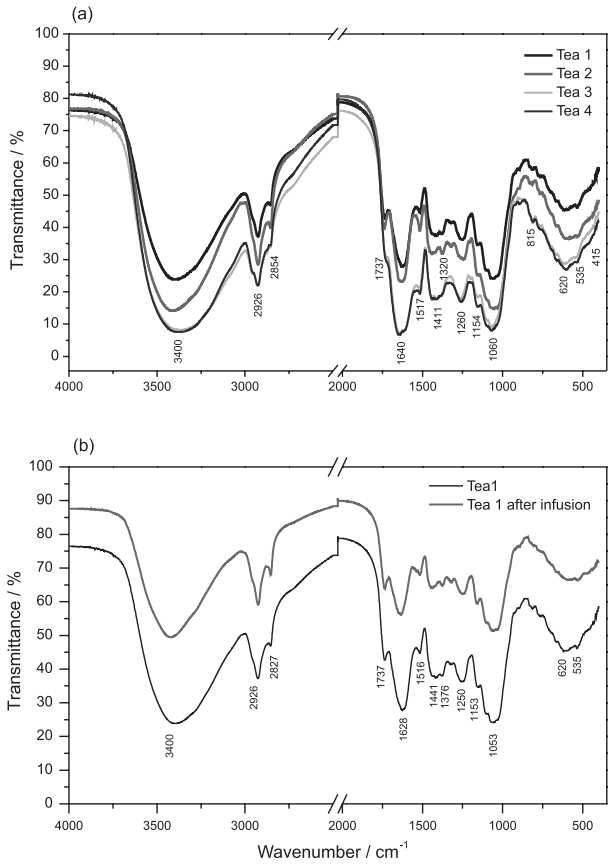

Figure 3. Comparison of FTIR spectra (a) tea 1: Organic Peppermint, USA, tea 2: Peppermint, Poland, tea 3: Organic Chamomile with Lavender, USA, and tea 4: Organic Chamomile & Lavender, UK.; and (b) the tea 1 and its infusion.

than in the raw teas, which indicates that the teas after brewing contain more aliphatic carbons. The percentage of carbon and nitrogen in the teas after infusion is slightly higher than in their raw teas. The O/C and (O + N)/C ratios in the teas after infusion are lower than in the raw teas, which indicates that the infusion process decreased the polarity of tea leaves.[35] A similar relationship was observed during Chinese tea leaf analysis.[35]

Conclusion

The analysis of four commercially available herbal tea products widely distributed in the international markets was presented and according to our best knowledge the peppermint teas (UK, Poland) and chamomile with lavender teas (USA, UK) were investigated for the first time.

Microwave digestion was the preferable method for tea sample preparation prior element measurements by ICP OES. Considering mineral composition of teas, it can be concluded that all of them are considered as a rich source of macroelements (Ca, Mg and P) as well as trace essential elements, i.e., Cu, Fe, Mn and Zn, and free from

Table 6. CHNS analysis of analyzed teas (Organic Peppermint, USA (tea 1), Peppermint, Poland (tea 2), Organic Chamomile with Lavender, USA (tea 3), and Organic Chamomile & Lavender, UK (tea 4))

Element	Content in raw teas / %						
	This study				Lin *et al.*[35]		
	Tea 1	Tea 2	Tea 3	Tea 4	Longjing tea	Yunnang large leaf tea	Huxiang tea
C	42.4	41.8	41.9	44.0	46.6	46.7	45.1
H	5.82	5.78	5.92	6.24	6.46	6.12	6.67
N	3.16	3.19	2.08	1.66	2.36	1.89	2.55
O[a]	48.2	48.7	49.8	47.9	38.7	39.5	43.2
S	0.46	0.52	0.29	0.19	–	–	–
H/C	1.63	1.65	1.68	1.69	1.67	1.57	1.78
C/N	15.7	15.3	23.5	30.9	23.0	28.8	20.6
O/C	0.85	0.87	0.89	0.82	0.62	0.63	0.72
(O + N)/C	0.92	0.94	0.93	0.85	0.67	0.67	0.77
Element	Tea after infusion / %						
	This study				Lin *et al.*[35]		
	Tea 1	Tea 2	Tea 3	Tea 4	Longjing tea	Yunnang large leaf tea	Huxiang tea
C		43.2	43.6		48.3	49.1	47.4
H		6.05	6.31		7.09	6.40	7.03
N		3.61	2.32		2.36	2.46	3.09
O[a]		46.9	47.7		37.4	37.5	38.0
S		0.25	0.10		–	–	–
H/C		1.68	1.72		1.76	1.56	1.78
C/N		14.0	21.9		23.9	23.3	17.9
O/C		0.81	0.82		0.58	0.57	0.60
(O + N)/C		0.88	0.87		0.62	0.61	0.66

toxic elements, including Cd and Pb. Concentrations of the former elements were below limit of detections.

The metal bioavailabilities were significantly different (0.2-87%). The highest percentages of releases from the teas to their infusions were noticed for Mg, P and Cu (up to 60%), while the lowest were devoted to Al, Ba, Fe, Ti and V. The daily intake of all elements from the analyzed herbal tea infusions did not exceed maximum permissible levels, therefore does not constitute a health risk. However, it is absolutely essential to permanently perform a process of control of the plant materials used for human consumption, especially in the case of commonly distributed products.

The BCR extraction procedure exhibited differences in the concentrations of metal bound to reducible and oxidizable fractions. Elements such as Ba and Cu were mostly extracted in oxidizable fractions. Extraction efficiencies for Zn and Sr were significantly lower in reducible fractions. In the case of macroelements (Ca, Mg and P), the extraction efficiencies for all teas were comparable. Al, Cr and Fe were found mainly in residues after extraction.

The N and S contents (CHNS analysis) in peppermint teas were found to be higher than in organic chamomile with lavender teas. In the case of N, the FTIR analysis presented a peak around 1320 cm^{-1} attributed to the C–N stretch vibration of aromatic primary and secondary amines that was quite well visible for the both peppermint teas and not recorded for both chamomile with lavender teas. Elemental analysis showed that sulfur was leached significantly from tea to infusion.

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