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The influence of citric acid on the extraction level of manganese in green and black tea infusions

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Original article

Abstract

Manganese concentration in green and black tea (10 samples of each type) was determined by means of graphite-furnace atomic absorption spectrometry. Both the dry leaves and the infusions were analysed. The concentration of manganese in dry leaves was in the range of 502–1277 mg · kg^{-1} for black tea and 798–1906 mg · kg^{-1} for green one. Since lemon juice is commonly added for tea to enrich its taste, citric acid was used to simulate lemon juice influence on manganese concentration in the infusions. The infusions prepared with and without citric acid addition were analysed and the results showed significant influence of citric acid on manganese leaching. The average extraction levels of manganese from black tea equal 16% (for non-acidified infusions) and 34% (for acidified ones) while these values for green tea equal 13% and 38%, respectively. Statistical evaluation of the results showed that the differences between acidified and non-acidified infusions were statistically significant. High manganese content makes the tea an important source of manganese in human diet.

Keywords

- manganese
- tea
- infusion
- extraction level
- citric acid

Authors contributions

A – Preparation of the research project
B – Assembly of data for the research undertaken
C – Conducting of statistical analysis
D – Interpretation of results
E – Manuscript preparation
F – Literature review
G – Revising the manuscript

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Conflict of interest

None declared.

Introduction

Tea is one of the most popular beverages worldwide with many benefits for human health [1]. It is well-known for its nutritional values – not only does it contain minerals, but it is also an important source of anti-oxidants [2–4]. Several types of tea can be distinguished but all of them origin from the *Camellia sinensis* plant and the differences between the types come from different methods of treatment at the production stage.

Lemon juice is a very common additive, used especially with black tea. Since it lowers the pH, it can facilitate leaching of metal ions from the tea leaves to the infusion. There are studies dealing with the leaching of metals from tea leaves into infusions [2,5–10] but there is no data on how leaching of metals is influenced by lemon juice additive. The leaching percentage of trace elements is also influenced by the tannins content in tea and higher tannins levels diminish the leaching efficiency [11].

Among other elements contained in tea, manganese plays an important role as a micronutrient in human body and is crucial for the proper growth, development and health maintenance [12]. It activates numerous enzymes [13] and it should be provided in sufficient amount with diet. On the other hand, when provided in large quantities, manganese cumulates in brain and can be neurotoxic [14], in severe cases causing parkinsonian-like symptoms – so called manganism [12]. The main risk group includes miners and steelworkers [15] but this disease was also observed in the case of infants fed with manganese-containing food [16].

Tea is an important source of manganese in human diet [14,17]. The main goal of this study was to determine the extraction level of manganese from tea leaves to tea infusions with and without the citric acid addition (which was supposed to simulate the addition of lemon juice). In order to do that, the content of manganese was determined in dry tea leaves and their infusions. The two types of tea: the green tea and the black one were analysed, in order to find possible correlations or significant differences between the two groups. The results were hoped to give more insight into the role of tea as a source of manganese in diet and for this reason, ordinary tea from groceries was used for this research.

Materials and methods

The set of 20 samples of tea was the subject of the study: 10 samples of the black tea and 10 samples of the green one. The tea was purchased in local tea shops and groceries in Tarnow (Poland) in 2020. The details of the samples are presented in Table 1.

Table 1. Tea samples

Tea type	Sample code	Name and brand
Black tea (leaves)	C01	Ceylion – Loyd
	C02	Assam – Tesco
	C03	Yunnan – Bastek
	C04	Earl Grey – Bastek
	C05	Madras – Posti
	C06	Yunnan – Cotterley
	C07	Assam – Lord Nelson
	C08	Black Screw
	C09	Assam TGF09-01 Keyhung
	C10	Pure Ceylon – Big Active
Green tea (leaves)	Z01	Green – Loyd
	Z02	Green – Tesco
	Z03	Green – Bastek
	Z04	Green – Posti
	Z05	Green – Cotterley
	Z06	Pure Green – Big Active
	Z07	Green – Lord Nelson
	Z08	China Chann Mex Moan Palace
	Z09	China Seneka
	Z10	Green – Home Relax

The samples were dried for 2.5 h in 100°C in a laboratory dryer and then stored in dry vials. In order to prepare the samples for the measurements, wet digestion was performed as described below.

Approximately 1 g of each sample (the exact mass was noted for further calculations) was placed in a 250 mL quartz beaker and 30 mL of concentrated nitric acid was poured in. Each beaker was then covered with a quartz watch glass and the samples were gently boiled on a hot plate until the red fumes were no longer given off (approximately 3 hours with occasional nitric acid additions). Next, the solutions were gently evaporated until only few mL were left and they were quantitatively transferred into 100 mL volumetric flasks and filled to the marks with water. For all operations, ultrapure nitric acid (Supelco by Sigma Aldrich, United States) and ultrapure water (18.2 MΩ · cm, Polwater, Poland) were used.

In order to monitor the proper analytical performance of the method, the Certified Reference Material (CRM) as well as blank sample (pure nitric acid, processed like other samples) were included into the analysis.

The tea infusions were prepared in the following way: approximately 1 g of dried tea leaves were put into a quartz beaker and 80 mL of boiling water was poured into it. The mixture was left for 10 minutes and the infusions were quantitatively transferred into a 100 mL volumetric flask and filled to the mark. The infusions with citric acid were prepared in the same way but, in addition, 1.4 mL of 10% citric acid solution was added to each sample. The amount of the citric acid was evaluated in a quick survey involving 10 people, who were asked to squeeze out a typical amount of lemon juice from a lemon, as they would do it with an ordinary tea. Next, the average amount of juice (based on the above survey) was added to one tea infusion and the pH change was noted. Finally, the volume of 10% citric acid solution, which gives the same pH change, was found to be 1.4 mL and this amount was used as an addition for all of the samples in the study. The pH of all infusions was measured.

Measurements

The measurements were performed using Agilent 240Z AA atomic absorption spectrometer (Agilent Technologies, USA) with a graphite-furnace atomization and the Zeeman background correction. As a light source, the hollow-cathode lamp was used and manganese was determined using 279.5 nm analytical line. Argon (99.995%) was used as an inert gas and the ashing and atomization temperature was 1100°C and 2400°C, respectively. As a matrix modifier, palladium nitrate(V) solution was used (1000 mg · L^{-1} of Pd). The calibration curve covered the range of 2–8 μg · L^{-1} Mn and was prepared using 1000 mg · L^{-1} Mn standard solution (Agilent Technologies, USA). The samples were diluted to roughly match the calibration range. The volume of the sample dispensed into the graphite furnace was 9 L and each sample was measured four times.

The pH of the tea infusions was measured using the Elmetron CP-551 pH meter (Elmetron, Poland).

Results and discussion

Quality control

As a quality control, the blank samples and two types of Certified Reference Materials were analysed: the NIES 07 (tea leaves, material by National Institute of Environmental Studies, Japan) and the ESH3 (EnviroMat Ground Water, by SPC Science, Canada). The tea leaves CRM (NIES 07) was used to confirm the accuracy of the whole analytical process: from wet digestion to the AAS measurements. The groundwater CRM (ESH3) was used during the tea infusion measurement, in order to confirm the accuracy of the calibration curve. The results are presented in Table 2 and they confirm sufficient accuracy of the methods. In order to estimate the precision of the method, standard deviation (SD) and relative standard deviation (RSD) were calculated. Low RSD values prove the precision of the method.

Table 2. The results of the quality control (the results are mean values of four replicates)

	Parameter	Mn [mg/kg]	SD*	RSD** [%]
NIES07 (Tea Leaves)	Certified value	700	–	–
	Experimental value	639	14.7	2.3
	Recovery [%]	91	–	–
ESH3 (Ground water)	Certified value	17.25	–	–
	Experimental value (three different samples)	17.3	0.33	1.9
		16.9	0.07	0.4
		18.3	0.90	4.9
	Recovery [%]	91–106	–	–

* SD – standard deviation;
**RSD – relative standard deviation.

Based on the blank samples, the limit of detection and the limit of quantification were calculated and they were more than two orders of magnitude lower than the lowest result in a given group (i.e. tea leaves and infusions).

Manganese in the samples

The concentration of manganese in the dry tea leaves and in the infusions, together with their expanded uncertainties, are presented in Table 3.

Based on the mean values it can be noticed, that green tea leaves contain more manganese than the black ones, although the difference is not substantial. The dispersion of the results is comparable between the two groups and the relative standard deviation equals 25% and 22% for black and green tea, respectively. Except for one sample, manganese concentration in green tea exceeds 1000 mg · kg^{-1}. Similarly, the difference between tea infusions (non-acidified) is also moderate and with higher dispersion of the results (RSD equals 42% and 34% for black and green tea infusions, respectively). When it comes to the acidified infusions, however, the difference is very clear with the green tea containing much more manganese than the black one. It is also interesting that the increase in manganese content in the infusions with a citric acid addition is much higher in case of the green tea than the black one. Comparison of the mean values shows, that the black tea infusions with citric acid contain approximately two times more manganese than without citric acid. In the case of the green tea infusions, the increase is three-fold.

Table 3. Manganese concentration in tea leaves and infusions (the results are mean values of four replicates)

Type	Sample code	Tea leaves		Tea infusions; nonacidified		Tea infusions; acidified	
		Mn [mg/kg]	U_{exp}*	Mn [µg/L]	U_{exp}	Mn [µg/L]	U_{exp}
Black tea	C01	538	25	891	90.7	1422	155
	C02	996	21	1679	90.8	2875	155
	C03	1126	7.2	1567	42.3	4193	354
	C04	932	36	1068	44.2	2983	111
	C05	885	49	1149	126	2658	125
	C06	1052	67	1864	144	4227	188
	C07	827	34	1478	143	3217	143
	C08	1277	81	2908	152	5470	331
	C09	917	47	1776	138	4043	186
	C10	502	23	674	36.4	1365	62.9
		Mean: 905; SD: 228**		**Mean:1506; SD: 632**		**Mean: 3245; SD: 1285**	
Green tea	Z01	1490	76	1963	122	6736	375
	Z02	1729	63	1389	117	7185	503
	Z03	1281	51	1921	162	5821	217
	Z04	1114	60	2121	105	6395	746
	Z05	1906	106	965	39.9	5405	361
	Z06	1561	57	1306	146	5422	538
	Z07	1381	48	2419	123	4399	229
	Z08	798	52	1522	144	3027	278
	Z09	1321	50	947	80.0	4565	272
	Z10	1354	73	2516	96.0	4931	159
		Mean: 1394; SD: 294		**Mean: 1707; SD: 565**		**Mean: 5389; SD: 1233**	

* U_{exp} – expanded uncertainty (coverage factor k = 2);
** SD – standard deviationw.

Table 4 presents the comparison of obtained results with the literature.

Table 4. Comparison of the presented data with the literature

Reference	Mean concentration or range [mg/kg]	
	Black tea	Green tea
Brzezicha-Cirocka, Grembecka, Szefer [18]	117–800	307–1600
Podwika, Kleszcz [19]	1094	660.7
Erdemir [20]	963–1270	1120–1240
Antakli, Sarkis, Al-Check [21]	225–1036	392–1633
Gajewska [22]	525–1078	369–961
Presented research	502–1277	798–1906

The pH of the infusions as well as the extraction levels, calculated as a ratio of the manganese content in the infusion to the content in the dry leaves, are presented in Table 5.

The pH of the infusions is consistent within the groups (low standard deviation) but the pH of the non-acidified green tea infusions is somewhat higher than of the black tea ones. As expected, the extraction levels in acidified infusions are clearly higher than in the non-acidified ones. In case of the black tea, citric acid addition doubles (on average) the extraction levels, while in case of the green tea the difference is by a factor of three.

The extraction level in regular (i.e. non-acidified) infusions was the subject of studies of other authors, too. According to Gajewska et al. [22], extraction level was in a range of 14–37% and 11–67% for black and green tea, respectively. These values overlap with the results from our work. Brzezicha-Cirocka et al. [18] reported extraction levels of 32.9 ± 12.4% for black tea and 29 ± 5.3% for green one which points out that there is no significant difference between these two types of tea. According to Antakli et al. [21], however, the extraction levels were significantly different, although the number of samples tested in that work was very low (27% and 11–18% for black and green tea, respectively; N = 3) so it's difficult to draw a clear conclusion.

According to Mn species research [23], the vast majority of manganese is extracted into the infusions in the Mn (II) form, which is well soluble in water. Citric acid addition may improve the solubility by complex formation which explains the observed phenomenon.

According to the Institute of Medicine [24], the Adequate Intake of manganese for adults (above 19 years old) equals 2.3 mg/day for men and 1.8 mg/day for

Table 5. The pH and the extraction levels of manganese for green and black tea

Type	Sample code	Extraction level [%]		pH	
		Non-acidified	Acidified	Non-acidified	Acidified
Black tea	C01	16.6	27.0	4.29	2.83
	C02	16.7	28.9	4.35	3.00
	C03	14.1	35.5	4.28	3.08
	C04	11.5	31.7	4.44	3.03
	C05	12.7	29.2	4.39	2.98
	C06	17.5	37.9	4.43	2.93
	C07	17.9	37.4	4.39	2.6
	C08	22.6	42.8	4.6	2.56
	C09	19.2	42.8	4.26	2.62
	C10	13.2	27.4	4.33	2.61
Mean		16.2	34.1	4.38	2.82
SD		3.4	6.1	0.10	0.21

Type	Sample code	Extraction level [%]		pH	
		Non-acidified	Acidified	Non-acidified	Acidified
Green tea	Z01	13.4	45.2	4.94	2.67
	Z02	8.0	39.2	4.78	2.75
	Z03	15.3	44.1	4.81	2.71
	Z04	19.0	54.2	4.79	2.83
	Z05	4.8	26.5	4.64	2.72
	Z06	8.0	33.4	4.77	2.82
	Z07	17.5	29.8	4.78	2.97
	Z08	18.5	37.6	4.98	2.91
	Z09	7.2	33.9	4.74	2.68
	Z10	18.2	36.4	4.83	2.84
Mean		13.0	38.0	4.81	2.79
SD*		5.5	8.1	0.10	0.10

* SD – standard deviation.

women. Assuming that the daily consumption of tea equals 0.5 L and taking the average values into account (both the Mn content and the extraction level) one can conclude that tea can be a major source of manganese in diet, as presented in Table 6.

Table 6. Contribution of manganese from tea consumption to the Adequate Intake

	Black tea		Green tea	
	Non-acidified	Acidified	Non-acidified	Acidified
Percent of adequate intake; men	33	71	30	117
Percent of adequate intake; women	42	90	39	150

Although green tea is usually consumed without citric acid addition, even a non-acidified one provides a elatively high amount of manganese, making tea an important source of manganese in diet.

Statistical evaluation of data

In order to find existing correlations among the results, the t-test was performed (each set of data has a parametric distribution). The calculations were performed using Statistica software (by StatSoft). The results are shown in Table 7.

The results show that there is a statistically significant difference between the manganese concentration in black and green tea leaves, which is also reflected in the acidified infusions. In both cases the *p*-value for the *t*-test is much lower than the 0.05 (the most common threshold for the statistical tests). However, non-acidified infusions (without the citric acid addition) show no significant difference in manganese content. The extraction levels comparison between the black and green tea infusions shows no significant difference, either.

On the other hand, citric acid addition has a clear impact on the manganese concentration. In all tested groups, the *t*-test showed significant differences ($p < 0.05$). In the last comparison, all the samples (black and green tea) were treated as one group, and the extraction levels for acidified vs. non-acidified infusions were compared. The result proved to be statistically significant.

Table 7. The results of the t-test for the studied parameters (S – significant; NS – non-signifficant)

	Tested parameters		*P*-values for the *t*-test
Black tea vs. Green tea	Mn in tea leaves BLACK	Mn in tea leaves GREEN	0.00097 (S)
	Mn in infusions (non-acidified) BLACK	Mn in infusions (non-acidified) GREEN	0.46 (NS)
	Mn in infusions (acidified) BLACK	Mn in infusions (acidified) GREEN	0.00129 (S)
	Extraction level (non-acidified) BLACK	Extraction level (non-acidified) GREEN	0.136 (NS)
	Extraction level (acidified) BLACK	Extraction level (acidified) GREEN	0.232 (NS)
Infusions (non-acidified) vs. Infusions (acidified)	Mn in infusions (non-acidified) BLACK	Mn in infusions (acidified) BLACK	0.0012 (S)
	Mn in infusions (non-acidified) GREEN	Mn in infusions (acidified) GREEN	<0.0001 (S)
	Extraction level (non-acidified) BLACK	Extraction level (acidified) BLACK	<0.0001 (S)
	Extraction level (non-acidified) GREEN	Extraction level (acidified) GREEN	<0.0001 (S)
	Extraction level NON-ACIDIFIED (all samples)	Extraction level ACIDIFIED (all samples)	<0.0001 (S)

Conclusion

Due to a relatively high manganese content, tea can be an important source of this element in diet, especially with lemon juice added, which strongly facilitates manganese leaching from tea leaves during preparation of the infusion. Green tea contains more manganese than the black one, both in leaves and infusions. The addition of citric acid increases the extraction level of manganese two-fold for black, and three-fold for green tea. As the difference in manganese content between green and black tea was proved to be statistically significant, it can be concluded that the green tea is a better source of manganese in everyday diet. Even without the addition of a lemon juice, tea covers up to 30–40% of the recommended daily intake of manganese.

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