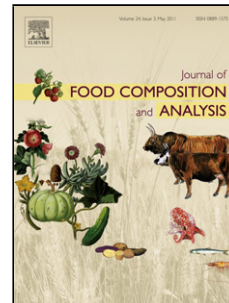# Journal Pre-proof

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Tereza Koláčková (Methodology) (Formal analysis) (Funding acquisition), Daniela Sumczynski (Investigation) (Methodology) (Funding acquisition), Vratislav Bednařík (Methodology) (Formal analysis) (Validation), Štěpán Vinter (Formal analysis), Jana Orsavová (Writing - review and editing), Kateřina Kolofiková (Formal analysis)

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Mineral and trace element composition after digestion and leaching into matcha ice tea infusions (*Camellia sinensis* L.)

Tereza Koláčková[a], Daniela Sumczynski[a,*], Vratislav Bednařík[b], Štěpán Vinter[b], Jana Orsavová[c], Kateřina Kolofiková[a]

a Department of Food Analysis and Chemistry, Tomas Bata University in Zlín, Vavrečkova 275, 760 01 Zlín, Czech Republic

b Department of Environmental Protection Engineering, Tomas Bata University in Zlín, Vavrečkova 275, 760 01 Zlín, Czech Republic

c Language Centre, Tomas Bata University in Zlín, Štefánikova 5670, 760 01 Zlín, Czech Republic

*Corresponding author.

E-mail address: sumczynski@utb.cz (D. Sumczynski).

Highlights

- High contents of K, Mg, Mn, Ca, Fe and Zn were recorded in matcha ice tea.

- Digestibility values of matcha reached 71.3%.

- Element amounts before digestion were K > Mg > Mn > Ca > Fe > Zn > Na > Se > Hg. Element values in non-digestible parts were K > Mg > Mn > Fe ≥ Ca > Zn > Na > Se > Hg.

- Low values of leaching factors for Na (18%), Fe (21%) and Se (37%) were identified.

ABSTRACT

Since matcha includes all leaf parts seems desirable to measure contents of elements before and after digestibility. This paper examines *in vitro* digestibility, mineral and trace element contents after digestion and their leaching ability during matcha ice tea processing. Digestibility values of matcha measured enzymatically reached 71.3%. High contents of K, Mg, Mn, Ca, Fe and Zn were recorded by FAAS/ETAAS. Interestingly, element concentrations in non-digestible parts of matcha decreased in the following order: $K > Mg > Mn > Fe \geq Ca > Zn > Na > Se > Hg$. Matcha ice teas were prepared using three different methods. Significant differences were found not only between the ice tea preparation methods, but also between the tea samples. Low values of leaching factors for Na, Fe and Se were identified (18, 21 and 37%, respectively).

Keywords: Camellia sinensis, in vitro digestibility, mineral and trace element, food composition

1. Introduction

Tea extracts from *Camellia sinensis* L. are commonly applied in the production of non-alcoholic beverages. Tea is generally classified into three main categories according to the oxidation level: black tea (fermented), oolong tea (semi-fermented tea) and green tea (non-fermented) (Senanayake, 2013; Rajchl et al., 2015). Concerning green teas, matcha tea occupies a privileged position due to the consumption of all leaf parts containing significant amounts of digestible and non-digestible bioactive substances. Precisely, it seems desirable to apply established enzymatic-gravimetric methods of digestibility and to monitor the concentrations of nutritional parameters and biologically active substances potentially

available by the human tract (Koláčková et al., 2020). The main constituents of green tea leaves include catechins, caffeine, organic acids, free amino acids, chlorophylls, vitamin C, minerals, fibres, enzymes and other important flavour compounds (Senanayake, 2013; Brzezicha-Cirocka et al., 2016; Dalipi et al., 2018). To date only a negligible number of studies explores contents of mineral and trace elements before and after *in vitro* digestibility. Moreover, the leaching processes from matcha leaves during the preparation of ice teas were not described well. Whereas contents of elements in plants are attributed to soil composition, fertilization, stage of growth and manufacturing processes (Erdemir, 2018; Karak and Bhagat, 2010) it seems to be desirable to analyse samples from different locations, producers etc. Therefore, the aim of this study was to establish basic nutritional characteristics as dry matter and ash contents, mineral and trace element concentrations and values of *in vitro* digestibility of three types of matcha teas. Furthermore, it proposes and investigates three different methods of matcha ice tea preparation to determine mineral and trace element amounts leaching to beverages. Mineral and trace element contents (sodium, magnesium, potassium, calcium, iron, manganese, zinc, selenium, cadmium and lead) were established using flame and electrothermal atomic absorption spectrometry (FAAS/ETAAS). Single-purpose atomic spectrometer AMA-254 was applied in mercury analysis.

2. Materials and methods

2.1 Samples

Three different kinds of matcha tea were selected to be analysed: Japan bio tea (country of origin Japan, bio-certified by OCIA), Allnature premium matcha tea and Wolfberry matcha tea (both originated in China). Commercial matcha tea samples were purchased in health food

stores in Zlín (CZ) in 2019 with their expiration dates of 2019 or 2020. They were stored in the original 0.25–1.00 kg packaging out of the sunlight in the air-conditioned laboratory (23±2°C) not longer than two weeks prior to the analyses.

2.2 Sample preparation

In case of powder matcha tea samples (in both forms before and after digestibility), 5 mL of 67% ultrapure HNO_3 and 1 mL of 30% ultrapure H_2O_2 (Analytika, Brno, CZ) were added to each sample (0.2 g) into teflon vessels. Then, the samples were decomposed by a microwave system Milestone Ethos One (Sorisole, Italy) with the parameters set as follows: 15 min, 200°C and 1500 W. After being cooled, the final volume was adjusted to 25 mL using ultrapure 18.2 MΩcm water (Purelab Classic Elga system, Labwater/VWS, London, UK) (Koláčková et al., 2020).

Matcha ice tea infusions were prepared using three different proposed methods (Table 1). Two grams of powder matcha teas were weighed and transferred into 250-mL plastic volumetric flasks. Then, 150 mL of ultrapure water were added and the mixture was allowed to infuse for the appropriate time while being stirred in a blender. Thereafter, 100 g of ice cubes were added to each sample and flasks were filled with ultrapure water to the mark. Finally, 20 mL of each sample were filtered through fluted paper filter and the obtained filtrate was transferred into plastic test tubes with 5 mL of 67% HNO_3 (Analytika, Brno, CZ). Each sample was analysed six times.

2.3 In vitro digestibility assay

Dry matter and ash contents, as a necessary parts of digestibility assay, were determined according to ISO 1573 (1980) and ISO 1575 (1980) methods employing *in vitro* digestibility values. *In vitro* digestibility of matcha teas in a powder form was established using enzymatic-gravimetric method in two consecutive digestion phases. First, the matcha samples were incubated in Daisy[II] incubator (Ankom Technology, MA, USA) in 0.1 M HCl with pepsin (E.C. 3.4.23.1) for 2 hours at 40°C in special sacks (type 57, Ankom Technology, MA, USA). Then, the samples were washed with redistilled water and re-incubated in phosphate buffer (pH 7.45) containing pancreatin (a mixture of protease – 350 FIG-U/g, amylase – 7500 FIG-U/g and lipase – 6000 FIG-U/g) for 24 hours (all enzymes were purchased from Merck, Darmstadt, Germany). After digestion, samples were rinsed with redistilled water and maintained in the oven (BMT Medical Technology, Brno, CZ) at 103°C for 24 hours and subsequently burned in a muffle furnace (LAC, Židlochovice, CZ) at 550°C for 5 hours, cooled and weighted (Koláčková et al., 2020). Organic matter digestibility (OMD) and dry matter digestibility (DMD) were calculated using these formulas (Mišurcová, 2008):

$$DMD(\%) = 100 - \frac{100 \times DMR}{m_2 \times DM}$$

$$OMD(\%) = 100 - \frac{(DMR - AR)}{m_2 \times DM \times OM} \times 100$$

$$DMR = m_3 - m_1 c_1$$

$$DM = \frac{DW \times m_s}{100}$$

$$AR = m_4 - m_1 c_2$$

$$OM = \frac{DW - A}{100}$$

$$c1 = \frac{ms}{m1}$$

$$c2 = \frac{mp}{m1}$$

where DMD denotes dry matter digestibility (%), OMD organic matter digestibility (%), DMR weight of the sample without the weight of the sack after digestion and drying (g), DM dry weight of the sample (g), DW dry weight of the sample (%), AR ash weight of the sample with the sack (g), OM organic matter content in dry matter of the samples (g), A ash content in the sample (g), m_s weight of the sample applied in dry matter determination (g), c_1 correction of the sack weight after the incubation (g), c_2 correction of the sack weight after the combustion (g), m_p weight of ash from the empty correction sack (g), m_1 weight of empty sack (g), m_2 weight of the sample (g) and finally, m_3 and m_4 weights of both the sample and sack after drying and combustion (g).

2.4 Determination of minerals and trace elements using FAAS/ETAAS method

Mineral and trace element contents were measured in a powder form of matcha tea and non-digestible parts of matcha samples. Determination of elements in ice tea samples was executed as well. Selected elements were identified using a high-resolution continuum source atomic absorption spectrometer ContrAA 800 (Analytik Jena AG, Jena, Germany) equipped with a xenon short-arc lamp. Electrothermal atomization was applied in determination of Cd and Pb in a graphite cuvette with the detection at 228.8018 and 283.3060 nm, respectively. The concentration ranges of the calibration solutions for Cd and Pb were 5–10 μg/L and 12.5–50.0 μg/L, respectively. Other minerals and trace elements (Na, Mg, K, Ca, Mn, Fe, Zn and Se) were subjected to atomization in acetylene-air flame. FAAS conditions are described in Table 2. All calibration standard solutions were prepared using 1 g/L primary certified standard solutions (Analytika, Brno, CZ). Reliability of the method was verified using

certified reference materials of tea leaves INCT-TL-1 (Institute of Nuclear Chemistry and Technology, Poland) and lichen (International Atomic Energy Agency (Vienna, Austria). The results of CRM measurements are shown in Table 3. Digestion of these materials was established by the same decomposition procedure as it was applied for powder matcha tea (Welz, 2005). The recoveries of the studied elements ranged between 88 and 109 %. Limits of the quantification (LOQs) of the method applied for minerals and trace elements were as follows: Cd (1.00 μg/g), Pb (1.00 μg/g), Na (1.50 μg/g), Mg (170 μg/g), K (130 μg/g), Ca (18 μg/g), Mn (50 μg/g), Fe (6.00 μg/g), Zn (5 μg/g) and Se (0.15 μg/g). LOQs were established according to Konieczka and Namieśnik (2009).

2.5 Mercury determination

Mercury content was determined using a single-purpose atomic absorption spectrometer AMA-254 (Advanced Mercury Analyzer, Altec, Prague, CZ) under these conditions: weight of the sample was 0.1 g, volume of the ice-tea sample 0.1 mL, drying time 60 s, decomposition time 150 s and waiting time 45 s. The range of calibration was from 1 to 850 ng of Hg standard (Hg in 2% HNO_3, Czech Metrology Institute, Olomouc, Czech Republic) per the analysed sample. The weight of mercury (ng) in the analysed volume of the sample was converted to the concentration in the original solid sample applying the formula:

$$C_{Hg} = \frac{(m_{Hg} - BL).f_D.V_0}{(V_A.m_0)}$$

where C_{Hg} is the concentration of Hg in the initial solid sample (ng/g), m_{Hg} mass of Hg in the analyzed sample (ng), BL mass of Hg in a blank sample (ng), f_D dilution factor, V_0 the total

volume of the sample solution (mL), V_A volume of the analysed sample and m_0 weight of the initial solid sample (g) (Száková et al., 2003).

2.6 Statistical analysis

All analyses were repeated 5-6 times and the results were expressed as mean ± standard deviation in dry weight basis. Dean-Dixon test and one-way analysis of variance (ANOVA) were applied. Subsequently, Tukey´s test was applied to identify the differences among the means. The level of significance was 5%.

3. Results and discussion

3.1 Results of in vitro digestibility assay

In Table 4, dry matter contents of matcha teas were recorded in the range from 96.2 to 97.3 %. According to the Ministry of Agriculture (CZ), moisture content for green tea leaves should not exceed 10% (Reg. No 330, 1997) as the moisture content is a major quality parameter. All the samples analysed in this study showed statistically significant differences in ash contents representing total mineral content of matcha tea. ISO 11287 (2011) defined 4 and 8 % as the minimal and maximal ash level for green tea leaves. In addition, the study provided by Topuz et al. (2014) indicated positive relationships between ash content and quality of green teas and proposed that ash content should be less than 5.54 % in order to maintain a good tea quality during the storage. However, the quality requirements for matcha tea have not been defined yet. As displayed in Table 4, OMD and DMD values reached 71.3 and 69.8%, respectively. Even though accessible data discussing digestibility values of

matcha teas is limited, the previous study reported OMD and DMD values of matcha teas of 69.7 and 66.0%, respectively (Koláčková et al., 2020). Furthermore, it is known that low digestibility values positively correlated with higher fibre contents and weak positive correlation between high protein content and digestibility values of matcha was observed (Koláčková et al., 2020). Moreover, Rehman et al. (2002) postulated that protein digestibility was greatly reduced if tannins from teas were included in diet as they decrease enzyme secretion in small intestine.

3.2 Minerals and trace elements evaluation

Concentrations of elements in leaves reflect local environmental conditions including a growth phase, soil composition, fertilization and processing procedures (Erdemir, 2018; Karak and Bhagat, 2010; Koch et al., 2018; Brzezicha-Cirocka et al., 2016). Since all leaf parts of matcha tea are consumed, many factors, including leaching processes of minerals and trace elements, digestibility and bioavailability, must be considered when evaluating its dietary intake levels (Koláčková et al. 2020; Dalipi et al., 2018). To assess mineral and trace element contents in non-digestible parts of matcha, determination of elements has been provided before and after *in vitro* digestibility.

Values of mineral and trace element contents established both in powder types of matcha samples and their non-digestible parts are presented in Tables 5 and 6. Na concentrations in matcha was 30.5 μg/g which was much lower than 123 μg/g published by Koch et al. (2018) and 160 μg/g presented by Yüksel et al. (2017). The K content (9.38– 9.71 mg/g) in matcha is higher than Mg value (2.68 mg/g), while Mg value is higher than Mn one (1.96–1.55 mg/g). Research results show that K content in matcha can reach 10.7 mg/g (Koch et al., 2018). Concerning green teas, Mn concentrations up to 1.4 mg/g have been recorded

(Szymczycha-Madeja et al., 2015) which is consistent with our study (1.16–1.55 mg/g). This study reveals high contents of Mg which is in accordance with the results published by Koch et al. (2018) and Erdemir (2018). Se and Hg levels (3.32–3.37 μg/g and 240–410 ng/g, respectively) have not shown statistically significant differences between their values. These results are in contrast with the values measured for Zn (30.0–40.5 μg/g) and Fe (70.0–220 μg/g). Potentially toxic elements, such as Pb, Cd and Hg, originating from the environment pollution can accumulate in leaves and emerge during tea manufacturing processes. Cd is considered to be a nephrotoxin and Pb is harmful to the nervous system (Dalipi et al., 2018). It should be highlighted that Cd and Pb concentrations were under their LOQs. Literature data discussing Hg values in matcha has been limited. Nonetheless, Kabata-Pendias (2011) identified the range of Hg concentration in tea leaves from 34.0 to 46.0 ng/g.

It is generally known that bioavailability of elements plays an important role (Dalipi et al., 2018). As matcha infusion contains all leaf parts, it is important to monitor leaching of the individual elements into the infusion and their contents in both digestible or indigestible portions as well. Data examining mineral and trace element amounts in non-digestible parts of matcha is limited as well. This research has shown that the concentrations of elements in non-digestible parts of matcha decreased in the following order: $K > Mg > Mn > Fe \geq Ca > Zn > Na > Se > Hg$. It is important to take into account that the rest contents of individual elements in non-digestible parts of matcha depend on the ability to be leached and digested by human tract and also on their original concentrations established in a powder form before digestibility. Differences in resulting mineral and trace element contents strongly reflect the geographical origin of tea samples and conditions of their cultivation (Brzezicha-Cirocka et al., 2016). Data obtained in this study describing non-digestible parts of matcha provides a new perspective on the contributions of individual elements to RDA (recommended daily allowance) or AI (adequate intake). Additionally, determining provisional tolerable weekly

(PTWI) and monthly intakes (PTMI) from digestible parts of matcha tea may be important for the future research.

Since data investigating leachability of elements in matcha ice teas is scarce, these results could be compared with the report of Brzezicha-Cirocka et al. (2016). When boiling water and infusion time of five minutes were applied to the green tea leaves, the values of leaching for individual elements decreased in this order: K (54.2–66.3%), Mg (26.8–40.0%), P (22.5–40.1%), Mn (24.2–31.4%) and Fe (7.85–13.4%). According to Brzezicha-Cirocka et al. (2016), the percentage of Na leaching from green tea leaves varied from 15.1 to 43.7%. In this study, leaching factor for Na from matcha into solutions of ice teas was relatively low, maximally 18% in method 3. Conversely, the percentages of leaching for K and Mg were 93 and 85 % in methods 2 and 1, respectively (Fig. 1). The results of this study could be compared with the investigation provided by Dambiec et al. (2013) where K was extracted to the infusion with the greatest extent which contrasted with the lowest percentage of Na leaching. Lower and comparable percentages of leaching in ice teas were recorded for Se, Fe and Mn (37, 21 and 19% in method 3, respectively). According to literature, tea infusions were characterized with low Cd contents which is consistent with this study published by Karak and Bhagat (2010). Low Fe leaching is explained by the formation of low soluble complexes. Tannic acid, tannins and the precipitation of metal chelates also decrease metal concentrations in infusions (Brzezicha-Cirocka et al., 2016). Tannic acid and tannin concentrations in matcha ice tea should be evaluated as these values correlate with leaching factors to assess the percentage of leaching of particular elements to infusions. Furthermore, some elements transferred into infusions depend on whether the compound is bound weakly to the organic matrix or less soluble in the solution (Dalipi et al., 2018). Extraction efficiency values are affected by further factors, such as pH, water temperature, time of extraction and ionic strength.

4. Conclusion

This study investigates dry matter and ash contents, digestibility, mineral and trace element contents before and after *in vitro* digestibility and also their leaching ability during matcha ice tea processing. Dry matter and ash contents of matcha were measured in the range 96.2–97.3 % and 4.58–5.23 %, respectively. Digestibility values of matcha teas reached 71.3%. This research has shown that concentrations of elements in matcha before digestion decreased in the following order: K > Mg > Mn > Ca > Fe > Zn > Na > Se > Hg ; and the values of Cd and Pb concentration were below their LOQs. Considering non-digestible parts of matcha tea, element concentrations decreased in this order: K > Mg > Mn > Fe ≥ Ca > Zn > Na > Se > Hg. Furthermore, this study has also proposed three different methods of matcha ice tea preparation and established leachability values for individual elements. Significant differences have been found between the ice tea preparation methods and also tea samples. Leaching factor for Na from matcha into ice tea solutions was relatively low (18%), as well as for Fe and Se (21 and 37%, respectively). Data examining non-digestible parts of matcha may provide new perspectives for producers and consumers not only by setting daily doses of matcha ice tea, but also by introducing RDA (recommended daily allowance) or AI (adequate intake) values of individual elements. What is more, evaluating the provisional tolerable weekly (PTWI) and monthly intakes (PTMI) from digestible parts of matcha tea may be important for the future research. Furthermore, an extended research will be performed employing more matcha samples and ICP-MS method to examine a wider range of elements.

CRediT authorship contribution statement

Koláčková Tereza: Methodology, Formal analysis, Funding acquisition. **Sumczynski Daniela:** Investigation, Methodology, Writing, Funding acquisition. **Kolofiková Kateřina:** Formal analysis. **Bednařík Vratislav:** Methodology, Formal analysis, Validation. **Vinter Štěpán:** Formal analysis. **Orsavová Jana**: Writing – review & editing.

Conflict of interest

The authors declare no competing financial and personal interest.

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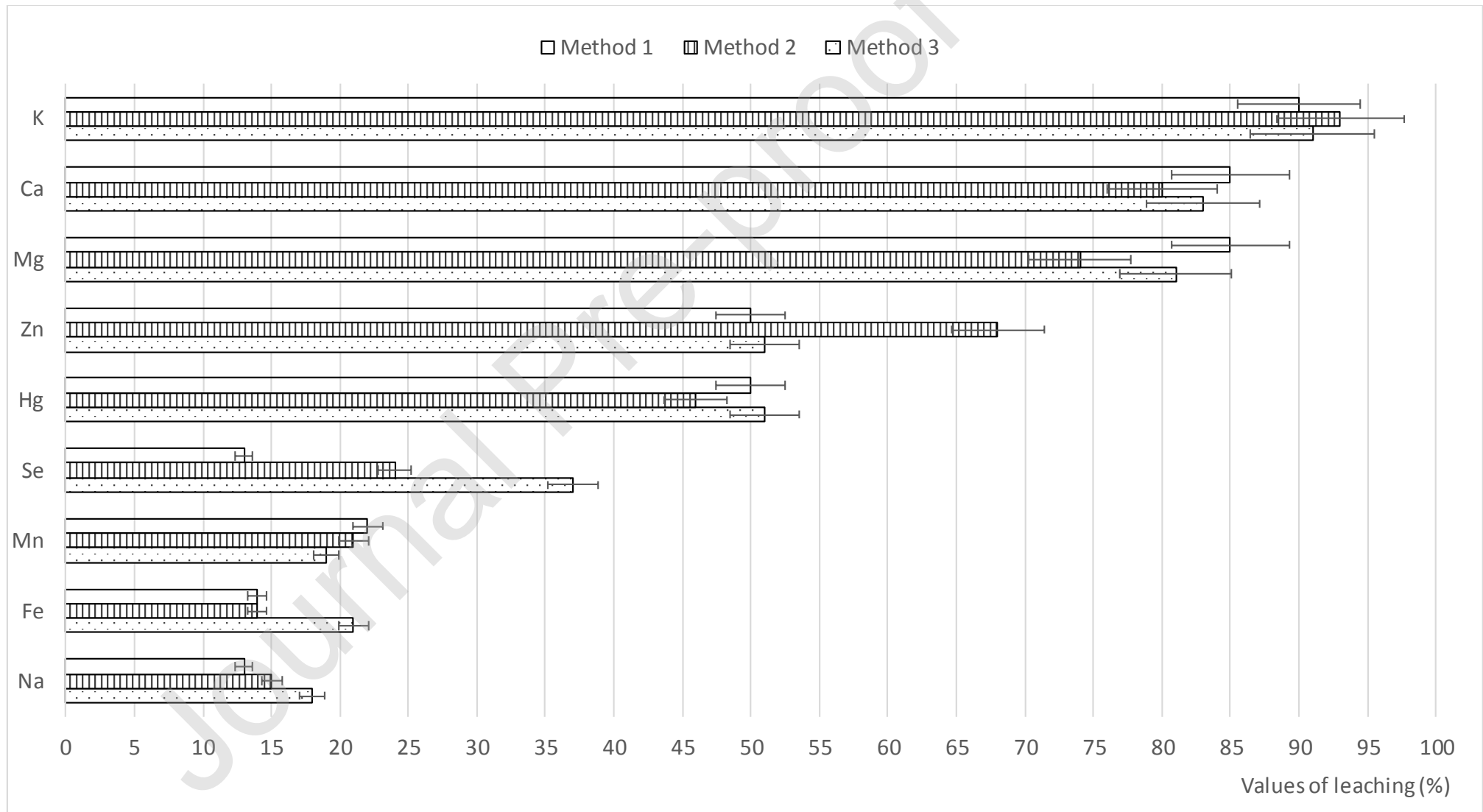
Fig 1. Maximum values of leachability of minerals and trace elements from matcha ice tea based on the method of preparation

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Table 1

Preparation of matcha ice teas.

Methods	Weight of matcha (g)	Temperature of water (°C)	Volume of water (mL)	Time of infusion	Weight of ice cubes (g)
1	2.00	70	150	5 minutes	100
2	2.00	20	150	15 minutes	100
3	2.00	20	150	10 hours in the fridge at 10°C	100

Table 2

Parameters used in FAAS analysis of the selected elements.

Elements	Wavelength (nm)	Air flow (L/h)	Acetylene flow (L/h)	Burner height (mm)	Calibration range (mg/L)
Na	330.2370	400	60	6	0–15
Mg	202.5820	400	70	6	0–30
K	404.4140	400	80	8	0–150
Ca	422.6728	400	80	6	0–15
Mn	280.1081	400	80	6	0–10
Fe	248.3270	400	60	6	0–10
Zn	213.8570	400	50	6	0–2
Se	196.0267	400	70	6	0–10

Table 3

The obtained values of certified materials measured by using FAAS/ETAAS method.

Reference materials	Elements	Analysed values[a] (mg/kg)	Reference values[a,b] (mg/kg)	Recovery (%)
Tea leaves (INC-TL-1)	Mg	2340±45	2240 ± 17	104
	Mn	1520±35	1570 ± 11	97
	Na	25.5±4.8	24.7 ± 3.2	103
	Zn	37.9±0.5	34.7 ± 2.7	109
Lichen (IAEA-336)	Fe	410±13	430 ± 50	95
	K	2000±55	1840 ± 200	108
	Mn	55.4±1.8	63.0 ± 7.0	88
	Na	333±29	320 ± 40	104
	Zn	33.1±1.4	30.4 ± 3.4	109

[a] Mean (mean value of five measurements) ± standard deviation.

[b] Confidence interval 95%.

Table 4

Results of dry matter and ash contents and *in vitro* digestibility values.

Matcha	Dry matter (%)	Ash (%)	DMD (%)	OMD (%)
Japan bio tea	97.3 ± 0.4^a	5.23 ± 0.03^a	67.2 ± 0.3^a	68.7 ± 0.1^a
Allnature	96.4 ± 0.3^b	4.73 ± 0.05^b	69.3 ± 0.3^b	70.8 ± 0.4^b
Wolfberry	96.2 ± 0.4^b	4.58 ± 0.04^c	69.8 ± 0.5^b	71.3 ± 0.5^b

Results are presented in dry weight basis as means ± SD, n=5 (the mean of five measurements). Means within a column with at least one

identical superscript do not differ significantly ($P{\geq}0.05$) while means with various superscripts show a significant difference ($P{<}0.05$).

DMD – Dry matter digestibility, OMD – Organic matter digestibility.

Table 5

Results of mineral and trace element contents in a powder form of matcha teas before and after *in vitro* digestibility.

Elements in matcha	Japan bio tea	Allnature	Wolfberry	Elements in non-digestible part of matcha	Japan bio tea	Allnature	Wolfberry
Na (µg/g)	20.0±0.1[a]	30.1±0.2[b]	30.5±0.2[c]	Na (µg/g)	5.01±0.04[a]	6.63±0.05[b]	6.40±0.03[c]
Mg (mg/g)	2.68±0.08[a]	2.21±0.05[b]	1.96±0.04[c]	Mg (mg/g)	0.50±0.03[a]	0.41±0.03[b]	0.84±0.02[c]
K (mg/g)	9.71±0.20[a]	9.63±0.10[b]	9.38±0.12[c]	K (mg/g)	1.02±0.05[a]	0.96±0.05[a]	0.88±0.04[b]
Ca (µg/g)	760±20a	680±12[b]	615±10[c]	Ca (µg/g)	87.2±1.2[a]	80.3±1.0[b]	85.2±1.2[a]
Fe (µg/g)	70.0±1.0[a]	220±5[b]	165±5[c]	Fe (µg/g)	35.4±1.5[a]	120±5[b]	90.1±2.0[c]
Mn (mg/g)	1.16±0.01[a]	1.55±0.03[b]	1.50±0.10[b]	Mn (µg/g)	200±20[a]	220±10[b]	205±10[a]
Zn (µg/g)	30.0±0.1[a]	40.5±0.2[b]	30.4±0.2[b]	Zn (µg/g)	17.4±0.3[a]	20.0±0.3[b]	18.9±0.4[c]
Se (µg/g)	3.33±0.10[a]	3.37±0.10[a]	3.32±0.10[a]	Se (µg/g)	2.57±0.10[a]	2.70±0.04[b]	2.61±0.10[a]
Cd (µg/g)	< 1.00	< 1.00	< 1.00	Cd (µg/g)	< 1.00	< 1.00	< 1.00
Pb (µg/g)	< 1.00	< 1.00	< 1.00	Pb (µg/g)	< 1.00	< 1.00	< 1.00

| Hg (ng/g) | 240 ± 10^a | 310 ± 20^a | 410 ± 30^a | Hg (ng/g) | 36.1 ± 0.3^a | 38.4 ± 1.0^b | 40.8 ± 1.0^c |

Results are presented in dry weight basis as means ± SD, n=6 (the mean of six measurements). Means within a line with at least one identical superscript do not differ significantly ($P \geq 0.05$) while means with various superscripts show a significant difference ($P < 0.05$).

LOQ: Cd and Pb 1.00 µg/g.

Table 6

Results of mineral and trace element contents in matcha ice teas and their values of leaching.

Elements	Method No.	Japan bio tea	Allnature	Wolfberry	Values of leaching (%)
Na (μg/g)	1	2.59±0.04[a,A]	3.35±0.05[b,A]	1.95±0.05[c,A]	6–13
	2	2.92±0.03[a,B]	2.24±0.04[b,B]	2.89±0.04[a,B]	7–15
	3	3.61±0.05[a,C]	2.60±0.04[b,C]	2.96±0.03[c,C]	9–18
Mg (mg/g)	1	2.27±0.02[a,A]	1.78±0.04[b,A]	1.64±0.02[c,A]	81–85
	2	1.97±0.03[a,B]	1.37±0.02[b,B]	1.45±0.02[c,B]	62–74
	3	2.03±0.05[a,C]	1.44±0.04[b,C]	1.58±0.04[c,C]	65–81
K (mg/g)	1	8.42±0.10[a,A]	8.55±0.10[b,A]	8.40±0.08[a,A]	87–90
	2	8.68±0.12[a,B]	8.40±0.10[b,B]	8.73±0.12[a,B]	87–93
	3	8.80±0.10[a,C]	7.99±0.11[b,C]	8.43±0.10[c,A]	83–91
Ca (μg/g)	1	640±5[a,A]	572±5[b,A]	525±10[c,A]	84–85
	2	515±5[a,B]	515±7[a,B]	490±5[b,B]	68–80

	3	$630\pm10^{a,C}$	$530\pm10^{b,C}$	$480\pm5^{c,C}$	78–83
Fe (µg/g)	1	$10.0\pm0.1^{a,A}$	$15.0\pm0.2^{b,A}$	$15.2\pm0.2^{b,A}$	7–14
	2	$10.0\pm0.1^{a,A}$	$20.0\pm0.3^{b,B}$	$20.0\pm0.2^{b,B}$	9–14
	3	$15.0\pm0.2^{a,B}$	$10.5\pm0.2^{b,C}$	$15.0\pm0.2^{a,A}$	5–21
Mn (µg/g)	1	$250\pm10^{a,A}$	$170\pm5^{b,A}$	$180\pm8^{c,A}$	11–22
	2	$240\pm7^{a,A}$	$223\pm10^{b,B}$	$225\pm6^{b,B}$	14–21
	3	$220\pm8^{a,B}$	$224\pm8^{a,B}$	$182\pm4^{b,A}$	12–19
Zn (µg/g)	1	$10.1\pm0.1^{a,A}$	$15.0\pm0.2^{b,A}$	$15.2\pm0.2^{b,A}$	34–50
	2	$20.4\pm0.2^{a,B}$	$15.2\pm0.4^{b,A}$	$19.5\pm0.3^{c,B}$	37–68
	3	$15.2\pm0.3^{a,C}$	$10.6\pm0.2^{b,B}$	$10.4\pm0.3^{b,C}$	26–51
Se (µg/g)	1	$0.20\pm0.02^{a,A}$	$0.41\pm0.03^{b,A}$	$0.42\pm0.02^{b,A}$	6–13
	2	$0.80\pm0.05^{a,B}$	$0.75\pm0.03^{b,B}$	$0.52\pm0.02^{c,B}$	16–24
	3	$1.10\pm0.10^{a,C}$	$1.25\pm0.12^{b,C}$	$1.20\pm0.07^{a,b,C}$	33–37
Cd (µg/g)	1	< 1.00	< 1.00	< 1.00	
	2	< 1.00	< 1.00	< 1.00	
	3	< 1.00	< 1.00	< 1.00	

Pb (μg/g)	1	< 1.00	< 1.00	< 1.00	
	2	< 1.00	< 1.00	< 1.00	
	3	< 1.00	< 1.00	< 1.00	
Hg (ng/g)	1	120±5[a,A]	154±5[b,A]	166±6[c,A]	40–50
	2	110±3[a,B]	132±4[b,B]	145±5[c,B]	36–46
	3	123±4[a,A]	153±6[b,A]	170±5[c,A]	41–51

Results are presented in dry weight basis as means ± SD, n=6 (the mean of six measurements). Means within a line with at least one identical small superscript (in case of individual samples) do not differ significantly ($P \geq 0.05$) while lines with various small superscripts show a significant difference ($P < 0.05$). Means within a column with at least one identical capital letter superscript (in case of leaching methods) do not differ significantly ($P \geq 0.05$) while columns with various capital letter superscript show a significant difference ($P < 0.05$).

LOQ: Cd and Pb 1.00 μg/g.