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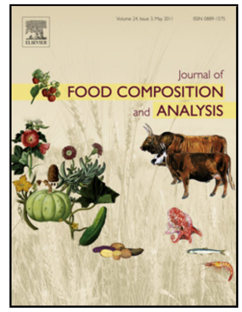

Free and bound amino acids, minerals and trace elements in matcha (*Camellia sinensis* L.): A nutritional evaluation

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Free and bound amino acids, minerals and trace elements in matcha (*Camellia sinensis* L.): A nutritional evaluation

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Highlights

- The study provides an overview of amino acids, minerals and trace elements in matcha.
- The amino acid score (AAS) of matcha (40.2%) is comparable to wheat protein.
- Ile and Thr were evaluated as limiting amino acids.
- Matcha contained substantial amounts of Mn, Cu, Fe, Mg and Zn.
- A daily portion of 5 g of matcha should not pose a health risk.

ABSTRACT

This study provides an overview of free and bound amino acids, minerals and trace elements content in matcha including evaluation of their dietary intakes. The analyses employed IEC and ICP-MS methods. Theanine followed by Glu, GABA, Thr and Me were the most abundant free amino acids. Considering bound amino acids, Glu, Asp, Leu, Lys, Arg and Val were the most frequent. The amino acid score (AAS) for matcha (40.2%) is comparable to the AAS for wheat and sunflower proteins. Ile and Thr were evaluated as limiting amino acids. Regarding recommended daily allowance (RDA), the contributions of Cys and Met were up to 8%. Matcha is contributor to Adequate Intake (AI) or RDA for males in the following order: Mn (up to 15%) > Cu > Fe (up to 7%). Similarly, for females, matcha contributes to RDA or AI values in this order: Mn (up to 19%) > Cu > Zn (up to 5%). It has not been proved that matcha is a significant source of Se and Cr. A daily serving portion of 5 g does not contribute to PTWI (Provisional tolerable weekly intake) and PTMI (Provisional tolerable monthly intake) for Al, Sn, Cd and Hg.

Keywords: Food analysis, Food composition, Matcha, Free and bound amino acid, Mineral and trace element, Dietary intake evaluation, Amino acid score, Nutrition

1. Introduction

Tea (*Camellia sinensis* L.) is a popular non-alcoholic beverage with significant contents of physiological and pharmacological metabolites affecting human health. These metabolites play an important role as antioxidants and possess stress-reducing functions with anti-inflammatory and neurological effects (Jeszka-Skowron et al., 2015; Das et al., 2019; Jiang et al., 2019). Particularly yang green tea leaves contain many bioactive compounds, such as catechins, caffeine, chlorophylls, amino acids, minerals and trace elements (Erdemir 2019; Brzezicha-Cirocka et al., 2016a; Brzezicha-Cirocka et al., 2016b; Milani et al., 2016).

Matcha tea is fine-powdered, non-oxidized and unfermented green tea produced from green tea leaves protected from sunlight using the unique shading technique. Compared to other kinds of tea, matcha is consumed in a form including all leaf parts containing substances that could be characterized as digestible/non-digestible in gastric tract (Topuz et al., 2014; Xu et al., 2016). Its chemical composition is affected by tea processing, geographical origin and agricultural practice. Given protection from sunlight, matcha has a high concentration of caffeine, chlorophylls and amino acids providing a higher level of umami taste. Generally, high levels of free amino acids in green tea is associated not only with umami taste but also with its quality; bound amino acids are constituents of tea proteins with their own nutritional significance (Zhu et al., 2016). Minerals, as well as essential and toxic trace elements, have attracted public attention regarding their possible impact on human health. However, their dietary intakes require monitoring to establish their safety. Even though studies on matcha phenolic composition, antioxidant activities and chlorophyll content exist (Jeszka-Skowron et al., 2015; Topuz et al., 2014; Xu eta al., 2016), precise data for amino acids and trace elements composition including their daily intakes has been insufficient. Since matcha is considered to be a new health-beneficial food, a growing focus on nutritional composition of matcha has been noted.

This study assesses the amino acid profile of matcha in both free and bound forms and determines the content of minerals and trace elements by ion-exchange chromatography (IEC) and inductively coupled plasma mass spectrometry (ICP-MS). This data was used to calculate the amino acid score (AAS), essential amino acid index (EAAI) and to evaluate the limiting amino acids (LAA). Subsequently, appropriate contributions to the recommended dietary allowance (RDA), adequate intake (AI), provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) have been established.

2. Material and method

2.1 Materials and reagents

In total, ten different kinds of matcha teas were analysed: eight of them were produced in Japan (Harmony, Don Matcha, Asagiri, Whittard, Mo Cha Fen, Royal Pharma, Day spa premium and Day spa organic); Shao Xing and Jeju originated from China and Korea, respectively. Shao Xing, Asagiri and Jeju matcha were provided by Oxalis (Slušovice, Czech Republic), while other commercial matcha samples were bought in health-food stores in 2017 and 2019. Samples of matcha teas were kept in their original packaging out of sunlight, no longer than four weeks prior to analysis.

Ninhydrin, methylcellosolve, acetate buffer (pH 5.5) and hydrindantin were bought from Ingos (Prague, Czech Republic) as a kit for ninhydrin reagent. Citric acid monohydrate, sodium citrate dihydrate, thiodiglycol, lithium citrate and sodium azide were also purchased from Ingos (Prague, Czech Republic). All amino acid standards were obtained from Sigma Aldrich (St. Louis, MO, USA). ICP-MS 19-standards (Be, Zn, Cu, Ni, Al, Ga, Mg, Co, Li, Ag, Mn, Sr, Ba, Tl, Ce, Cs, Ho, Ta and U), ICP-MS 13-standards (As, Ca, Cd, Cr, Fe, Hg, K, P, Na, Pb, Se, Sn and Ti) and ICP-MS internal In and Rh-standards were purchased from Analytika (Prague, Czech Republic). Ar and He were obtained from Linde Gas (Zlín, Czech Republic) and ultrapure water was supplied by Purelab Classic Elga water system (Labwater/VWS Ltd., London, UK). Certified reference materials (CRM) of Metranal®8 and NIST rice flour 1568b were purchased from Analytika Ltd. (Prague, Czech Republic), CRM tea leaves INCT-TL-1 were supplied by the institute of Nuclear Chemistry and Technology (Warsaw, Poland) and CRM lichen was bought via International Atomic Energy Agency (Vienna, Austria). Tune 7 and 8 solutions were purchased from Analytika (Prague, Czech Republic).

2.2 Basic chemical analysis

Determination of dry matter and ash content was performed according to the ISO 1573 (1980) and ISO 1575 (1980) methods. Total nitrogen determination was accomplished using the Kjeldahl method; nitrogen content was multiplied by factor of 6.25 and expressed as crude protein.

2.3 Determination of free amino acids using IEC

To determine free amino acids, the samples (0.4 g) were accurately weighted into vials. Four millilitres of 0.6 M perchloric acid were added to shaken for 30 min and centrifuged at 12300 × *g* (Velocity 13 μ; Dynamica Scientific Ltd., Newport Pargnell, UK). The extraction procedure was repeated three times, when 2.5, 2.5 and 0.6 mL of 0.6 M perchloric acid was added. Then the 10 mL-volumetric flask was filled up using 0.6 M perchloric acid. Afterwards, the extract solution was diluted with lithium citrate loading buffer (Pachlová et al., 2011).

Free amino acids were assessed using IEC. The amount of 100 μL of the mixture was injected into the analyzer AAA400 (Ingos, Prague, Czech Republic) equipped with a column of 150 × 3.7 mm filled with an ion-exchanger Polymer AAA. The post-column ninhydrin derivatization and spectrophotometric detection were performed at 570 nm. Free amino acids were eluted according to the following program: 0–2 min buffer A, 2–46 min buffer B, 46–83 min buffer C, 83–101 min buffer D, 101–162 min buffer E. The temperature of the column was set at 40°C

(0–46 min), 65°C (46–83 min) and 74°C (83–162 min). The chemical composition of the buffers is shown in Supplementary Table S1 (Buňková et al., 2009).

2.4 Determination of bound amino acids using IEC

Acidic hydrolysis of samples was used to determine bound amino acids. Samples (50 mg) were weighted accurately, mixed with 15 mL of 6 M HCl and purged with Ar for 30 s in vials. Next, vials were placed into a heating block (Labicom, Olomouc, Czech Republic) to hydrolyse at 117 °C for 23 h. After the hydrolysis, the vials were cooled, HCl was evaporated and the residue was diluted in sodium citrate buffer at pH 2.2.

To determine methionine and cysteine, the oxidative acid hydrolysis process was applied. Samples (500 mg) were submitted to oxidation in the fridge with a mixture of 15 mL of 30% H_2O_2 and 85% HCOOH for 16 h. Then, 50 mL of 6 M HCl were added to the sample and samples were placed in an oil bath where the oxidative hydrolysis was performed at 118 °C for 23 h. Subsequently, the samples were transferred into a 250-mL volumetric flask using 0.1 M HCl. 25 mL of this volume was removed and evaporated. The residue was redissolved in redistilled water, evaporated again; and then quantitatively transferred with buffer (sodium citrate buffer, pH 2.2) into a 25-mL volumetric flask.

Amino acids were determined using IEC with a post-column ninhydrin derivatization. Their contents were identified using the analyser AAA 400 with spectrophotometric detection (440 nm for Pro and 570 nm for other amino acids). The amount of 100 µL of the sample in loading buffer was injected into an analyzer equipped with a column of 370 × 3.7 mm filled with an ion-exchanger Ostion LG ANG. Analytes were eluted according to the program: 0–5 min buffer A, 5–32 min buffer B, 32–44 min buffer C, 44–75 min buffer D. A flow rate was 0.3 mL/min for buffers and 0.2 mL/min for ninhydrin reagent. The composition of individual citrate buffers are given in Supplementary Table S2 (Buňka et al., 2009).

2.5 ICP-MS analysis

Thirty-two minerals and trace elements were measured in all analysed samples. Matcha powdered teas (0.2 g) were weighted into teflon vessels with 7 mL of 67% Analpure® HNO_3 and 1 mL of 30% Analpure® H_2O_2. Then, the samples were decomposed by a microwave system Milestone Ethos One (Sorisole, Italy) with the parameters set as follows: 500 W for 5 min, 1500 W for 15 min and 500 W for 10 min. After being cooled, the sample final volume was adjusted to 25 mL using high purity 18.2 MΩcm water (Purelab Classic Elga system, Labwater/VWS Ltd., London, UK). The final samples were promptly analysed using ICP-MS (Sumczynski et al., 2018).

Daily performance of ICP-MS in terms of sensitivity and background signals was checked using Tune 7 and 8 solutions with Ag, Al, Ba, Be, Bi, Ce, Co, Cs, Cu, Ga, Ho, In, Li, Mg, Mn, Ni, Rh, Sc, Sr, Ta, Tb, Tl, U, Y, Zn containing 1 µg/L of each element in 2% HNO_3; and Ba, Bi, Ce, Co, In, Li and U containing 1 µg/L of each element in 2% HNO_3 + 0.5% HCl. To obtain calibration curves, two lines of standard concentrations were prepared to be matched with the expected concentration ranges in matcha samples: ICP-MS 19-standard series with concentrations of 2–30 µg/L and ICP-MS 13-standard series with concentrations of 0.2–1.0 µg/L. ^{103}Rh and ^{115}In at the concentration of 100 and 50 µg/L were used as an internal standard, respectively. Certified reference materials (CRM) of green algae Metranal®8, tea leaves, rice flour 1568b and lichens were applied to assess the measurement accuracy. The resulting values of certified materials measured using ICP-MS are shown in Supplementary Table S3.

Minerals and trace elements concentrations were measured using a quadrupole-based Thermo Scientific iCAP Qc inductively coupled plasma-mass spectrometer (ICP-MS) (Thermo Scientific, Waltham, MA, USA) equipped with a collision cell (QCell) containing He to remove undesirable molecule ions by distinguishing their kinetic energy (CCT, Collision Cell Technology; KED, Kinetic Energy Discrimination mode). Specific working parameters were set as follows: power of 550 W, sampling depth of 5-mm, cool gas flow rate of 14.0 L/min, auxiliary gas flow rate of 0.8 L/min, nebulizer gas flow rate of 1.015 L/min, He flow rate of 4.1 mL/min, nebulizer pump speed of 40.00 rpm and chamber temperature of 2.7 °C. Samples were analysed five times in total (Sumczynski et al., 2018).

2.6 Evaluation of the contribution of amino acids, minerals and trace elements to the RDA, AI, PTWI and PTMI

AAS, EAAI and RDA of amino acids were calculated according to Friedman et al. (1996) and FAO/WHO/UNU (Supplementaty Table S4) (WHO/FAO/UNU, 2007). A LAA was determined to be the essential amino acid in matcha protein showing the greatest difference in AAS value from the amino acid in standard protein. Daily dietary intake levels for minerals and trace elements from matcha were established and compared with the appropriate RDA or AI values as recommended by the Institute of Medicine (IOM 1997; 2000; 2001; 2005). Intake levels of toxic elements were also evaluated and compared with the PTWI or PTMI as suggested by the FAO/WHO (FAO/WHO 2006; 2011a; 2011b; 2013). Intake levels were determined for adults aged between 31 and 50, both males with the average weight of 80 kg and females weighing 65 kg. Since there is no recommendation for daily intake of matcha, the daily serving size was set to 5 g (approx. two cups of matcha tea per day).

2.7 Statistical analysis

Free and bound amino acid, mineral and trace element analyses were repeated 5 times and their results were reported as mean ± standard deviation on a dry weight basis. Results of all analyses were statistically evaluated using one-way analysis of variance (ANOVA). Subsequently, Tukey´s test was applied to identify the differences among the means. The level of probability for significance was 5%.

3. Results and discussion

3.1 Free and bound amino acid evaluation

The total content of free amino acids in matcha are displayed in Table 1 (results expressed in mg/g are in Supplementary Table S5). To the extent of our knowledge, a comparison of data based on amino acid contents has not been possible due to the limited studies on matcha tea. What is more, existing results of commercial matcha tea analyses may be affected by several factors, such as the characteristics of the growing environment (soil, climate, geographical area), shading, harvesting season and cultivar variety (Jiang et al., 2019; Horanni and Engelhardt, 2013).

It is evident that considerable differences in free amino acid composition were established. The most abundant amino acid was theanine accounting 16–69% (73.0–457 mg/16 g N) of TFAAs (Total free amino acids). This is not entirely consistent with the study by Wang et al. (2010) who determined theanine concentrations in green teas exceeding 50% of TFAAs values. For instance, Shao Xing, Harmony, Day spa organic and premium matcha samples were below this limit. As theanine participates in the protection of leaves from sunlight, it is lower in matcha grown in the shade than in other green teas grown in direct sunlight. Consumption of green tea with high theanine content has provided several health benefits and significant medicinal potential. It also plays a significant role in the inhibition of the neurotransmitter GABA (Zhu et al., 2016). Furthermore, brothy sweet umami taste of green tea stems from the presence of theanine and Glu. Also Asp and Glu may affect that; they act synergistically with Thr to contribute to umami taste (Jiang et al., 2019). It should be emphasized that low concentrations of theanine corresponded to undetectability of Ala in the Shao Xing, Day spa organic and Day spa premium samples, but not for the Asagiri sample. It may be related to the steaming of fresh leaves during matcha tea processing, since Wang et al. (2010) declared Ala content only in fermented teas. Subsequently, Glu (from 10.1 to 41.3 mg/16 g N), GABA (from 1.61 to 38.6 mg/16 g N), Ser (from 2.00 μg to 30.4 mg/16 g N), Asp (from 8.42 to 20.9 mg/16 g N), Thr (from 1.00 μg to 56.5 mg/16 g N) and Met (from 1.00 μg to 44.7 mg/16 g N) were detected in higher concentrations than other amino acids which is compliant with the study by Das et al. (2019). Day spa organic and premium samples were rich in Ile and ornithine, even though Jiang et al. (2019) detected lower concentrations of ornithine in green teas (less than 0.03 mg/g). Ornithine, a non-proteinogenic free amino acid, attenuates fatigue by increasing the efficiency of energy consumption and by promoting the excretion of ammonia. Hence, it has been suggested that ornithine can be taken as a nutritional supplement in cases of physical fatigue (Sugino et al., 2008).

Concentrations of bound amino acids in matcha teas are presented in Table 2 (appropriate concentrations in mg/g are in Supplementary Table S6). Published results discussing bound amino acid in matcha are limited similarly as in other types of tea leaves. The results of this study showed that the most abundant bound amino acids were Glu (from 0.47 to 0.68 g/16 g N), Asp (from 0.31 to 0.42 g/16 g N), Leu (from 0.28 to 0.37 g/16 g N), Lys (from 0.23 to 0.32 g/16 g N), Arg (from 0.19 to 0.30 g/16 g N) and Val (from 0.19 to 0.27 g/16 g N). In contrast, the lowest concentrations of Met and Cys were identified (0.09–0.12 and 0.07–0.09 g/16 g N, respectively). The highest EAAs (Essential amino acids) content of bound amino acids was evaluated in Day spa premium matcha (1.71 g/16 g N).

AAS and EAAI scores and indexes were calculated from the obtained data as the sum of both free and bound amino acids (Table 2). AAS shows the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirement at the safe level of protein intake. This is achieved by a comparison of the content of the essential amino acids in protein with their content defined in the required standard (WHO/FAO/UNU, 2007). Day spa premium sample showed the highest AAS score (40.2%), as well as the highest EAAI value (5.7%). On the other hand, the lowest values of AAS and EAAI were observed in Mo Cha Fen sample (30.4 and 4.3%, respectively). As data evaluating AAS for matcha tea protein is limited, AAS values for kidney beans (84%), lentils (62%), wheat gluten (26%), sunflower protein (39%) and whole wheat (44%) were clearly declared (FAO/WHO, 1991). Generally, sulphur amino acid Met is considered as a limiting amino acid of legumes similarly to Lys and Trp in cereals (WHO/FAO/UNU, 2007). Regarding individual contribution of essential amino acid to AAS, Ile and Thr were identified as limiting amino acids in matcha teas.

3.2 Minerals and essential trace elements evaluation

Since data discussing the content of minerals and trace elements in matcha teas is limited, the results are compared mostly with green and black teas. Generally, the content of elements in plant foods are attributed to various local environmental conditions including soil composition, fertilization, stage of growth and manufacturing processes. Contents of trace elements of teas may have both beneficial and adverse effects on human health. However, individual element concentrations may not reflect the availability by the human tract (Erdemir, 2018; Karak and Bhagat, 2010).

Mineral and trace element content in matcha are presented in Table 3. Sodium concentrations reached 185 µg/g, which exceeded the value of 123 µg/g presented by Koch et al. (2018) and 13.9 µg/g published by Szymczycha-Madeja et al. (2015) in green tea samples. It influences blood pressure and regulates the body water balance (Derun, 2014). AI value was set to 1500 mg/day for both females and males (IOM, 2005). High levels of magnesium (up to 2400 µg/g) were established in matcha, which is in accordance with the results of the studies by Erdemir (2018) and Koch et al. (2018). According to Szymczycha-Madeja et al. (2015) magnesium content in black and green teas may reach only 1.76 and 1.66 µg/g, respectively. Regarding its deficiency, symptoms may include a muscle weakness and nervous malfunction (Derun, 2014). RDA values for males and females have been estimated to 420 and 320 mg/day, respectively (IOM, 1997). The phosphorus content in matcha reached 4180 µg/g. In contrast, green tea leaves originated from Japan and China contained 2360 and 3370 µg/g phosphorus (Brzezicha-Cirocka et al. (2016b). Apart from bone stiffening, it plays an important role in transport of cellular energy by means of ATP. RDA for males and females is 700 mg/day (IOM, 1997). Szymczycha-Madeja et al. (2015) indicated potassium content in green and black teas of 11.3 and 16.4 µg/g, respectively. Our results are in accordance with the study provided by Koch et al. (2018) whereas potassium content in matcha reached 10750 µg/g. The current study shows slightly lower amounts (less than 4730 µg/g). Potassium acts as a regulator in the cell osmotic balance and regulates the heartbeat (Derun, 2014). AI value for females and males is 4700 mg/day (IOM, 2005). The calcium content in matcha reached 2650 µg/g, which is in agreement with the study by Koch et al. (2018). In contrast, calcium content in Pu-erh tea reached 14370 µg/g (Brzezicha-Cirocka et al. (2016a). Mainly, Ca is stored in bones and acts as an essential nutrient for muscle contractions. RDA value for females and males aged 31–50 has been established at 1000 mg/day (IOM, 1997).

Mn values in matcha teas ranged between 17.1 and 68.4 µg/g. Brzezicha-Cirocka et al. (2016b) published Mn concentration in green teas up to 1380 µg/g, while Szymczycha-Madeja et al. (2015) reported only 1.68 µg of Mn per g of green tea leaves. If black tea is mentioned, Mn content was measured in a wide range (from 108 to 1960 µg/g) (Milani et al., 2016). Manganese is involved in a bone formation and acts as an activator for enzyme complexes. The IOM (2001) has set its AI values for females and males to 1.8 and 2.3 mg/day, respectively. Most of the iron amount is detected in heme proteins as an essential element for red blood cell formation (Derun, 2014). Generally, the amount of available Fe from plants is low due to the presence of phytic acid. According to Szymczycha-Madeja et al. (2015), Fe contents in green and black teas were determined in the range from 63.6 to 150 and from 39.0 to 117 µg/g, respectively, which is in accordance with the results of this study. RDA values for males and females have been set to 8 and 18 mg/day, respectively (IOM, 2001). Copper content in matcha varied from 6.12 to 25.3 µg/g, which is similar to the studies by Brzezicha-Cirocka et al. (2016a) and Szymczycha-Madeja et al. (2015) where Cu content in black teas reached 19.4 and 22.3 µg/g, respectively. Considering green tea leaves, Cu content was measured in the range of 4.99 to 11.4 µg/g (Szymczycha-Madeja et al., 2015). The RDA value for Cu has been set to 900 µg/day for both females and males (IOM, 2001). It is known that excessive Zn consumption results in the reduction of Cu concentration in the human body; furthermore, the Zn-chelating effect of phytic and tannic acids has to be considered as well. Koch et al. (2018) determined Zn content in matcha three times lower than in this study, similarly, Milani et al. (2016) provided Zn level in green teas up to 23.0 µg/g. Zn deficiency causes hair loss and protracted wound healing (Derun, 2014). The RDA intake values for females and males are declared to 8 and 11 mg/day, respectively (IOM, 2001). It has been confirmed that Cr^{3+} enhances insulin activity and participates in the metabolism of carbohydrates (Kabata-Pendias, 2010). Specifically, Cr plays an important role in glucose metabolism (Derun, 2014). Its content in matcha teas ranged between 2.12 and 21.1 ng/g, whereas Brzezicha-Cirocka et al. (2016a) identified Cr content in black teas up to 3.8 μg/g. Similarly, Milani et al. (2016) published Cr content in black, green and white teas up to 3.38, 2.33 and 2.92 μg/g, respectively. AI for both males and females aged 31–50 has been defined as 35 and 25 μg/day, respectively (IOM, 2001). It has been recognized that Se, as an essential component of glutathione peroxidase, acts as cellular antioxidant and its RDA value for females and males, aged 31–50 years has been set to 55 μg/day (IOM, 2000). The selenium level in matcha was determined in the range from 7.82 to 17.6 ng/g, whereas green, black and white types of teas can contain 370, 140 and 2850 ng/g, respectively (Milani et al., 2016). Since most plants contain only low Se levels of around 25 ng/g (Kabata-Pendias, 2010), matcha were not expected to be a quality source of Se.

3.3 Toxic trace elements evaluation

Daily tea drinking is very popular. Therefore, it is important to monitor the quantity of harmful contaminants in tea beverages. Potentially toxic elements, such as Pb, As, Cd, Hg, Sn and Al, originate from pollution of the growth environment or emerge during tea manufacturing processes and can accumulate in leaves (Street et al., 2006; Dalipi et al., 2018). Hence, they received much research interest. The results of measurements are presented in Table 3.

Neurotoxicity could be connected with a potential toxicity of Al. Even though many studies have referred to a link between Alzheimer’s disease and aluminium, an appropriate relationship has not been established yet. Since *Camellia sinensis* is one of the plants that can strongly accumulate Al in concentrations exceeding 16000 mg/kg in leaves, its determination should be highly encouraged (Dalipi et al., 2018; Karak and Bhagat, 2010). The lowest and highest Al concentrations in matcha teas were 5.42 and 113 μg/g, respectively. If compared with green tea, Szymczycha-Madeja et al. (2015) assessed the Al content at only 1.48 to 2.36 μg/g and Milani et al. (2016) published Al content of green and black teas up to 3470 and 3940 μg/g, respectively. PTWI value for Al has been set to 2 mg/kg bw (FAO/WHO, 2013). Cadmium is considered to be nephrotoxin and thus it has to be monitored. PTMI value has been set to 25 μg/kg bw (FAO/WHO, 2013). In this study, Cd concentrations reached 1.52 ng/g. Koch et al. (2018) established Cd concentration in matcha up to 2.6 ng/g, whereas green, black and white types of teas can accumulate up to 13, 13 and 54 ng of Cd per g (Milani et al., 2016). Reflecting black teas, Brzezicha-Cirocka et al. (2016b) assessed Cd concentrations below 0.2 ng/g. Tin amount was determined in the range from 0.42 to 1.51 ng/g. Literature data examining tin content in matcha and other types of teas is scarce. Commonly, tin values assessed in plants ranged to 29 ng/g (Kabata-Pendias, 2010). It is evident that the results of this study were below this limit. The accumulation of Sn in tissues is rather limited due to its movement through the tract. However, tin may cause acute gastrointestinal tract issues. PTWI for tin has been determined at 14 mg/kg bw (FAO/WHO, 2006). Considering mercury, due to toxic effects of its compounds, PTWI value of 4 μg/kg bw has been set (FAO/WHO, 2011a). Symptoms of an excessive Hg intake include neurological and cardiovascular diseases. The range of Hg contents in matcha was identified from 1.31 to 2.63 ng/g. Literature data discussing mercury values in matcha has been limited so far. However, Kabata-Pendias (2011) showed a range of mercury content from 34 to 46 ng/g in tea leaves. Despite of the fact that Pb is harmful to the nervous system, the PTWI value for lead of 25 μg/kg bw was withdrawn in 2011 (Sumczynski et al., 2018). In this study, low Pb concentrations were determined. It seems that white tea has the ability to accumulate higher levels of lead compared to other types of teas (Milani et al., 2016). Arsenic content were in the range from 14.2 to 31.5 ng/g. When compared to different types of teas, green, black and white teas can contain up to 63, 48 and 270 ng/g of As, respectively

(Milani et al., 2016). The JECFA committee has reported that current PTWI values for As of 2.1 µg/kg bw per day has not been health protective any longer as BMDL value of 0.5 (Benchmark dose lower confidence limit for 0.5% increase in the incidence of lung cancer in humans) is in the same range as PTWI. Therefore, PTWI for As has been withdrawn (FAO/WHO, 2011a). In this analysis, lower concentrations of nickel (up to 434 ng/g) were determined in all samples. In contrast, Koch et al. (2018) and Szymczycha-Madeja et al. (2015) established Ni content in matcha and green tea in the magnitude of µg/g (specifically 2.13 and 4.36 µg/g, respectively). Nickel is a carcinogenic metal documented to initiate epigenetic alteration of a normal cell into a cancerous one. Therefore, it is important to monitor nickel concentrations in food.

3.4 Estimation of dietary intakes of amino acids, minerals and trace elements from matcha

Dietary intake levels of essential amino acids from matcha have not been published yet. As matcha tea is consumed with all leaf parts, it is difficult to analyse data discussing amino acids in infusions from other types of teas and compare appropriate RDA values. As daily intakes of essential amino acids from matcha tea could contribute not only to the quality of diet, but might also exert biological effects on brain function and inflammation (Das et al., 2019), appropriate contributions to RDA values for amino acids of matcha have been evaluated. Dietary intake levels of amino acids were evaluated applying the recommendations of the WHO/FAO/UNU (2007). Table 4 summarizes the contributions to individual RDA values for essential amino acids. It is evident that individual contributions of amino acids to RDA are rather low, although it is essential to consider the fact that a daily serving size of matcha tea is only 5 g. The highest daily intake of Cys may reach 8 and 7% of RDA for females and males, respectively. The results show that matcha is a contributor to RDA for both, females and males, in the following order:

Cys > Met, Met+Cys > Phe+Tyr and Thr > His, Ile, Lys and Val > Leu, respectively. Cys plays a substantial role in cellular homeostasis as an intermediate in protein synthesis and in the production of GSH (glutathione), taurine and H_2S. Cell signalling is affected by dietary intake of sulphur amino acids via modulating intracellular amounts of cysteine and cystine, as well as cysteine/cystine redox state, in the postprandial period. Therefore, in the last decade, a growing interest in the use of Cys to enhance health in humans has been witnessed (Yin et al., 2015).

Data regarding dietary intake levels of minerals obtained from matcha has been limited. When evaluating matcha dietary intake levels, many factors such as the leaching process, digestibility and bioavailability of minerals must be considered as matcha is consumed in a form including leaf parts (Dalipi et al., 2018; Koláčková et al., 2020). Furthermore, anti-nutrient phytic and tannic acids influence bioavailability of Zn, Fe and Mn. Appropriate dietary intakes values from matcha were estimated and compared with RDA or AI values for adults aged 31–50 (Table 5). It can be seen that matcha contributes negligibly to AI of Na, K and Cr, and to RDA of Se and Ca. This should be applied in the recommendations for consumers as a benefit to obtain less than 0.1% of AI value of Na to prevent hypertension. In contrast, it provides high amounts of Mn and Cu. This research has shown that matcha is a contributor to AI or RDA for females in the following order: Mn (up to 19%) > Cu (up to 14%) > Zn (up to 5%) > Mg (up to 4%) > Fe (up to 3.2%) > P (up to 3.0%) and Ca (up to 1.3 %); and for males: Mn (up to 15%) > Cu (up to 14%) > Fe (up to 7.3%) > Mg ≈ P ≈ Zn (all up to 3%) > and Ca (up to 1.3%). It confirms that matcha is a valuable source of Mn (Yin et al., 2015). Green tea infusions were a significant source of Mn as the consumption of one cup could contribute as much as 1/3 of the daily need for Mn (up to 5–32% of RDA) (Koch et al., 2018). What is more, Koch et al. (2018) claimed green teas as an important source of dietary Cr; however, this has not been proved in this study. The contribution of matcha to Al, Sn and Hg intakes was calculated as the weekly intake based on PTWIs; and its contribution to Cd was calculated as the monthly intake based on PTMI set by the JECFA (FAO/WHO 2006; 2011a; 2011b; 2013) when portion size was set to 5 g per day. PTWIs and PTMIs values are displayed in Table 6. The estimated matcha tea contribution to PTWI for Al is below 0.5% for males and 0.6% for females; its contribution to PTMI for Cd is less than 0.1% for both males and females. Concerning Hg and Sn, PTWI is lower than 0.03% for both males and females. PTWI (or PTMI) expresses the long-term exposure risk for contaminants that may accumulate in the human body.

5. Conclusion

This study provides meaningful data on free and bound amino acids, minerals and trace elements of matcha that has not been published yet. It may be valuable particularly in the evaluation of essential scores and indices regarding amino acid contents, quality of protein and detection of toxic elements. It has confirmed that theanine was the most abundant free amino acid of matcha followed by Glu, GABA, Met and Thr. Similarly, Glu, Asp, Leu, Lys and Arg have been identified as the most abundant bound amino acids. What deserves further attention is low concentrations of theanine corresponding to the absence of Ala in some samples. That might be due to poor quality steaming and drying of fresh leaves during matcha tea processing. Data evaluating AAS for matcha protein shows the values comparable with AAS scores of wheat and sunflower proteins. Regarding AAS scores, Ile and Thr has been confirmed as limiting amino acids. Furthermore, high concentrations of Mg, P, K, Ca, Fe, Zn, Mn and Cu in matcha teas were observed. This study has confirmed that matcha contained substantial amounts of minerals and trace elements for males and females in the following orders: Mn > Cu > Fe > Mg and Mn > Cu > Zn > Mg, respectively. It has not been confirmed that matcha, as a type of green tea, is a good source of dietary Se or Cr. The results of this study have confirmed that a daily serving portion of 5 g matcha tea does not contribute to PTWIs for Al, Sn and Hg or PTMIs for Cd. Therefore, the daily consumption of 5 g matcha should not pose a health risk. On the contrary, it may possess many valuable health benefits.

CRediT authorship contribution statement

Tereza Koláčková: Investigation, Formal analysis. **Daniela Sumczynski:** Investigation, Writing – review & editing, Designed the study. **Ludmila Zálešáková, Lenka Šenkárová** and **Nikoleta Lanczová**: Formal analysis. **Jana Orsavová:** Writing – review & editing.

Conflict of interest

The authors declare no conflict of interest.

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Table 1

Concentrations of free amino acids analysed in matcha using ion-exchange chromatography.

Analyte mg/16 g N	Mo Cha Fen	Whittard	Shao Xing	Asagiri	Harmony	Jeju	Don Matcha	Day spa organic	Day spa premium	Royal Pharma
Asp	20.9±0.2^{a}	11.6±0.02^{b}	15.2±0.3^{c,g}	13.6±0.3^{d}	11.7±0.03^{b}	14.5±0.3^{e}	12.4±0.2^{f}	14.9±0.2^{g}	19.2±0.3^{h}	8.42±0.10^{i}
Glu	37.9±0.4^{a}	18.1±0.2^{b}	30.9±0.4^{c}	28.0±0.2^{d}	18.4±0.3^{b}	22.0±0.4^{e}	10.1±0.2^{f}	31.9±0.3^{g}	41.3±0.5^{h}	19.6±0.3^{i}
hPro	3.38±0.05^{a}	2.35±0.10^{b}	1.71±0.12^{c}	1.25±0.10^{d}	2.73±0.05^{e}	2.75±0.10^{e}	2.96±0.15^{e}	ND	1.47±0.10^{f}	2.72±0.10^{e}
Ser	9.85±0.09^{a}	18.6±0.2^{b}	7.04±0.10^{c}	9.82±0.15^{a}	10.2±0.3^{a}	15.3±0.4^{d}	30.4±0.4^{e}	91.6±1.0^{f}	5.60±0.10^{g}	ND
Gly	2.77±0.05^{a}	4.34±0.10^{b}	1.28±0.10^{c}	1.07±0.05^{d}	2.73±0.07^{a}	2.25±0.04^{e}	ND	ND	2.95±0.06^{f}	10.1±0.1^{g}
His	7.38±0.20^{a}	8.49±0.20^{b}	ND	ND	ND	ND	ND	14.1±0.2^{c}	5.60±0.20^{d}	ND
Arg	4.92±0.10^{a}	ND	ND	2.14±0.10^{b}	8.18±0.20^{c}	7.75±0.20^{d}	28.9±0.3^{e}	7.06±0.20^{f}	17.7±0.4^{g}	ND
Thr	9.23±0.10^{a}	9.93±0.20^{b}	6.83±0.10^{c}	2.32±0.10^{d}	8.43±0.20^{e}	8.00±0.30^{e}	6.18±0.25^{f}	56.5±0.30^{g}	ND	2.72±0.10^{h}
GABA	6.46±0.20^{a}	5.96±0.30^{b}	9.81±0.30^{c}	1.61±0.10^{d}	4.71±0.30^{e}	4.00±0.20^{f}	3.71±0.20^{g}	31.4±0.4^{h}	38.6±0.4^{i}	4.62±0.20^{e}
Ala	13.9±0.20^{a}	0.54±0.05^{b}	ND	3.21±0.15^{c}	1.98±0.10^{d}	18.0±0.20^{e}	14.6±0.20^{f}	ND	ND	2.99±0.10^{c}
Pro	1.85±0.10^{a}	2.17±0.10^{b}	2.13±0.10^{b}	ND	26.3±0.30^{c}	ND	1.73±0.20^{a}	3.92±0.40^{d}	1.18±0.20^{e}	39.4±0.30^{f}
Theanine	166±5^{a}	280±10^{b}	73.0±5^{c}	94.6±4^{d}	156±5^{e}	345±10^{f}	457±12^{g}	94.7±6^{d}	128±7^{h}	272±5^{b}
Tyr	3.38±0.05^{a}	4.15±0.05^{b}	2.56±0.14^{c}	1.07±0.10^{d}	2.98±0.15^{e}	3.50±0.25^{a}	6.42±0.12^{f}	ND	1.18±0.10^{d}	ND
Cys	2.46±0.11^{a}	ND	ND	2.50±0.10^{a}	2.73±0.10^{b}	2.25±0.12^{c}	1.98±0.20^{d}	16.2±0.3^{e}	ND	ND
Val	2.46±0.10^{a}	4.34±0.15^{b}	2.99±0.15^{c}	0.71±0.10^{d}	14.1±0.3^{e}	3.00±0.20^{c}	4.94±0.25^{f}	ND	1.47±0.14^{g}	2.17±0.12^{h}

Met	2.46±0.10[a]	34.9±0.3[b]	15.4±0.3[c]	10.5±0.2[d]	5.21±0.20[e]	34.3±0.3[f,b]	44.7±0.3[g]	ND	1.47±0.07[h]	ND
Phe	0.31±0.05[a]	ND	0.64±0.06[b]	ND	3.97±0.12[c]	ND	ND	47.6±0.2[d]	ND	34.8±0.3[e]
Ile	2.77±0.03[a]	5.60±0.10[b]	1.92±0.10[c]	ND	ND	ND	6.18±0.22[d]	45.8±0.3[e]	30.4±0.3[f]	2.72±0.3[a]
Lys	2.46±0.10[a]	5.78±0.20[b]	2.77±0.17[c]	1.96±0.11[d]	97.2±0.6[e]	8.00±0.20[f]	9.14±0.30[g]	ND	1.18±0.10[h]	29.9±0.4[i]
Leu	2.77±0.10[a]	5.24±0.07[b]	2.13±0.03[c]	1.61±0.10[d]	3.47±0.20[e]	4.50±0.14[f]	9.63±0.22[g]	15.2±0.4[h]	15.6±0.3[h]	3.26±0.10[e]
Ornithine	2.77±0.12[a]	4.34±0.22[b]	3.63±0.16[c]	ND	ND	ND	9.88±0.23[d]	100±6[e]	67.0±4[f]	2.99±0.10[g]
Trp	4.31±0.23[a]	5.96±0.21[b]	4.48±0.21[a]	ND	ND	5.25±0.20[c]	7.66±0.17[d]	ND	2.06±0.05[e]	ND
Met+Cys	4.92±0.11[a]	34.9±0.3[b]	15.4±0.3[c]	13.0±0.2[d]	7.94±0.20[e]	36.6±0.30[f]	46.7±0.3[g]	16.2±0.3[h]	1.47±0.07[i]	ND
Phe+Tyr	3.69±0.05[a]	4.15±0.05[b]	3.20±0.12[c]	1.07±0.10[d]	6.95±0.14[e]	3.50±0.25[a,c]	6.42±0.12[f]	47.6±0.2[g]	1.18±0.10[d]	34.8±0.03[h]
EAAs	26.8±0.3[a]	71.8±0.5[b,j]	37.2±0.4[c]	17.1±0.3[d]	132±7[e]	63.1±5.0[f]	88.4±6.0[g]	165±7[h]	52.2±4.0[i]	75.6±4.0[j]
NEAAs	284±4[a]	360±8[b,j]	147±5[c]	159±4[d]	249±6[e]	437±9[f]	580±10[g]	406±5[h]	330±6[i]	362±4[j]
TFAAs	311±5[a]	432±10[b,i]	184±5[c]	176±4[d]	381±7[e]	500±10[f]	668±12[g]	571±6[h]	382±7[e]	438±5[i]

Results are presented in dry weight basis as means ± SD, n=5 (mean value of five measurements). Means within a line with at least one identical superscript do not differ significantly ($P \geq 0.05$), while means with different superscripts show a significant difference ($P < 0.05$).

hPro – hydroxyprolin, GABA – γ-amino-*n*-butyric acid, EAAs – Essential amino acids, NEAAs – Non-essential amino acids, TFAAs – Total free amino acids.

LOQ: hPro, Gly, His, Thr, Pro, Tyr, Val, Met, Phe, Ile, Lys, ornithine, Trp – 1.0 µg/g; Ser, Arg, Ala, Cys – 2.0 µg/g.

Table 2

Concentrations of bound amino acids analysed in matcha using ion-exchange chromatography.

Analyte g/16 g N	Mo Cha Fen	Whittard	Shao Xing	Asagiri	Harmony	Jeju	Don Matcha	Day spa organic	Day spa premium	Royal Pharma
Asp	0.31±0.02^{a}	0.37±0.03^{b}	0.40±0.05^{b,c}	0.37±0.04^{b}	0.39±0.06^{b,c}	0.42±0.04^{c}	0.40±0.06^{c}	0.37±0.07^{b}	0.41±0.05^{c}	0.40±0.05^{c}
Thr	0.15±0.02^{a,b}	0.15±0.02^{a,b}	0.16±0.03^{a,c}	0.14±0.02^{b}	0.18±0.02^{c,d}	0.17±0.03^{c}	0.17±0.02^{c}	0.16±0.03^{a,c}	0.19±0.02^{d}	0.18±0.02^{d}
Ser	0.16±0.03^{a,b}	0.15±0.02^{a,c}	0.17±0.03^{b,d}	0.14±0.02^{c}	0.18±0.03^{d}	0.18±0.02^{d}	0.16±0.02^{a,b}	0.16±0.03^{a,b}	0.18±0.03^{d}	0.18±0.02^{d}
Glu	0.47±0.04^{a}	0.62±0.04^{b,e}	0.58±0.03^{c,e}	0.64±0.05^{b}	0.58±0.04^{c,e}	0.68±0.03^{d}	0.60±0.06^{e}	0.62±0.07^{b,e}	0.65±0.07^{b}	0.52±0.06^{f}
Pro	0.18±0.02^{a,c}	0.16±0.04^{b}	0.18±0.02^{a,c}	0.16±0.03^{b}	0.18±0.03^{a,c}	0.19±0.02^{c}	0.18±0.03^{c}	0.17±0.02^{b,c}	0.18±0.02^{c}	0.19±0.03^{c}
Gly	0.18±0.01^{a}	0.18±0.02^{a}	0.21±0.01^{b}	0.19±0.03^{a}	0.22±0.03^{b,c}	0.23±0.02^{c,d}	0.22±0.03^{b,c}	0.20±0.03^{a,b}	0.24±0.03^{d}	0.22±0.0^{b,c}
Ala	0.18±0.02^{a}	0.19±0.02^{a}	0.21±0.01^{b}	0.19±0.02^{a}	0.23±0.01^{c,d}	0.23±0.03^{b,c,d}	0.22±0.03^{b,c,d}	0.21±0.03^{b,c}	0.24±0.04^{d}	0.22±0.02^{b,c}
Val	0.19±0.03^{a,d}	0.20±0.03^{a,d}	0.23±0.01^{b,d}	0.22±0.04^{a,b,d}	0.24±0.03^{b,c}	0.25±0.03^{c,e}	0.25±0.02^{c,e}	0.21±0.03^{d}	0.27±0.01^{e}	0.24±0.03^{b,c}
Ile	0.15±0.03^{a}	0.18±0.03^{a,b}	0.18±0.02^{a,b}	0.16±0.02^{a}	0.18±0.02^{a,b}	0.18±0.02^{a,b}	0.19±0.03^{b,c}	0.16±0.01^{a}	0.20±0.01^{c}	0.18±0.02^{a,b}
Leu	0.28±0.03^{a}	0.28±0.03^{a}	0.32±0.03^{a,b}	0.29±0.03^{a,b}	0.33±0.02^{b}	0.34±0.05^{b}	0.34±0.04^{b}	0.29±0.04^{a,b}	0.37±0.01^{c}	0.36±0.06^{c,d}
Tyr	0.12±0.01^{a,b}	0.11±0.01^{a}	0.13±0.02^{a,b}	0.11±0.01^{a}	0.14±0.02^{b}	0.12±0.01^{a,b}	0.14±0.01^{b}	0.12±0.02^{a,b}	0.13±0.02^{a,b}	0.13±0.02^{a,b}
Phe	0.19±0.02^{a}	0.18±0.02^{a}	0.20±0.02^{a,b}	0.19±0.03^{a}	0.22±0.02^{b,c}	0.22±0.02^{b,c}	0.22±0.01^{b,c}	0.20±0.03^{a,b}	0.24±0.03^{c}	0.22±0.01^{b,c}
His	0.08±0.01^{a}	0.09±0.01^{a,b}	0.10±0.01^{b,c}	0.09±0.01^{a,b}	0.11±0.10^{c,d}	0.11±0.01^{c,d}	0.10±0.02^{b,c}	0.09±0.01^{a,b}	0.12±0.02^{d}	0.11±0.01^{c,d}
Lys	0.23±0.03^{a}	0.26±0.03^{a}	0.30±0.04^{b,c}	0.26±0.02^{a}	0.29±0.02^{b}	0.30±0.02^{b,c}	0.30±0.02^{b,c}	0.26±0.02^{a}	0.32±0.03^{c}	0.28±0.03^{a,b,c}
Arg	0.19±0.02^{a}	0.26±0.02^{b,c}	0.29±0.05^{b,d}	0.29±0.04^{b,d}	0.28±0.03^{b,d}	0.29±0.04^{b,d}	0.26±0.04^{b,c}	0.24±0.03^{c}	0.30±0.04^{d}	0.27±0.04^{b}

CysH	0.08±0.01^{a,b}	0.07±0.021^{a}	0.08±0.01^{a,b}	0.07±0.01^{a}	0.08±0.01^{a,b}	0.08±0.02^{a,b}	0.07±0.01^{a}	0.09±0.01^{b}	0.09±0.02a,^{b}	0.09±0.02^{a,b}
MetS	0.10±0.02^{a}	0.10±0.01^{a}	0.11±0.02^{a,b}	0.09±0.02^{a}	0.11±0.01^{a,b}	0.11±0.02^{a,b}	0.11±0.02^{a,b}	0.12±0.02^{b}	0.12±0.02^{b}	0.11±0.02^{a,b}
Met+Cys	0.18±0.02^{a}	0.17±0.01^{a,b}	0.19±0.02^{a,c}	0.16±0.01^{b}	0.19±0.01^{a,c}	0.19±0.02^{a,c}	0.18±0.02^{a}	0.21±0.02^{c}	0.21±0.02c	0.20±0.02^{c}
Phe+Tyr	0.31±0.02^{a,c}	0.29±0.02^{a}	0.33±0.02^{b,c}	0.30±0.01^{a,c}	0.36±0.03^{b,d}	0.34±0.02^{b,c}	0.36±0.02^{b,d}	0.32±0.03^{c}	0.37±0.03^{d}	0.35±0.02^{d}
EAAs	1.29±0.03^{a}	1.35±0.03^{b}	1.50±0.04^{c}	1.35±0.04^{b}	1.55±0.03^{c,d}	1.56±0.04^{c,d}	1.58±0.03^{d}	1.40±0.04^{d}	1.71±0.03^{e}	1.57±0.04^{d}
NEAAs	1.95±0.04^{a}	2.20±0.04^{b}	2.25±0.05^{b}	2.25±0.03^{b}	2.39±0.03^{c}	2.47±0.04^{d}	2.35±0.03^{c}	2.27±0.05^{b}	2.54±0.05^{d}	2.33±0.04^{c}
TBAAs	3.24±0.04^{a}	3.55±0.04^{b}	3.85±0.05^{c}	3.60±0.04^{b,e}	3.94±0.03^{d}	4.02±0.04^{d}	3.93±0.03^{d}	3.67±0.05^{e}	4.25±0.05^{f}	3.90±0.04^{d}
AAS (%)	30.4±0.5^{a}	31.8±0.4^{b}	34.9±0.4^{c}	30.9±0.3^{a}	37.9±0.5^{d}	36.8±0.5^{e}	38.4±0.6^{d}	36.4±0.6e	40.2±0.7^{f}	37.0±0.5^{e}
EAAI (%)	4.30±0.03^{a}	4.50±0.04^{b}	5.00±0.04^{c}	4.40±0.03^{d}	5.40±0.05^{e}	5.20±0.06^{f}	5.40±0.05^{e}	5.10±0.04f	5.70±0.07^{g}	5.20±0.05^{f}
LAA	Ile	Thr	Thr	Thr	Ile	Ile	Thr	Thr	Thr	Thr
Crude protein (%)	20.2±0.6^{a}	34.6±0.5^{b}	29.4±0.4^{c}	35.0±0.6^{b}	23.4±0.6^{d}	25.0±0.5^{e}	25.3±0.6^{e}	23.9±0.5^{d}	21.2±0.6^{a}	23.0±0.5^{d,f}
Ash (%)	5.10±0.10^{a}	6.30±0.20^{b}	7.90±0.20^{c}	8.00±0.10^{c}	6.00±0.20^{b}	8.20±0.20^{c}	5.70±0.20^{d}	5.40±0.10^{e}	5.00±0.10^{a}	5.50±0.10^{e}
Dry matter (%)	97.0±0.5^{a}	97.3±0.4^{a}	96.7±0.2^{a,b}	97.0±0.3^{a}	96.5±0.5^{b}	95.8±0.3^{c}	97.3±0.4^{a}	96.3±0.5^{b}	97.1±0.3^{a}	97.4±0.3^{a}

Results are presented in dry weight basis as means ± SD, n=5 (mean value of five measurements). Means within a line with at least one identical superscript do not differ significantly ($P \geq 0.05$), while means with different superscripts show a significant difference ($P<0.05$).

EAAs – Essential amino acids, NEAAs – Non-essential amino acids, TBAAs – Total bound amino acids, AAS – Amino acid score, EAAI – Essential amino acid index, LAA – Limiting amino acid.

Table 3

Contents of selected minerals and trace elements in matcha evaluated by inductively coupled plasma mass spectrometry (ICP-MS).

Analyte	Mo Cha Fen	Whittard	Shao Xing	Asagiri	Harmony	Jeju	Don Matcha	Day spa organic	Day spa premium	Royal Pharma
µg/g										
^{23}Na	130±2^{a}	160±8^{b}	135±2^{c}	125±2^{d}	161±3^{b}	185±2^{e}	131±2^{a}	135±2^{c}	155±2^{f}	185±1^{e}
^{24}Mg	1890±20^{a}	2290±20^{b}	1850±20^{c}	1760±10^{d}	2400±30^{e}	2090±20^{f}	2080±30^{f}	1850±30^{c}	2300±20^{b}	2090±20^{f}
^{27}Al	9.11±0.10^{a}	5.42±0.12^{b}	11.5±0.1^{c}	12.1±0.1^{d}	113±1^{e}	10.8±0.1^{f}	9.11±0.20^{a}	11.5±0.1^{c}	11.2±0.3^{c}	10.8±0.1^{f}
^{31}P	3850±20^{a}	4070±20^{b}	4160±30^{c}	3740±30^{d}	4040±20^{b}	3740±30^{d}	3770±20^{d}	4160±20^{c}	4180±30^{c}	3740±30^{d}
^{39}K	2970±20^{a}	4150±30^{b}	4290±30^{c}	2750±20^{d}	2970±10^{a}	4190±30^{b}	2980±20^{a}	4290±30^{c}	4730±30^{e}	4190±30^{b}
^{44}Ca	1980±20^{a}	1650±20^{b}	2090±10^{c}	2310±20^{d}	2640±20^{e}	2650±20^{e}	1760±20^{f}	2090±10^{c}	2650±30^{e}	2650±30^{e}
^{55}Mn	40.1±1.0^{a}	59.7±2.1^{b}	68.4±2.4^{c}	17.1±1.0^{d}	19.1±1.2^{e}	23.5±1.0^{f}	17.8±1.2^{d}	17.1±1.3^{d}	17.4±1.0^{d}	24.6±1.1^{f}
^{57}Fe	89.4±1.5^{a}	87.5±1.4^{a}	94.1±2.8^{b}	112±3^{c}	96.4±2.5^{b}	115±2^{c}	83.6±1.5^{d}	112±3^{c}	111±3^{c}	87.5±1.8^{a}
^{63}Cu	19.6±1.2^{a}	6.21±0.20^{b}	6.12±0.10^{b}	23.6±2.0^{c,d}	22.9±0.6^{c}	20.1±1.0^{a}	25.3±1.0^{d}	23.7±2.1^{c,d}	7.04±0.2^{e}	6.69±0.20^{f}
^{66}Zn	68.5±1.5^{a}	21.5±0.5^{b}	23.5±0.6^{c}	73.0±1.4^{d}	34.7±1.1^{e}	24.7±0.3^{f}	68.5±1.8^{a}	73.0±1.5^{d}	24.7±0.7^{f}	38.7±1.0^{g}
^{137}Ba	4.42±0.08^{a}	4.40±0.10^{a}	2.21±0.10^{b}	7.02±0.10^{c}	3.84±0.10^{d}	5.33±0.10^{e}	2.22±0.1^{b}	7.02±0.10^{c}	3.61±0.1^{f}	4.41±0.1^{a}
ng/g										
^{7}Li	15.4±0.3^{a}	24.8±1.0^{b}	31.6±1.5^{c}	39.1±0.5^{d}	34.3±1.0^{e}	19.6±1.0^{f}	34.7±0.3^{e}	31.6±1.5^{c}	21.5±1.1^{g}	19.6±1.0^{f}
^{9}Be	12.2±0.4^{a}	3.80±0.10^{b}	15.5±0.2^{c}	5.30±0.10^{d}	2.40±0.08^{e}	12.2±0.1^{a}	6.21±0.11^{f}	15.5±0.20^{c}	6.72±0.10^{g}	12.2±0.20^{a}

^{48}Ti	771±10[a]	421±8[b]	621±6[c]	658±9[d]	459±10[e]	663±7[f]	370±10[g]	658±9[d]	452±8[e]	380±6[f]
^{52}Cr	10.9±0.3[a]	5.60±0.10[b]	18.9±0.1[c]	21.1±0.5[d]	4.80±0.10[e]	20.3±0.2[f]	9.61±0.20[g]	21.1±0.4[d]	2.12±0.10[i]	7.93±0.20[j]
^{59}Co	32.6±1.0[a]	30.4±1.0[b]	31.6±0.8[a]	22.3±0.5[c]	31.8±1.1[a]	29.0±0.5[d]	35.5±1.0[e]	22.3±0.2[c]	32.9±1.0[a]	23.7±1.2[c]
^{60}Ni	268±3[a]	205±2[b]	326±2[c]	228±2[d]	366±4[e]	296±4[f]	343±2[g]	228±52[d]	434±5[h]	281±3[i]
^{71}Ga	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
^{75}As	30.1±1.0[a]	31.5±1.2[a]	31.2±0.4[a]	24.2±0.4[b]	14.2±0.3[c]	30.0±1.3[a]	21.2±0.7[d]	24.2±0.4[b]	28.6±0.3[e]	28.0±0.5[e]
^{77}Se	12.5±0.2[a]	17.6±0.3[b]	8.92±0.12[c]	15.9±0.3[d]	16.3±0.3[d]	14.3±0.4[e]	8.83±0.20[f]	15.9±0.3[d]	7.82±0.10[g]	10.2±0.2[h]
^{88}Sr	233±2[a]	288±2[b]	195±2[c]	255±3[d]	388±2[e]	194±2[c]	205±3[f]	256±3[d]	258±3[d]	408±5[g]
^{107}Ag	17.3±0.5[a]	14.2±0.3[b]	25.6±1.0[c]	30.4±0.5[d]	31.4±0.4[e]	18.2±0.5[f]	25.8±0.4[c]	30.4±0.5[d]	15.4±0.3[g]	23.8±0.4[h]
^{111}Cd	1.31±0.10[a]	1.12±0.10[b]	1.03±0.10[c]	1.41±0.10[a]	1.52±0.10[d]	1.11±0.10[b]	1.03±0.02[c]	1.43±0.03[a]	1.23±0.10[a,b]	1.11±0.10[b]
^{118}Sn	1.12±0.10[a]	0.81±0.10[b]	0.62±0.10[c]	0.62±0.08[c]	0.53±0.10[c,e]	1.51±0.10[d]	0.42±0.03[e]	0.63±0.10[c]	1.10±0.10[a]	1.10±0.10[a]
^{133}Cs	27.4±1.1[a]	22.8±0.4[b]	27.6±0.1[a]	36.5±1.5[c]	38.9±1.0[d]	27.6±0.3[a]	54.3±0.4[e]	36.5±2.0[c]	25.6±0.2[f]	25.1±0.2[f]
^{140}Ce	21.4±0.3[a]	43.8±1.0[b]	37.5±1.2[c]	41.3±0.9[d]	44.6±1.4[e]	36.9±1.0[c]	25.6±0.3[f]	41.3±0.5[d]	16.3±0.3[g]	22.3±0.4[h]
^{165}Ho	0.92±0.10[a]	0.91±0.10[a]	1.32±0.10[b]	1.63±0.11[c,e]	1.01±0.11[d]	1.52±0.05[c]	1.54±0.02[c]	1.62±0.04[c,e]	0.81±0.05[a]	2.22±0.04[f]
^{181}Ta	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
^{202}Hg	1.32±0.10[a]	1.41±0.10[a]	2.14±0.05[b]	1.71±0.10[c]	1.72±0.10[c]	1.31±0.10[a]	2.63±0.10[d]	1.70±0.05[c]	1.50±0.05[e]	1.50±0.10[e]
^{205}Tl	1.01±0.10[a]	2.23±0.10[b]	2.03±0.10[c]	1.02±0.10[a]	1.21±0.10[d]	2.20±0.10[b]	1.90±0.05[c,e]	1.00±0.10[a]	1.81±0.10[e]	1.81±0.10[e]
^{208}Pb	1.82±0.10[a]	ND	0.07±0.01[b]	0.05±0.01[c]	0.07±0.01[b]	ND	1.04±0.05[d]	ND	1.33±0.10[e]	ND
^{238}U	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Results are presented in dry weight basis as means ± SD, n=5 (mean value of five measurements). Means within a line with at least one identical superscript do not differ significantly (*P*≥0.05), while means with different superscripts show a significant difference (*P*<0.05).

ND – not detected. Values of LOQ: Ga, Ta, Pb and U < 0.01 ng/g.

Table 4

Daily intake estimations for the essential amino acids, histidin and cystein in matcha.

Analyte	Range (mg/g)	Daily intake (mg/day)	RDA (mg/kg)	RDA (%) F, 65 kg	RDA (%) M, 80 kg
His	2.96–5.48	14.8–27.4	10	2–4	2–3
Ile	4.95–10.20	24.8–51.0	20	2–4	2–3
Leu	9.07–16.20	45.4–81.0	39	2–3	1–3
Lys	7.49–15.62	37.5–78.1	30	2–4	1–3
Met + Cys	6.12–11.57	30.6–57.9	15	3–6	3–5
Met	3.29–7.42	16.5–37.1	10	3–6	2–5
Cys	2.83–4.15	14.2–20.8	4	6–8	4–7
Phe + Tyr	10.00–16.93	50.0–84.7	25	3–5	3–4
Thr	5.03–9.01	25.2–45.1	15	3–5	2–4
Val	6.36–12.24	31.8–61.2	26	2–4	2–3

RDA – Recommended daily allowance according to WHO/FAO/UN (2007), F – female, 65 kg, M – male, 80 kg. A daily serving size of matcha tea was set to 5 g.

Table 5

Daily intake estimations for essential minerals and trace elements in matcha.

Analyte	Range µg/g	Daily intake mg/day	RDA or AI* (F) mg/day	RDA or AI* (M) mg/day	RDA or AI* (F) %	RDA or AI* (M) %
Mg	1760–2400	8.8–12.0	320	420	3.0–4.0	2.0–3.0
P	3740–4180	18.7–20.9	700	700	2.7–3.0	2.7–3.0
K	2750–4730	13.8–23.7	4700*	4700*	0.3–0.5*	0.3–0.5*
Ca	1650–2650	8.3–13.3	1000	1000	0.8–1.3	0.8–1.3
Na	124–185	0.6–0.9	1500*	1500*	< 0.1*	< 0.1*
Mn	17.1–68.4	0.09–0.34	1.8*	2.3*	5.0–19.0*	4.0–15.0*
Fe	83.6–115.0	0.42–0.58	18	8	2.3–3.2	5.3–7.3
Cu	6.21–25.3	0.03–0.13	0.9	0.9	3.3–14.0	3.3–14.0
Zn	21.5–73.0	0.11–0.37	8	11	1.0–5.0	1.0–3.0
Cr	0.0021–0.0210	0.00001–0.00010	0.025*	0.035*	0.1–0.4*	0.1–0.3*
Se	0.0078–0.0176	0.00005–0.00009	0.055	0.055	0.1–0.2	0.1–0.2

RDA – Recommended daily allowance is written in ordinary type without an asterisk; AI* – Adequate intake is followed by an asterisk (*), M – male 31–50 years old, F – female 31–50 years old. A daily serving size of matcha tea was set to 5 g.

Table 6

Intake estimations for toxic elements in matcha.

Analyte	Range µg/g	Daily intake µg/day	Weekly/Monthly* intake µg	PTWI, PTMI* µg/kg	PTWI, PTMI* (F, 65 kg) %	PTWI, PTMI* (M, 80 kg) %
Al	5.42–113.0	27.1–565.0	190–791	2000	0.2–0.6	0.1–0.5
Cd	0.0010–0.0015	0.005–0.008	0.15–0.24	25*	< 0.1*	< 0.1*
Sn	0.0004–0.0015	0.002–0.008	0.014–0.056	14000	< 0.01	< 0.01
Hg	0.0013–0.0026	0.006–0.013	0.042–0.091	4	< 0.03	< 0.02

PTWI – Provisional tolerable weekly intake, PTMI – Provisional tolerable monthly intake is followed by an asterisk (*), F – female, 65 kg, M – male, 80 kg. A daily serving size of matcha tea was set to 5 g.