Journal of Trace Elements and Minerals 12 (2025) 100217

Contents lists available at ScienceDirect

Journal of Trace Elements and Minerals

journal homepage: www.elsevier.com/locate/jtemin

Trace element and mercury speciation analysis in yerba mate (*Ilex paraguariensis*)

María Victoria Panzl [a,c], Jarol R. Miranda-Andrades [b,*], Wendy J. Sandoval Rojano [b], Joseane A. Mendes [b], Tatiana D. Saint'Pierre [b], Alejandra Rodríguez-Haralambides [c], Ricardo Q. Aucelio [b]

[a] Graduate Program in Chemistry, Facultad de Química, Universidad de la República, Uruguay
[b] Chemistry Department, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Rio de Janeiro, RJ 22453-900, Brazil
[c] Bioanalytical Chemistry Laboratory, Instituto Polo Tecnológico de Pando, Facultad de Química, Universidad de la República, Pando 91000, Uruguay

ARTICLE INFO

Keywords:
Yerba mate
Speciation analysis
Essential and toxic elements
Cold vapor techniques
Inductively coupled plasma mass spectrometry
Optical emission

ABSTRACT

Background: Yerba mate, a popular beverage in South America, is rich in bioactive compounds and minerals. However, yerba mate leaves may contain toxic metals and environmental contaminants. This study performed multi-element analyses using ICP-MS and ICP OES and mercury speciation by GC-CV-AFS and CV–AAS to profile infusions of ten yerba mate samples consumed in Brazil and Uruguay.

Materials and methods: For ICP OES and ICP-MS analyses, 500 mg infusions of samples were prepared and diluted with 10 % v/v HNO_3. Ultrasonic-assisted extractions of mercurial species were made followed by distillation and dilution with water for CV-AAS. For GC-CV-AFS speciation, samples were extracted and distilled, using 200 µL aliquots for analysis.

Results: The most abundant elements in the digested solid samples were K, Ca, and Mg, whereas in infusions the ranges were up to 9.18 mg g^{-1} for K, 0.68 mg g^{-1} for Ca, and 2.74 mg g^{-1} for Mg. Pb presented a mean concentration of 260 µg kg^{-1} in leaves but it was not found in infusions. Hg^{2+} was found from 0.67 to 0.96 µg kg^{-1} in leaves with one sample presenting 0.67 µg kg^{-1} of CH_3Hg.

Conclusion: K, Ca, and Mg were the most abundant elements in the yerba mate samples. Four samples showed ultra-trace concentrations of Hg^{2+} and one of CH_3Hg.

1. Introduction

Mercury is a highly toxic and persistent contaminant, widely present in environmental sources [1,2]. According to the World Health Organization (WHO), Hg is ``one of the top ten chemicals or groups of chemicals of great concern for public health'' [3]. The toxicity of mercury depends heavily on its chemical form, which includes predominantly elemental mercury (Hg^0), inorganic mercury (Hg^{2+} and its inorganic complex ions) and organic mercury [4]. Organic mercury such as CH_3Hg bioaccumulate in human tissues [5] and its toxicological effects on plant and animal life have long been recognized [6].

The availability of analytical information is crucial to prevent human exposure to toxic levels of metals through the ingestion of foods and beverages [7]. Therefore, the development of effective and sensitive methods for the determination of mercury species at ultra-trace levels in food samples is of great importance.

One of the most studied sources of mercury contamination in humans is through fish and seafood [7], where mercury biomagnification in the food chain reveals high concentrations in top predators [8]. Studies in other types of food revealed total mercury concentrations from 75.2 to 158.7 µg kg^{-1} in rice, and from 55.6 to 343.9 µg kg^{-1} in vegetables [9]. In bottled natural mineral waters, a total mercury concentration has been reported reaching up to 1.0 ng L^{-1} [10]. Studies have also reported the presence of mercury in some fertilizers used in agricultural activities [11], which can influence the accumulation and transfer of this toxic metal in the soil-plant system.

Yerba mate (*Ilex paraguariensis*) is a perennial specie, native of South America southern cone [12], whose leaves are consumed as hot or cold infusions by large part of the population [13]. For instance, in Uruguay the consumption is about 12 kg of the dried yerba mate /person /year

* Correspondent author.
E-mail address: jmirandaa83@live.com (J.R. Miranda-Andrades).

https://doi.org/10.1016/j.jtemin.2025.100217
Received 4 September 2024; Received in revised form 8 January 2025; Accepted 2 February 2025
Available online 3 February 2025

[14]. Different authors reports that consumption of yerba mate is beneficial to humans due to the great variety of bioactive compounds [15–17], source of macroelements (Ca, Mg, Na, K), and microelements (Cu, Zn, Fe, and Mn) [18–22]. Recently, a research reported by Welna et al. [23] determined the concentration in yerba mate samples of Ca, Mg, K, and Na ranging between 16 and 118 mg kg^{-1}, and small amounts of the essential microelements (Cu, Fe, Mn, and Zn, 0.032–2.40 mg kg^{-1}). The consumption of yerba mate is also a way of exposure to toxic elements (Al, Cd, and Pb) [24–26], given that the yerba mate tree can be a bioaccumulator of toxic metals, and can potentially act as a route for these elements to enter the food chain [27]. Accordingly, maximum accepted limits for Cd, As and Pb were set at 0.6 μg $g^{-1}$and 0.4 μg g^{-1} respectively [28,29]. The main sources of contaminants in yerba mate leaves are related to the environment or to anthropological activities, being the soil an important source for cadmium and lead intake [30]. Several studies has been conducted in order to determine the content of minerals and metals in leaves and infusions using spectrometric-based techniques, like inductively coupled plasma optical emission spectrometry (ICP OES) [31], and inductively coupled plasma mass spectrometry (ICP-MS) [32], both techniques employed in the present study.

In 2011, WHO established 4 μg kg^{-1} as the tolerable weekly intake limit of mercury, but the panel of Contaminants in the food chain (CONTAM) of the European Food Safety Authority (EFSA) recommend a tolerable weekly intake of Hg of 1.3 μg kg^{-1} body weight [33]. Likewise, the Committee of Specialists in Food Additives (JECFA) established two values, one for CH_3Hg (1.6 μg kg^{-1} of body weight), and another for inorganic Hg (4 μg kg^{-1} of body weight) [11]. In 2018, the Regulation (EU) 2018/73 updated the maximum residue levels (MRL) for mercury compounds in teas or infusions (tea, coffee, infusions and cocoa beans, etc.) to 20 μg kg^{-1} [34]. In Brazil, the National Sanitary Surveillance Agency (ANVISA), in 2013, established 50 μg kg^{-1} as the MRL for mercury in any food [35].

The methods for analyzing mercury (Hg) and identifying its forms in environmental, food, and biological samples are well-established [36]. They usually involve separating the mercury from other substances and then detecting it using spectrometric techniques [37–42]. Other methods can also be used, such as using inductively coupled plasma mass spectrometry (ICP-MS) combined with high-performance liquid chromatography (HPLC) [43–47], capillary electrophoresis [48], total-reflection X-ray fluorescence [49], electrochemical methods [50], or direct mercury analysis [51].

Recent studies have measured mercury levels in herbal and fruit infusions, finding 10 μg kg^{-1} in tea leaves, but none in tea infusions [52]. Another study found an average of 2.47 μg kg^{-1} in tea and 5.67 μg kg^{-1} in yerba mate [53]. These studies measured total mercury without considering its different chemical forms, which vary in toxicity. This is important because methylmercury is highly toxic and poses significant health risks.

To identify the different forms of mercury, sensitive methods like gas chromatography with cold-vapor atomic fluorescence spectrometry (GC-CV-AFS) are recommended by the United States Environmental Protection Agency (EPA) as the most reliable approach [54]. Ensuring the safety and quality of yerba mate requires precise measurements, as mercury can transform and become more toxic in the environment [55]. Understanding how mercury is in terms of the chemical form is crucial for assessing its toxicity, which is an important but underexplored area in yerba mate research [56].

This study aims to analyze the overall metal content, including mercury, in commercial yerba mate from southern Brazil using ICP OES and ICP-MS, helping to address gaps in food safety research. By using GC-CV-AFS with improved extraction and distillation methods, the study ensures accurate and precise mercury measurements. Understanding mercury in yerba mate is vital for public health, especially since yerba mate is a popular beverage in South America.

2. Material and methods

2.1. Reagents and materials

Ultrapure deionized water (resistivity ≥18.2 MΩ cm) was obtained from a Milli-Q A10 Gradient system (Millipore, USA). Nitric acid (65 % p.a.) and hydrogen peroxide (30 % Suprapur) were obtained from Merck (Germany). Nitric acid was purified by sub-boiling bi-distillation in quartz still (Duo-PUR, Milestone, USA). Brooks Rand Instruments (USA) provided sodium tetra (n-propyl) borate ($NaBPr_4$), and high purity standard solutions (1 mg L^{-1}) of methylmercury chloride (CH_3HgCl), ethylmercury chloride (CH_3CH_2HgCl) and mercury chloride ($HgCl_2$). Multi-elemental standard PE-29 (Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn) elements was obtained from PerkinElmer (USA). Acetate buffer solution (pH 4.5) was also from Brooks Rand Instruments. Tin chloride was obtained from Vetec (Brazil). Sulfuric acid, hydrochloric acid, sodium chloride, sodium nitrate, ammonium hydroxide, Triton X-100, Triton X-114 were purchased from Merck (Germany). The nitrogen gas used were 99.99 % purity, and argon was of high purity (99.996 %). The solution of simethicone (about 75 mg mL^{-1}), used as an antifoam, was purchased from Prati-Donaduzzi (Brazil) and acquired at a local pharmacy. Samples were filtered through a 0.22 μm filter (13 and 47 mm diameter) made of hydrophilic polyvinylidene fluoride (PVDF) acquired from Waters (USA).

2.2. Commercial samples of yerba mate (*Ilex paraguariensis*) and standard reference material

Ten commercial samples of yerba mate were used in this study. The information of the samples is summarized in Table 1. Standard Reference Material (NIST CRM 1515 - Apple Leaves, National Institute Standards and Technology, USA) was used to evaluate efficiency of the extraction methods.

2.3. Instruments

Total mercury determinations were made on a model RA-915 portable mercury cold vapor (CV) atomic absorption spectrometry (AAS) analyzer with the Zeeman background correction and a multi-pass cell (LUMEX, Russia). A RP-92 chemical reduction accessory for aqueous solutions (LUMEX) was adapted to the analyzer. Mercury speciation analysis was made on a MERX automated methyl mercury system (Brooks Rand Instruments) composed by a trap system, a GC system and a CV atomic fluorescence detector. The GC-CV-AFS system has modules for purging and retention/pre-concentration, which are used respectively to purge the volatile derivatized mercury species into the system where they are retained in pre-concentration traps to be further desorbed. A chromatographic module performs the separations of the mercury-derivatized species, while the pyrolysis module promotes thermal decomposition generating the Hg^0, which is detected by the

Table 1
Commercial samples of yerba mate (*Ilex paraguariensis*).

Identification	City/State	Country	Type of process/product
M_1	Barão de Cotegipe-RS	Brazil [a]	Aged yerba mate
M_2	Barão de Cotegipe-RS	Brazil [a]	Aged yerba mate with herbs
M_3	Encantado-RS	Brazil [a]	Aged yerba mate
M_4	Encantado-RS	Brazil [a]	Aged yerba mate
M_5	Tuparendí-RS	Brazil [a]	Aged yerba mate
M_6	Rio Grande-PR	Brazil	Roasted yerba mate
M_7	Nova Prata-RS	Brazil [a]	Aged yerba mate with herbs
M_8	Barão de Cotegipe-RS	Brazil [a]	Aged yerba mate with herbs
M_9	Arvoreziha-RS	Brazil [a]	Aged yerba mate
M_{10}	Ilópolis-RS	Brazil	Green yerba mate

Reference: [a] Produced only for its consumption in Uruguay.

fluorescence detector. The GC separation column (Brooks Rand Instruments) was a U-shaped glass column (4 mm internal diameter) packed with 15 % OV-3 (10 % phenylmethyl-dimethylsilicone) in *Chromosorb* white solid support. The determination of the others elements was made by ICP-MS using a NexIon 300X mass spectrometer (PerkinElmer, USA), and by ICP OES employing an Optima 7300 DV spectrometer (PerkinElmer, USA).

2.3.1. Cleaning procedures for ultra-trace determination of mercurial species

In order to prevent possible sources of external contamination of residual mercury, all materials (made of glass, Teflon and quartz) were previously subjected to a thorough cleaning. First, the materials were immersed, for 12 h, in sub-boiled HNO_3 solution (5 % v /v), washed with Extran detergent solution and then with distilled water, before rinsing three times with ultrapure water. The materials used in the distillation were subsequently washed with acetone, then with dichloromethane, and finally using hexane before drying in an oven. Quartz tubes (used for photocatalytic oxidation of mercurial species) were placed in a muffle furnace for 6 h at 400 °C. Occasionally, it was also necessary to place material in detergent solution inside an ultrasonic bath. All reagents were evaluated for original traces of mercury using the CV-AAS system. For this, 1 mL aliquots of a reagent solution were diluted 10 times and mixed with 3 mL of $SnCl_2$ (20 % m/v) in the RP−92 chemical reduction accessory. Any residual mercury measured from each reagent was considered in the final calculation of the mercury found in the samples.

2.4. Standards solutions

2.4.1. Multi-elemental standards

Standard stock solutions were prepared in acid medium (10 % v/v HNO_3) at concentrations of 10, 100 and 1000 μg L^{-1}. The PE-29 solutions were diluted to the correct extent in an acidic medium to adjust analytical curves within proper ranges for ICP-MS: from 0.1 to 0.8 μg L^{-1}, 1.0 to 8.0 μg L^{-1} and from 10 to 80 μg L^{-1}. For ICP OES the elements Al, B, Ba, Ca, Fe, K, Mg, Mn, Na, P, Sr, and Zn were diluted in a range of 10 to 1000 μg L^{-1}.

2.4.2. Methyl mercury solutions

The CH_3HgCl intermediary solutions (1000 ng L^{-1}) were prepared by the direct dilution of 1 mg L^{-1} commercial standard solutions in ultrapure water. Working solutions (10 ng L^{-1} and 100 ng L^{-1}) were prepared by dilution of the intermediary solution in water. The derivatization reagent solution (1 % v/v) was prepared by dissolving 50 mg of $NaBPr_4$ in 5 mL of the ultrapure KOH (1 % v/v) aqueous solution. The $SnCl_2$ (2 % v/v) solution was prepared by dissolving 20 g of the salt in 100 mL of concentrated HCl. This solution was heated to evaporate half of the volume, in order to eliminate mercury contamination (decreasing analyte background and preventing contamination of the system). After cooling, ultrapure water was added to adjust final volume.

2.5. Sample preparation

2.5.1. Hot aqueous infusions procedure

As described by Pozebon et al. [21] for the infusion, a mass about 500 mg of sample was placed into a polypropylene tube along with 20 mL of ultrapure water at 100 °C standing for 5 min, in order to obtain the sample infusion. The mixture was filtered using a Whatman N° 542 (diameter 100 mm) filter paper (Whatman, USA) under forced vacuum system. Finally, the samples were diluted 10-fold and 1000-fold, before analysis, using a 10 % v/v HNO_3 solution.

2.5.2. Acid dissolution procedures

As described by Pozebon et al. [21] for elemental determinations, the targeted elements were extracted by mixing (200.0 ± 0.1) mg samples with 2.5 mL of HNO_3 in a polypropylene tube. Subsequently, the tube was closed with a screw cap and placed on a heating block for 4 h at 90 °C. Then: i) 1.0 mL of H_2O_2 was added to the mixture, ii) the mixture was further heated, on the heating block, for 30 min at 90 °C, iii) the obtained solution was left to cool to room-temperature, iv) the volume of the solution was adjusted to 25 mL using ultrapure water and v) and then 10-fold diluted with 10 % (v/v) HNO_3 before analyses. The efficiency of the procedure was checked using the NIST CRM 1515 and expressed as recovery percentage (Table S1), with results varying from 54 % (Ca) to 109 % (B).

2.5.3. Acid dissolution procedures for the determination of mercurial species

For the analysis of total mercury (comparative method), it was necessary to perform an acid dissolution (in closed vessels). In this case, 50.0 ± 0.1 mg of yerba mate samples were digested with 2 mL of HNO_3 (concentrated and sub-boiled distilled) in a Teflon tube at 80 °C for 2 h in an automated digestion system. After digestion, samples were cooled to room temperature and the volume adjusted to 10 mL with ultrapure water. For determinations of total mercury, aliquots of 1 mL of this solution were diluted to 10 mL and then analyzed in the CV-AAS system. Five replicates of 50 mg of reference material NIST CRM 1515 were digested with 2 mL of HNO_3. The same CV-AAS analysis protocol was followed for total mercury determinations in the reference material.

2.5.4. Ultrasonic-assisted extraction process for mercurial species

Sample extracts were prepared using macerated and homogenized yerba mate with an agate mortar and pestle, an established technique that ensures sample uniformity prior to extraction. However, the innovation lies in the use of ultrasound-assisted extraction with a nonionic surfactant, followed by distillation. This procedure was adjusted based on an existing protocol in the literature [57] but introduces a new methodological contribution by applying the combination of processes, in the efficiency of extraction of mercury species from yerba mate matrices. In brief, 50.0 ± 0.1 mg of yerba mate sample was weighed and transferred to Teflon tubes. Aliquots of Triton X-114 solution (5 % w/v) and H_2SO_4 solution (2 mol L^{-1}) were added to adjust the pH to 3. The application of an ultrasonic water bath at 40 °C to extract mercury species from the leaves highlights the use of modern and innovative technology in the extraction.

Subsequently, 50.0 mL of yerba mate extracts, 0.5 mL of H_2SO_4 aqueous solution (8.0 mol L^{-1}), and 50 μL of simethicone (75 g L^{-1}) were added to a Teflon tube used in the distillation system for methylmercury. Here, the EPA−1630 method [58] was applied, representing an established technique for the distillation of methylmercury. Distillation was performed at 130 °C under argon flow, adjusted between 50 and 90 mL min^{-1}, concluding in approximately 120 min or until 40 mL of distillate were collected. Finally, the distillates were stored at 5 °C in the dark in the refrigerator, according to established practices for the preservation of the extracted compounds. This procedure stands out for eliminating the need for toxic solvents, thus reducing the environmental impact. The incorporation of Triton X-114 as a nonionic surfactant replaces harmful solvents, demonstrating a commitment to more environmentally friendly practices.

2.6. Procedures for mercury determination

2.6.1. Measurements of Hg^{2+} and total mercury by cold vapor atomic absorption spectrometry

For preliminary studies regarding the determination of mercurial species, aliquots (10 mL of infusion) of yerba mate were used. After filtering the sample infusions (using a 0.22 μm PVDF filter), a 1 mL aliquot was diluted to 10 mL in volumetric flasks with ultrapure water. To determine inorganic mercury, the total volume of the sample was transferred to the glass reaction cell (bubbler) of the CV-AAS system.

Preliminary analyzes of total mercury in yerba mate samples were

carried out using 1 mL of filtered samples of (digested liquid/solid infusions) yerba mate were placed in 10 mL volumetric flasks, containing 1 mL of H_2O_2 solution (1 % in volume) with pH adjusted to 4.5 (by adding aliquots of 0.1 mol HCl L^{-1} solution), then the total volume was transferred to a quartz tube (15 mL). The quartz tubes were placed for 15 min in a photoreactor under UV irradiation [59] to convert organic mercury into inorganic mercury. Then, the solution was placed in the glass reaction cell of the CV-AAS system to be reduced with 3 mL of $SnCl_2$ solution (20 % w/v) and then determined.

2.6.2. Speciation analysis of mercury by gas-chromatography cold vapor atomic fluorescence spectrometry

An aliquot (200 μL) from a 40 mL (of distilled sample) solution was transferred to a sample flask (amber with 40 mL volume and septum cap) containing 20 mL of ultrapure water, then mixed with 200 μL of the acetate buffer solution, and 100 μL of the derivatization reagent solution ($NaBPr_4$ at 1 % v/v). After 15 min, the flask was placed in the automatic sampler to be transferred to a purge vessel, forcing the liberation of the volatile propylated mercurial species that were carried by an argon flow (312 μmL μmin^{-1}) to a dry trap, where they were adsorbed and then thermally desorbed to be carried by an argon flow (34 μmL μmin^{-1}), through the chromatographic column. After separation, each of the species passed sequentially through a tube section, where heated by a resistance produce the Hg vapor detected.

2.7. Analytical conditions for ICP-MS and ICP OES

The analytical performance was evaluated through the following analytical figures of merit: sensitivities, response linearity by fitting twelve concentration points of analytical curves (evaluated as coefficient of determination or R^2), instrumental and methods limits of detection (LOD) and limits of quantification (LOQ) using respectively three and ten times the blank signal (comprising of water, hydrogen peroxide and nitric acid) and precision (expressed as relative standard deviation or %RSD for authentic triplicates) expressing inter-day and intra-day variations. Blank and triplicate samples were analyzed to provide quality control. The concentration of Al, B, Ba, Ca, Fe, K, Mg, Mn, Na, P, Sr, and Zn were determined by ICP OES. The concentration of Co, Cu, Mg, Ni, Sr, V, Zn, La and Ce were measured by ICP-MS. ^{103}Rh was used as internal standard (IS) to correct and/or compensate non-spectral interferences and matrix effects. Operating conditions for sample analysis by ICP-MS and ICP OES are presented in Table 2.

Table 2
Operational conditions used for REE analysis.

Parameter	ICP-MS	ICP OES
RF power	1100 W	1400 W
Plasma gas	17 L min^{-1}	15 L min^{-1}
Auxiliary gas	1 L min^{-1}	0.6 L min^{-1}
Nebulizer gas	0.92 L min^{-1}	0.6 L min^{-1}
Sample uptake rate	1.5 mL min^{-1}	1.5 mL min^{-1}
Replicates	3	3
Dwell time	50 ms	–
Plasma view mode	–	Axial (Al, B, Ca, K, Mg, Na, P) and Radial (Ba, Fe, Mn, Sr, Zn)
Monitored isotopes	^{75}As, ^{138}Ba, ^{59}Co, ^{112}Cd, ^{65}Cu, ^{24}Mg, ^{55}Mn, ^{60}Ni, ^{208}Pb, ^{88}Sr, ^{51}V, ^{66}Zn, ^{139}La and ^{140}Ce.	–
Emission lines (nm) and state	–	Al 396.153(I), B 249.677(I), Ba 455.403(II), Ca 422.673 (I), Fe 259.939(II), K 766,490 (I), Mg 285.213(I), Mn 257.610(II), Na 589.592(I), P 213,617(I), Sr 421.552(II), Zn 206.200(II).

3. Results and discussion

3.1. Mercurial species

3.1.1. Preliminary studies

Preliminary studies to assess the content of mercury (Hg^{2+} and total mercury) were made by CV-AAS (with multi-pass optical system), using aliquots of infusions from each of the different yerba mate samples (10 different samples). Results indicated that sample infusions identified as M_3, M_4, M_6, and M_9 presented ultra-trace levels of Hg^{2+}. The results for total mercury (after photochemical treatment) indicated that sample M_9 also presented ultra-trace residues of organic mercurial species (probably CH_3Hg) as seen in Fig. 1A.

In order to confirm the results obtained from the preliminary study using infusions, yerba mate leaves from each sample were submitted to acid dissolution using sub-boiled HNO_3. Thus, the determinations of Hg^{2+} (where organomercurial species transformed in Hg^{2+} during acid dissolution) were made by CV-AAS. The results for total mercury found in these digested yerba mate closely aligns with the observed results in yerba mate infusions, as samples identified M_3, M_4, M_6, and M_9 presented measurable ultra-trace levels of Hg^{2+} (Fig. 1B). Therefore, further speciation analysis studies were performed in samples M_3, M_4, M_6 and M_9. Temporal profiles obtained by CV-AAS for the calibration curve used in the determination of total mercury is shown in Fig. 1C.

3.1.2. Study of artifact formation and matrix effect of yerba mate samples on mercury speciation analysis

One of the challenges involved in carrying out mercury determinations in samples of yerba mate leaves is their organic content [60–62] that imposes interferences in speciation analysis [41]. For this reason, it was decided to separate the mercurial species from the sample matrix using ultrasonic extraction with Triton X-114, followed by derivatization and distillation assisted with argon.

Organomercurials artifacts tend to be formed during chemical derivatization [63] and extraction/distillation [57]. In addition, the presence of high amounts of purine alkaloids (methylxanthines such as caffeine and theophylline), polyphenols (chlorogenic acids and their derivatives), saponins and flavonoids in yerba mate, could promote methylation of inorganic mercury species when they are distilled, leading to erroneous results [57]. In order to study artifacts and provide conditions to minimize its formation, studies were conducted with the yerba mate sample M_2 (which presented a low amount of mercury detected by the CV-AAS method). The formation of artifacts of CH_3Hg and CH_3CH_2Hg was evaluated in function of the amount of sample mass (25; 50; 75; 100 and 200 mg); transferring the content of the yerba to Teflon tubes (70 mL) followed by the addition of 50 mL of water and fortification with 800 pg L^{-1} of Hg^{2+}. The extraction procedure was performed following conditions reported in the literature: pH 3.0 adjusted with H_2SO_4 and 40 min of ultrasonic agitation at 40 °C [57]. In order to monitor the efficiency of sample processing (ultrasonic assisted extraction-distillation), a standard solution with 800 pg de Hg^{2+} (called control solution) was submitted to distillation (bypassing the ultrasonic step). In all cases, distillation proceeded until about 90 % of the aqueous sample mixture volume had been collected as a distilled solution. Results showed that the CH_3Hg artifact appeared with the experiments carried out with 50 mg of yerba mate sample or more (2 pg, equivalent to 0.25 % in mass of the fortified amount of Hg^{2+}). The interference progressively increases when the experiments were carried out with 75 mg yerba mate (15 pg CH_3Hg); 100 mg (37 pg CH_3Hg); 150 mg (71 pg CH_3Hg), and 200 mg (117 pg CH_3Hg), as seen in Fig. 2A. In contrast, the CH_3CH_2Hg content remained constant (about 3 pg, where source was identified as the derivatization reagent). In order to find a compromise between minimization of artifacts and the mass of sample needed to provide measurable quantities of mercury, further experiments were made using 50 mg of yerba mate.

It is also known that formation of methylated compounds tends to

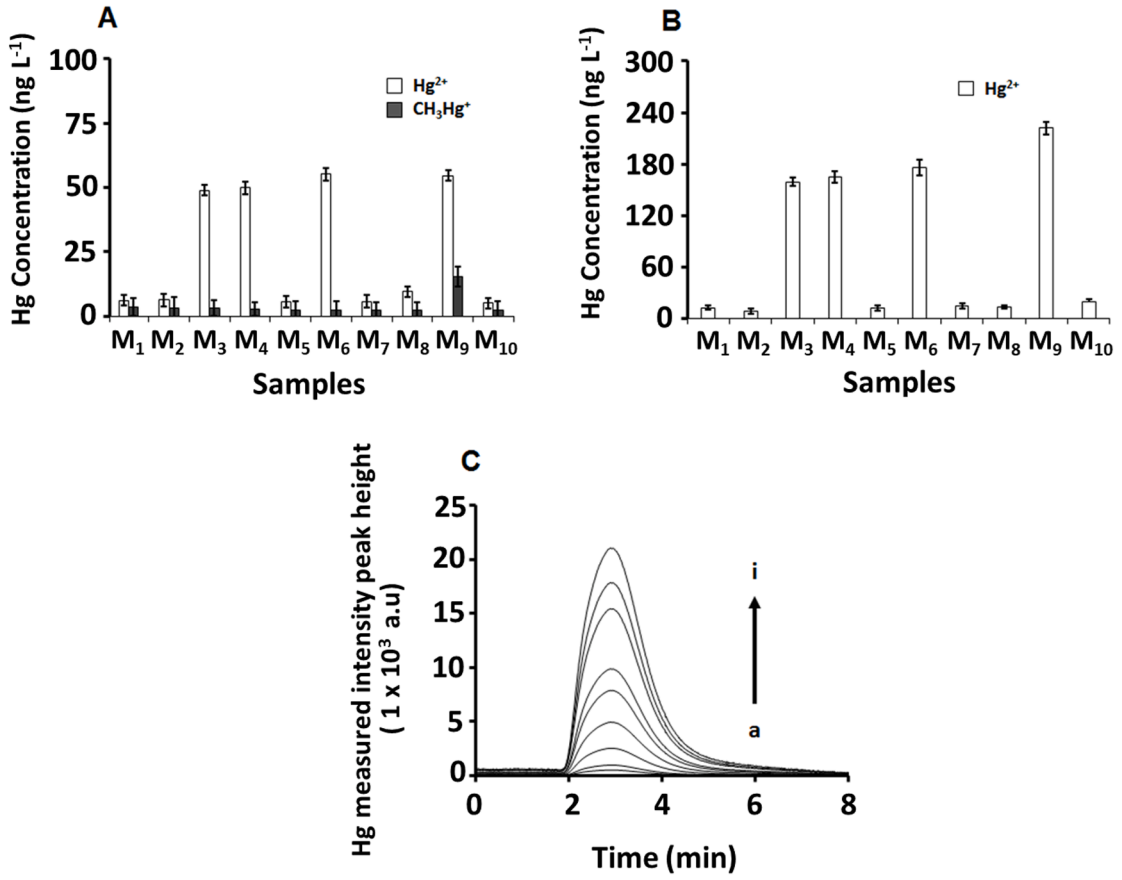

Fig. 1. Evaluation of mercury content (Hg^{2+} and other mercurial species) in samples (M_1–M_9) of yerba mate. A) Aliquots of infusions. B) Aliquots of acid solubilized samples. C) Hg^0 temporal profiles of increasing Hg^{2+} concentrations using CV-AAS with a multipass cell.

increase as the total organic content (TOC) increases, particularly at the end of the distillation stage, when organic matter and Hg^{2+} are concentrated in the distillation flask [57]. For this reason, the formation of organic mercurial species artifacts was also evaluated in function of the relative volume of the collected distilled solution (60 %, 70 % and 80 % of the original aqueous mixture). Results showed the formation of a constant amount of CH_3Hg artifact (about 0.20 % of the quantity measured for Hg^{2+} in control solution) no matter the distilled fraction collected (Fig. 2B); in contrast to about 0.25 % of artifact found when distilled collection was processed up to 90 % of the aqueous sample mixture. Therefore, in order to minimize artifact formation, the extraction-distillation procedure adopted employed 50 mg of yerba mate with distillation carried out until 80 % of the aqueous sample mixture was collected.

As the experiments carried out with 50 mg of yerba mate produced insignificant amounts of CH_3Hg as artifacts compared to those reported in the literature [63], it was necessary to evaluate matrix effect imposed by sample. Hence, fortifications of 800 pg of both Hg^{2+} and CH_3Hg were made in Teflon tubes containing 50 mg of sample (yerba mate M_2). In one set of samples ($n = 3$), a volume of 50 mL of water was added with no further pH adjustment and no addition of Triton X-114 as an aid for extraction. In a second set of samples ($n = 3$), besides water, an aliquot of a concentrated hydrochloric acid solution was added, to adjust to pH 3, along with Triton X-114 (aiming a final concentration of 0.5 %) before adjusting final volume to 50 mL. Control solutions, containing 800 pg of Hg^{2+} and CH_3Hg (without pH adjustment and without surfactant) were directly distilled, bypassing ultrasonic extraction, and used to evaluate efficiency of the distillation process.

Extractions were carried out following the protocol reported in the literature [57], using an ultrasonic bath for 40 min at 40 °C, and then, the Teflon tubes containing the extracts were placed in the distiller with the distillation ended right after the collection of about 80 % in volume of the distilled extract. This extract was transferred to amber tubes of the GC-CV-AFS system, where, shortly after the addition of the derivatizing reagent and pH adjustment, they were analyzed. The results from the speciation analysis performed on the yerba mate extracts, without pH adjustment and in absence of surfactant, showed recoveries of 15.8 % and 9.4 % for the Hg^{2+} and CH_3Hg respectively (Fig. 3B). In contrast, extractions carried out with the proper pH adjustment and using Triton X-114 produced recoveries yields of 79 % and 67.6 %, for the Hg^{2+} and CH_3Hg respectively (Fig. 3C), compared with the results obtained by analyzing the control samples (Fig. 3A). This indicated that the use of acidic pH and surfactant decreased sample matrix interferences after the distillation process.

3.1.3. Optimization of ultrasonic agitation and temperature for the determination of mercurial species in yerba mate

In order to achieve maximum recovery of mercurial species in the presence of vegetal matrix, an optimization was performed for the ultrasonic agitation and temperature during the extraction/distillation process. For these studies, 50 mg of yerba mate M_2 was used along with 50 mL of an aqueous solution at pH 3 and containing 0.5 % Triton X-114 and fortification compounds (800 pg of Hg^{2+} and 800 pg of CH_3Hg). The closed Teflon tubes were submitted to ultrasonic agitation during 30 min at different temperatures (30; 40; 50; 60; and 70 °C) before transferring the extract to the distiller. The GC-CV-AFS analyses were performed with distillates, and results showed better recoveries of the mercury species when ultrasonic agitation was made, at 50 °C and 60 °C. In experiments made at higher temperatures, the concentration of Hg^{2+} decreased while the concentration of CH_3Hg increased (Fig. 4A),

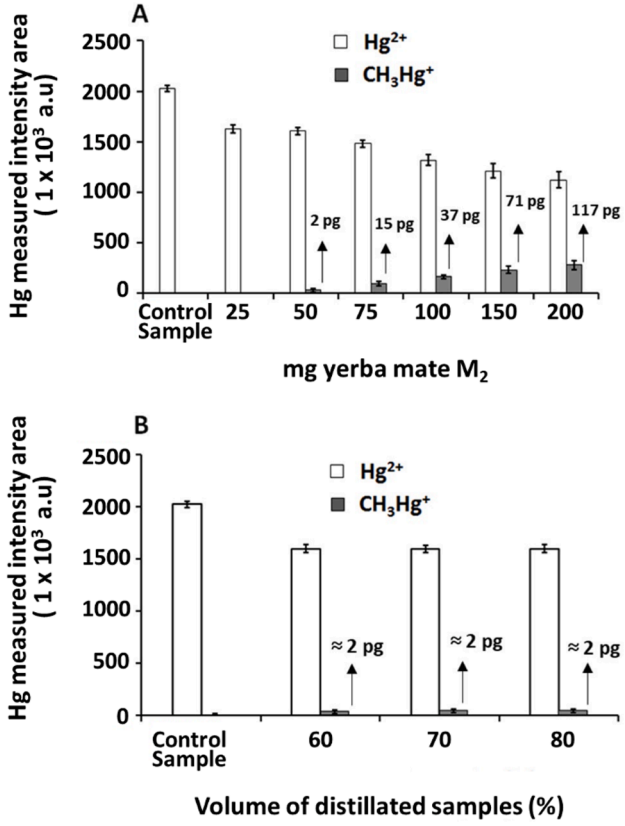

Fig. 2. Study of the formation of CH_3Hg artifact in samples extracts fortified with 800 pg of Hg^{2+}, according to: (A) mass in mg of yerba mate M_2. (B) volume of sample collected from distillation.

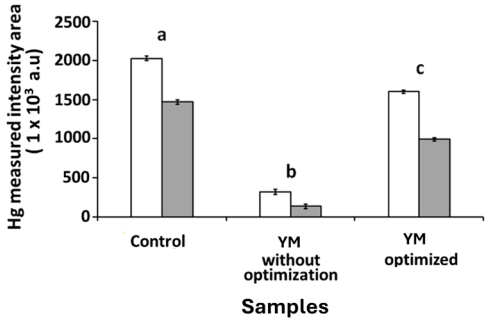

Fig. 3. Study of extraction/distillation of mercury species (800 pg of Hg^{2+} and CH_3Hg): a) control samples in water; b) A 50 mg of yerba mate (YM) and water; c) A 50 mg of yerba (YM) mate in pH 3 aqueous solution containing 0.5 % of Triton X-114. For (b) and (c), it was used ultrasonic agitation (40 min) at 40 ° C.

indicating a probable methylation of Hg^{2+} due to interactions with the organic material of the sample [57].

The ultrasonic agitation time (20; 30; 40; 50; 60; and 70 min) was further studied at 50 °C in order to evaluate the recovery of analytes in the presence of sample matrix. A gradual increase in recovery of mercury species occurred as samples were placed under prolonged ultrasonic agitation (Fig. 4B), reaching maximum yields after 50 min (recoveries of 91 % for Hg^{2+} and 85 % for CH_3Hg, using as reference the results obtained for distilled control standard solutions). As a conclusion the selected conditions to perform the analytical validation were: 50 mg of yerba mate, plus the addition of a concentrated hydrochloric acid solution to adjust to pH 3, along with Triton X-114 (0.5 %) solution, before adjusting final volume to 50 mL. Extractions were carried out using an ultrasonic bath for 40 min at 40 °C, and then, the Teflon tubes containing the extracts were placed in the distillation apparatus. The distillation end when collection of distilled reached about 80 % of the original volume.

3.2. Analytical figures of merit and method application for mercurial species

The sensitivity of the analytical curves prepared with standard solutions of Hg^{2+} in water and combined with 50 mg of yerba mate matrix M_2 are shown in Fig. 5. The yerba mate matrix imposes interference even after extraction and distillation, as can be evaluated by the decreasing of 7.6 % in curve sensitivity for Hg^{2+} and of 15.3 % for CH_3Hg (Fig. 5A and B). A two-tailed Student's *t*-test was used to determine the statistical significance of results, indicating that both decreases in sensitivity were statistically significant, with *p*-values of 0.028 and 0.035 (less than the critical significance level of 0.05). This confirms that the observed differences are not due to chance, but rather to true matrix interference.

Therefore, matrix-matched standards were used to improve the accuracy of determinations. Thus the analytical curves were constructed using the standards of Hg^{2+} and CH_3Hg mixed with 50 mg M_2 (sample with no detectable amounts of the mercurial species), so to achieve a matrix matching calibration within the absolute range from 30 to 800 pg (0.75 ng L^{-1} to 20 ng L^{-1}). These standards were also submitted to the ultrasonic treatments and distillation. Analytical curve equations for Hg^{2+} and for CH_3Hg using matrix-matched standards were, respectively, $Y = (2.3 \times 10^6 \pm 7.6 \times 10^4\ L\ pg^{-1})\ X - (325 \pm 43)$ and $Y = (1.4 \times 10^6 \pm 5.2 \times 10^4\ L\ pg^{-1})X - (443 \pm 68)$ with a R^2 of 0.9997 (Hg^{2+}) and 0.9998 (CH_3Hg).

Limit of detection (LOD) (pg/50 mg mass of sample) and limits of quantification (LOQ) were based on the amount of the measured mercury (as Hg^0) that produced, respectively 3 times and 10 times the standard deviation of the average baseline ($n = 10$) [64]. The LOD values were 9 pg per 50 mg mass of sample (or 0.2 ng L^{-1}) for Hg^{2+}, and 12 pg per 50 mg mass of sample (or 0.3 ng L^{-1} for CH_3Hg) while the LOQ for Hg^{2+} and CH_3Hg were 20 pg per 50 mg mass of sample (or 0.5 ng L^{-1}) and 23 pg per 50 mg mass of sample (or 0.6 ng L^{-1}) respectively. The LOD and LOQ values presented for Hg^{2+} and CH_3Hg are within the expected range of mercury in environmental samples and are considered acceptable. Achieved LOQ values are low enough to detect and quantify small concentrations of mercury, which is crucial for environmental monitoring and health risk assessment. The ability to detect and quantify mercury at such low levels indicates that the method is sensitive and suitable for applications that require high accuracy and reliability in the detection of contaminants in complex samples. Furthermore, these values are achieved thanks to an exhaustive cleaning protocol for the materials used in the analyses, ensuring that there is no cross-contamination and that the results are highly accurate and reliable.

As matrix interference from vegetal matrix was found, recovery of the method was evaluated by analyzing the CRM 1515 (Apple Leaves, with certified total mercury value of 43.2 ± 2.3 μg kg^{-1}). Matrix matching was made by performing distillation of standards in the presence of the yerba mate M_2, a dried vegetal sample whose matrix is supposed to impose similar interference that one expected with the dried apple leaves matrix. The value obtained using the GC-CV-AFS system was $(39.6 \pm 6.3) \times 10^3$ μg kg^{-1} (91.6 % recovery with only inorganic mercury, as Hg^{2+}, detected), after the CRM were subjected to the extraction and distillation process. Statistical similarity of results was proved using a two-tailed Student-*t*-test = 0.314 ($\alpha = 0.05$ and $n_1 = n_2 = 3$), which can be considered very satisfactory, considering the large factor of dilution applied to the sample to perform the analysis with GC-CV-AFS.

The precision of the developed method was also evaluated as intra-day and inter-day precision. For this, mercury speciation and determinations were evaluated using the GC-CV-AFS system, after the extraction and distillation of three replicates of samples containing 50

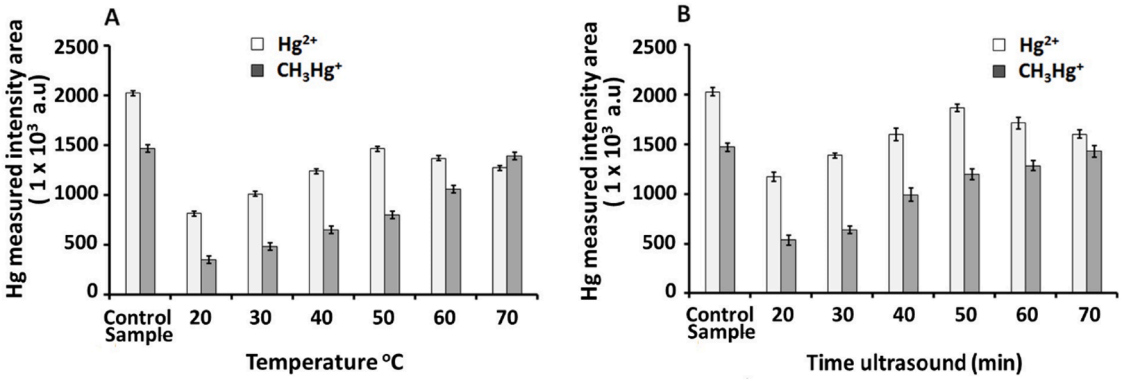

Fig. 4. (A) Effect of temperature on recoveries of mercury species (fortification of 800 pg of Hg^{2+} and 800 pg of CH_3Hg) in presence of 50 mg of yerba mate using ultrasonic-assisted extraction (30 min) using Triton $X-114$ (0.5 %) and solution at pH 3. Measurements made by GC-CV-AFS distillation and propylation. (B) Effects of ultrasonic agitation on recoveries of mercurial species (800 pg L^{-1} of Hg^{2+} and CH_3Hg) in presence of 50 mg of yerba mate. Extraction made using Triton $X-114$ (0.5 %); solution at pH 3 and at 50 °C. Measurements made by GC-CV-AFS distillation and propylation (standard deviation for $n = 3$).

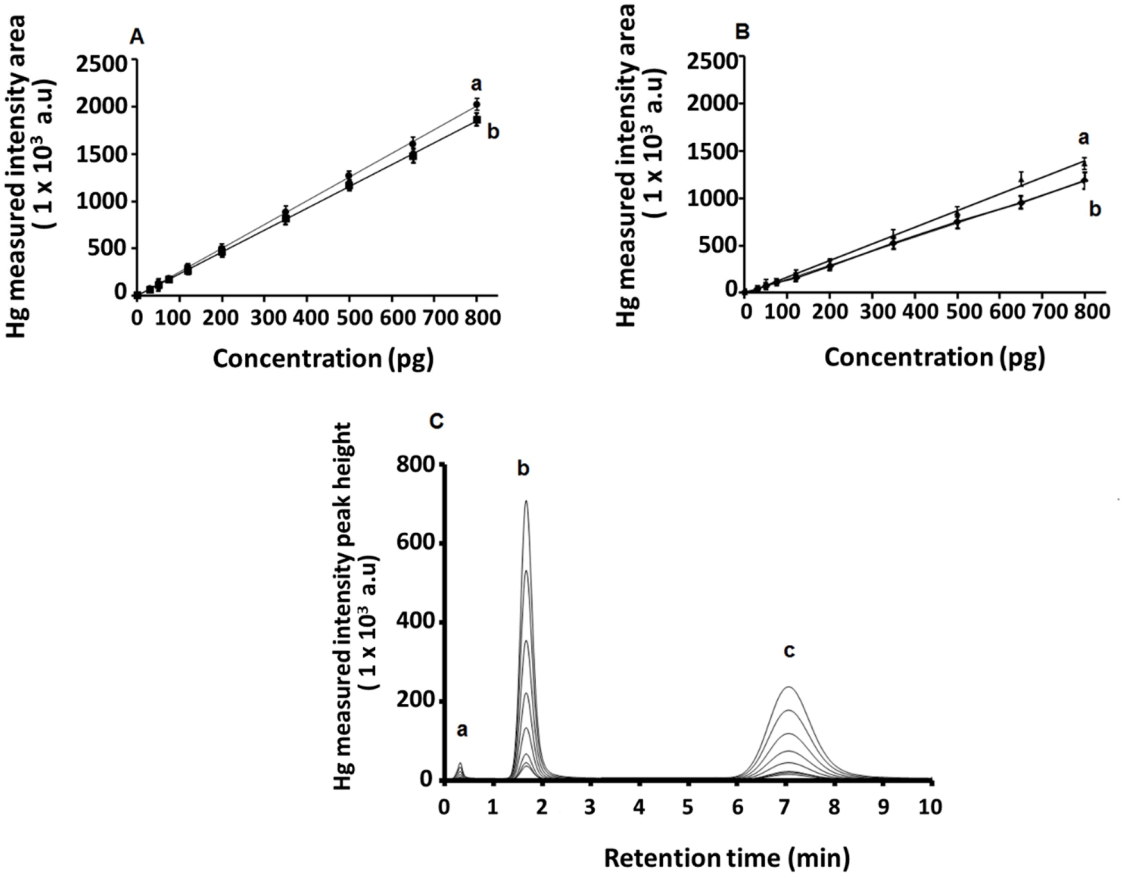

Fig. 5. A) Analytical curves (from 30 to 800 pg L^{-1}): a) using standard solution of Hg^{2+} and b) using standard solution of Hg^{2+} in the presence of 50 mg yerba mate M_2. B) Analytical curves: a) using standard solution of CH_3Hg and b) using standard solution of CH_3Hg in the presence of 50 mg yerba mate M_2; (C) Typical GC-CV-AFS chromatographic profiles of mercurial species (a) residual Hg^0 (b) CH_3Hg; (c) Hg^{2+}.

mg yerba mate M_2 fortified with three different concentrations (3.0 μg L^{-1}; 9.0 μg L^{-1}; and 12.0 μg L^{-1}) of either Hg^{2+} or CH_3Hg^+. Measurements were made in three consecutive days. The obtained precisions (relative standard deviation) was less than 1 % (Table S9), ensuring that the proposed method is reproducible.

The proposed method was applied to samples of yerba mate identified with the codes M_3, M_4, M_6, and M_9. The analysis of these samples were performed using optimized conditions to minimize artifact formation and maximize analyte accuracy (using matrix-matched standards with 50 mg of yerba mate M_2). For the quantification studies, 50 mg of the samples (M_3, M_4, M_6 and M_9) were used ($n = 3$), submitting them to the ultrasonic extraction and distillation process. The results (corrected by the dilution factor) are summarized in Table 3, indicating the presence of the Hg^{2+}, which ranges from 0.67 μg kg^{-1} or 34 pg in absolute value (for sample M_6), to 0.96 μg kg^{-1} or 48 pg in absolute value (for sample M_3). Besides, sample M_9 was the only one that presented ultra-trace levels of organic mercury (as CH_3Hg) at 0.07 μg kg^{-1} or 3.5 pg in absolute value.

Table 3
Concentrations of Hg^{2+} and CH_3Hg in yerba mate samples determined by the proposed method based on GC-CV-AFS[a], and the determination of total mercury found in yerba mate samples using CV-AAS[b].

Samples	Hg^{2+} (μg kg^{-1})[a] (absolute value in pg)	CH_3Hg (μg kg^{-1})[a] (absolute value in pg)	Total mercury[b]* (μg kg^{-1})	$t_{calculated}$**
M_3	0.96 ± 0.02 (48)	–	0.91 ± 0.08 (46)	0.982
M_4	0.74 ± 0.03 (37)	–	0.72 ± 0.07 (36)	0.743
M_6	0.67 ±0.03 (34)	–	0.63 ± 0.07 (32)	0.674
M_9	0.82 ± 0.01 (41)	0.07 ± 0.02 (3.5)	0.85 ± 0.05 (43)	0.877

*Samples after the acid digestion process.
**t*-critical (two-tailed *Student-t*-test; α = 0.05 (d.f. = $n_1 + n_2 - 2 = 4$) = 2.77).

The results of this study were compared with the results obtained from the quantification of total mercury in samples M_3, M_4, M_6 and M_9 after acid dissolution using CV-AAS. These total mercury results were compared to the ones obtained by GC-CV-AFS and they were statistically similar (Table 3). In case of sample M_9, the value used in the comparison was the sum of the GC-CV-AFS results found for Hg^{2+} and CH_3Hg.

Yerba mate samples were also fortified with analytes at three concentration levels (Table 4). The recovery percentages of analyte fortification (discounting the original concentrations reported in Table 3) were close to 100 %. The total concentrations recovered, at the different levels of fortification, allowed estimating the original concentrations in the samples (M_3, M_4 and M_6), and also estimate the content of CH_3Hg and Hg^{2+} in sample M_9. Results were similar to those previously determined and presented in Table 4 (according to two-tailed *Student-t*-test at the 95 % confidence limit).

According to the results, four of the yerba mate samples were found to contain Hg^{2+} concentrations between 0.67 and 0.96 μg kg^{-1} based on dry weight. Only one sample of yerba mate presented a CH_3Hg concentrations of 0.67 μg kg^{-1}, these results did not exceed the maximum Hg limit (50 μg kg^{-1}) established by the National Sanitary Surveillance Agency (ANVISA, Brazil) for any kind food. It is important to highlight that in the literature there are only works that report the total mercury content in yerba mate [53], not focusing on the speciation analysis of mercury in this type of matrix, which is of extreme importance, considering the risk to human health of organomercurials. In addition, the use of ultrasound-assisted extraction method in a medium containing surfactant (Triton X-114) does not involve the use of toxic organic solvents, traditionally used for extraction processes, which makes it an environmentally friendly method. Finally, distillation of samples, followed by speciation analysis and Hg determination by GC-CV-AFS, is a breakthrough on EPA Method 1630, allowing accurate determinations of mercury in this type of samples.

Table 4
Quantification study using fortification of yerba mate samples with estimation of the original analyte content.

Sample	Hg^{2+} fortification level (μg L^{-1})	Total Hg^{2+} found (μg L^{-1})	Original Hg^{2+} recovered in the fortification experiment [a] (μg L^{-1})	Average original Hg^{2+} (μg L^{-1})
	3.00	3.97±0.04	0.97±0.04	
M_3	9.00	9.98±0.06	0.98±0.06	0.97±0.04
	12.00	12.96±0.07	0.96±0.07	
	3.00	3.74±0.03	0.74±0.03	
M_4	9.00	9.75±0.05	0.75±0.05	0.74±0.03
	12.00	12.74±0.05	0.74±0.07	
	3.00	3.65±0.06	0.65±0.06	
M_6	9.00	9.64±0.07	0.64±0.07	0.64±0.07
	12.00	12.65±0.08	0.65±0.08	
M_9	3.00	3.83±0.07	0.83±0.08	
	9.00	9.84±0.05	0.84±0.05	0.83±0.05
	12.00	12.83±0.07	0.83±0.07	

Sample	CH_3Hg fortification level (μg kg^{-1})	Total CH_3Hg found (μg kg^{-1})	Original CH_3Hg recovered in the fortification experiment (μg kg^{-1})	Original CH_3Hg recovered in the fortification experiment (μg kg^{-1})
M_9	0.50	0.49±0.05	0.07 ± 0.03	
	1.00	1.07±0.04	0.07±0.03	0.07±0.02
	2.00	2.07±0.06	0.07±0.02	

3.5. Comparative study with others matrices

The results obtained in the present study are compared with those reported by others authors in Table 5. In terms of elemental and total mercury analysis, the required time is comparable if considering sample and instrumental preparations. It should be noted that LOD and LOQ for the general elements are in accordance with literature using ICP OES and ICP-MS. In the case of mercurial species, the present study details LOD and LOQ for both Hg^{2+} and CH_3Hg, and no other work reports values for CH_3Hg. The diversity of sample preparation procedures adds complexity to the interpretation of the results, but, in the case of mercurial species, the optimization and distillation have been employed specifically to guarantee high level of cleanliness in order to minimize contamination and avoid artifacts. The achieved results for yerba mate samples indicate that the average value was 0.797 μg kg^{-1}, being about 10 times smaller compared to the only value reported by a single author. Furthermore, in two of the articles the data reflect only a result derived from acid digestion, while in our case, we work with both digestion and infusion. The Hg content range in others studies was 2.5 to 15.8 μg/100 and was significantly higher than in our determinations (0.67 to 0.92 μg Hg/100 g). Our findings suggest that Hg contamination could come from a later stage such as roasting, so in this case it would be of environmental contamination.

3.6. Multi-element analysis of yerba mate and yerba mate infusions

The overall elemental composition of the ten different samples of commercial mate leaves was evaluated through ICP-MS and ICP OES. In terms of analytical validation, the analytical curves for all the elements revealed a good linearity with a R^2 higher than 0.99. The instrumental LOD were between 0.06 and 50 μg L^{-1} (Table S2). The average recoveries for CRM 1515 (apple leaves) were in the range between 54 % and 109 % (Table S1). Low recovery for some of the elements can be attributed to several factors. One potential cause is the incomplete dissolution of the plant matrix during the acid digestion process. Calcium, for example, can form stable compounds with organic plant components, making it difficult to be completely released during digestion [68]. Pequerul et al., [69] point out that the pH of the digestion solution and the presence of other ions can promote the precipitation of calcium compounds, decreasing their availability for analysis [69]. Matrix effects, including the presence of co-dissolved organic matter, may also influence element recovery affecting specially results using ICP-MS. Liu et al., discuss how organic compounds present in the sample can interact with analytes during digestion and detection, leading to increased or decreased signals, depending on the nature of the element and matrix [70]. In fact, Trimmel et al., demonstrated that Ca does not have an optimal recovery even when using more than one different CRM [71]. In our study, using different CRMs under acid digestion (Tomato leaves 1573a and Peach leaves 1547) the recovery for Ca was 52 and 69 % respectively (data not shown). In addition, other factors affecting the results are the quantity amount of matrix, being ICP-OES prone to better matrix tolerance and long-term stability [72].

Elements such as Mg, P, K, Ca, Mn, Fe, Co, Cu, Zn, Fe, Sr, V, Pb and Cd were determined in mate leaves (Table 6, Fig. S1.A and B) and in the

Table 5
Comparative study with previous reported data of mercury.

Nª of study	Type of methodology	N° of samples	Type of extraction	Mean concentration found (ng g^{-1})	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)	Analysis time per sample in the instrument (min)	Ref
1	AAS	86-Tea (black, green, white, Pu-erh) and Yerba Mate	50 mg with 250 mL of boiling water	Teas: 2.86 (range 0.36–10.76) Yerba mate: 6.87	0.01	Not reported	92.2 (Mixed Polish Herbs INCT-MPH-2)	1 to 3	[53]
2	CV-AFS	129-Green tea	2 g with 150 mL of boiling water and 2 g with 150 mL of boiling water (repeated to obtain 10 tea infusions) samples	6.3 (range 1.8–102.9)	Not reported		93–97 (CRM; GBW10020 and 10,048- citrus leaf)	30	[65]
3	CV-AFS	10-Drinking water and food	1 g with 5 mL of 60 % HNO_3, 2 mL of 33 % H_2O_2, and 1 mL of H_2O	Drinking water: 0.002 Food (eggs, beans, meat and garlic): 0.0012–0.0018	0.002	0.006	Not reported	30	[66]
4	HPLC-CV-AFS	3 water (sea, river water and sewage)	1 mL of the infusion was diluted to 50 mL with double-distilled water. The separate second extraction was carried out in 5 mL TMAH (25 % solution) for 20 min at 55 °C and another 20 min at 60 °C The extract was ultra-centrifuged and 1 mL of sample was diluted in water to a final volume of 50 mL and acidified with 0.55 % HCl (0.75 mL) to a pH of around 0.8	< LOD	0.00004	Not reported	83–98 (Human hair samples NIES CRM N° 13 and IAEA-085) 91–102	22	[67]
5	GC-CV-AFS	5-Yerba mate (aged, green and roasted)	50 mg with 50 mL of an aqueous solution at pH 3 in a ultrasonic agitation for 50 min at 50 °C	0.797	0.002 for Hg^{2+} 0.003 for CH_3Hg	0.005 for Hg^{2+} 0.006 for CH_3Hg	91.2 (CRM 1515) 100	30	This study

Table 6
Results (mean ± SD, $n = 3$) of elements in 10 samples of yerba mate leaves.

Yerba Mate Leaves (mg g^{-1})										
Element	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}
K [a]	11.56 ± 0.18	14.01 ± 0.11	12.78 ± 0.22	13.33 ± 0.34	15.03 ± 0.46	11.2 ± 0.39	13.95 ± 0.29	14.06 ± 0.39	12.74 ± 0.15	16.0 ± 0.35
Ca [a]	7.19 ± 0.18	7.32 ± 0.13	7.46 ± 0.63	7.17 ± 0.51	7.42 ± 0.19	7.53 ± 0.46	8.79 ± 0.18	7.62 ± 0.15	7.14 ± 0.21	7.26 ± 0.25
Mg [a]	5.16 ± 0.09	4.85 ± 0.10	5.60 ± 0.16	5.42 ± 0.07	5.46 ± 0.20	5.32 ± 0.28	6.01 ± 0.09	5.54 ± 0.13	5.40 ± 0.07	4.71 ± 0.12
P [a]	1.19 ± 0.3	1.46 ± 0.09	1.04 ± 0.02	1.10 ± 0.04	1.38 ± 0.03	1.35 ± 0.05	1.65 ± 0.07	1.10 ± 0.005	1.05 ± 0.02	1.23 ± 0.05
Mn [b]	1.04 ± 0.11	1.13 ± 0.03	1.22 ± 0.05	1.22 ± 0.05	1.09 ± 0.08	0.77 ± 0.02	1.23 ± 0.03	0.98 ± 0.03	1.25 ± 0.07	0.97 ± 0.02
Yerba Mate Leaves (mg kg^{-1})										
Element	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}
Fe [a]	164 ± 12	197 ± 58	99.7 ± 15.2	123 ± 3.0	144 ± 8.0	62.0 ± 3.7	215 ± 44	129 ± 44	134 ± 4.0	73.1 ± 6.3
Ba [a]	60.4 ± 0.7	56.2 ± 4.7	58.2 ± 4.2	61.3 ± 2.6	65.3 ± 1.9	72.5 ± 12.8	74.3 ± 6.9	61.8 ± 5.4	56.9 ± 3.0	62.4 ± 3.8
Zn [b]	59.1 ± 6.4	77.64 ± 1.7	50.4 ± 9.6	58.2 ± 7.8	78.1 ± 6.5	72.0 ± 5.7	90.5 ± 3.9	50.7 ± 2.7	65.3 ± 6.1	46.9 ± 3.6
Sr [a]	33.2 ± 1.3	31.1 ± 0.4	39.8 ± 2.7	43.4 ± 2.1	33.1 ± 0.6	26.7 ± 2.1	34.4 ± 1.2	45.0 ± 0.9	31.1 ± 0.7	30.7 ± 1.1
Na [a]	<LOD	92.1 ± 43.1	<LOD	<LOD	<LOD	<LOD	47.1 ± 12.4	<LOD	15.8 ± 7.5	<LOD
Cu [b]	10.4 ± 0.7	10.1 ± 0.2	10.9 ± 0.5	10.8 ± 0.2	10.9 ± 0.4	8.75 ± 0.34	9.70 ± 0.39	10.7 ± 0.3	10.3 ± 0.7	8.89 ± 0.48
Ni [b]	2.99 ± 0.30	2.70 ± 0.08	3.40 ± 0.29	3.86 ± 0.11	3.71 ± 0.09	2.38 ± 0.09	3.20 ± 0.11	3.04 ± 0.18	3.28 ± 0.23	1.39 ± 0.11
V [b]	0.314 ± 0.019	0.504 ± 0.032	0.178 ± 0.012	0.257 ± 0.010	0.291 ± 0.026	0.177 ± 0.001	0.491 ± 0.004	0.227 ± 0.006	0.305 ± 0.013	0.113 ± 0.005
Pb [b]	0.234 ± 0.041	0.183 ± 0.013	0.279 ± 0.014	0.317 ± 0.023	0.209 ± 0.020	0.209 ± 0.037	0.157 ± 0.017	0.457 ± 0.02	0.206 ± 0.055	0.275 ± 0.027
Co [b]	0.192 ± 0.005	0.279 ± 0.035	0.106 ± 0.013	0.151 ± 0.012	0.392 ± 0.016	0.270 ± 0.015	0.374 ± 0.037	0.101 ± 0.006	0.251 ± 0.012	0.162 ± 0.018
Ce [b]	0.223 ± 0.006	0.334 ± 0.012	0.218 ± 0.023	0.278 ± 0.002	0.236 ± 0.010	0.147 ± 0.018	0.266 ± 0.013	0.369 ± 0.082	0.251 ± 0.017	0.166 ± 0.004
La [b]	0.137 ± 0.002	0.284 ± 0.021	0.129 ± 0.007	0.182 ± 0.006	0.269 ± 0.008	0.238 ± 0.006	0.244 ± 0.018	0.239 ± 0.033	0.187 ± 0.006	0.114 ± 0.004

References: [a] analyzed by ICP OES, [b] analyzed by ICP-MS.

infusions (Table 7, Fig. S2.A and B). In general, elements content were in agreement with what was found in yerba mate by other authors, reported in Table S3 (alkaline elements and P), Table S4 (alkaline earth elements), Table S5 (essential trace elements) and Table S6 (toxic elements) [18,20–22,24,32,73–94]. As expected, K, Ca and Mg were the most abundant elements in the original samples, on the other hand, the infusion values ranged from 5.46 to 9.18 for K, 0.19 to 0.68 for Ca and 1.39 to 2.74 mg g^{-1} for Mg. However, the sample analyzed by Olivari

Table 7
Results (mean ± SD, $n = 3$) of elements in 10 samples of yerba mate infusion.

Yerba Mate Infusion (mg g^{-1})										
Element	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}
K	9.18 ± 2.06	9.06 ± 0.54	5.55 ± 0.30	9.17 ± 1.47	6.91 ± 0.96	6.43 ± 0.60	8.47 ± 1.01	8.48 ± 1.41	5.46 ± 0.49	6.67 ± 0.81
Mg	2.55 ± 0.46	2.06 ± 0.03	1.74 ± 0.04	2.74 ± 0.42	1.65 ± 0.31	1.71 ± 0.06	2.45 ± 0.19	2.30 ± 0.36	1.51 ± 0.07	1.39 ± 0.16
Mn	0.59 ± 0.02	0.72 ± 0.03	0.76 ± 0.05	0.79 ± 0.05	0.57 ± 0.03	0.23 ± 0.02	0.61 ± 0.04	0.57 ± 0.02	0.68 ± 0.02	0.46 ± 0.05
P	0.60 ± 0.13	0.54 ± 0.004	0.26 ± 0.02	0.45 ± 0.08	0.39 ± 0.06	0.49 ± 0.05	0.64 ± 0.05	0.38 ± 0.07	0.26 ± 0.01	0.37 ± 0.05
Ca	0.50 ± 0.08	0.43 ± 0.03	0.37 ± 0.02	0.68 ± 0.12	0.26 ± 0.03	0.21 ± 0.01	0.48 ± 0.04	0.37 ± 0.06	0.31 ± 0.001	0.19 ± 0.02
Yerba Mate Infusion (mg kg^{-1})										
Element	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}
Zn	25.7 ± 0.2	31.8 ± 5.6	21.5 ± 0.7	26.1 ± 2.7	28.9 ± 1.7	8.06 ± 0.26	32.2 ± 2.6	22.7 ± 2.7	27.1 ± 2.7	22.3 ± 1.6
Cu	7.01 ± 0.28	6.72 ± 0.79	6.92 ± 0.23	8.21 ± 0.66	6.25 ± 0.60	0.27 ± 0.024	4.35 ± 0.13	7.12 ± 0.05	6.71 ± 0.50	5.56 ± 0.25
Ni	3.59 ± 0.71	2.89 ± 0.38	3.37 ± 0.14	3.77 ± 0.35	3.34 ± 0.24	1.06 ± 0.18	2.94 ± 0.19	3.01 ± 0.05	3.04 ± 0.15	1.11 ± 0.07
Fe	4.53 ± 0.40	3.16 ± 0.10	2.14 ± 0.09	1.96 ± 0.46	2.49 ± 0.28	1.44 ± 0.27	3.45 ± 0.25	2.92 ± 0.37	1.73 ± 0.26	2.59 ± 0.25
Ba	2.86 ± 0.56	1.54 ± 0.11	2.53 ± 0.12	4.03 ± 0.44	3.00 ± 0.55	3.87 ± 0.37	2.80 ± 0.24	1.48 ± 0.26	2.36 ± 0.07	2.44 ± 0.34
Sr	2.50 ± 0.40	1.97 ± 0.06	2.20 ± 0.06	3.81 ± 0.68	1.51 ± 0.16	1.33 ± 0.15	2.33 ± 0.22	2.44 ± 0.39	1.46 ± 0.03	1.04 ± 0.10
Co	0.134 ± 0.01	0.193 ± 0.017	0.098 ± 0.005	0.130 ± 0.009	0.276 ± 0.018	0.088 ± 0.016	0.225 ± 0.010	0.079 ± 0.006	0.177 ± 0.009	0.116 ± 0.008

References: [a] analyzed by ICP OES, [b] analyzed by ICP-MS. Pb and Cd were not detected (n.d.) in all the samples.

et al. [95] (i.e. sample U1, for consumption in Uruguay) shows a content of K and Mg below (ten times less) the values reported in the present work. Such a difference in reported concentrations can be attributed to environmental and agricultural factors as soil quality plays a crucial role, and mineral composition, including heavy metals, affects plant uptake. The use of fertilizers and pesticides further affects the elemental profile of yerba mate plants and climatic conditions, such as rainfall and temperature, influence the nutrient. It is important to clarify that yerba mate is not cultivated in Uruguay, and this generates the loss of traceability of the quality of the sample. The amount of minerals in the infusions depends on the extraction efficiencies of the brewing conditions. According to Natesan and Ranganathan [96] the analyte elements can be classified into three groups: highly extractable elements (>55 %); moderately extractable elements (22–55 %), and poorly extractable elements (<20 %). The extraction efficiency of each element was estimated following the formula of Olivari et al. [95]. High extractable element were found in yerba mate, that the case of K (42 % to 79 %); but in the case of Mg is moderately extractable (28 % to 50 %). The very high solubility of K is explained by the fact that it is present in plants in readily soluble forms. Moreover, K is known to be more abundant outside the plant cells [91]. In contrast, Matsuura et al. [97] suggested that most of the Ca is accumulated inside the cells and is hardly extracted during brewing, which is consistent with the data obtained for Ca. The poorest leached elements were Ba, Ca, Fe and Sr with solubility below 10 % (poorly extractable). In general terms, Ca concentration was lower, K, Mg, Mn were similar, iron was about half, and zinc was almost a third of the values reported in other study dealing with similar samples (Table S7) [21]. Others elements such as P and Mn are in very low concentration. The leached content for these elements are between 19 and 51 % (moderately extractable). This variability can be attributed, for example, to factors such as elaboration process. In fact, it has been verified in previous investigations [60], that the process and the particle size are also important factors. Both samples, M_6 and M_{10} are the ones with the lowest levels of all the determined elements, and they were produced using different processes (M_6 is not aged and M_{10} is roasted) and present a different particle size distribution.

Considering minor elements (Co, Cu, Fe, Ni, Zn, Ce and La), the highest concentration was found for Zn, Cu and Fe, corroborating previous reports [31,98]. In the case of the infusion, values varied from 8.0 to 32 (1×10^{-3} μg kg^{-1}, Zn), from 0.27 to 8.2 (1×10^{-3} μg kg^{-1}, Cu) and from 1.4 × 10^{-3} to 4.5 × 10^{-3} μg kg^{-1}, Fe). Finally, the concentration found of rare earths (Ce, La) are in agreement with concentration reported in other studies. Co as well as Ni were leached to solution in percentages that ranges from 33 to 100 % (high extractable), except for sample M_6. Lead presented a mean concentration of 260 μg kg^{-1} in leaves (ranging between 180 and 450 μg kg^{-1}), showing that the samples do not surpass the maximum limit allowed for this inorganic contaminants in food; being our results in agreement with values reported in other studies [21,23,99]. However, lead was not found in infusions.

Researches carried out by Baran et al. [100], Pozebon et al. [21] and Ulbrich et al. [99] on yerba mate samples showed similar mineral compositions for dried leaves and infusions. In terms of agreement with this work, the elements were arranged in the following descending order: K > Ca > Mg > P > Mn > Fe > Zn > Sr > Cu > Ni > V > Pb > Co > Cd (leaves) and K > Mg > P > Mn > Ca > Zn > Cu > Fe > Ni > Sr > Co (infusions). Reporting the level of concentration leached to the infusion (Table S8), is an information of great importance for the habitual consumer. However, limitation in the small sample size may affect statistical and the ability to detect subtle differences in analyte concentrations, besides the geographic scope is limited, hindering the capture of the full range of environmental conditions, such as differences in soil and agricultural practices.

4. Conclusions

In this study, the total content of Hg and the speciation analysis was determined in ten widely consumed commercial yerba mate brands. For the first time, the condition of extraction and distillation were optimized and successfully used in a complex matrix rich in active compounds and organic material. The mean Hg concentration for all samples was 0.797 μg kg^{-1}. This results indicate the potential for Hg accumulation in yerba mate but the exposure to human's consumption is extremely low. Furthermore, ICP OES and ICP-MS were used for quality control of elements in yerba mate. Magnesium and potassium were presents in high concentration, with high solubility, which reinforces that yerba mate has a great capacity to accumulate these elements and is a relevant dietary source. Future research should include a wider range of samples and consider increasing the sample size to reinforce reliability and applicability.

Funding statement

This work was supported by the Brazilian Scientific and Education Agencies: National Council for Scientific and Technological Development (CNPq), Carlos Chagas Filho Foundation for Research Support of Rio de Janeiro State (FAPERJ) and Coordination of Higher Education Personnel Improvement (CAPES) and Funding from PEDECIBA and UDELAR (Uruguay).

Ethical statement

Nothing to declare. The presented work did not involve any humans nor animal subjects as objects of the study.

CRediT authorship contribution statement

María Victoria Panzl: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Conceptualization. **Jarol R. Miranda-Andrades:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Wendy J. Sandoval Rojano:** Formal analysis. **Joseane A. Mendes:** Writing – original draft, Validation. **Tatiana D. Saint'Pierre:** Writing – original draft, Conceptualization. **Alejandra Rodríguez-Haralambides:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Ricardo Q. Aucelio:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ricardo Queiroz Aucelio reports financial support was provided by National Council for Scientific and Technological Development. Ricardo Queiroz Aucelio reports financial support was provided by Carlos Chagas Filho Foundation for Research Support of Rio de Janeiro State. Ricardo Queiroz Aucelio reports financial support was provided by Coordination of Higher Education Personnel Improvement.

Acknowledgments

This work was supported by Programa de Desarrollo de las Ciencias Básicas (PEDECIBA) -Ministerio de Educación y Cultura de Uruguay (Rodriguez-Haralambides). Panzl thanks Agencia Nacional de Investigación e Innovación de Uruguay (ANII) for her scholarship (POS_NAC_2016_1_130367). Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES-Brazil) – Finance Code 001 is acknowledged. Miranda-Andrades thanks Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (E-26/201.952/2020–SEI-260003/002294/2020) and (E-26/201.951/2020–SEI-260003/002294/2020) for scholarship. Aucelio thanks scholarships from Brazilian agencies FAPERJ (E-26/200.810/2021 and E-26/204.226/2024) and CNPq (306108/2021-6) and also scientific grants from FAPERJ (E-26/210.726/2021 and E-26/210.525/2023). Analytical facilities from the Chemistry Department at PUC-Rio (CALPH PUC-Rio) were used.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtemin.2025.100217.

References

[1] P. De Almeida Rodrigues, R.G. Ferrari, L.N. dos Santos, C.A. Conte Junior, Mercury in aquatic fauna contamination: a systematic review on its dynamics and potential health risks, J. Environ. Sci. (China) 84 (2019) 205–218, https://doi.org/10.1016/j.jes.2019.02.018.

[2] M. Jaishankar, T. Tseten, N. Anbalagan, B.B. Mathew, K.N. Beeregowda, Toxicity, mechanism and health effects of some heavy metals, Interdiscip. Toxicol. 7 (2014) 60–72, https://doi.org/10.2478/intox-2014-0009.

[3] Mercury and health, (n.d.). https://www.who.int/news-room/fact-sheets/detail/mercury-and-health (accessed July 2, 2020).

[4] R.M. Blanco, M.T. Villanueva, J.E.S. Uría, A. Sanz-Medel, Field sampling, preconcentration and determination of mercury species in river waters, Anal. Chim. Acta 419 (2000) 137–144, https://doi.org/10.1016/S0003-2670(00)01002-3.

[5] K. Leopold, M. Foulkes, P. Worsfold, Methods for the determination and speciation of mercury in natural waters—A review, Anal. Chim. Acta 663 (2010) 127–138, https://doi.org/10.1016/j.aca.2010.01.048.

[6] P. Grandjean, H. Satoh, K. Murata, K. Eto, Adverse effects of methylmercury: environmental health research implications, Environ. Health Perspect. 118 (2010) 1137–1145, https://doi.org/10.1289/ehp.0901757.

[7] L.S. Keith, O.M. Faroon, B.A. Fowler, Uranium, fourth ed., Elsevier, 2015 https://doi.org/10.1016/B978-0-444-59453-2.00059-7.

[8] R.S. Matias, H.R. Guímaro, P. Bustamante, J. Seco, N. Chipev, J. Fragão, S. Tavares, F.R. Ceia, M.E. Pereira, A. Barbosa, J.C. Xavier, Mercury biomagnification in an Antarctic food web of the Antarctic Peninsula, Environ. Pollut. 304 (2022), https://doi.org/10.1016/j.envpol.2022.119199.

[9] T.R. Sanga, K.K. Maseka, M. Ponraj, C. Tungaraza, M.E. Mng'ong'o, E. B. Mwakalapa, Accumulation and distribution of mercury in agricultural soils, food crops and associated health risks: a case study of Shenda gold mine-Geita Tanzania, Environ. Chall. 11 (2023), https://doi.org/10.1016/j.envc.2023.100697.

[10] M. Varde, A. Servidio, G. Vespasiano, L. Pasti, A. Cavazzini, M. Di Traglia, A. Rosselli, F. Cofone, C. Apollaro, W.R.L. Cairns, E. Scalabrin, R. De Rosa, A. Procopio, Ultra-trace determination of total mercury in Italian bottled waters, Chemosphere 219 (2019) 896–913, https://doi.org/10.1016/j.chemosphere.2018.12.020.

[11] X. Zhao, D. Wang, Mercury in some chemical fertilizers and the effect of calcium superphosphate on mercury uptake by corn seedlings (*Zea mays* L.), J. Environ. Sci. 22 (2010) 1184–1188, https://doi.org/10.1016/S1001-0742(09)60236-9.

[12] D. Hao, X. Gu, P. Xiao, Z. Liang, L. Xu, Y. Peng, Research progress in the phytochemistry and biology of Ilex pharmaceutical resources, Acta Pharm. Sin. B 3 (2013) 8–19, https://doi.org/10.1016/j.apsb.2012.12.008.

[13] C.M. Pagliosa, S.M. Pereira, M.A. Vieira, L.A. Costa, E. Teixeira, R.D.D.E.M. C. Amboni, E.R. Amante, Bitterness in yerba mate (*Ilex paraguariensis*) leaves, J. Sens. Stud. (2009), https://doi.org/10.1111/j.1745-459X.2009.00218.x.

[14] V. Márquez, N. Martínez, M. Guerra, L. Fariña, E. Boido, E. Dellacassa, Characterization of aroma-impact compounds in yerba mate (*Ilex paraguariensis*) using GC-olfactometry and GC–MS, Food Res. Int. 53 (2013) 808–815, https://doi.org/10.1016/j.foodres.2013.02.016.

[15] C.P. Croge, F.L. Cuquel, P.T.M. Pintro, Yerba mate: cultivation systems, processing and chemical composition. A review, Sci. Agric. 78 (2020) 1–11, https://doi.org/10.1590/1678-992x-2019-0259.

[16] N. Bracesco, A.G.G. Sanchez, V. Contreras, T. Menini, A. Gugliucci, Recent advances on *Ilex paraguariensis* research: minireview, J. Ethnopharmacol. 136 (2011) 378–384, https://doi.org/10.1016/j.jep.2010.06.032.

[17] C.I. Heck, E.G. De Mejia, Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations, J. Food Sci. 72 (2007), https://doi.org/10.1111/j.1750-3841.2007.00535.x.

[18] R. Heinrichs, E. Malavolta, Composição mineral do produto comercial da erva-mate (*Ilex paraguariensis* St. Hil.), Ciência Rural 31 (2001) 781–785, https://doi.org/10.1590/s0103-84782001000500007.

[19] C.B. Reissmann, M.I. Radomski, R.M.B. De, Chemical Composition of Ilex paraguariensis St . Hil, Under different management conditions in seven localities of Paraná State, Brazilian Arch. Biol. Technol. 42 (1999) 2, https://doi.org/10.1590/S1516-89131999000200009.

[20] M.G. Maiocchi, L.A. Del Vitto, M.E. Petenatti, E.J. Marchevsky, R.G. Pellerano, E. M. Petenatti, Multielemental composition and nutritional value of and their commercial mixture in different forms of use, Rev. La Fac. Ciencias Agrar. UNCuyo 48 (2016) 145–159.

[21] D. Pozebon, V.L. Dressler, M.C.A. Marcelo, T.C. de Oliveira, M.F. Ferrão, Toxic and nutrient elements in yerba mate (*Ilex paraguariensis*), Food Addit. Contam. Part B Surveill. 8 (2015) 215–220, https://doi.org/10.1080/19393210.2015.1053420.

[22] A.C.V. Motta, J.Z. Barbosa, E. Magri, G.Q. Pedreira, D. Santin, S.A. Prior, R. Consalter, S.D. Young, M.R. Broadley, E.L. Benedetti, Elemental composition of yerba mate (*Ilex paraguariensis* A.St.-Hil.) under low input systems of southern Brazil, Sci. Total Environ. 736 (2020), https://doi.org/10.1016/j.scitotenv.2020.139637.

[23] M. Welna, A. Szymczycha-Madeja, P. Pohl, Novel ICP-OES-based method for the reliable determination of the total content of 15 elements in Yerba Mate drinks along with the determination of caffeine and the In vitro bioaccessibility of the compounds, Molecules 28 (2023) 1–22, https://doi.org/10.3390/molecules28083374.

[24] J.Z. Barbosa, L.M. Zambon, A.C.V. Motta, I. Wendling, Composição, solubilidade de elementos em água quente e valor nutricional de frutos e folhas de erva-mate, Cienc. Agrotecnologia 39 (2015) 593–603, https://doi.org/10.1590/S1413-70542015000600006.

[25] A.T. Valduga, I.L. Gonçalves, E. Magri, J.R.D. Finzer, Chemistry, pharmacology and new trends in traditional functional and medicinal beverages, Food Res. Int. 120 (2019) 478–503, https://doi.org/10.1016/j.foodres.2018.10.091.

[26] R.B. Pardinho, P. Dalla Vecchia, A.L.G. Mendes, C.A. Bizzi, P.A. Mello, F. A. Duarte, E.M.M. Flores, Determination of toxic elements in yerba mate by ICP-MS after diluted acid digestion under O_2 pressure, Food Chem. 263 (2018) 37–41, https://doi.org/10.1016/j.foodchem.2018.04.112.

[27] R.B. Pardinho, P.D. Vecchia, C.M.A.C. Alves, N. Pimentel, D. Gazzana, R. C. Bolzan, F.A. Duarte, D.A. Bisognin, E.M.M. Flores, *Ilex paraguariensis* exposition to As and Cd in a closed soilless system, Chemosphere 258 (2020) 127284, https://doi.org/10.1016/j.chemosphere.2020.127284.

[28] ANVISA, Agência Nacional de Vigilância Sanitária. Resolução da Diretoria Colegiada - RDC No 42, de 29 de Agosto de 2013, (2013) 17.

[29] Mercosur, Reglamento Técnico MERCOSUR sobrel límites máximos de contaminantes inorgánicos en alimentos, 2014 (2014) 1–5. https://doi.org/10.1007/s13398-014-0173-7.2.
[30] E. Magri, A.T. Valduga, I.L. Gonçalves, J.Z. Barbosa, D. de Oliveira Rabel, I.M.N. R. Menezes, P. de Andrade Nascimento, A. Oliveira, R.S. Corrêa, A.C.V. Motta, Cadmium and lead concentrations in yerba mate leaves from agroforestry and plantation systems: an international survey in South America, J. Food Compos. Anal. (2020), https://doi.org/10.1016/j.jfca.2020.103702.
[31] M.G. Maiocchi, M.V. Avanza, R.G. Pellerano, L.A. Del Vitto, M.E. Petenatti, E. M. Petenatti, E.J. Marchevsky, Multielemental composition and nutritional value of "dumosa" (*Ilex dumosa*), "yerba mate" (*I. paraguariensis*) and their commercial mixture in different forms of use, Rev. Fac. Cienc. Agrar. 48 (2016) 145–159.
[32] L.M.G. dos Santos, S.A. Vicentini Neto, G. Iozzi, S.do C. Jacob, Arsenic, cadmium and lead concentrations in Yerba mate commercialized in Southern Brazil by inductively coupled plasma mass spectrometry, Ciênc. Rural 47 (2017), https://doi.org/10.1590/0103-8478cr20170202.
[33] FAO/WHO, Safety evaluation of certain food additives and contaminants, Food Addit. Ser. 58 (2007) 269–315.
[34] Council of European Union, REGLAMENTO (UE) 2018/73 DE LA COMISIÓN - de 16 de enero de 2018 - por el que se modifican los anexos II y III del Reglamento (CE) n.o 396/2005 del Parlamento Europeo y del Consejo en lo relativo a los límites máxim, 2018.
[35] ANVISA, Technical regulation Mercosul on maximum limits of inorganic contaminants in food (in Portuguese), 2013.
[36] A. Alilović, I. Živković, M. Horvat, Optimisation of distillation as an isolation method for the determination of low methylmercury concentrations in urine samples, Talanta 264 (2023) 124765, https://doi.org/10.1016/j.talanta.2023.124765.
[37] M.F. Silva, I.V. Tóth, A.O.S.S. Rangel, Determination of mercury in fish by cold vapor atomic absorption spectrophotometry using a multicommuted flow injection analysis system, Anal. Sci. 22 (2006) 861–864, https://doi.org/10.2116/analsci.22.861.
[38] P. Cava-Montesinos, E. Ródenas-Torralba, Á. Morales-Rubio, M.L. Cervera, M. De La Guardia, Cold vapour atomic fluorescence determination of mercury in milk by slurry sampling using multicommutation, Anal. Chim. Acta 506 (2004) 145–153, https://doi.org/10.1016/j.aca.2003.11.023.
[39] E. Bramanti, C. Lomonte, M. Onor, R. Zamboni, A. D'Ulivo, G. Raspi, Mercury speciation by liquid chromatography coupled with on-line chemical vapour generation and atomic fluorescence spectrometric detection (LC-CVGAFS), Talanta 66 (2005) 762–768, https://doi.org/10.1016/j.talanta.2004.12.031.
[40] H. Pyhtilä, P. Perämäki, J. Piispanen, M. Niemelä, T. Suoranta, M. Starr, T. Nieminen, M. Kantola, L. Ukonmaanaho, Development and optimization of a method for detecting low mercury concentrations in humic-rich natural water samples using a CV-ICP-MS technique, Microchem. J. 103 (2012) 165–169, https://doi.org/10.1016/j.microc.2012.02.010.
[41] J.R. Miranda-Andrades, S. Khan, C.A.T. Toloza, R.M. Maciel, R. Escalfoni, M.L. B. Tristão, R.Q. Aucelio, Speciation and ultra trace determination of mercury in produced waters from offshore drilling operations using portable instrumentation and matrix-matching calibration, Microchem. J. 146 (2019) 1072–1082, https://doi.org/10.1016/j.microc.2019.02.045.
[42] Z. Yao, J. Liu, X. Mao, G. Chen, Z. Ma, B. Li, Ultratrace mercury speciation analysis in rice by in-line solid phase extraction—liquid chromatography—atomic fluorescence spectrometry, Food Chem. 379 (2022) 132116, https://doi.org/10.1016/j.foodchem.2022.132116.
[43] C.S. Chiou, S.J. Jiang, K.S. Kumar Danadurai, Determination of mercury compounds in fish by microwave-assisted extraction and liquid chromatography-vapor generation-inductively coupled plasma mass spectrometry, Spectrochim. Acta - Part B At. Spectrosc. 56 (2001) 1133–1142, https://doi.org/10.1016/S0584-8547(01)00180-X.
[44] J.L. Rodrigues, S.S. de Souza, V.C. de Oliveira Souza, F. Barbosa Jr., Methylmercury and inorganic mercury determination in blood by using liquid chromatography with inductively coupled plasma mass spectrometry and a fast sample preparation procedure, Talanta 80 (2010) 1158–1163, https://doi.org/10.1016/j.talanta.2009.09.001.
[45] B.L. Batista, J.L. Rodrigues, S.S. De Souza, V.C. Oliveira Souza, F. Barbosa, Mercury speciation in seafood samples by LC-ICP-MS with a rapid ultrasound-assisted extraction procedure: application to the determination of mercury in Brazilian seafood samples, Food Chem. 126 (2011) 2000–2004, https://doi.org/10.1016/j.foodchem.2010.12.068.
[46] S. Zhang, H. Luo, Y. Zhang, X. Li, J. Liu, Q. Xu, Z. Wang, In situ rapid magnetic solid-phase extraction coupled with HPLC-ICP-MS for mercury speciation in environmental water, Microchem. J. 126 (2016) 25–31, https://doi.org/10.1016/j.microc.2015.11.040.
[47] L. Li, Z. Wang, S. Zhang, M. Wang, Directly-thiolated graphene based organic solvent-free cloud point extraction-like method for enrichment and speciation of mercury by HPLC-ICP-MS, Microchem. J. 132 (2017) 299–307, https://doi.org/10.1016/j.microc.2017.02.011.
[48] Y. Zhao, J. Zheng, L. Fang, Q. Lin, Y. Wu, Z. Xue, F. Fu, Speciation analysis of mercury in natural water and fish samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry, Talanta 89 (2012) 280–285, https://doi.org/10.1016/j.talanta.2011.12.029.
[49] M. Musielak, M. Serda, E. Talik, A. Gagor, J. Korzuch, R. Sitko, Highly selective and sensitive determination of mercury ions by total-reflection X-ray fluorescence spectrometry, J. Anal. At. Spectrom. 36 (2021) 1533–1543, https://doi.org/10.1039/D1JA00084E.
[50] D. Martín-Yerga, M.B. González-García, A. Costa-García, Electrochemical determination of mercury: a review, Talanta 116 (2013) 1091–1104, https://doi.org/10.1016/j.talanta.2013.07.056.
[51] R.F.L. Ribeiro, A. Germano, Development and validation of a method for the determination of Hg in animal tissues (equine muscle, bovine kidney and swine kidney, and poultry muscle) by direct mercury analysis (DMA), Microchem. J. 121 (2015) 237–243, https://doi.org/10.1016/J.MICROC.2015.03.005.
[52] G. Schulzki, B. Nüßlein, H. Sievers, Transition rates of selected metals determined in various types of teas (*Camellia sinensis* L. Kuntze) and herbal/fruit infusions, Food Chem. 215 (2017) 22–30, https://doi.org/10.1016/j.foodchem.2016.07.093.
[53] B. Brodziak-Dopierała, A. Fischer, Analysis of mercury content in various types of tea (*Camellia sinensis*) and Yerba mate (*Ilex paraguariensis*), Int. J. Environ. Res. Public Health 19 (2022), https://doi.org/10.3390/ijerph19095491.
[54] D. Gibičar, M. Logar, N. Horvat, A. Marn-Pernat, R. Ponikvar, M. Horvat, Simultaneous determination of trace levels of ethylmercury and methylmercury in biological samples and vaccines using sodium tetra(n-propyl)borate as derivatizing agent, Anal. Bioanal. Chem. 388 (2007) 329–340, https://doi.org/10.1007/s00216-007-1208-0.
[55] D.M. Templeton, H. Fujishiro, Terminology of Elemental Speciation—An IUPAC Perspective, Elsevier B.V., 2017, https://doi.org/10.1016/j.ccr.2017.02.002.
[56] Y. Gao, Z. Shi, Z. Long, P. Wu, C. Zheng, X. Hou, Determination and speciation of mercury in environmental and biological samples by analytical atomic spectrometry, Microchem. J. 103 (2012) 1–14, https://doi.org/10.1016/j.microc.2012.02.001.
[57] J.R. Miranda-Andrades, S. Khan, M.J. Pedrozo-Penãfiel, K.de C.B. Alexandre, R. M. Maciel, R. Escalfoni, M.L.B. Tristão, R.Q. Aucelio, Combination of ultrasonic extraction in a surfactant-rich medium and distillation for mercury speciation in offshore petroleum produced waters by gas chromatography cold vapor atomic fluorescence spectrometry, Spectrochim. Acta - Part B At. Spectrosc. 158 (2019) 105641, https://doi.org/10.1016/j.sab.2019.105641.
[58] E.P. Agency, Method 1631, revision E: mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry, United States environ, Prot. Agency (2002) 1–34.
[59] E. Lara, C. Junior, C. Morand, Interest of mate (*Ilex paraguariensis* A. St. -Hil.) as a new natural functional food to preserve human cardiovascular health—A review, J. Funct. Foods 21 (2016) 440–454, https://doi.org/10.1016/j.jff.2015.12.010.
[60] M.V. Panzl, D. Menchaca, A. Rodríguez-Haralambides, Analysis of polyphenols and xanthines in yerba mate (*Ilex paraguariensis*) infusions by high-pressure extraction and ultra-high performance liquid chromatography, Appl. Food Res. 2 (2022) 100192, https://doi.org/10.1016/j.afres.2022.100192.
[61] E. Rzasa-Duran, A. Kryczyk-Poprawa, D. Drabicki, A. Podkowa, K. Sułkowska-Ziaja, A. Szewczyk, K. Kała, W. Opoka, P. Zięba, M. Fidurski, B. Muszyńska, Yerba Mate as a source of elements and bioactive compounds with antioxidant activity, Antioxidants 11 (2022) 1–13, https://doi.org/10.3390/antiox11020371.
[62] J.H. Huang, Artifact formation of methyl- and ethyl-mercury compounds from inorganic mercury during derivatization using sodium tetra(n-propyl)borate, Anal. Chim. Acta 532 (2005) 113–120, https://doi.org/10.1016/j.aca.2004.10.057.
[63] J.-H. Huang, Artifact formation of methyl- and ethyl-mercury compounds from inorganic mercury during derivatization using sodium tetra(n-propyl)borate, Anal. Chim. Acta 532 (2005) 113–120, https://doi.org/10.1016/J.ACA.2004.10.057.
[64] S.A. Gegenschatz, F.A. Chiappini, C.M. Teglia, A.M. de la Peña, H.C. Goicoechea, Binding the gap between experiments, statistics, and method comparison: a tutorial for computing limits of detection and quantification in univariate calibration for complex samples, Anal. Chim. Acta 1209 (2022), https://doi.org/10.1016/j.aca.2021.339342.
[65] Q. Wang, D. Wang, Z. Li, Y. Wang, Y. Yang, M. Liu, D. Li, G. Sun, B. Zeng, Concentrations, leachability, and health risks of mercury in green tea from major production areas in China, Ecotoxicol. Environ. Saf. 232 (2022) 113279, https://doi.org/10.1016/j.ecoenv.2022.113279.
[66] M. Dos Santos, F.M.R. da Silva Júnior, D.V. Zurdo, P.R.M. Baisch, A.L. Muccillo-Baisch, Y. Madrid, Selenium and mercury concentration in drinking water and food samples from a coal mining area in Brazil, Environ. Sci. Pollut. Res. (2019), https://doi.org/10.1007/s11356-019-04942-4.
[67] C.C. Brombach, B. Chen, W.T. Corns, J. Feldmann, E.M. Krupp, Methylmercury in water samples at the pg/L level by online preconcentration liquid chromatography cold vapor-atomic fluorescence spectrometry, Spectrochim. Acta - Part B At. Spectrosc. 105 (2015) 103–108, https://doi.org/10.1016/j.sab.2014.09.014.
[68] K.L. Sahrawat, G.R. Kumar, J.K. Rao, Evaluation of triacid and dry ashing procedures for determining potassium, calcium, magnesium, iron, zinc, manganese, and copper in plant materials, Commun. Soil Sci. Plant Anal. 33 (2002) 95–102, https://doi.org/10.1081/CSS-120002380.
[69] A. Pequerul, C. Pérez, P. Madero, J. Val, E. Monge, A rapid wet digestion method for plant analysis, Optim. Plant Nutr 2 (1993) 3–6, https://doi.org/10.1007/978-94-017-2496-8_1.
[70] S. Liu, Z. Han, X. Kong, J. Zhang, Z. Lv, G. Yuan, Organic matrix effects in inductively coupled plasma mass spectrometry: a tutorial review, Appl. Spectrosc. Rev. 57 (2022) 461–489, https://doi.org/10.1080/05704928.2021.1897991.
[71] S. Trimmel, T.C. Meisel, S.T. Lancaster, T. Prohaska, J. Irrgeher, Determination of 48 elements in 7 plant CRMs by ICP-MS/MS with a focus on technology-critical elements, Anal. Bioanal. Chem. 415 (2023) 1159–1172, https://doi.org/10.1007/s00216-022-04497-3.

[72] T.H. Hansen, K.H. Laursen, D.P. Persson, P. Pedas, S. Husted, J.K. Schjoerring, Micro-scaled high-throughput digestion of plant tissue samples for multi-elemental analysis, Plant Methods 5 (2009) 1–11, https://doi.org/10.1186/1746-4811-5-12.
[73] A.P.F. Saidelles, R.M. Kirchner, É.M.de M. Flores, N.R.Z. dos Santos, J. K. Benedetti, L.C. da Cruz, Determinação de Cu, Ni e Zn por ICP-MS em infusões de erva-mate comercializadas nas regiões do Sul do Brasil, Alim. Nutr.= Braz. J. Food Nutr. 24 (2010) 283–289.
[74] M.C.A. Marcelo, C.A. Martins, D. Pozebon, V.L. Dressler, M.F. Ferrão, Classification of yerba mate (*Ilex paraguariensis*) according to the country of origin based on element concentrations, Microchem. J. 117 (2014) 164–171, https://doi.org/10.1016/j.microc.2014.06.027.
[75] B. de Fátima Pereira Schmite, A. Bitobrovec, A.C.M. Hacke, R.P. Pereira, P. L. Weinert, V.E. dos Anjos, In vitro bioaccessibility of Al, Cu, Cd, and Pb following simulated gastro-intestinal digestion and total content of these metals in different Brazilian brands of yerba mate tea, Food Chem. 281 (2019) 285–293, https://doi.org/10.1016/j.foodchem.2018.12.102.
[76] P. Konieczynski, A. Viapiana, M. Wesolowski, Comparison of infusions from black and green teas (*Camellia sinensis* L. Kuntze) and erva-mate (*Ilex paraguariensis* A. St.-Hil.) based on the content of essential elements, secondary metabolites, and antioxidant activity, Food Anal. Methods 10 (2017) 3063–3070, https://doi.org/10.1007/s12161-017-0872-8.
[77] E. Magri, E.K. Gugelmin, F.A.P. Grabarski, J.Z. Barbosa, A.C. Auler, I. Wendling, S.A. Prior, A.T. Valduga, A.C.V. Motta, Manganese hyperaccumulation capacity of *Ilex paraguariensis* A. St. Hil. And occurrence of interveinal chlorosis induced by transient toxicity, Ecotoxicol. Environ. Saf. (2020) 203, https://doi.org/10.1016/j.ecoenv.2020.111010.
[78] C.N. Carducci, P.C. Dabas, J.O. Muse, Determination of inorganic cations by capillary ion electrophoresis in *Ilex paraguariensis* (St.H.), a plant used to prepare tea in South America, J. AOAC Int. 83 (2000) 1167–1173.
[79] P.F.T. Schunk, I.C. Kalil, E.F. Pimentel-Schmitt, D. Lenz, T.U. de Andrade, J. S. Ribeiro, D.C. Endringer, ICP-OES and micronucleus test to evaluate heavy metal contamination in commercially available Brazilian herbal teas, Biol. Trace Elem. Res. 172 (2016) 258–265, https://doi.org/10.1007/s12011-015-0566-2.
[80] E. Rusinek-Prystupa, Z. Marzec, I. Sembratowicz, W. Samolińska, B. Kiczorowska, M. Kwiecień, Content of selected minerals and active ingredients in teas containing Yerba Mate and rooibos, Biol. Trace Elem. Res. 172 (2016) 266–275, https://doi.org/10.1007/s12011-015-0588-9.
[81] V.L.C. Bragança, P. Melnikov, L.Z. Zanoni, Trace elements in different brands of Yerba mate tea, Biol. Trace Elem. Res. 144 (2011) 1197–1204, https://doi.org/10.1007/s12011-011-9056-3.
[82] R.F. Milani, L.K. Silvestre, M.A. Morgano, S. Cadore, Investigation of twelve trace elements in herbal tea commercialized in Brazil, J. Trace Elem. Med. Biol. 52 (2019) 111–117, https://doi.org/10.1016/j.jtemb.2018.12.004.
[83] I.R.C. Vulcano, J.N. Silveira, E.M. Alvarez-Leite, Teores de chumbo e cádmio em chás comercializados na região metropolitana de Belo Horizonte, Rev. Bras. Cienc. Farm. J. Pharm. Sci. 44 (2008) 425–431, https://doi.org/10.1590/S1516-93322008000300012.
[84] A.M.G. Da Costa, E.M. Nogami, J.V. Visentainer, N.E. De Souza, E.E. Garcia, Fractionation of aluminum in commercial green and roasted yerba mate samples (*Ilex paraguariensis* St. Hil.) and in their infusions, J. Agric. Food Chem. 57 (2009) 196–200, https://doi.org/10.1021/jf802808h.
[85] B.K. De Campos, J.P. Dos Prazeres, Y.R. Torres, V.E. Dos Anjos, S.P. Quináia, Avaliação da labilidade de alumínio em infusões de erva-mate empregando voltametria adsortiva de redissolução catódica, Quim. Nova 37 (2014) 1479–1486, https://doi.org/10.5935/0100-4042.20140226.
[86] M.V. Barrella, O.A. Heringer, P.M.M. Cardoso, E.F. Pimentel, R. Scherer, D. Lenz, D.C. Endringer, Metals content in herbal supplements, Biol. Trace Elem. Res. 175 (2017) 488–494, https://doi.org/10.1007/s12011-016-0776-2.
[87] A.R. Borges, D.N. Bazanella, Á.T. Duarte, A.V. Zmozinski, M.G.R. Vale, B. Welz, Development of a method for the sequential determination of cadmium and chromium from the same sample aliquot of yerba mate using high-resolution continuum source graphite furnace atomic absorption spectrometry, Microchem. J. 130 (2017) 116–121, https://doi.org/10.1016/j.microc.2016.08.010.
[88] M. Theuma, E. Attard, From herbal substance to infusion: the fate of polyphenols and trace elements, J. Herb. Med. 21 (2020), https://doi.org/10.1016/j.hermed.2020.100347.
[89] K. Janda, K. Jakubczyk, A. Łukomska, I. Baranowska-Bosiacka, E. Rębacz-Maron, K. Dec, J. Kochman, I. Gutowska, Effect of the Yerba mate (*Ilex paraguariensis*) brewing method on the content of selected elements and antioxidant potential of infusions, Polish J. Chem. Technol. 22 (2020) 54–60, https://doi.org/10.2478/pjct-2020-0008.
[90] C.C. Pereira, A.O. Souza, E.Q. Oreste, M.J.A. Cidade, C. Solange Cadore, A. S. Ribeiroa, M.A. Vieira, Acid decomposition of Yerba mate (*Ilex paraguariensis*) using a reflux system for the evaluation of Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Pb and Zn contents by Atomic spectrometric techniques, J. Braz. Chem. Soc. 27 (2016) 685–693.
[91] K. Wróbel, K. Wróbel, E.M. Urbina, Determination of total aluminum, chromium, copper, iron, manganese, and nickel and their fractions leached to the infusions of black tea, green tea, Hibiscus sabdariffa, and *Ilex paraguariensis* (mate) by ETA-AAS, Biol. Trace Elem. Res. 78 (2000) 271–280, https://doi.org/10.1385/BTER:78:1-3:271.
[92] R. Giulian, C.E.I. Dos Santos, S. De Moraes Shubeita, L.M. Da Silva, J.F. Dias, M. L. Yoneama, Elemental characterization of commercial mate tea leaves (*Ilex paraguariensis* A. St.-Hil.) before and after hot water infusion using ion beam techniques, J. Agric. Food Chem. 55 (2007) 741–746, https://doi.org/10.1021/jf062456r.
[93] R. Giulian, C.E. Iochims dos Santos, S. de Moraes Shubeita, L. Manfredi da Silva, M.L. Yoneama, J.F. Dias, The study of the influence of industrial processing on the elemental composition of mate tealeaves (*Ilex paraguariensis*) using the PIXE technique, LWT - Food Sci. Technol. 42 (2009) 74–80, https://doi.org/10.1016/j.lwt.2008.05.007.
[94] A.P.F. Saidelles, R.M. Kirchner, N.R.Z. Dos Santos, É.M.de M. Flores, F.R. Bartz, A. Paula, F. Saidelles, R.M. Kirchner, F.R. Bartz, Análise de metais em amostras comerciais de erva-mate do sul do Brasil, Alim. Nutr. 21 (2010) 259–265.
[95] I. Olivari, S. Paz, Á.J. Gutiérrez, D. González-Weller, A. Hardisson, G. Sagratini, C. Rubio, Macroelement, trace element, and toxic metal levels in leaves and infusions of yerba mate (*Ilex paraguariensis*), Environ. Sci. Pollut. Res. 27 (2020) 21341–21352, https://doi.org/10.1007/s11356-020-08681-9.
[96] S. Natesan, V. Ranganathan, Content of various elements in different parts of the tea plant and in infusions of black tea from Southern India, J. Sci. Food Agric. 51 (1990) 125–139, https://doi.org/10.1002/jsfa.2740510112.
[97] H. Matsuura, A. Hokura, F. Katsuki, A. Itoh, H. Haraguchi, Multielement determination and speciation of major-to-trace elements in black tea leaves by ICP-AES and ICP-MS with the aid of size exclusion chromatography, Anal. Sci. 17 (2001) 391–398, https://doi.org/10.2116/analsci.17.391.
[98] D. Kara, Evaluation of trace metal concentrations in some herbs and herbal teas by principal component analysis, Food Chem. 114 (2009) 347–354, https://doi.org/10.1016/j.foodchem.2008.09.054.
[99] N.C.M. Ulbrich, L.L. do Prado, J.Z. Barbosa, E.M. Araujo, G. Poggere, A.C. V. Motta, S.A. Prior, E. Magri, S.D. Young, M.R. Broadley, Multi-elemental analysis and health risk assessment of commercial Yerba Mate from Brazil, Biol. Trace Elem. Res. 200 (2022) 1455–1463, https://doi.org/10.1007/s12011-021-02736-9.
[100] A. Baran, A. Gruszecka-Kosowska, A. Kołton, C. Jasiewicz, P. Piwowar, Content and health risk assessment of selected elements in the Yerba mate (*Ilex paraguariensis*, St. hillaire), Hum. Ecol. Risk Assess. 24 (2018) 1092–1114, https://doi.org/10.1080/10807039.2017.1406304.