



Multielement Determination in Green, Black, and White Tea Leaves (*Camellia sinensis*) Using Ultrasound-Assisted Extraction with Natural Deep Eutectic Solvents

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Abstract

Multielement determination of essential and potentially toxic elements in tea leaves requires efficient and sustainable sample preparation strategies. An ultrasound-assisted extraction (UAE) method using natural deep eutectic solvent (NADES) was developed for the determination of As, Cd, Cu, Fe, Mn, Pb, and Zn in *Camellia sinensis* leaves. The NADES, composed of choline chloride, oxalic acid, and water (1:1:1 molar ratio), showed high extraction efficiency. Multivariate optimization established conditions of 40 min extraction, 80 °C, and 3 mL solvent. Method performance was evaluated with certified reference material, with recoveries of 73% to 107% and relative standard deviation < 10%. Limits of quantification ranged from 0.019 to 9.70 mg kg⁻¹, depending on the element. Application to tea samples revealed concentration ranges (mg kg⁻¹) of 0.021–0.130 (As), 0.024–0.523 (Cd), 6.67–18.03 (Cu), 50–714 (Fe), 56–3203 (Mn), 0.063–1.02 (Pb), and 17.2–62.6 (Zn). Estimated daily intake values (EDI, mg day⁻¹) ranged from 0.03–0.19 (Cu), 0.01–2.71 (Fe), 0.21–33.5 (Mn), and 0.07–0.65 (Zn), underscoring the nutritional and toxicological relevance of the analyzed elements. In addition, self-organizing maps (SOM) were applied as an exploratory multivariate tool, enabling the identification of multielement patterns among tea types and highlighting the combined influence of environmental, botanical, and processing-related factors on elemental composition. The NADES-based method provided results comparable to the reference method and proved simple, efficient, safe, and low-cost, complying with Green Analytical Chemistry principles (AGREE, 0.68; AGREEprep, 0.58) as a sustainable alternative for multi-element analysis of complex food matrices.

Keywords Natural deep eutectic solvents · *Camellia sinensis* · Elemental analysis · Self-organizing maps · Green Analytical Chemistry · Food matrices

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Introduction

Tea is among the most consumed beverages worldwide and exhibits multiple bioactivities, including antioxidant, anti-inflammatory, and antitumor activities (Koláčková et al. 2021). This infusion is made from the leaves of the *Camellia sinensis* plant, which have several beneficial properties for human health, such as relieving flu symptoms, helping reduce cholesterol, controlling blood sugar levels, and aiding weight loss (Li et al. 2022; Xu et al. 2023). However, tea leaves can also accumulate toxic elements such as arsenic, cadmium, and lead, raising concerns about food safety and consumer health (Barrella et al. 2017; Alnaimat et al. 2021). Monitoring the elemental composition of tea is therefore crucial to ensure product quality and compliance with regulatory standards.

Sensitive techniques, such as inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), are widely used for multi-element analysis of plant matrices (Milani et al. 2016; Barrella et al. 2017; Santana et al. 2020; Costa et al. 2022). However, these methods require a critical sample preparation step, usually based on acid decomposition in closed systems under high pressure and temperature (Smichowski and Londonio 2018). Furthermore, these procedures require large volumes of aggressive reagents, such as concentrated acids or mixtures of acids with hydrogen peroxide, which can pose risks to the operator's health and the environment, in addition to contradicting the principles of Green Analytical Chemistry (GAC) (Gałuszka et al. 2013; Rocha et al. 2013).

To address these challenges, natural deep eutectic solvents (NADES) have emerged as environmentally friendly alternatives in sample preparation (Zhao et al. 2023; Costa et al. 2024a; Marco et al. 2025; Soares et al. 2025). These solvents are formed by combining hydrogen bond donors and acceptors in appropriate proportions and generally derive from natural, biodegradable, and non-toxic compounds such as choline salts, organic acids, and sugars (Zhang et al. 2012; Luján et al. 2024). In addition to being non-volatile, NADES are easy to prepare, which significantly reduces environmental and operational risks (Armenta et al. 2019; Esteve-Turrillas et al. 2024). When combined with ultrasound-assisted extraction (UAE), which promotes the release of analytes from the matrix through acoustic cavitation, they enable the development of simple, cost-effective, safe, and sustainable extraction methods (Moreira et al. 2022).

In addition to adopting green solvents and facilitating techniques, optimizing the extraction process is essential to ensure reproducibility, analytical efficiency, and cost reduction (Santana et al. 2024). In this context, chemometric tools, such as factorial design and central composite design (CCD),

have been widely used to systematically evaluate the effects of multiple experimental variables, allowing for the refinement of methods based on robust statistical criteria (Pacheco-Fernández and Pino 2019; Solaberrieta et al. 2022; Munjanja et al. 2025). Although numerous studies have addressed the determination of elemental concentrations in tea leaves (Herador 2001; Alnaimat et al. 2021; Koláčková et al. 2021; Li et al. 2021; Długaszek and Mierczyk 2024), the application of NADES-based extraction for multielement determination remains limited. In addition, data interpretation in these studies is often restricted to univariate comparisons. Such approaches may overlook complex multielement relationships arising from the combined influence of environmental conditions, plant physiology, and processing steps. From this perspective, exploratory multivariate tools, such as self-organizing maps (SOM), can provide complementary insight into the structure and variability of elemental datasets.

This study proposes an ultrasound-assisted extraction method using natural deep eutectic solvents (UAE-NADES) for the determination of As, Cd, Cu, Fe, Mn, Pb, and Zn in *Camellia sinensis* leaves by ICP-OES and ICP-MS. Experimental design was used as a multivariate optimization tool, and the proposed method was applied to tea leaves (green, white, and black), with the resulting dataset evaluated using SOM.

Experimental

Instrumentation

The analyte extraction procedure in plant leaves samples was performed in an Elmasonic Select ultrasonic bath at 37 kHz and 350 W (Elma, Germany). The samples were agitated in a Vortex Basic (Kasvi, Brazil) and separated in a centrifuge, model Rotofix 32 A (Hettich, Germany). In the decomposition, the samples were digested in a microwave radiation oven, model Multiwave Go Plus, with a DMC system (Anton Paar, Austria). Before the decomposition and extraction procedures, the samples were dried in an oven (Ethik, Brazil) and then comminuted in an analytical mill (model A11 basic, IKA, Germany).

The characterization of the prepared solvents was performed by Fourier transform infrared spectroscopy (ATR-FTIR), using the Agilent MicroLab software and the Cary 630 spectrometer (Agilent, United States). The determination of Fe, Mn, and Zn was performed using an Optima 7000DV ICP-OES (PerkinElmer, United States). In contrast, the determination of As, Cd, Cu, and Pb was performed using an ICP-MS model 7850 (Agilent, United States) equipped with a collision cell in KED mode. The operational conditions are presented in Table S1 (Supplementary data).

Reagents and Materials

Throughout the analytical procedure, deionized water with a resistivity of 18.2 M Ω cm, purified using a PURELAB Ultra system (ELGA, United Kingdom), was used. All glassware and flasks were decontaminated by immersion in a 5% v v⁻¹ Extran MA 02 Neutral solution (Merck, Germany) for 24 h, followed by immersion in a 15% v v⁻¹ HNO₃ solution for an additional 24 h, and subsequently rinsed with ultrapure water.

For the decomposition of the tea samples, nitric acid (HNO₃ 65% w w⁻¹, Merck, Germany), previously purified in a sub-boiling acid distiller, model BSB-939-IR (Berg-hof, Germany), and hydrogen peroxide (H₂O₂ 30% v v⁻¹, Sigma-Aldrich, United States) were used. For the extraction procedure, the following reagents were used: choline chloride, min. 99% purity (ACS, Brazil); xylitol, min. 99% purity (ACS, Brazil); DL-malic acid 99% purity (Neon, Brazil); and oxalic acid dihydrate, min. 99% purity (ACS, Brazil).

The calibration curves were prepared from the multi-element stock solution (Q.C. Standard 3) in 5% v v⁻¹ HNO₃ (SCP Science, Brazil), with a concentration of 10 mg L⁻¹ for the elements As, Cd, Cu, and Pb; and mono-element standards in 5% v v⁻¹ HNO₃, with concentrations of 100 mg L⁻¹ and 1000 mg L⁻¹ for Fe (Supelco, Brazil), Mn (SCP Science, Brazil), Rh (Sigma-Aldrich, Brazil), Y (PlasmaCal, Brazil), and Zn (SCP Science, Brazil). The analytical curves were prepared in the ranges of 0–60 μ g L⁻¹ for As, Cd, Cu, and Pb; 0–20 mg L⁻¹ for Fe and Mn; and 0–1 mg L⁻¹ for Zn. All the standards mentioned had 99.9% purity.

The reference materials used to evaluate the accuracy and precision of the proposed method were peach leaves (NIST 1547, NIST, USA), tomato leaves (NIST 1573a, NIST, USA), and *Brachiaria brizantha* cv. Marandu (Embrapa, Brazil).

Samples and Sample Pretreatment

Green, black, and white tea samples, obtained from the leaves of *Camellia sinensis*, were acquired from herbal shops, supermarkets, and online stores. Thirteen green tea samples were obtained (four from herbal shops, three from supermarkets, and six through online purchases); thirteen black tea samples (two from herbal shops, four from supermarkets, and seven through online purchases); and four white tea samples (all acquired through online purchases), totaling 30 samples. Before the extraction procedure, the samples were dried in an oven for 72 h at 60 °C, comminuted in an analytical mill, and then stored in a desiccator until the time of analysis. The tea samples were analyzed in triplicate, and results were expressed as mean $\pm$ standard deviation ($n = 3$).

NADES Preparation and Characterization

Initially, three NADES were prepared from mixtures of the following: (1) choline chloride, oxalic acid, and water (ChCl:Ox:Wa); (2) choline chloride, malic acid, and water (ChCl:Ma:Wa); and (3) choline chloride, xylitol, and water (ChCl:Xy:Wa). The solvents were prepared in a 1:1:3 molar ratio. The components were weighed, mixed, and heated at 80 °C for 2 h on a heating plate without agitation. The resulting mixtures were then stored in polypropylene tubes.

The extraction efficiency of each solvent under UAE conditions was evaluated using a univariate approach, employing 150 mg of the reference material *Brachiaria brizantha* cv. Marandu, 3 mL of solvent, a temperature of 65 °C, and 30 min of sonication time. The evaluated molar ratios were selected based on preliminary laboratory tests and previous studies involving similar NADES systems. After selecting the most suitable solvent, further studies were carried out using different molar ratios (2:1:1, 1:2:1, 1:1:1, 1:1:3, and 1:1:5), following the same experimental procedure (Costa et al. 2022; Moreira et al. 2022).

The characterization of each NADES and the corresponding starting materials was performed by ATR-FTIR in transmittance mode, over the range of 4000–600 cm⁻¹, with a resolution of 4 cm⁻¹ and 16 scans.

Multivariate Experimental Design

Multivariate optimization (screening and modeling) was performed for the parameter's temperature, extraction time in the ultrasonic bath, and volume of the previously selected NADES. In the screening, a full 2³ factorial design was used, and in the modeling, a face-centered central composite design was adopted. The analytical response considered was the recovery (%), calculated based on the concentration of the analytes in the reference material *Brachiaria brizantha* cv. Marandu (E1001a). To determine the optimal condition, the multiple response method (MR) was applied, in which the results are transformed into dimensionless scale values (Bezerra et al. 2019).

The mathematical model was evaluated using analysis of variance (ANOVA), which included the statistical significance of the regression (F -test), the lack of fit test, and the coefficients of determination (R^2). The dataset was processed using Statistica software (version 12, StatSoft, Tulsa, USA) at a 95% confidence level.

UAE-NADES Extraction Method

Approximately 150 mg ($n = 3$) of tea leaf samples were weighed into 15 mL polypropylene tubes, followed by the addition of 3 mL of NADES (ChCl:Ox:Wa) in a 1:1:1 molar ratio. The tubes were vortexed for 30 s and then placed in an ultrasonic bath at 80 °C. After sonication for

40 min, 3 mL of deionized water was added, and the tubes were vortexed again for 10 s. Finally, the mixture was centrifuged at 4000 rpm (2.68 thousand g, $r = 15$ cm) for 10 min. An aliquot of the supernatant was taken for 10- to 25-fold-dilutions before ICP-OES and ICP-MS analyses.

MW-AD Reference Method

Approximately 200 mg ($n = 3$) of tea leaf samples were weighed directly into the microwave decomposition PTFE tubes. Then, 6 mL of HNO_3 solution (7 mol L^{-1}) and 2 mL of H_2O_2 ($30\% \text{ v v}^{-1}$) were added, and a pre-decomposition step was performed for 30 min at room temperature. The heating program consisted of the following steps: (1) 10 min to reach a temperature of 100°C ; (2) 10 min maintaining heating at 100°C ; (3) 10 min to reach a temperature of 200°C ; (4) 20 min maintaining heating at 200°C ; and (5) 10 min of cooling (Moreira et al. 2022). After reaching room temperature, the solutions were quantitatively transferred to 50 mL polypropylene tubes, with the final solution volume measured to 15 mL. Preparation blanks were performed concurrently, following the same procedure. All resulting solutions were stored in a refrigerator at 3 to 5°C until analysis.

Analytical Performance Parameters

The reference material of *Brachiaria brizantha* cv. Marandu grass and the certified reference materials (CRM) of NIST 1547 (peach leaves) and NIST 1573a (tomato leaves), for inorganic elements in plant leaves, were used to verify the accuracy of the analytical extraction and decomposition methods. The agreement between the CRM value and the value determined by the method was evaluated using the IRMM (Institute for Reference Materials and Measurements) statistical test, as defined in Eq. 1 (Linsinger 2010).

$$|C_m - C_{CRM}| \leq k \sqrt{u_m^2 + u_{CRM}^2} \quad (1)$$

C_m is the determined concentration, C_{CRM} is the certified concentration in the CRM, k is the coverage factor given by the CRM, u_m^2 is the uncertainty (standard deviation) obtained by the determination, and u_{CRM}^2 is the certified uncertainty in the CRM. If the condition of Eq. 1 is met, there is no statistical difference with 95% confidence between the determined and certified values. On the other hand, if the condition is not met, it can be stated that there is a statistical difference between the determined and certified values. A paired Student's t -test (95% confidence level) was applied to evaluate whether the concentrations obtained by the UAE-NADES method differed significantly from those obtained by the reference MW-AD method.

The precision was evaluated using the relative standard deviation (RSD%). The limits of detection (LOD) and quantification (LOQ) were determined according to IUPAC recommendations, corresponding to $3\sigma/s$ and $10\sigma/s$, respectively, where σ is the standard deviation of independent measurements of the blank solution, and s is the slope of the calibration curve (IUPAC 1978; Currie 1999). A comparison between calibration curves prepared in HNO_3 and in $1\% \text{ v v}^{-1}$ NADES was performed to evaluate the matrix effect.

The greenness of the UAE-NADES and MW-AD methods was evaluated using the AGREE and AGREEprep metrics, considering the principles of Green Analytical Chemistry and Green Sample Preparation, respectively (Pena-Pereira et al. 2020; Wojnowski et al. 2022). The evaluations were performed using the corresponding freeware calculators, and the final scores were used for comparative assessment of the proposed and reference methods.

Recommended Daily Intake (RDI)

Equation 2 of the estimated daily intake (EDI) was used to calculate the assessment of exposure to elements from tea consumption.

$$EDI_e = \frac{(C_e \times RDI \times TR_e)}{BW \times 1000} \quad (2)$$

In this equation, C_e represents the concentration of the element in tea samples (mg kg^{-1}), and RDI is the recommended daily intake of tea for adults ($11.4 \text{ g/person/day}$). TR_e is the rate of transfer of the element from the tea sample to the infusion (Zhang et al. 2018). BW is body weight (60 kg for adults) (Krstić et al. 2021). The TR_e values used in this study were 20% and 50% for Cu, Mn, and Zn, and 1% to 20% for Fe (Szymczycha-Madeja et al. 2012; Krstić et al. 2021).

SOM-Based Exploratory Multivariate Analysis

A SOM was applied as an exploratory analysis tool. SOM allows the visualization of high-dimensional data in a simpler and more dynamic two-dimensional map (Vesanto and Alhoniemi 2000). The variables used in the map were the concentrations obtained in the tea samples (black, green, and white) tea samples. The two-dimensional map, the component planes, and the unified distance matrix (U -matrix) were obtained using the SOM Toolbox package, version 2.1 (Helsinki University of Technology, Finland) (Vatanen 2015). The tool was implemented in its routine using the Matlab software version R2014a (MathWorks, USA) under standard conditions (Vesanto et al. 1999).

Results and Discussion

NADES Composition and Components Molar Ratio

The first step in univariate optimization consisted of selecting the eutectic mixture. The solvents ChCl:Ox:Wa, ChCl:Ma:Wa, and ChCl:Xy:Wa were applied to the Marandu reference material for analyte extraction using an ultrasonic bath under preliminary conditions established based on literature reports (Costa et al. 2022; Moreira et al. 2022), and the analytical response was expressed as recovery (%). It was observed that combinations with oxalic acid and malic acid generally provided better recovery values for the analytes. On the other hand, the mixture with xylitol presented the least satisfactory results, possibly due to the lower capacity for intermolecular interaction between the constituents of the eutectic mixture. This can be attributed to the absence of carbonyl groups in the xylitol molecule, unlike oxalic and malic acids, which possess this functional group. The presence of the carbonyl group contributes to greater polarization of the molecules, favoring intermolecular interactions between the components and, consequently, increasing the efficiency of solvent extraction in the plant matrix (Costa et al. 2022; Omar and Sadeghi 2023).

Thus, the mixture composed of choline chloride, oxalic acid, and water (ChCl:Ox:Wa) showed the best recovery values, ranging from 39 to 73% for Fe and Mn, and from 65 to 109% for As, Cd, Cu, Pb, and Zn, as illustrated in Fig. 1. According to the acceptance criteria recommended by INMETRO (2020) and AOAC (2016), recovery values

between 80 and 110% are generally considered acceptable for analyte concentrations at the mg kg^{-1} level. Therefore, although the ChCl:Ox system presented the best performance in this first step, some analytes still did not meet the recommended recovery criteria. Iron, for example, showed the greatest variation among the analytes, suggesting that the extraction of this element from the plant matrix is more complex. Considering the overall performance of the three solvents prepared for all the elements determined, the ChCl:Ox:Wa mixture was therefore selected to proceed to the optimization step.

In the subsequent step, a univariate evaluation of the molar ratio of the previously selected NADES was performed. The solvents prepared at the different molar ratios were applied to the Marandu reference material for analyte extraction, and the analytical response was expressed as recovery (%), as shown in Fig. 1. In this step, an improvement in the extraction performance was observed. In general, the 1:1:1 molar ratio showed the best recovery levels, with a consistent average for all analytes, ranging from 56 to 92% for Fe and Mn and from 98 to 108% for As, Cd, Cu, Pb, and Zn. Lower recovery values were observed for Fe, which may be associated with its interaction with polyphenols and structural components of the plant matrix, reducing its solubility and hindering solvent access during extraction. In addition, the fibrous structure of plant tissues may contribute to Fe retention in cellulose and hemicellulose, making its extraction more challenging under mild extraction conditions (Dambiec et al. 2013; Lin et al. 2016; Benvenuti et al. 2020; Pohl et al. 2020).

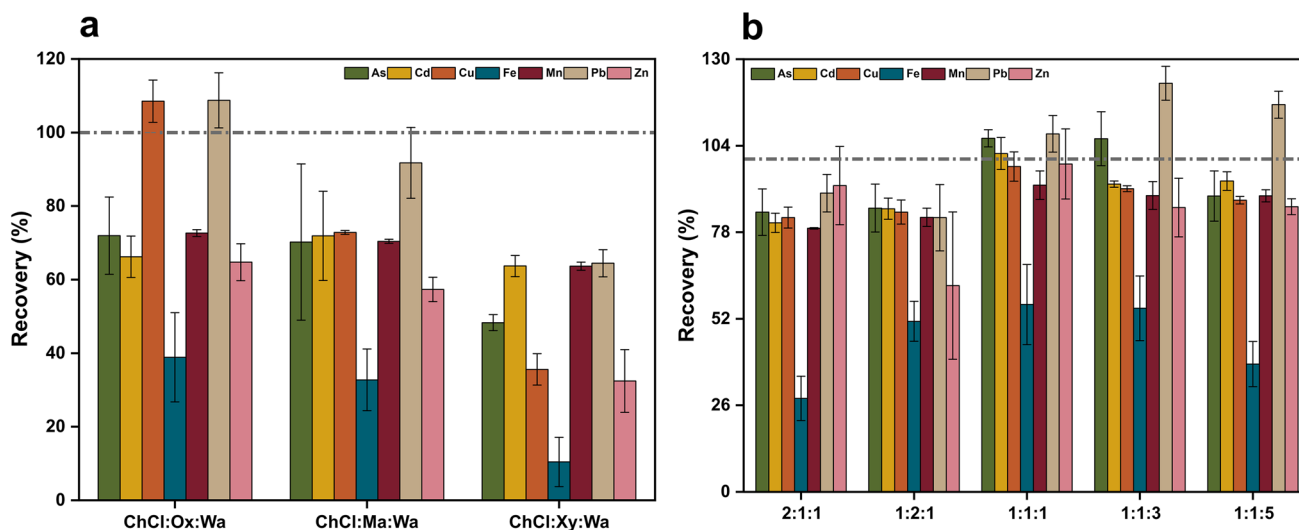


Fig. 1 Recovery (%) for As, Cd, Cu, Fe, Mn, Pb, and Zn obtained during the optimization of the natural deep eutectic solvent (NADES) applied to the Marandu reference material: **a** selection of the NADES composition prepared at a 1:1:3 molar ratio, and **b** optimization of the

molar ratio of the selected ChCl:Ox:Wa system for UAE-NADES. Experimental conditions: extraction time of 30 min, temperature of 65 °C, and NADES volume of 3 mL

NADES FT-IR Characterization

FT-IR analysis of the selected NADES (ChCl:Ox:Wa) showed key spectral features indicative of solvent formation. These spectra revealed characteristic functional groups (Figure S1, Supplementary Data). The absorption band at 950 cm^{-1} was attributed to the C–N group of choline chloride's quaternary ammonium. It was observed that this band persists in the formed solvent, but with lower intensity; this can be explained by solvation effects in the medium and the reorganization of hydrogen bonds. The characteristic absorption band in the $3400\text{--}3200\text{ cm}^{-1}$ region corresponds to the elongation and displacement of the –OH group, the main indicator of hydrogen bond formation (Moreira et al. 2022).

Additionally, a band corresponding to the stretching of the C=O group of oxalic acid was observed, shifted from 1609 to 1720 cm^{-1} . These changes arise from the formation of hydrogen bonds between the hydroxyl groups of choline chloride and the carboxyl groups of oxalic acid, altering the vibrational frequencies due to changes in the electronic environment. This affects the polarization of the molecules and, consequently, intensifies the intermolecular forces of the medium (Omar and Sadeghi 2023; Costa et al. 2024b). Thus, the formation of NADES ChCl:Ox:Wa was confirmed mainly through the presence of hydrogen bonds between their components (Moreira et al. 2022; Lai et al. 2023). The ChCl:Ox:Wa (1:1:1) system prepared with oxalic acid dihydrate contains approximately 19% water (w/w), including the water of crystallization. Previous studies have demonstrated that moderate water contents can reduce viscosity and improve mass transfer without completely disrupting the hydrogen-bonding network responsible for the physicochemical properties of NADES (Dai et al. 2015; Savi et al. 2019).

Experimental Multivariate Optimization

After completing the univariate optimization step, a multivariate approach was applied to define the optimal experimental conditions for the UAE-NADES method. The significant variables identified in the screening step were selected for the construction of a predictive model (modeling) and obtaining the response surface.

The variables chosen for the screening step were time and temperature of the ultrasonic bath and NADES volume. For this, a full 2^3 factorial design was used, with the central point values set at 30 min, $65\text{ }^\circ\text{C}$, and 3 mL of NADES. Table S2

(Supplementary Data) presents the experimental matrix of the design, with the real and coded values of the variables, as well as the analytical response (multiple response) corresponding to each experiment.

The ANOVA results (Table S3, Supplementary Data) confirmed the statistical significance of the regression model ($F_{\text{calculated}} = 43.2 > F_{\text{tabulated}} = 19.4$), indicating that the investigated factors explain the variability of the response. The quadratic regression model showed a high coefficient of determination ($R^2 = 0.994$), indicating good agreement between the experimental data and the fitted model.

Further analysis using the Pareto chart (Fig. 2) revealed that the independent variables volume and temperature exerted the greatest positive effects, indicating that increasing these parameters improves analyte extraction. However, the experimental condition of the ultrasonic bath temperature is a limiting variable, as the equipment used allows a maximum temperature of $80\text{ }^\circ\text{C}$. Therefore, due to an experimental limitation, the temperature was fixed at this value, and the variables NADES volume and extraction time were considered significant and taken to the modeling stage to determine the optimal condition.

The second part of the multivariate optimization was performed to achieve an optimal condition by constructing a response surface, based on a quadratic model, using a face-centered central composite design. According to the results of the full 2^3 factorial design, the central point was defined at 35 min with 5 mL of solvent. Thus, Table S4 (Supplementary Data) represents the design matrix with the experimental and coded values and the multiple responses corresponding to each experiment.

The ANOVA, presented in Table S3 (Supplementary Data), indicated that the quadratic model was statistically significant, with a value $F_{\text{calculated}} (46.5) > F_{\text{tabulated}} (19.3)$. The regression of the quadratic model showed that the model is well fitted to the data, explaining a substantial part of the observed variation ($R^2 = 0.838$). In addition, the lack-of-fit test was not significant ($p > 0.05$), indicating that the residual variation not explained by the model is comparable to the pure experimental error.

Based on the maximum condition predicted by the model, the contour plot shown in Fig. 3 was obtained. Under this condition, the model predicts an optimal extraction time of 40 min and a NADES volume of 3 mL. Equation 3 presents the quadratic model obtained, where T represents time and V represents NADES volume.

$$MR = 5.52 + 0.0275T - 2.76 \times 10^{-4}T^2 + 0.179V - 0.0158V^2 - 0.00201TV \quad (3)$$

The statistical validity of the mathematical model was experimentally evaluated by performing replicates under the optimal condition, for which an MR value of 6.62 ± 0.13 ($n=3$) was obtained. This value does not differ statistically from the predicted MR (6.33), since $T_{\text{calculated}} (3.85) < T_{\text{tabulated}} (4.30)$ at the 95% confidence level ($n=3$). These results demonstrate good agreement between the experimental data and the model prediction, confirming the suitability of the model within the studied experimental domain.

Analytical Performance

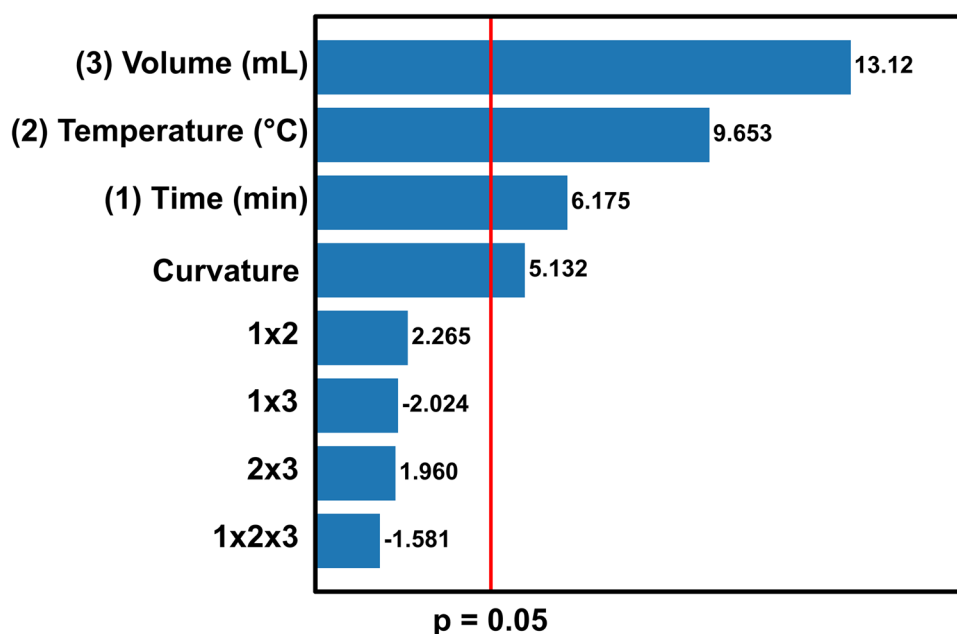
Under the optimized conditions, the performance of UAE-NADES was determined. Accuracy was assessed using reference and certified reference materials, and the results are presented in Table 1. The agreement, ranging from 73 to 90% for Fe and Mn, and 80% to 107% for As, Cd, Cu, Pb, and Zn, was obtained. The levels of agreement obtained with the UAE-NADES method follow the concentration criteria accepted by the AOAC for As, Cd, Cu, Mn, and Pb; however, it was not possible to achieve the appropriate concentration range for Fe (AOAC 2016). The agreement between the certified and determined values was assessed using the IRMM statistical test for both methods. The test condition was met for most elements (As, Cd, Cu, Mn, Pb, and Zn), indicating that there is no statistically significant difference, with 95% confidence, between the determined and certified values. In the UAE-NADES method, Fe and Mn in tomato leaves (NIST 1573a) and peach leaves (NIST 1547) showed this statistical difference, and for MW-AD, Fe,

Mn, Pb, and Zn also showed a statistical difference, although not pronounced.

Although plants efficiently absorb iron from the soil, extracting it from plant materials, such as leaves, is not a simple process. This is because the absorbed iron is often complexed with other molecules, such as polyphenols. As a result, iron shows a low solubility in plant tissues. These complexes facilitate the transport, utilization, and storage of iron within the plant. They play a crucial role in processes like photosynthesis and respiration. The plant cell wall also acts as a physical and chemical barrier and hinders solvent access. A more fibrous plant can help absorb and retain iron in cellulose and hemicellulose (Dambiec et al. 2013; Lin et al. 2016; Benvenuti et al. 2020; Pohl et al. 2020). Therefore, even though the extraction is a milder process that does not completely decompose the organic matter, the results obtained for Fe were considered satisfactory under the conditions of the developed method. This is due to the intrinsic difficulty of extracting this analyte from plant leaves.

An additional study was performed to assess the quantitative recovery of the analytes between the developed extraction method and the reference decomposition method (AOAC 2016; Moreira et al. 2022). The recoveries obtained ranged from 79 to 110%, which could be considered satisfactory for these concentration levels. A paired t-test was conducted to assess systematic differences between the results obtained by the UAE-NADES and MW-AD methods using all 20 paired results. No statistically significant difference was observed at the 95% confidence level ($t = 2.00$, $p = 0.059$, $df = 19$), highlighting that UAE-NADES provides results comparable to

Fig. 2 Pareto plot of the full 2^3 factorial design showing the effects of extraction time (min), ultrasonic bath temperature ($^{\circ}\text{C}$), NADES volume (mL), and their interactions



MW-AD, with the additional advantage of using an eco-friendly solvent. The RSD% obtained ($n=6$) in the UAE-NADES ranged from 2.8% to 9.9%, which is compatible with the deviations obtained in the MW-AD reference method ($n=6$), where the range was 1.7% to 11%.

The other analytical parameters evaluated are shown in Table 2. The calculated LODs ranged from 0.006 to 2.91 mg kg⁻¹, and the LOQs ranged from 0.019 to 9.70 mg kg⁻¹. The low values obtained for LOD and LOQ are acceptable, since they are below the values established by the Brazilian National Health Surveillance Agency (ANVISA) for the Recommended Daily Intake (RDI) of the nutrients Cu, Fe, Mn, and Zn, as well as for the non-essential elements As, Cd, and Pb (ANVISA 2005, 2022a, b). These results indicate that the method has adequate sensitivity and allows for the reliable determination of these elements.

Finally, potential matrix effects were evaluated by comparing the slope of the calibration curves. Greater sensitivity was observed in the curves with natural deep eutectic solvent

compared to the curves with nitric acid for most elements, and more pronouncedly for arsenic (As). This enhanced sensitivity observed for As when using NADES can be explained by the well-known carbon-induced signal enhancement effect in ICP-MS (Allain et al. 1991; Hu et al. 2004). Arsenic is a difficult-to-ionize element (ionization potential approximately 9.8 eV), and its ionization efficiency is significantly improved in the presence of carbon species in the plasma. According to the literature, the introduction of carbon leads to a systematic increase in the ⁷⁵As⁺ signal, which is mainly attributed to charge transfer reactions between ionized carbon species, particularly C⁺ and CO⁺, and neutral As atoms (Grindlay et al. 2013; Moreira et al. 2022). This mechanism increases the population of As⁺ ions in the plasma and, consequently, the analytical signal. Experimental and theoretical studies have demonstrated that this effect is dependent on the carbon load and can result in signal enhancement factors of several times compared to carbon-free solutions. Therefore, the improvement in As sensitivity observed in this study is consistent with

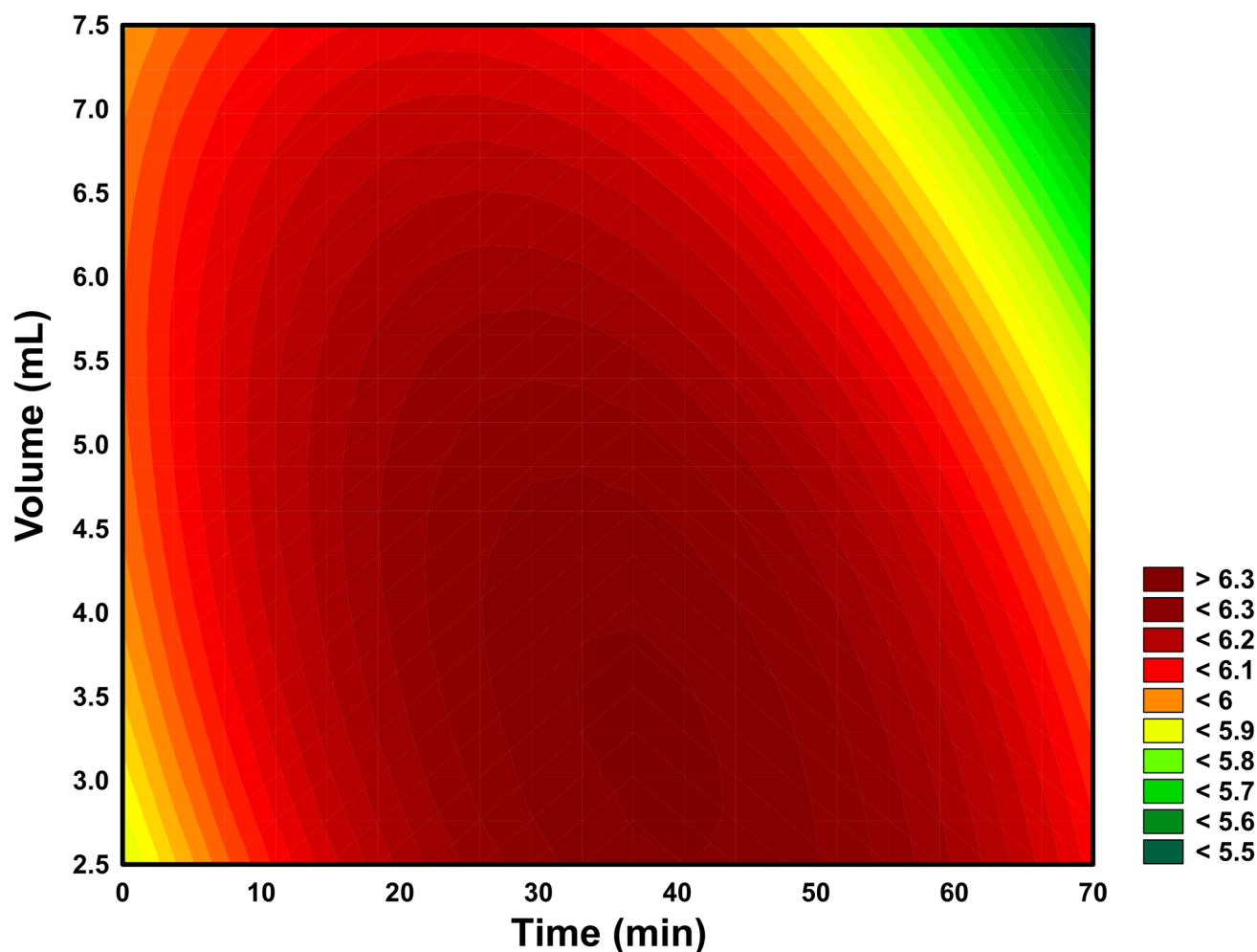


Fig. 3 Contour plot obtained from the multivariate optimization of As, Cd, Cu, Fe, Mn, Pb, and Zn, generated using the quadratic model fitted to the face-centered central composite design. A multiple-response function was used as the experimental response

Table 1 Analytical performance parameters of ultrasound-assisted extraction using a natural deep eutectic solvent (UAE-NADES) and microwave-assisted acid digestion (MW-AD) for the determination of As, Cd, Cu, and Pb by ICP-MS and Fe, Mn, and Zn by ICP-OES in certified reference materials

CRM/MR	Analyte	Certified value (mg kg ⁻¹)	UAE-NADES				MW-AD			
			Concentration (mg kg ⁻¹ ± SD)	RSD (%)	Agreement (%)	IRMM	Concentration (mg kg ⁻¹ ± SD)	RSD (%)	Agreement (%)	IRMM
Tomato leaves (NIST 1573a)	As	0.1126 ± 0.0024	0.111 ± 0.008	7.2	99	0.0021 ≤ 0.0167	0.124 ± 0.007	5.5	110	0.0109 ≤ 0.0144
	Cd	1.517 ± 0.027	1.373 ± 0.045	3.3	91	0.065 ≤ 0.1101	1.504 ± 0.096	6.4	99	0.0130 ≤ 0.1994
	Cu	4.70 ± 0.14	5.05 ± 0.22	4.3	107	0.3800 ≤ 0.5385	4.58 ± 0.17	3.7	98	0.1200 ≤ 0.4405
	Fe	367.5 ± 4.3	296.1 ± 8.9	3.0	81	74.10 ≥ 21.40	362 ± 18	5.0	99	5.50 ≤ 37.01
	Mn	246.3 ± 7.1	218.6 ± 3.5	2.8	89	27.70 ≥ 15.83	245 ± 14	5.6	99	1.30 ≤ 31.40
	Zn	30.94 ± 0.55	28.0 ± 1.4	4.9	92	2.94 ≤ 3.01	28.0 ± 1.8	6.3	91	2.04 ≤ 3.96
Peach leaves (NIST 1547)	As	0.062 ± 0.014	0.062 ± 0.006	9.9	100	0.0000 ≤ 0.0305	0.066 ± 0.005	7.5	106	0.0040 ≤ 0.0297
	Cd	0.0261 ± 0.0022	0.024 ± 0.001	5.3	90	0.0029 ≤ 0.0052	0.030 ± 0.003	11	114	0.0038 ≤ 0.0078
	Cu	3.75 ± 0.37	3.69 ± 0.21	5.7	98	0.1200 ≤ 0.9044	3.47 ± 0.08	2.3	93	0.2800 ≤ 0.7571
	Fe	219.8 ± 6.8	159.4 ± 8.3	5.2	73	59.80 ≥ 21.31	199.8 ± 4.1	2.0	91	20.00 ≥ 15.88
	Mn	97.8 ± 1.8	87.9 ± 3.3	3.8	90	9.20 ≥ 7.52	88.3 ± 1.6	1.8	90	9.50 ≥ 4.82
	Pb	0.869 ± 0.018	0.786 ± 0.038	4.8	90	0.0830 ≤ 0.0841	0.718 ± 0.016	2.2	83	0.1510 ≥ 0.0482
Marandu (E1001a)	Zn	17.97 ± 0.53	15.7 ± 1.0	6.2	89	2.17 ≤ 2.21	15.09 ± 0.34	2.3	84	2.87 ≥ 1.26
	As	1.69 ± 0.70	1.46 ± 0.14	9.4	86	0.29 ≤ 1.43	1.71 ± 0.15	8.5	101	0.02 ≤ 1.43
	Cd	19.9 ± 5.1	15.9 ± 1.0	6.4	80	4.00 ≤ 10.39	18.2 ± 1.2	6.4	91	1.70 ≤ 10.48
	Cu	4.0 ± 0.7	3.3 ± 0.3	7.7	83	1.00 ≤ 1.52	4.2 ± 0.7	16	104	0.20 ≤ 1.98
	Fe	91 ± 13	75 ± 2	3.1	83	16.00 ≤ 26.31	88 ± 2	2.7	97	3.00 ≤ 26.31
	Mn	76.0 ± 18.5	68.6 ± 2.2	3.2	90	8.00 ≤ 37.26	75.4 ± 1.3	1.7	99	0.60 ≤ 37.09
	Pb	4.0 ± 1.8	4.3 ± 0.3	7.0	106	0.30 ≤ 3.65	4.2 ± 0.3	7.6	106	0.20 ≤ 3.65
	Zn	9.9 ± 1.6	9.0 ± 0.6	7.2	91	0.90 ≤ 3.42	9.1 ± 0.4	4.3	92	0.20 ≤ 3.30

Mean concentration ($n=3$), standard deviation (SD), relative standard deviation (RSD%), and Institute for Reference Materials and Measurements (IRMM) statistical test at the 95% confidence level

previously reported carbon-induced enhancement ionization mechanism promoted by the NADES matrix.

Greenness Assessment

The deep natural eutectic solvent extraction method was assessed for its alignment with Green Analytical Chemistry (GAC) principles using the analytical greenness metric (AGREE) (Wojnowski et al. 2022) and analytical greenness metric for sample preparation (AGREEprep) analytical sustainability calculators (Pena-Pereira et al. 2020). The AGREE and AGREEprep scores for the proposed method were 0.68 and 0.58, respectively, surpassing those achieved by the conventional MW-acid digestion method (0.41 and 0.37, respectively) (Fig. 4; Figure S2, Supplementary Data).

According to AGREE and AGREEprep scores, the UAE-NADES method is more environmentally sustainable than the MW-AD method. The UAE-NADES method exhibits strong adherence to Green Analytical Chemistry (GAC) principles, particularly regarding choice of reagents, minimal energy consumption, enhanced safety, and reduced waste.

A relevant advantage in the AGREE metric when comparing the UAE-NADES method and the MW-AD method is related to waste generation, the use of hazardous materials, and operator safety. The UAE-NADES method resulted in only 5 mL of waste for an extracted sample, in contrast, the MW-AD method generated 14 mL of waste for the digested sample. The MW-AD method received penalty points due to the use of a hazardous reagent (nitric acid) and presents lower operator safety when using this type of reagent (Pena-Pereira et al. 2020; Wojnowski et al. 2022; Esteve-Turrillas et al. 2024).

In general, the AGREEprep metric is more suitable for the developed method, since it is directly linked to sample preparation, which is the focus of this study, specifically evaluating energy consumption, operator safety, and the use of renewable sources. The UAE-NADES method was considered green because it obtained an overall score higher than 0.60 for AGREE and a value close to this for AGREEprep. The parameters demonstrated that the use of NADES as an alternative solvent is a highly efficient

extraction method. For contextual purposes, Table 3 presents a comparative overview of selected NADES-based methods reported for elemental analysis of plant matrices, including information on extraction strategies, optimization approaches, and greenness assessment.

Elemental Composition of the *Camellia sinensis* Leaves

The optimized method was applied to 30 commercial tea samples to evaluate multielemental variability among tea types. The concentrations of As, Cd, Cu, Fe, Mn, Pb, and Zn in commercially available *Camellia sinensis* leaves used for tea preparation (green, black, and white) are presented in Table 4. The following concentration ranges (mg kg⁻¹) were observed in the samples: 0.021–0.130 (As), 0.024–0.523 (Cd), 6.67–18.03 (Cu), 50–714 (Fe), 56–3203 (Mn), 0.063–1.02 (Pb), and 17.2–62.6 (Zn). This dispersion of values can be attributed mainly to differences in environmental growing conditions and leaf processing stages, factors that directly influence the elemental composition of teas (Milani et al. 2016; Kolářková et al. 2021; Peng et al. 2024).

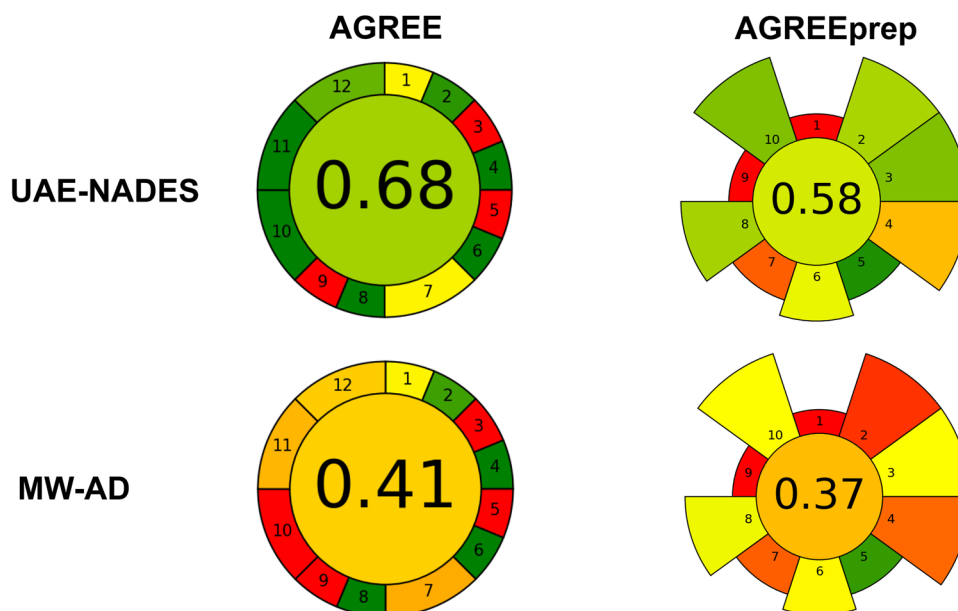
In general, samples of green and white teas exhibited higher elemental concentrations than black tea samples. This behavior is consistent with literature reports and can be directly related to the degree of oxidation/fermentation and to the specific processing steps applied to the leaves. White and green teas are minimally processed and therefore retain a larger fraction of the natural compounds present in fresh leaves, including phenolic and proteinaceous constituents capable of binding metal ions. As a consequence, elements such as Fe, Zn, Mn, and Cu tend to remain in more soluble or extractable forms. In contrast, black tea undergoes complete fermentation/oxidation of catechins (flavonoids), a process during which part of these complexes is degraded, leading to a decrease in the concentrations of several elements (Pohl et al. 2020; Długaszek and Mierczyk 2024).

Considering the nutritionally essential elements, Fe and Mn showed the greatest variations in concentration among the analyzed samples, indicating a strong influence of both plant physiology and environmental availability. Copper and

Table 2 Calibration curve parameters in nitric acid and natural deep eutectic solvent (NADES) media, including slope, coefficient of determination (R^2), limit of detection (LOD), and limit of quantification (LOQ)

Analyte	Slope/NADES	R^2	Slope/HNO ₃	R^2	LOD (mg kg ⁻¹)	LOQ (mg kg ⁻¹)
As	5250	0.9998	3581	0.9999	0.006	0.021
Cd	24,550	0.9999	22,510	0.9999	0.006	0.019
Cu	39,351	0.9999	36,848	0.9999	0.05	0.16
Fe	1,876,473	0.9998	1,702,378	0.9998	2.91	9.70
Mn	10,715,259	0.9999	10,412,408	0.9999	0.97	3.23
Pb	41,319	0.9999	40,042	0.9999	0.019	0.062
Zn	527,994	0.9998	488,653	0.9999	0.64	2.12

Fig. 4 Evaluation of UAE-NADES and MW-AD sample preparation methods using AGREE and AGREEprep metrics for *Camellia sinensis* leaves



zinc, although present at lower absolute levels, also exhibited high variability across tea types. A similar behavior was observed for arsenic (As), which, despite being present in generally low concentrations, showed marked variability among the samples. This general pattern suggests that the elemental composition of *Camellia sinensis*, including both essential and toxic elements, is influenced by the combined effects of growing conditions, leaf selection, and processing steps, rather than being governed by a single dominant factor (Szymczycha-Madeja et al. 2012; Martín-Domingo et al. 2017).

From a toxicological perspective, arsenic, cadmium, and lead deserve special attention, as they are considered toxic to humans and their exposure must be carefully monitored (Martín-Domingo et al. 2017; Li et al. 2021). According to Brazilian ANVISA regulations (RDC 722/2022 and IN 160/2022), the maximum tolerated limits for As, Cd, and Pb in tea, yerba mate, and other plants for infusion are 0.6, 0.4, and 0.6 mg kg⁻¹, respectively (ANVISA 2022a, b). These results were interpreted in the context of current international recommendations provided by the Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed (CXS 193–1995, as amended in 2025) (Codex Alimentarius Commission 2025).

Considering these limits, all analyzed samples presented arsenic concentrations within the established regulatory limits. In contrast, two samples showed Cd concentrations above the permitted value, while four exceeded the established limit for Pb. The elevated levels of Cd and Pb observed in some samples can be attributed to the well-known bioaccumulation capacity of *Camellia sinensis*, combined with environmental factors such as possible soil and water contamination in the cultivation regions. Indeed, the

literature indicates that this plant is particularly efficient in absorbing toxic elements, which highlights the importance of controlling the geographic origin of the raw material and the agricultural management practices employed (Zhuang et al. 2022).

To assess potential dietary exposure, the estimated daily intake (EDI) was calculated from the metal concentrations determined in the tea intake ($T_i = 11.4 \text{ g day}^{-1}$) (Krstić et al. 2021), using the recommended daily intake (RDI) values established by ANVISA (ANVISA 2005). The EDIs (Table S5, Supplementary Data) ranged from (mg day⁻¹): 0.03–0.19 (Cu), 0.01–2.71 (Fe), 0.21–33.5 (Mn), and 0.07–0.65 (Zn). According to ANVISA Resolution RDC No. 269/2005, the daily intake values for Fe, Cu, and Zn are within the regulated limits of 14 mg day⁻¹, 0.9 mg day⁻¹, and 7 mg day⁻¹, respectively (ANVISA 2005). However, for most samples, the estimated intake of Mn exceeded the recommended limit (2.3 mg day⁻¹). This may indicate that Mn is an element that accumulates significantly in *Camellia sinensis*, in agreement with its important role in plant physiological processes such as photosynthesis and growth (Li et al. 2017).

The consumption of infusions prepared from *Camellia sinensis* can therefore contribute to meeting nutritional requirements, since Cu, Fe, Mn, and Zn are essential elements for the human body, as they act in various physiological and enzymatic processes. However, in the case of these tea samples, they can become potentially toxic elements depending on the absorption of these analytes by the human body. Nevertheless, depending on their levels and bioavailability, these elements may also pose potential health risks (Bessone et al. 2022). For this reason, reliable sample preparation and analytical methods for

Table 3 Comparative overview of NADES-based methods reported for elemental analysis of plant matrices

Element	Sample	NADES (molar ratio)	Heating	Sample preparation method	Optimization strategy	Extraction time (min)	NADES volume (mL)	Detection technique	LOQ ^a (mg kg ⁻¹)	Recovery (%)	Green assessment	Reference
Mn	Vegetable	ChCl:Ta (1:1); ChCl:Ox (1:1); ChCl:Ca (1:1)	Heating		Univariate	120	NR	ICP-OES	1.13–4.10 (μg L ⁻¹)	82–114	NR	(Bağda et al. 2017)
As, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, and Zn	Plant	Ca:Xy:Wa (1:1:10); Ma:Xy:Wa (1:1:10); Ca:Ma:Wa (1:1:10)	UAE		NR	45	9.0	ICP-MS and ICP-OES	NR	70–120	NR	(Santana et al. 2019)
As, Cd, Hg, Pb, Se, and V	Plant	Ca:Xy:Wa (1:1:10); Ma:Xy:Wa (1:1:10); Ca:Ma:Wa (1:1:10)	UAE or MAE	Mixture design		45 (UAE); 18 (MAE)	9.0	ICP-MS	1.3 × 10 ⁻⁵ –1.04 × 10 ⁻² (UAE); 5.7 × 10 ⁻⁶ –7.98 × 10 ⁻³ (MAE)	80–120	Eco-Scale (MAE 96; UAE 97)	(Santana et al. 2020)
Cd, Cu, Fe, Mn, and Zn	Medicinal herbs	ChCl:Ox:Wa (1:1:1)	MAE	Doehlert design		35 s	3.0	ICP-OES	0.0229–1.10	87–109	AGREE (0.81)	(Costa et al. 2022)
Co, Cr, Cu, Ni, and Zn	Plant	ChCl:Ox (1:2)	UAE and stirred	Univariate		25	2.0	ICP-OES	1.2–8.2	91–112	NR	(Yang et al. 2022)
Ca, Fe, K, Mg, Mn, Na, P, S, and Zn	Plant	ChCl:Ma (1:1.5)	UAE and digester	Univariate		50	NR	ICP-OES	0.12–7.04	87–115	NR	(Zhao et al. 2023)
As	Medicinal herbs	β-Ala:Ca:Wa (1:2:17); β-Ala:Xy:Wa (1:2:17)	UA-MSPD or MAE	Doehlert design		60 (UA-MSPD); 40 (MAE)	NR (UA-MSPD); 5.0 (MAE)	ICP-MS	0.011–0.130	77–101	Eco-Scale (MAE 92; UA-MSPD 94); RGB model (MAE 84; UA-MSPD 85)	(Guimarães et al. 2023)
Cd, Mn, Pb, and Zn	Vegetable	L-Me:Fa (1:3)	S-NADES-DLLME	Univariate		7	1.25	FAAS	0.08–2.5 (ng mL ⁻¹)	94–96	AGREE (0.66); BAGI (67.5)	(Biggin et al. 2025)
Ca, Cd, Cu, Mg, Mn, Pb, and Zn	Plant	DL-Me:Pa (0.85:0.15)	DLLME	Univariate		NA	2.0	LIBS	NR	94–122	AGREEprep (0.66)	(Soares et al. 2025)

Table 3 (continued)

Element	Sample	NADES (molar ratio)	Sample preparation method	Optimization strategy	Extraction time (min)	NADES volume (mL)	Detection technique	LOQ ^a (mg kg ⁻¹)	Recovery (%)	Green assessment	Reference
As, Cd, Cu, Fe, Mn, Pb, and Zn	<i>Camellia sinensis</i> leaves	ChCl:Ox:Wa (1:1:1)	UAE	Factorial design, face-centered, composite design, and multivariate exploratory analysis (SOM)	40	3.0	ICP-OES and ICP-MS	0.019–9.70	73–107	Agree (0.68); AGREEprep (0.58)	This study

NA not applicable, NR not reported, LOQ limit of quantification, ChCl choline chloride, Ta tartaric acid, Ox oxalic acid, Wa water, β -Ala β -alanine, L-Me L-menthol, Fa formic acid, DL-Me DL-menthol, Pa palmitic acid, UAE ultrasound-assisted extraction, microwave-assisted extraction, UA-MSPD ultrasound-assisted matrix solid-phase dispersion, S-NADES-DLME simultaneous natural deep eutectic solvent-based dispersive liquid-liquid microextraction, DLLME dispersive liquid-liquid microextraction, ICP-OES inductively coupled plasma optical emission spectrometry, ICP-MS inductively coupled plasma mass spectrometry, FAAS flame atomic absorption spectrophotometry, LIBS laser induced breakdown spectroscopy, Eco-Scale Analytical Eco-Scale, AGREE Analytical Greenness Calculator, AGREEprep Analytical Greenness Metric for Sample Preparation, and BAGI Blue Applicability Grade Index

^aLOQ values are reported as originally published; unit conversions were performed only when methodologically justified

elemental determination are essential, and knowledge of the elemental composition of tea leaves represents a fundamental step for both nutritional assessment and toxicological risk management.

Finally, although these univariate comparisons reveal general trends associated with tea type and processing, the considerable variability observed among samples indicates that the elemental composition is controlled by multiple interacting factors. This complexity suggests that multivariate approaches are required to better understand the underlying structure of the dataset and the combined behavior of the elements.

Exploratory Data Analysis

The SOM analysis demonstrated a high capacity for classifying and interpreting multielemental patterns, enabling the visualization of complex relationships within the dataset that may not be readily captured by conventional linear multivariate approaches. Recent studies have highlighted that SOM performs better in identifying complex structures in chemical datasets, especially when multiple environmental and biological factors act simultaneously on the samples (Basu et al. 2025; Ghosh et al. 2025).

In this study, SOM allowed the identification of distinct patterns of elemental accumulation among the different types of tea leaves evaluated (Fig. 5). The map structure revealed four well-defined clusters, corresponding to black teas (Cluster A), white teas (Cluster B), green tea—group 1 (Cluster C), and green tea—group 2 (Cluster D). The spatial distribution of intensities in the component planes showed consistent chemical contrasts between the groups, confirming that the elemental composition of *Camellia sinensis* leaves is not governed by a single factor, but results from the interaction between botanical characteristics, environmental conditions, and post-harvest processing steps, as also observed in previous studies on commercial teas (Szymczycha-Madeja et al. 2012; Milani et al. 2016).

Cluster D, corresponding to the second group of green teas, showed the highest concentrations of As, Fe, and Pb. The literature indicates that these elements are strongly associated with edaphic characteristics and availability in the soil, being influenced by factors such as geological origin, agricultural practices, and fertilizer use (Szymczycha-Madeja et al. 2012). Large-scale studies also demonstrate that *Camellia sinensis* has a high capacity for absorbing chemical species from the soil, which explains the occurrence of distinct elemental profiles even within the same type of tea (Li et al. 2021). Thus, the behavior observed for Cluster D suggests greater sensitivity to environmental growing conditions.

Cluster B, formed by white teas, showed the highest concentrations of Cd and Mn. The enrichment in Mn is consistent

Table 4 Concentrations (mg kg⁻¹, mean ± standard deviation, *n*=3) of As, Cd, Cu, Fe, Mn, Pb, and Zn in *Camellia sinensis* leaves determined by ultrasound-assisted extraction using a natural deep eutectic solvent (UAE-NADES)

Tea sample	Sample ID	As	Cd	Cu	Fe	Mn	Pb	Zn
Black	S1	<LOQ	<LOQ	10.38 ± 0.22	91.5 ± 3.5	1220 ± 24	0.08 ± 0.01	17.2 ± 2.7
	S2	<LOQ	0.080 ± 0.002	6.67 ± 0.22	166 ± 13	1938 ± 110	0.115 ± 0.002	24.6 ± 1.2
	S3	<LOQ	<LOQ	11.37 ± 0.17	71.7 ± 1.9	74.9 ± 2.0	<LOQ	22.4 ± 1.0
	S4	<LOQ	<LOQ	11.04 ± 0.29	77.5 ± 4.2	73.8 ± 1.4	0.063 ± 0.001	19.9 ± 0.9
	S5	<LOQ	<LOQ	11.85 ± 0.10	80.6 ± 3.2	1435 ± 17	0.08 ± 0.01	20.3 ± 1.3
	S6	<LOQ	<LOQ	11.67 ± 0.72	69.6 ± 2.3	79.3 ± 3.9	<LOQ	21.5 ± 1.3
	S7	<LOQ	<LOQ	11.26 ± 0.08	60.8 ± 0.8	1006 ± 14	0.149 ± 0.002	21.4 ± 0.4
	S8	<LOQ	0.038 ± 0.001	11.18 ± 0.13	122 ± 21	1752 ± 16	0.28 ± 0.01	17.6 ± 1.2
	S9	<LOQ	<LOQ	11.38 ± 0.53	114.5 ± 4.8	1507 ± 26	<LOQ	21.3 ± 0.7
	S10	<LOQ	0.034 ± 0.001	14.57 ± 0.29	197 ± 33	612 ± 15	0.37 ± 0.09	23.5 ± 2.2
	S11	<LOQ	0.031 ± 0.002	18.03 ± 0.12	60.3 ± 1.3	114.1 ± 0.4	0.120 ± 0.005	30.3 ± 0.3
	S12	<LOQ	0.026 ± 0.001	11.81 ± 0.10	59.5 ± 1.0	1093 ± 12	0.180 ± 0.003	20.6 ± 0.1
	S13	<LOQ	0.042 ± 0.002	11.79 ± 0.37	167.4 ± 9.7	876 ± 28	0.13 ± 0.01	22.4 ± 2.3
Green	S1	0.102 ± 0.007	0.302 ± 0.008	11.98 ± 0.44	714 ± 94	2268 ± 116	0.91 ± 0.12	46.2 ± 1.5
	S2	0.081 ± 0.010	0.062 ± 0.008	8.41 ± 0.56	239 ± 36	1369 ± 114	0.69 ± 0.09	20.8 ± 1.5
	S3	0.050 ± 0.001	0.176 ± 0.007	7.43 ± 0.23	164.8 ± 1.2	898 ± 14	0.50 ± 0.02	32.3 ± 0.3
	S4	0.130 ± 0.005	0.068 ± 0.003	8.49 ± 0.36	259.0 ± 6.3	1347 ± 23	1.02 ± 0.06	21.5 ± 1.5
	S5	<LOQ	<LOQ	9.44 ± 0.77	83.3 ± 4.5	69.4 ± 4.8	<LOQ	20.3 ± 1.2
	S6	<LOQ	<LOQ	9.10 ± 0.37	88.2 ± 2.0	84.7 ± 0.9	<LOQ	20.4 ± 0.7
	S7	<LOQ	<LOQ	11.93 ± 0.28	142.9 ± 2.0	1283 ± 26	0.114 ± 0.002	25.9 ± 0.9
	S8	0.027 ± 0.001	0.306 ± 0.013	9.87 ± 0.21	233 ± 19	1762 ± 69	0.52 ± 0.04	62.6 ± 1.2
	S9	<LOQ	<LOQ	10.06 ± 0.67	100 ± 12	78.8 ± 6.0	<LOQ	22.6 ± 2.1
	S10	<LOQ	0.020 ± 0.001	11.48 ± 0.62	49.9 ± 2.4	56.1 ± 1.5	<LOQ	22.1 ± 0.7
	S11	<LOQ	0.194 ± 0.011	9.20 ± 0.16	113.2 ± 3.2	1656 ± 30	0.51 ± 0.05	27.3 ± 0.6
	S12	<LOQ	0.024 ± 0.001	13.21 ± 0.32	159.4 ± 4.0	1427 ± 18	0.27 ± 0.06	22.2 ± 0.3
	S13	0.075 ± 0.007	0.082 ± 0.002	9.16 ± 0.79	202 ± 17	1287 ± 40	0.90 ± 0.06	18.5 ± 0.4
White	S1	0.025 ± 0.007	0.033 ± 0.002	8.36 ± 0.39	510 ± 24	2229 ± 106	0.10 ± 0.02	18.4 ± 2.5
	S2	0.030 ± 0.008	0.489 ± 0.015	9.63 ± 0.33	123.4 ± 9.1	3157 ± 166	0.22 ± 0.01	55.0 ± 2.7
	S3	0.021 ± 0.006	0.523 ± 0.018	9.71 ± 0.24	108.9 ± 5.9	3204 ± 74	0.20 ± 0.02	54.7 ± 2.3
	S4	<LOQ	0.026 ± 0.001	12.17 ± 0.34	142.1 ± 6.7	1297 ± 49	0.17 ± 0.01	24.5 ± 0.8

Limit of quantification (LOQ, mg kg⁻¹): As (0.021), Cd (0.019), Cu (0.16), Fe (9.70), Mn (3.23), Pb (0.062), and Zn (2.12)

with the predominant use of very young buds and leaves in the production of these teas, since this element plays an essential role in physiological processes associated with photosynthesis and plant growth (Li et al. 2021). In the case of Cd, its accumulation has been largely related to availability in the soil and competitive absorption by root systems, reinforcing the influence of regional environmental factors on the chemical profile of white teas (Szymczycha-Madeja et al. 2012; Li et al. 2021).

Cluster A, corresponding to black teas, stood out mainly for its higher Cu content. Previous studies indicate that this element may be associated with both agricultural practices, such as the use of copper fungicides, and the processing of black tea itself, which involves enzymatic fermentation and chemical transformations of polyphenols, capable of

influencing the retention and extraction of chemical species (Szymczycha-Madeja et al. 2012; Milani et al. 2016). Thus, both agronomic aspects and the processing method itself can explain the greater accumulation of Cu in this group.

For Zn, a diffuse pattern was observed between Clusters B and D, indicating that its distribution is controlled by multiple factors, including availability in the soil and the plant's physiological demand. This behavior is consistent with reports in the literature, which indicate Zn as an essential micronutrient with high variability in teas of different origins and types (Milani et al. 2016; Li et al. 2021).

Finally, Cluster C, corresponding to group 1 of green teas, showed the lowest concentrations for all elements evaluated. This profile suggests cultivation carried out under more

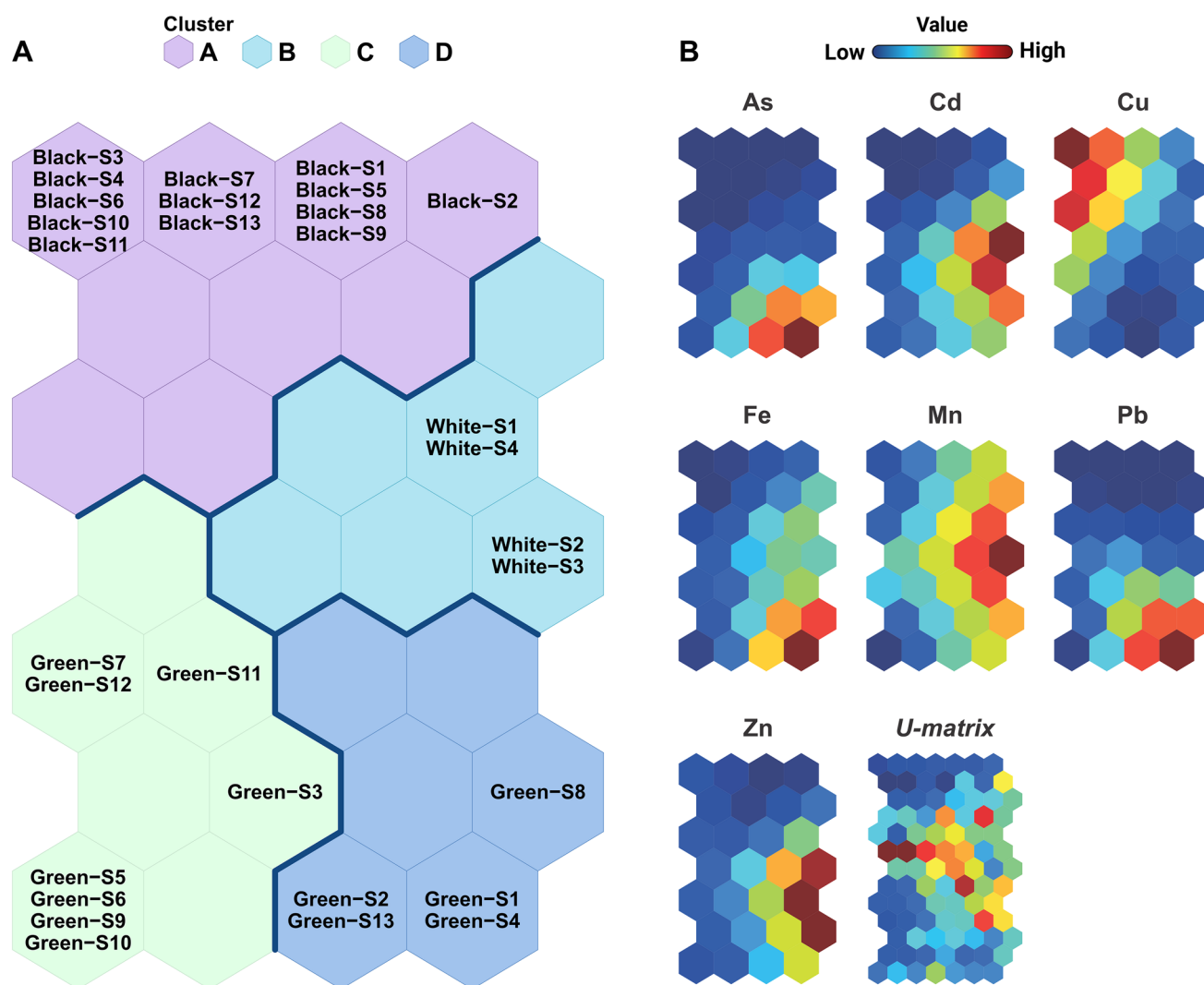


Fig. 5 Two-dimensional map generated by self-organizing maps (SOM) from the multielemental dataset of *Camellia sinensis* leaf samples (Panel A). The map shows the clustering of samples according to their elemental profiles, with clusters A–D highlighted. Sample labels indicate tea type (black, green, and white). Component planes

for As, Cd, Cu, Fe, Mn, Pb, and Zn illustrate the spatial distribution of elemental concentrations across the map, from low to high intensity. The unified distance matrix (*U*-matrix) highlights the distances between neighboring neurons and delineates the boundaries between clusters (Panel B)

controlled environmental conditions, less exposure to contaminants, or soils with lower mineral availability. These characteristics stand out in the SOM as a region of low intensity in practically all component planes.

The results confirm that the SOM is a robust tool for exploring multi-elemental patterns in tea samples. The approach enables the discrimination of tea samples into chemically distinct groups, highlighting the concurrent influence of environmental conditions, botanical characteristics, and processing-related factors on their elemental composition. The patterns observed in this study reinforce the relevance of elemental characterization combined with multivariate methods for the chemical characterization of complex plant-derived matrices.

Conclusions

The UAE-NADES proved to be a promising alternative to MW-AD in sample preparation for the elemental determination in tea samples by ICP-OES and ICP-MS. The application of univariate and multivariate optimization steps (screening and modeling) was fundamental to achieving ideal experimental conditions.

The combination of NADES and UAE represents a green approach to determining chemical species, contributing to the advancement of more sustainable sample preparation methodologies. This viable alternative to conventional methods showed a simple, effective, and safe application, aligning with the principles of Green Analytical Chemistry.

The determination of essential (Cu, Fe, Mn, and Zn) and non-essential (As, Cd, and Pb) elements in *Camellia sinensis* leaves is particularly relevant considering the widespread consumption of this plant in the form of infusions. In this context, the application of SOM complemented the univariate evaluation by enabling the identification of multielement patterns and chemically distinct groups of tea samples. Overall, the results demonstrate that the proposed UAE-NADES method, combined with multivariate data analysis, provides a reliable and informative approach for the elemental characterization of tea leaves, supporting both nutritional assessment and toxicological evaluation.

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Author Contribution **H.B.F.**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft; **L.S.M.**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing; **F.S.C.**: Data curation, Formal analysis, Software, Visualization, Writing – review & editing; **E.K.B.G.**: Data curation, Investigation, Validation; **G.P.B.**: Funding acquisition, Resources; **D.C.E.**: Conceptualization, Funding acquisition, Methodology, Resources; **M.T.W.D.C.**: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Data Availability Data will be made available on request.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing interests The authors declare no competing interests.

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