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# Application of Hot Water Extraction Techniques and the Principal Component Analysis to Study the Influence of Cultivation of Commercial Yerba Mate Samples on Their Mineral Profiles

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## Abstract

Consumption of organic food has grown much around the world in the last 20 years. Change in the profile of consumers who have increasingly sought a healthy diet is the major contributor to this phenomenon. In scientific literature, some studies have already shown the nutritional superiority of organic food in the individual evaluation of metabolites. However, few studies have assessed interaction among metabolites, especially the one between minerals and the food matrix. This information may have great relevance in determining the extractability of minerals, especially in food consumed through infusion, such as yerba mate, since this interaction can directly influence their solubility. Thus, this study aimed to use hot water extraction techniques (infusion and decoction) and the principal component analysis (PCA) to evaluate the differences between organic and conventional cultivation systems in absorption and availability of Ca, Mg, Fe, Mn, and Zn in yerba mate leaves and stems. The PCA showed that cultivation influences total mineral contents found in both leaves and stems. Results of extractability only showed differences in conventional leaf samples, from which all minerals under study were better extracted. Results point out a different interaction between minerals and the matrix, depending on the cultivation, and to the stronger interaction between the matrix and minerals in organic samples, a fact which leads to low availability of minerals for consumption.

**Keywords** Yerba mate · Organic food · Mineral nutrients · Decoction · Infusion

## Introduction

Organic products, which derive from the organic production system, are produced and processed in agreement with international standards of organic agriculture and are usually certified by an independent certificate authority. Essential requirements of organic production are the non-use of organisms and products generated by genetic engineering and replacement of synthetic chemical products, such as pesticides and fertilizers, used for maintaining agricultural production, with sustainable agricultural practices based on ecological principles, whenever possible (Yu et al. 2018).

The IFOAM (International Federation of the Organic Agriculture Movement), an organization that was founded in the 1970s, is in charge of harmonizing standard concepts of organic agriculture and the basis for the regulation of various certifying entities of organic products. There are several organic regulations in the world, both governmental and private ones. In Brazil, the legislation is made up of many laws which permeate several governments and were updated in 2021. Products that comply with the legislation receive an organic product identification seal from the Brazilian Ministry of Agriculture, Livestock and Supply (Muñoz et al. 2016).

Worldwide retail sales of organic products totaled €15 billion in 2000. It reached €106.4 billion in 2019, which means increase of about 600% in the period. The USA was responsible for the movement of €44.7 billion, which represents more than 40% of the global organic market. However, the coronavirus pandemic has caused a profound impact on the organic food industry due to numerous factors, such as changes in consumers' eating habits in pursuit of personal health and wellness. Thereby, a rapid increase in the organic market is expected all over the world, mainly in developing countries, such as Brazil, as the result of the development of

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regional markets, which was also accelerated by the coronavirus crisis (Willer et al. 2021).

Changes in eating habits related to concern for health are usually associated with consumers' perception of the nutritional superiority of a certain type of food. Regarding organic food, this perception can be linked mainly to the following issues: (1) more beneficial nutrition components in organic food, such as vitamins, polyunsaturated fatty acids, and antioxidants and (2) lower levels of heavy metals, fungal toxins and pesticide residues in organic food (Niggli, 2009). It was corroborated by Yu et al. (2018) in a recent review article. According to the authors, in general, organic food have several beneficial properties, such as antioxidant and anti-cancer activities, related to compositional characteristics, such as the profile of phenolic compounds. In addition, they exhibit high levels of high-quality proteins and polyunsaturated fatty acids in bovine meat, besides low levels of nitrate and heavy metals in fruit and vegetables and no pesticide residues (Yu et al. 2018).

Although there are some studies in the literature that compare different aspects of nutritional profiles of organic and conventional food (Vigar et al. 2020), few have addressed the influence that their management exerts on the correlation among components of the food matrix, mainly minerals and metabolites. This type of study becomes significant when food under study is consumed through aqueous extracts, such as coffee, tea, and yerba mate since the interaction between minerals and the matrix may influence their solubility and, therefore, their availability in the diet. It is known that a balanced diet needs to supply all essential minerals required by the body. Minerals play key roles in the human body, from building strong bones (Ca, Mg, Mn, and others) to constituting enzymes (Cu, Fe, Mn, Mg, and others) and functioning in nerve cells (K and others) (Gharibzahedi & Jafari 2017).

Yerba mate (*Ilex paraguariensis St. Hil*) is a forest tree native to South America which is consumed as a tea-like beverage in many Latin American countries, such as Argentina, Paraguay, Brazil, and Uruguay. The main consumers are Brazil and Uruguay (Bastos et al. 2018; Pardinho et al. 2018; Rocha et al. 2017). Consumption of yerba mate products has recently expanded to different countries, such as Australia, France, Germany, Italy, Japan, Korea, Russia, Spain, Syria, and the USA (Cardozo Junior, Morand 2016). Regarding its cultivation, yerba mate is a culture that has its leaves and stems removed regularly, which implies massive export of nutrients and requires nutritional replacement to maintain productivity (Gaiad, Lopes 1986). Nevertheless, organic brands have been easily found in markets nowadays.

The mineral content of yerba mate has been extensively studied by the scientific literature over the years with different approaches. Not only total concentration and hot water extraction (Pandolfo et al. 2003; Pereira et al. 2016) and in vitro bioaccessibility of micronutrients and inorganic contaminants (Schmite et al. 2019) but also the influence of agronomic variables on the mineral profile have been reported (Barbosa et al. 2015, 2018; Reissmann et al. 1999). However, although the influence of agronomic variables has already been considered, the impact of the set of standards regulated by organic legislations on the mineral profile and on its extractability has not yet been evaluated.

Hot water extraction techniques have been applied to study the extraction of components of various herbs consumed through solution. Infusion and decoction are methods that have often been cited by this type of study (Grzesik et al. 2008; Ozcan et al. 2008; Kaneria et al. 2011). There are some studies that compare the efficiency of both techniques; regarding minerals, decoction usually exhibits better extraction efficiency (Pereira Junior et al. 2016; Randjelovic et al. 2013). The difference in the contact between the extractor and the sample may be a determining factor in results since the more intense contact that occurs in decoction could lead to extraction of compounds that are more strongly bound to the matrix while, in infusion, only minerals of lesser interaction would be extracted (Dias et al. 2015). Therefore, these techniques could be useful tools to understand the interaction between minerals and the food matrix, particularly with the aid of chemometric tools, such as the principal component analysis (PCA).

Thus, this study aims at investigating the influence of techniques standardized by the Brazilian legislation of organics on the mineral profile of commercial samples of yerba mate and at evaluating the use of different hot water extraction techniques combined with the PCA to understand this influence on macro and micronutrient interaction with the matrix and extractability.

## Material and Methods

### Samples

Samples of green yerba mate were randomly purchased in a market in the Rio Grande, Rio Grande do Sul (RS) state, Brazil, and on sales websites, totaling 12 samples (7 of conventional origin and 5 of organic origin). All samples were from southern Brazil and were within the validity period specified by the supplier. They were composed of leaves and stems in the ratio of 70:30, respectively, except for one made for export that was only composed of leaves. All organic samples were certified by the Brazilian Ministry of Agriculture, Livestock, and Supply.

### Instrumentation

Determinations of Fe, Mg, Mn, and Zn were performed by flame atomic absorption spectrometry (FAAS). Calcium (Ca) and K were determined by flame atomic emission spectrometry (FAES). All determinations were performed by a Perkin Elmer Analyst-800 atomic spectrometer equipped with a 10-cm air-acetylene burner head and a single element hallow-cathode lamp was used for all measurements. Selected spectral lines (nm) of all metals were as follows: Ca 422.7, Fe 248.3, K 766.5, Mg 285.2, Mn 279.5, and Zn 213.9. Deuterium lamp background correction was employed to correct for the non-specific absorbance. Analytical parameters of determinations were limit of detection and limit of quantification (Ca = 0.34 mg $L^{-1}$ and 1.00 mg $L^{-1}$; Fe = 0.23 mg $L^{-1}$ and 0.70 mg $L^{-1}$; K = 0.03 mg $L^{-1}$ and 0.09 mg $L^{-1}$; Mg = 0.02 mg $L^{-1}$ and 0.05 mg $L^{-1}$; Mn = 0.04 mg $L^{-1}$ and 0.12 mg $L^{-1}$; Zn = 0.06 mg $L^{-1}$ and 0.19 mg $L^{-1}$); linear range (Ca = 1.00–3.00 mg $L^{-1}$; Fe = 0.75–6.00 mg $L^{-1}$; K = 0.10 – 1.50 mg $L^{-1}$; Mg = 0.06–0.30 mg $L^{-1}$; Mn = 0.15–1.50 mg $L^{-1}$; Zn = 0.20–0.75 mg $L^{-1}$).

## Reagents and Analytical Solutions

All glassware and polypropylene flasks were washed with soap, soaked in 10% v $v^{-1}$ $HNO_3$ for 24 h, and rinsed with deionized water prior to use. Analytical grade reagents and milli-Q water (Millipore, Bedford, MA, USA) were employed. All stock standard solutions used to prepare element standard solutions were certified with AAS grade (SpecSol, Brazil). Working standard solutions were prepared daily by diluting appropriate aliquots of the stock solution in Milli-Q water. Nitric acid (65% m $v^{-1}$) was purchased from Vetec (Brazil). Perchloric acid (37%) was purchased from Dinamica (Brazil).

Stems and leaves were manually separated, portions were weighed and the finest part was sifted through a sieve whose mesh size was 0.2 mm. Stems were manually collected with the use of plastic tweezers, ground by a blender with stainless steel blades, and sifted through a sieve whose mesh size was 0.2 mm. After separation, both leaves and stems in each sample were ground, sifted through a 32-mesh sieve, oven-dried with forced air circulation at 40 °C until constant weight, stored individually, and identified in polyethylene bottles for later analyses.

## Sample Preparation

### Total Mineral Determination

The sample decomposition method was performed in agreement with recommendations issued by the Brazilian Agricultural Research Corporation (EMBRAPA). It consisted of two steps in a heating program by a block digester. The mixture consisted of a 200-mg sample (leaf or stem), 2.5 mL $HNO_3$:$HClO_4$ (2:1 ($vv^{-1}$)), and 1.0 mL ultrapure water. After the 4-h pre-decomposition step at room temperature, the mixture was slowly heated until 120 °C, which was kept until $NO_2$ release (brown vapor) ceased completely. Then, the temperature was slowly raised to 210 °C and kept for 25 min. After the solution cooled down, it was diluted to the final volume of 50.0 mL with ultrapure water (Nogueira et al. 2005).

### Hot Water Extraction

**Infusion** Fifty milliliters of ultrapure water heated to 100 °C was poured over a 1-g sample, homogenized for 1 min, and kept in contact for 15 min. During the extraction time, the temperature of the solution gradually returned to room temperature. Samples were centrifuged at 17,920 × G for 3 min and the supernatant was filtered (average pore size of 8 um). Finally, extracts were diluted with ultrapure water to 100 mL, stored in polyethylene bottles, and kept under refrigeration for further analysis in FAAS and FAES.

**Decoction** 50 ml ultrapure water heated to 100 °C was poured over 1 g sample in a glass beaker and placed on a heating plate. The mixture was boiled for 15 min, and then, the sample was centrifuged at 17,920 × *g* for 3 min. Volume variations due to evaporation were not considered. The supernatant was filtered (average pore size of 8 um), diluted with ultrapure water to 100 mL, stored in polyethylene bottles, and kept under refrigeration for further analysis in FAAS and FAES.

Aqueous extracts resulting from infusion and decoction were all directly injected into the equipment nebulization system without any treatment. All measurements were performed in triplicate.

## Exploratory Analyses

Data matrices composed of rows with yerba mate leaf and/or stem samples and analyte concentrations in columns were organized and the PCA was carried out by the Scilab 6.0.1 software (GNU General Public License 2.0), in agreement with Ferreira et al. (1999). Data were auto-scaled due to different orders of magnitude among variables.

The results of total content and extractability were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test ($p < 0.05$) with the aid of statistical software 5.0.

## Accuracy

To evaluate the accuracy of the digestion procedure, peach leaves certified reference material (NIST 1547) was analyzed. Moreover, to evaluate the matrix effect on all determinations, addiction and recovery tests were performed. Final concentrations added to each element in addiction and recovery tests were as follows: Ca (1.50 mg $L^{-1}$ for digestion and 0.25 mg $L^{-1}$ for extraction), Fe (1.00 mg $L^{-1}$ for digestion and extraction), K (0.25 mg $L^{-1}$ for digestion and 0.50 mg $L^{-1}$ for extraction), Mg (0.10 mg $L^{-1}$ for digestion and extraction), Mn (1.00 mg $L^{-1}$ for digestion and extraction), and Zn (0.25 mg $L^{-1}$ for digestion and extraction).

## Results and Discussion

### Accuracy

The accuracy of determinations of Fe, Mn, Mg, Zn, Ca, and K was estimated for their total values. Agreement for determination of total contents of Fe, Mn, Mg, Zn, Ca, and K in the acid digestion solution of the certified reference material (NIST 1547, peach leaves) ranged from 85.2 to 105.9%. Addiction and recovery tests were also performed. Results ranged from 94.7 to 119.0% in leaves and from 82.0 to 116.5% in stems.

Concerning aqueous extracts, addiction, and recovery tests were carried out for both infusion and decoction. Results of infusion of leaves and stems ranged from 80.1 to 114.8% and from 81.8 to 102.2%, respectively. Results of decoction of leaves and stems ranged from 80.1 to 118.7% and from 84.1 to 112.2%, respectively.

### Principal Component Analysis (PCA)

To evaluate the mineral profile of yerba mate (leaves and stems), FAAS and FAES analysis of sample solutions provided by acid decomposition (Table 1), infusion and decoction extraction were performed. To avoid that certain factors, such as variation in concentrations of minerals in the soil, could affect results, the ones of mineral extractability were expressed as relative values of total concentrations (percentages of extractability) (Table 2).

PCA and ANOVA were applied to the results. Comparison between trials shows general agreement. However, some observations made in the PCA are not clear in the ANOVA test, mainly due to the high dispersion of groups around the mean value, as can be observed, for example, by Mn in total contents of leaves. The PCA showed that the analyte characterizes organic samples; however, despite visualizing this behavior in the average of organic and conventional groups, no statistical difference could be found between them. This result is not surprising since PCA reduces the dimensionality of the dataset while retaining most of its variation, allowing to group samples and visualize characteristics that sometimes do not emerge univariately (Ringnér, 2016). Thus, the PCA is a very interesting tool for this exploratory study.

### Total Concentration

First, the PCA was carried out to visualize all resulting data on the total concentration of minerals. Results are shown in Fig. 1a scores and 1b loadings. The PCA demonstrated that a small number of variables dominate total data variability since the two first principal components account for 80% of the total variability. The first component was responsible for 62% while the second accounted for 18% of total information. Considering the parts of plants in PC1 and PC2 plots, two visual clusters appeared. All data on leaf samples were located at negative PC1 scores (squares), well separated from stem samples, which had positive PC1 scores (circles). The profile of PC1 loadings exhibits negative values in all descriptors, thus characterizing leaf samples. It is well known that mineral elements are extracted from the soil by roots, cross it via apoplastic (extracellular) and/or symplastic (intracellular) pathways until the xylem; so, they are delivered to transpiring leaf tissues, where they are stored or remobilized by phloem, depending on their mobility characteristics (White & Brown 2010). With respect to cultivation, a well-defined separation between conventional (black

**Table 1** Ca, Fe, K, Mn, and Zn average total contents (mg $kg.^{-1}$) in organic and conventional samples of yerba mate leaves and stems

| Element | Conventional | | Organic | |
|---|---|---|---|---|
| | Leaf | Branch | Leaf | Branch |
| Fe | $279^{c}\pm54$ | $101^{a}\pm45$ | $337^{d}\pm85$ | $151^{b}\pm40$ |
| Zn | $64^{a}\pm18$ | $54^{a}\pm15$ | $54^{a}\pm15$ | $52^{a}\pm17$ |
| Mn | $2118^{c}\pm322$ | $871^{a}\pm196$ | $2281^{c}\pm753$ | $1295^{b}\pm190$ |
| K | $20{,}096^{b}\pm2391$ | $14{,}734^{a}\pm1553$ | $16{,}224^{a}\pm238$ | $13{,}701^{a}\pm488$ |
| Mg | $5877^{b}\pm937$ | $2862^{a}\pm1059$ | $5496^{b}\pm627$ | $3655^{a}\pm775$ |
| Ca | $6825^{b}\pm600$ | $6925^{b}\pm635$ | $5545^{a}\pm1217$ | $4839^{a}\pm912$ |

[*] Values represent mean concentration for each group of samples ± standard deviation

[**] Equal letters in the same line symbolize similarity at the 95% significance level while different letters symbolize difference at the 95% significance level

**Table 2** Ca, Fe, K, Mn, and Zn average aqueous extractability (%) in organic and conventional samples of yerba mate leaves and stems extracted by infusion and decoction

| Element | Extraction | Conventional | | Organic | |
|---|---|---|---|---|---|
| | | Leaf | Stem | Leaf | Stem |
| Fe | Decoction | $1.4^{a,A} \pm 0.7$ | $3.0^{a,A} \pm 1.4$ | $0.8^{a,A} \pm 0.3$ | $1.2^{a,A} \pm 0.3$ |
| | Infusion | $2.3^{a,B} \pm 0.6$ | $3.8^{b,A} \pm 1.3$ | $0.9^{a,A} \pm 0.3$ | $1.3^{a,A} \pm 0.3$ |
| Zn | Decoction | $40.0^{b,A} \pm 11.2$ | $25.2^{a,A} \pm 6.7$ | $21.3^{a,A} \pm 6.0$ | $19.0^{a,A} \pm 5.8$ |
| | Infusion | $13.5^{a,B} \pm 2.4$ | $9.6^{a,B} \pm 3.0$ | $14.0^{a,B} \pm 5.0$ | $9.0^{a,B} \pm 3.0$ |
| Mn | Decoction | $58.3^{b,A} \pm 5.4$ | $51.4^{b,A} \pm 8.8$ | $31.2^{a,A} \pm 5.0$ | $48.2^{b,A} \pm 4.7$ |
| | Infusion | $56.0^{a,A} \pm 4.8$ | $55.0^{a,A} \pm 8.8$ | $45.9^{a,B} \pm 9.2$ | $57.1^{a,A} \pm 10.8$ |
| K | Decoction | $78.3^{b,A} \pm 14.7$ | $76.6^{b,A} \pm 13.0$ | $87.7^{b,A} \pm 11.0$ | $58.7^{a,A} \pm 10.1$ |
| | Infusion | $80.5^{a,A} \pm 10.2$ | $78.1^{a,A} \pm 8.8$ | $69.2^{a,B} \pm 7.4$ | $71.5^{a,A} \pm 10.8$ |
| Mg | Decoction | $25.2^{a,A} \pm 19.4$ | $17.5^{a,A} \pm 7.5$ | $24.8^{a,A} \pm 3.4$ | $20.2^{a,A} \pm 1.7$ |
| | Infusion | $19.4^{a,A} \pm 3.9$ | $18.1^{a,A} \pm 7.6$ | $21.2^{a,A} \pm 5.0$ | $19.5^{a,A} \pm 2.6$ |
| Ca | Decoction | $37.5^{b,A} \pm 6.6$ | $15.1^{a,A} \pm 3.4$ | $14.2^{a,A} \pm 2.2$ | $12.3^{a,A} \pm 1.3$ |
| | Infusion | $25.6^{c,B} \pm 3.4$ | $21.7^{b,A} \pm 3.0$ | $14.5^{a,A} \pm 0.4$ | $14.9^{a,A} \pm 2.0$ |

[*] Values represent mean extractability for each group of samples ± standard deviation

[**] Equal lowercase letters in the same line, for each element, symbolize similarity at the 95% significance level while different lowercase letters symbolize difference at the 95% significance level

[***] Equal uppercase letters in the same column, for each element, symbolize similarity at the 95% significance level while different uppercase letters symbolize difference at the 95% significance level

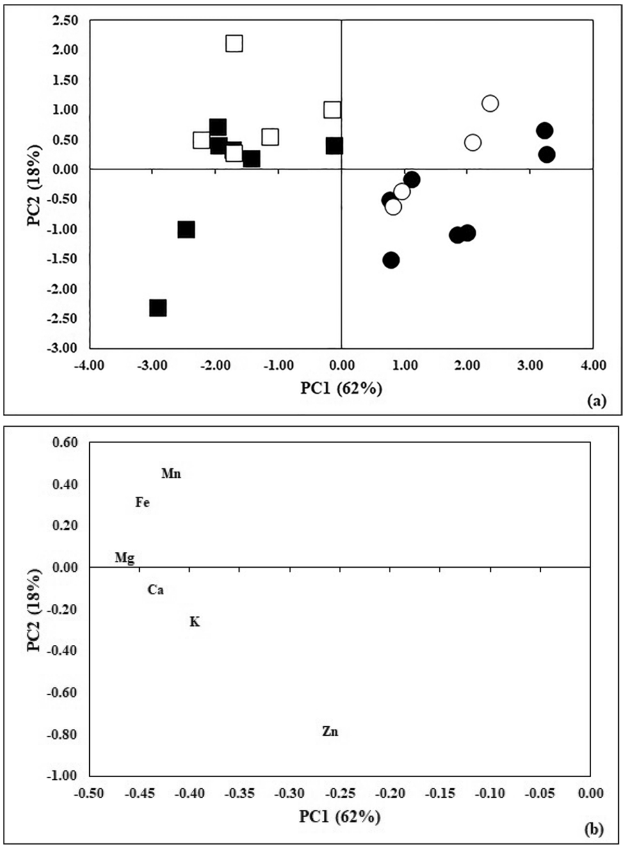

**Fig. 1** PC1 and PC2 **a** scores and **b** loading plots of all yerba mate samples, showing a comparison of part of the plant and cultivation. Stems (squares), leaves (circles), conventional cultivation (black circles and squares), organic cultivation (white circle and squares)

circles and squares) and organic (white circles and squares) ones could not be observed.

The first PCA clearly shows that leaves and stems have different characteristics regarding the mineral profile. Therefore, to better understand the results, a second study was carried out to analyze leaves and stems separately. Firstly, data on leaf samples (squares) were used to perform a new classification by the PCA. The two first components accounted for 61% of total data variability. The first and second components were responsible for 34% and 27% of total information, respectively. Figure 2a and b show score and loading scatter plots of PC1 and PC2.

The analysis of cultivation classes suggested the existence of two groups based on the distribution of samples along PC1. Conventional samples (black squares) had negative PC1 scores while organic ones (white squares) had positive PC1 scores. The profile of PC1 loadings exhibits negative values for Ca, K, Mg, and Zn (conventional sample descriptors) and positive values for Mn and Fe (organic sample descriptors).

When stem samples (circles) were analyzed, the two first components accounted for 76% of total data variability. The first and second components were responsible for 44% and 32% of total information, respectively. Again, two groups were observed along PC1. Conventional samples (black circles) had negative PC1 scores and organic samples (white circles) were located around the positive quadrant of PC1 and the negative quadrant of PC2. The loading profile shows negative values of PC1 for K (conventional sample descriptors), positive value of PC1 and negative of PC2 for Mn and

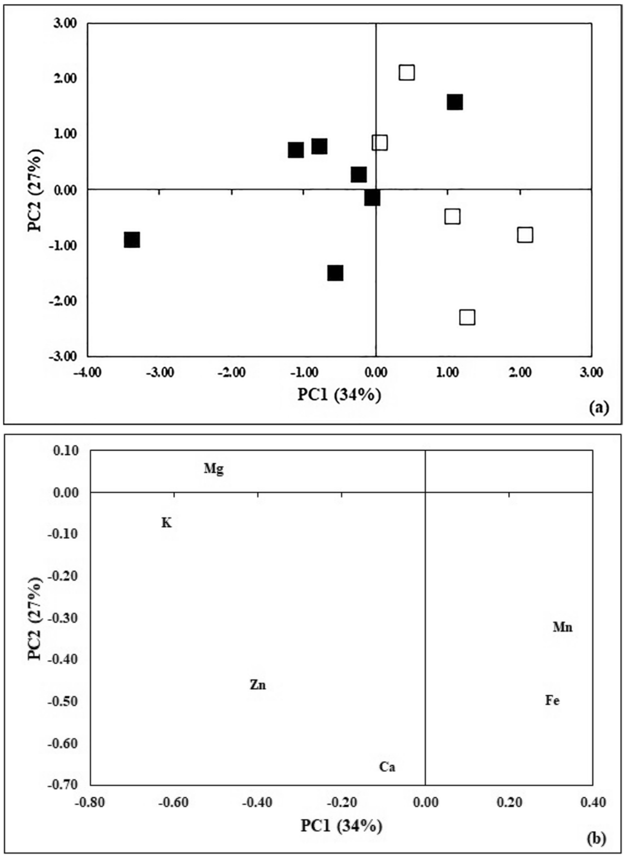

**Fig. 2** PC1 and PC2 **a** scores and **b** loading plots of yerba mate leaf samples, showing a comparison of cultivation. Conventional cultivation (black squares), organic cultivation (white squares)

Fe (organic sample descriptors), and positive values of PC1 and PC2 for Ca, Mg, and Zn (both group descriptors).

The first observation that should be highlighted is the high concentration of Fe and Mn in both stems and leaves in organic samples. Contributors could be related to some practices, such as soil management and organic fertilization in organic cultivation, that lead to an increment in organic acid concentration in the soil. Organic compounds, through the chelation process, increase Fe and Mn solubility and, consequently, their availability for absorption by plants (Hue, Vega & Silva 2001, Worthington 2001). Moreover, high organic matter concentration could lead to a reducing environment which also increases the availability of these elements (Hue et al. 2001, Pandey 2015).

Potassium had the opposite behavior of Mn and Fe since higher concentration was found in leaves and stems in conventional samples. The most likely explanation for this observation is related to the use of potassium-based fertilizer, commonly employed by the conventional cultivation system, which leads to the high availability of this element for plant absorption (Worthington 2001). Besides, the great mobility of K in the plant system justified its similar behavior in leaf and stem samples.

High concentrations of Ca, Mg, and Zn were found in conventional leaf samples, but no difference could be found between cultivation and stem samples. Ca is a highly immobile mineral in plants, i.e., once deposited in leaves, it becomes virtually unavailable for remobilization. As a result, plants need a continuous supply of these elements for new organs (Loneragan et al. 1976). Wherefore, the results of this study suggest that Ca found in stems does not come from relocation but from continuous uptake from the soil, which has not been influenced by any cultivation process. On the other hand, conventional leaves exhibited higher concentrations of Ca than organic ones. An explanation could be related to the high concentration of organic matter in the soil in organic cultivation, including previously discussed organic acids, that could increase soil-Ca affinity, consequently making it less available to plant absorption and enabling a smaller amount of this nutrient to reach leaves.

Magnesium tends to accumulate in transpiring organs, such as leaves (Merhaut 2007). A significant part is bounded to chlorophyll or insoluble species, such as malate and citrate; however, the largest fraction is in the inorganic form, highly mobile, that is redistributed to other parts of the plant. Therefore, results show that, since there is no magnesium deficiency in the cultivation system and its quantity for remobilization is enough to supply the stem demand, the cultivation system did not exert much influence. On the contrary, the results of leaves show high content of this element in conventional samples. Magnesium absorption is largely influenced by the competitive effect of other minerals. The main interfering elements are $K^+ > NH_4^+ > Ca^{2+} > Na^+$; however, Mn, in divalent form, when at elevated concentrations, can also compete with Mg for retention sites in soil or plant membranes (Merhaut 2007). Quartin et al. (2001) corroborate this information since they reported that an increase in manganese (Mn) accumulation in triticale leaves led to a significant decrease in Mg contents. Thus, the results of this study show that a possible determinant factor of high Mg contents in conventional leaves is the low Mn content absorbed by conventional samples, a fact that may be confirmed by the results of both stems and leaves described in Table 1.

In soils richly supplied with Zn, this element is highly mobile and broadly remobilized to the meristematic region of the plant (Loneragan et al. 1976). Therefore, similar to Mg, demand for Zn in stem samples seems to be supplied by its remobilization, without the great influence of the cultivation process. However, differences found in analyses of leaf samples show higher content of Zn in conventional leaves, probably due to its high affinity for sites of organic matter, which is found in high quantity in organic soils.

Results discussed so far concern levels of minerals absorbed by plants. However, they neither enable to infer how minerals are linked to leaves and stems nor provide information about their extractability and availability for ingestion (Fig. 3).

### Mineral Extractability

As clearly shown by the analysis of total contents, the behavior of leaves and stems regarding the accumulation of nutrients is different. Therefore, the PCA of extractability results was done separately for leaves and stems. However, score plots were classified both by cultivation (organic and conventional) and by the extraction method (infusion and decoction) simultaneously.

In the analysis of leaf samples (squares), the first three components accounted for 85% of total data variability. The first component was responsible for 41%, the second, for 25%, and the third, for 19% of total information. Since the second and third components exhibit similar data variability, the three components were evaluated by the study. Although both PC1 and PC2 and PC1 and PC3 score plots show good separation related to the variable cultivation, the PC1 and PC3 score plots exhibit better separation regarding extraction methods. Thus, it was shown by this study. Figure 4a and b shows score and loading scatter plots of PC1 and PC3.

The analysis of cultivation classes suggested the existence of two groups based on the distribution of samples by cultivation along PC1. In addition, separation by extraction methods along PC3 may be observed. The infusion group is represented by white (organic samples) and black (conventional samples) squares while the decoction group is represented by light gray (organic samples) and dark gray (conventional samples) squares. Figure 4b shows that all descriptors of loading plots are in the negative quadrant of PC1; therefore, they characterize conventional cultivation samples. Besides, Fe and K were found to be better extracted by the infusion method while Ca, Mg, Mn, and Zn were better extracted by the decoction method. Results suggest that all minerals under study are more strongly bound to the matrix in organic samples; therefore, less extracted by the applied methods.

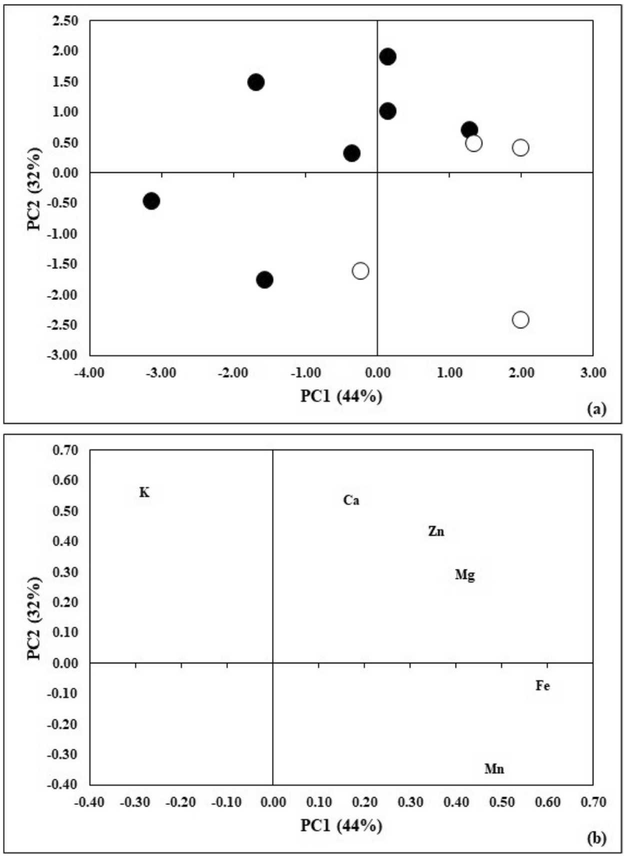

**Fig. 3** PC1 and PC2 **a** scores and **b** loading plots of yerba mate stem samples, showing a comparison of cultivation. Conventional cultivation (black circles), organic cultivation (white circles)

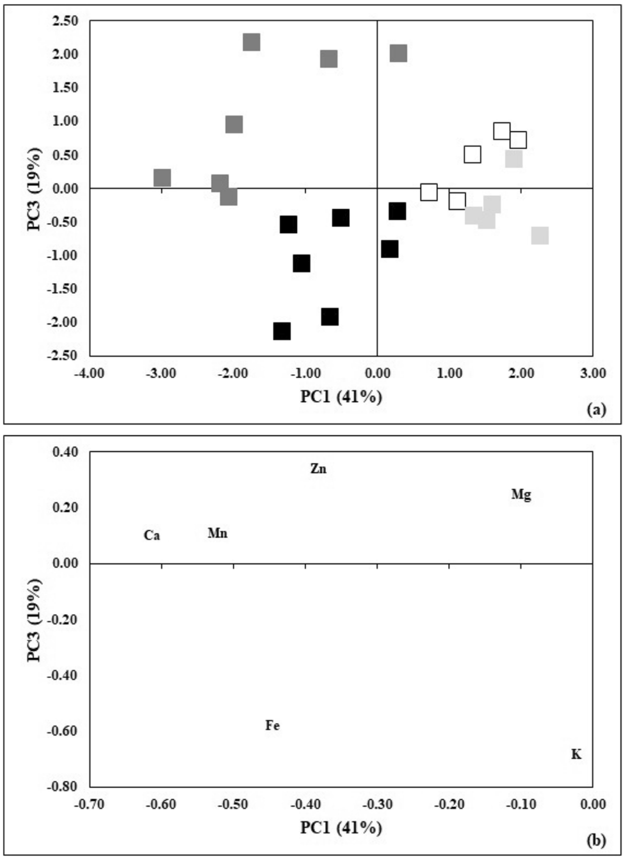

**Fig. 4** PC1 and PC3 **a** scores and **b** loading plots of yerba mate leaf samples, showing a comparison of cultivation. Conventional sample infusion (black squares), organic sample infusion (white squares), conventional sample decoction (dark gray squares), organic sample decoction (light gray squares)

Moreover, since decoction is a more aggressive extraction method, it seems that, in conventional samples, extractable fractions of Ca, Mg, Mn, and Zn are more strongly bound to the matrix than Fe and K.

Firstly, it should be highlighted that, although Mn and Fe are found at higher concentrations in organic samples, they are more easily extracted from conventional samples. Fe is the least extracted element by the applied methods, as shown in Table 2. However, the behavior observed in the PCA, i.e., that Fe is more effectively extracted by the infusion method, may indicate that its extractable fraction is stored in soluble species and that exposure to high temperature in the decoction method seems to decrease extractability. An explanation for this observation is that storage of soluble iron as inorganic species and more abrupt conditions to which samples are subjected in decoction can make these species react with insoluble matrix concomitants. Phenolic compounds are a viable hypothesis, firstly because they have an affinity for metal complexation (Hynes, O'Coinceanainn 2004). Secondly, classes of insoluble phenolic compounds (conjugated and bounded) could justify a reduction in Fe extractability (Hynes, O'Coinceanainn 2004). Finally, organic samples usually have high quantity of phenolic compounds, as means of defense, because organic cultivation does not use pesticides. All these facts may be the reasons for low Fe extraction from organic samples, indicating that, in organic samples, high phenolic content may be an important factor in the low solubility of Fe. Another possibility is reduction in solubility due to oxidation of inorganic Fe in the decoction process. Potassium, predominantly found as an inorganic soluble species, may have a similar explanation to Fe but, in decoction, it may have been precipitated by adsorption by other compounds.

Results of Ca, Mg, and Mn may be related to biomineralization processes performed by plants. The formation of oxalate crystals of Ca, Mg, and Mn in leaf tissues has already been reported by the literature. He et al. (2012), for example, reported the formation of oxalate crystals of Ca, Mg, Sr, and Ba in Acacia species. Pongrac et al. (2020) pointed out the formation of oxalate crystals of Ca and Mn in sample of tea leaves (*Camellia sinensis* (L.) Kuntze). In yerba mate leaves, crystals have also been reported. Barbosa et al. (2019) stated that the formation of calcium oxalate crystals is the most likely explanation for crystals found in yerba mate leaves, even though they highlighted the need for further experiments to confirm it (Barbosa et al. 2019). The biomineralization process may have different functions for each element investigated by this study. In the case of Mn, it would probably be related to detoxification due to its high absorption, mainly in organic samples. For Ca and Mg, it could be a set of factors, such as mechanical support and protection against herbivores; the latter is very relevant to this study since it may explain the low extractability of these elements in organic samples. Finally, the low solubility of oxalate salts may be the reason for the great extractability of these elements by the decoction process.

There are several ways in which plants accumulate zinc. Some examples are complexes coordinated with organic acids, such as oxalic, citric and malic ones, zinc crystals with high sulfur or phosphorus contents and glutathione, phytochelatins, or metallothioneins-linked zinc compounds (Gupta et al. 2016; Dinh et al. 2015). Although the influence of cultivation may be observed in the assimilation of this element through the PCA, unlike the other elements analyzed by this study, no hypothesis can be raised from the results.

Finally, in the PCA of stem samples, the first three components accounted for 80% of total data variability. The first component was responsible for 39%, the second, for 24%, and the third, for 17% of total information. The formation of groups related to cultivation and type of extraction could not be observed by the analyses considering PC1and PC2, PC1 and PC3, or PC2 and PC3 (results not shown). Results may be evidence of the fact that differences in metabolism of elements resulting from differences in cultivation are more evident in storage organs, such as leaves. It may be due to the supply of nutrients to stem from remobilization processes from storage organs.

## Conclusion

Differences between mineral profiles of organic and conventional yerba mate could be found. Leaf samples exhibited higher concentrations of all elements than stem ones. Mn and Fe characterized all organic samples while potassium characterized all conventional samples. Ca, Mg, and Zn characterize conventional leaves, but no differences related to cultivation could be found in stem samples. Remobilization of nutrients seems to be an important issue to understand the results, mainly the ones of stem samples. It may be inferred that organic matter of the soil is an important factor in the absorption of minerals, influencing the absorption of Fe and Mn positively and Ca and Zn, negatively. Competition among nutrients was determinant for Mg contents while Mn was the most significant competitor in samples under study. The use of fertilizers may be the determining factor for the behavior of K. It should be emphasized that observations made by this study were carried out in commercial samples with no control of variables; therefore, they reflect the influence of rules controlled by the Brazilian legislation for production of organic food on final products sold in the market.

Regarding results of hot water extraction, elements under study were found to be less extractable from samples of organic leaves, thus, indicating a greater connection with the matrix in this group. Fe and K are more extracted by the infusion technique, showing that exposure to agitation and temperature in decoction leads to their precipitation process. On the other hand, Mg, Mn, Ca, and Zn are more extractable by the decoction technique. The formation of oxalate crystals is a hypothesis for the behavior of the first three elements. In the case of Zn, despite showing similar behavior, no hypothesis could be formulated. In stem samples, no difference was found between organic and conventional groups. It corroborates what was previously described about the nutrient supply in stems being influenced by remobilization processes.

Finally, the application of the PCA to results of both analyses of total and hot water extraction showed the potential of preliminary studies of the influence of agricultural practices on freely traded food. However, based on these results, it is necessary to propose fractionation studies or more specific speciation to deepen knowledge in this area.

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**Data Availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Informed Consent** Not applicable.

**Ethical Approval** Not applicable.

**Conflict of Interest** Monize Morgado Neves declares that she has no conflict of interest. João Batista dos Santos Espinelli Junior declares that he has no conflict of interest. Michele Moraes de Souza declares that she has no conflict of interest. Rodolfo Carapelli declares that he has no conflict of interest.

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