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# Effect of the Yerba mate (Ilex paraguariensis) brewing method on the content of selected elements and antioxidant potential of infusions

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Yerba mate is a source of biologically active substances. The aim was to determine whether the place of origin of Yerba and the brewing method have any influence on the levels of Ca, Mg and Fe and antioxidant activity of infusions. Samples were steeped in cold water (25°C) and hot water (three consecutive infusions with 85°C water). Infusions had a high antioxidant activity and high Mg level. The levels of elements and the antioxidant activity were influenced by the brewing method. There were no significant differences in the examined parameters depending on the country of origin. The results on the levels of elements and the antioxidant activity indicate that the most efficient brewing method was infusion in hot water. The highest levels of elements were found in first infusions, with the highest antioxidant activity in the third infusions.

**Keywords:** Yerba, antioxidant activity, infusions, minerals

## INTRODUCTION

Yerba mate, brewed from the dried leaves of *Ilex paraguariensis*, is one of the most widely consumed drinks in the world. Its natural range includes countries such as Brazil, Argentina, Paraguay, and Uruguay[1]. There are many commercially available Yerba mate blends, with different compositions and from different regions of the world. They are prepared either in hot or cold water and the leaves can be infused repeatedly[2]. 50 g of dried plant per liter of water is an average daily portion[2]. There are many reports on the positive effect of Yerba mate on the human body, which may result from the fact that its leaves have a high antioxidant activity associated with the occurrence of numerous biologically active substances such as antioxidants[1, 3–7], polyphenols, xanthine alkaloids, flavonoids, amino acids, and vitamins[2, 8–10]. It has been shown that drinking Yerba mate infusions decreases blood cholesterol and has protective effects on the liver[8, 11]. Additionally, it can stimulate the central nervous system[12, 13]. Some studies indicate that Yerba mate helps in the prevention of cardiovascular diseases[14] and obesity[1, 15–19]. Yerba mate extracts have been shown to have a strong antimicrobial effect, especially against *Escherichia coli* 0157:H7, *Staphyllococcus aureus*[12, 13, 20]. Pre-treatment with yerba mate extract may protect the small intestine from gamma radiation damaging effects showed in the measured biochemical parameters also maintained the microbial flora of lactic acid bacteria and removed pathogenic bacteria and may replace antibiotics description for radiotherapy patients since it showed antimicrobial effect against pathogenic[21, 22]. Tests on human cancer hepatoma cells (HepG2) showed the cytotoxicity of Yerba mate extract associated with increased apoptosis and inhibition of topoisomerase II[5]. On the other hand, some epidemiological studies have indicated a positive correlation between the consumption of Yerba mate and the incidence of oral, oropharyngeal, esophageal, laryngeal, and bladder cancers[2, 23, 24].

Elements are essential in human nutrition, with levels in the body depending primarily on the occurrence in soil, drinking water, and food[25]. A deficient or excess intake of any of these chemical elements may cause adverse effects on the human body[26], especially in children[27]. Elemental composition analysis of Yerba mate revealed the presence of many microelements and macroelements[1, 7, 28, 29], including fluoride[30]. There are few works on the impact of the place of origin of and brewing method on Yerba mate's antioxidant activity and the amount of elements released into the infusion. In addition, there are no reports regarding the correlation between the levels of elements and the antioxidant activity of Yerba infusions. Therefore, the aim of the work was to determine the Fe, Ca and Mg levels and the Yerba infusion effect, including the origin of the raw material and brewing method. We also examined the potential dependencies between the levels of individual elements and between the levels of elements and antioxidant activity of the infusions.

## EXPERIMENTAL

### Raw materials

The following chemical reagents were used for the tests: ethanol (analytical grade, Chempur); acetone (analytical grade, Chempur); DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma Aldrich); 65% $HNO_3$ (Suprapur, Merck); 30% $H_2O_2$ solution (Suprapur, Merck); 1% Triton (Triton X-100, Sigma); 0.075% nitric acid (Suprapur, Merck).

### Plant material

We analyzed 16 Yerba mate tea blends. The samples were mixtures of dried leaves and stalks of different varieties of *Ilex paraguariensis*. Four samples originated in Brazil, six from Argentina, and six from Paraguay.

### The preparation of the infusions

Cold infusion: Ten gram test portions were placed in plastic cups. 50 mL of water at room temperature (25°C) was poured over the samples and allowed to stand for 10 min. After that time, the infusions were filtered into 50 mL tubes.

Hot infusion: Ten gram test portions were placed in plastic cups; 50 mL of water at 85°C was poured over the tea and left to stand for 10 min. After that time, the infusions were filtered into 50 mL tubes. The procedures were repeated two more times, flooding the same Yerba mate tea samples. Infusions from each filtration were placed in separate tubes.

### Determining Antioxidant Activity of Yerba mate infusions

The antioxidant activity of samples was measured with spectrophotometric method using synthetic radical DPPH (2,2diphenyl-1-picrylhydrazyl, Sigma) and spectrophotometer Agilent 8453UV. All assays were performed in triplicate. Antioxidant potential (antioxidant activity, inhibition) of tested solutions has been expressed by the percent of DPPH inhibition[31].

### Determining Fe, Ca and Mg content in Yerba mate infusions

Sample preparation: The samples were mineralized using microwave digestion system MARS 5, CEM. The volume of the sample given to research was 0.8 ml. The samples were transferred to clean polypropylene tubes. 0.6 mL of 65% $HNO_3$ (Suprapur, Merck) was added to each vial and each sample was allowed 30 minutes pre reaction time in the clean hood. After completion of the pre-reaction time, 0.6 mL of non-stabilized 30% $H_2O_2$ solution (Suprapur, Merck) was added to each vial. Once the addition of all reagents was complete, the samples were placed in special Teflon vessels and heated in microwaved digestion system for 35 minutes in 180°C (15min ramp to 180°C and maintained at 180°C for 20 min). At the end of digestion all samples were removed from the microwave and allowed to cool to room temperature. In the clean hood, samples were transferred to acid-washed 15 mL polypropylene sample tubes. A further 10-fold dilution was performed prior to ICP-OES measurement. The volume of 1 mL was taken from each digest. The samples were spiked with an internal standard to provide a final concentration of 0.5 mg/L Ytrium, 1ml of 1% Triton (Triton X-100, Sigma) and diluted to the final volume of 10 mL with 0.075% nitric acid (Suprapur, Merck). Samples were stored in a monitored refrigerator at a nominal temperature of 8°C until analysis. Blank samples were prepared by adding concentrated nitric acid (500 $\mu$L) to tubes without sample and subsequently diluted in the same manner described above. Multielement calibration standards (ICP multi-element standard solution IV, Merck) were prepared with different concentrations of inorganic elements in the same manner as in blanks and samples. Deionized water (Direct Q UV, Millipore, approximately 18.0 MΩ) was used for preparation of all solutions.

Sample determination: All samples were transferred into tubes and stored at –20°C until processed. Samples were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, ICAP 7400 Duo, Thermo Scientific) equipped with a concentric nebulizer and cyclonic spray chamber to determine their Ca, Fe, Mg content. Analysis was performed in both radial and axial mode. The wavelengths (nm) were 393.366 (Ca), 238.204 (Fe) and 279.553 (Mg).

### Statistical analysis

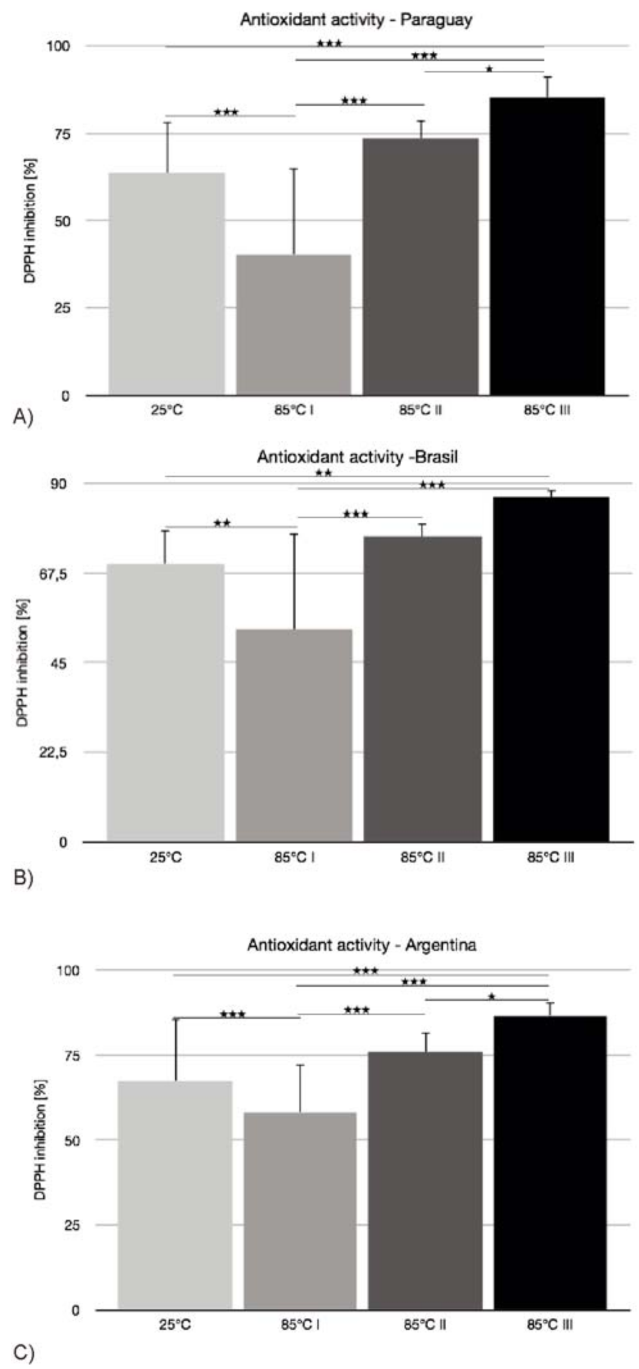
In all the experiments, three samples were analysed and all the assays were carried out at least in triplicate. The statistical analysis was performed using Stat Soft Statistica 13.0 and Microsoft Excel 2010. The results are expressed as mean values and standard deviation (SD). To assess the differences between the studied groups, the Mann–Whitney test and Tukey test were used. Correlation analysis was performed by Spearman coefficient. The level of significance was $p \leq 0.05$.

## RESULTS AND DISCUSSION

### Antioxidant activity of Yerba mate infusions

The antioxidant activity of Yerba mate infusions expressed as a percentage of DPPH inhibition depended on the temperature of the infusion preparation, ranging from 40.2% to 86.5%. For Yerba mate from Paraguay (Fig. 1A), the antioxidant activity ranged from 40.2% to 85.3%, for Brazilian Yerba mate from 53.1% to 86.3% (Fig. 1B), and for Argentinian Yerba mate from 58.1% to 86.5% (Fig. 1C) The lowest antioxidant activity was recorded for the first infusion at 85°C, regardless of the origin of the raw material. The antioxidant activity increased with further infusions as the result of more bioactive substances with antioxidant activity being released into the infusion. The highest antioxidant activity was recorded for the third infusions in 85°C water. Water temperature and the number of infusions were therefore significant for the antioxidant activity of the Yerba mate infusions, and in most cases statistically significant differences were found between the different brewing methods for Yerba mate, regardless of the place of origin.No statistically significant differences were found between infusions prepared in cold water (25°C) and the second infusions with 85°C water, regardless of the place of origin. In the case of Yerba mate from Brazil, there was no statistically significant difference between infusions resulting from the second and third infusion at 85°C. There were no statistically significant differences in antioxidant activity between the individual steeps (1$^{st}$ vs 1$^{st}$, 2$^{nd}$ vs 2$^{nd,}$ etc.) depending on the place of origin with one exception during the first brewing with 85°C water between material originating from Argentina and Paraguay ($p < 0.01$). In other cases, the origin of the raw material did not influence its antioxidant activity.

The antioxidant activity of Yerba mate infusions is similar to coffee[31] or green tea[8], commonly considered substantial sources of antioxidants. The antioxidant activity of *I. paraguariensis* has also been found to be higher




significant differences: * – $p < 0.05$; *** – $p < 0.001$

**Figure 1.** Antioxidant activity of Yerba mate infusions from Paraguay (A), Brazil (B) and Argentina (C)

than many types of red and white wine[33]. In a study by Dudonne et al.[21] aqueous extracts from Yerba mate leaves showed an antioxidant activity of 71.75% inhibition of DPPH. Despite the considerable differences in the research methods used to demonstrate the antioxidant activity of Yerba mate, the results obtained in all available studies clearly indicate its high antioxidant activity. The results of our study (from 40.23% to 86.47% inhibition of DPPH) are consistent with literature data.

Important is maturity of the raw material or its age. Research by Blum-Silva et al.[34] showed that the antioxidant activity of Yerba mate leaves decreases with the age of the plant. The extraction method is another factor influencing the antioxidant activity, as evidenced by research carried out by Grujic et al.[35]. A higher antioxidant activity was obtained using liquid $CO_2$ extraction in combination with ethanol than when ethanol was the sole extractant. The levels of antioxidants in the extract are also higher with higher extraction pressure and a higher concentration of ethanol used as a solvent[35]. The effect of the solvent used was also observed by Bastos et al.[8] where aqueous and ethanolic extracts from green and roasted Yerba mate had an antioxidant activity of about 90% inhibition of DPPH, while the extract obtained from ethyl ether showed the ability to neutralize free radicals at 30–35% DPPH inhibition. However, no statistically significant differences were found between the antioxidant activity of green and burned leaf extracts[8]. In turn, Türkmen Erol et al.[6], observed that extracts from roasted mate leaves had statistically significantly higher free radical scavenging ability than green leaf extracts. The concentration of the test extracts is another factor that significantly affects their antioxidant activity. Boaventura et al.[28] observed that the antioxidant activity of aqueous extracts from *I. paraguariensis* increases during cryoconcentration, resulting in significant differences at every stage of the process. The initial extracts had an EC50 of 127.7 μg/ml, whereas in concentrated extracts it was 3.88 μg/ml. Huang et al.[18] observed that the inhibition of DPPH in aqueous extracts increased in proportion to the concentration used. Bassani et al.[36] examined the antioxidant activity of extracts prepared at 60°C, 75°C and 90°C and obtained 53%, 57% and 75% inhibition of DPPH, respectively, thus showing that the extraction temperature influenced the Yerba mate antioxidant activity. Those results are partly consistent with the conclusions of our study in which we observed statistically significant differences between infusions obtained at 25°C and 85°C. However, contrary to Bassani et al.[36] the antioxidant activity in the first infusion with 85°C water was lower than that for the 25°C extract. Colpo et al.[23] investigated the antioxidant activity of water extracts from Brazilian and Argentinian Yerba mate, also taking into account the number of infusions. They observed that in the majority of cases the first infusions were characterized by the highest free radical scavenging ability, which gradually decreased in subsequent infusions. Only in the case of some types of Yerba mate, there was a slight increase in the antioxidant activity in the second and/or the fifth infusion. Although our research showed that the antioxidant activity grew with each subsequent infusion, it should be remembered we investigated only three consecutive infusions, while Colpo et al.[23] studied the effect of up to 15 infusions. It is probable that if we had studied the antioxidant activity in further Yerba mate infusions, the initial increase in the potential may have been followed by a decline, as observed by Colpo et al.[23]. Those researchers also noted a statistically significant difference in the antioxidant activity between Yerba mate from different countries[23], unlike our study where no relationship between the antioxidant activity and the origin of the raw material were observed. This discrepancy may result from differences in the methodology of the conducted research, but also be related to the differences in raw material processing by Yerba mate manufacturers (both studies are based on commercially available Yerba mate).

**Fe, Ca and Mg levels in Yerba mate infusions**

Both the water temperature and the number of infusions influenced the levels of Fe in Yerba mate extracts (Fig. 2A).In Yerba mate from Paraguay and Brazil, a statistically significantly higher level of this element was observed in infusions prepared with 85°C water compared to 25°C water. There was no difference in Fe level between the infusions prepared at 25°C and 85°C for Yerba mate from Argentina.

Steeping the raw material with 85°C water was repeated three times in order to compare Fe levels in the subsequent infusions. The first infusion contained the highest level of Fe with each subsequent infusion containing significantly less. Changes in the Fe levels in the tested infusions are shown in Table 1.

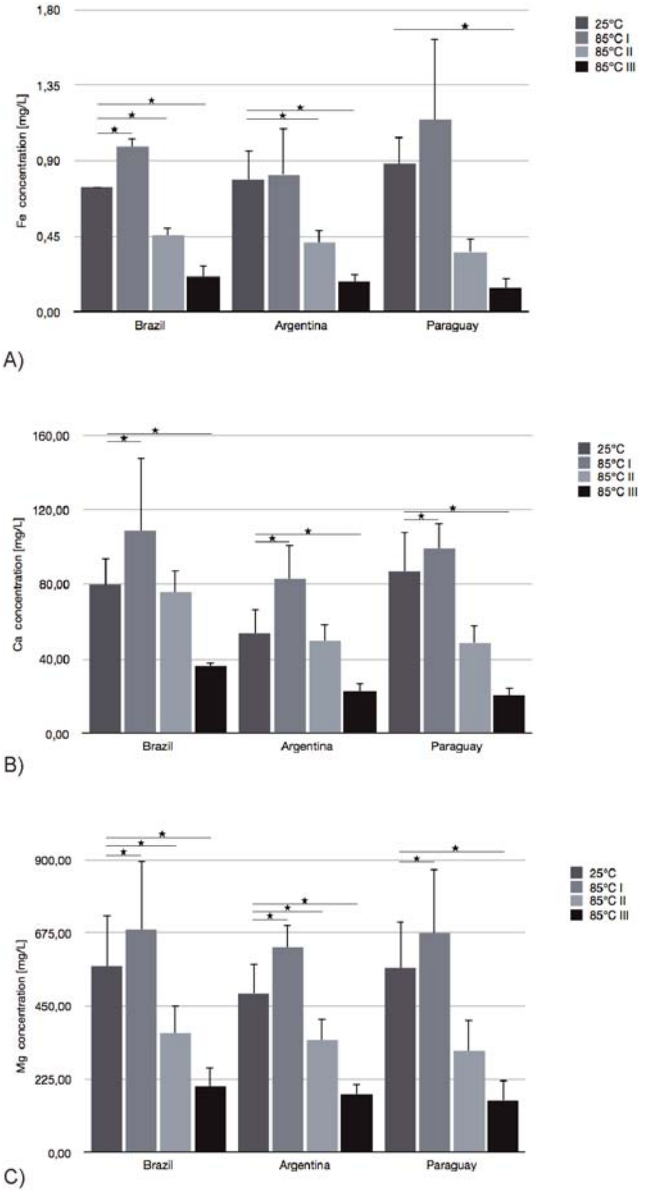
Also, in the case of Ca content, the analysis showed that the temperature of the water and the number of infusions at 85°C had an effect on the levels of this element in the Yerba mate infusions (Fig. 2B).

A statistically significantly higher Ca level in 85°C infusions was found compared to 25°C water. In Yerba mate from Brazil and Argentina, in the case of 85°C infusions, the first infusion was the most efficient, with the highest Ca concentration. The subsequent infusions showed significantly decreased Ca levels. A significant increase in Ca was observed only for the second infusion of Yerba mate from Paraguay. Changes in the Ca levels in the infused brews are shown in Table 2.

The temperature of water and number of 85 infusions had an effect on Mg levels (Fig. 2C). Mg levels in 85°C infusions were statistically significantly higher compared to 25°C infusions. The most efficient was the first infusion, and in each subsequent infusion the levels of this element dropped significantly.Changes in the Mg levels in the studied extracts are shown in Table 3.

Yerba mate infusions have high levels of microelements and macroelements that can assist in the proper functioning of organs and the preservation of homeostasis. According to the commonly accepted concept, all mineral compounds contained in food, subjected to extraction in water, show high bioavailability, therefore the analysis of the levels of individual elements in the extracts of Yerba mate presented here is a source of information on their bioavailability[37].

The analysis examined the levels of Ca, Mg and Fe in cold (25°C) and hot (85°C) infusions of Yerba mate originating from Brazil, Argentina and Paraguay. The supply of these elements in the diet is essential because they regulate the basic metabolic processes in cells. The obtained results clearly indicate that the best method of preparing Yerba mate is a first infusion at 85°C, to extract the highest levels of microelements and macroelements, compared to cold extraction or subsequent infusions at 85°C. Garcia et al.[36] showed the same dependence




significant differences: * – $p < 0.05$

**Figure 2.** Fe (A), Ca (B) and Mg (C) content in Yerba mate infusions in dependence on temperature of infusion and place of origin

**Table 1.** The influence of the number of infusions with 85°C water on Fe levels

| Origin of Yerba mate | The number of infusions with 85°C water | | |
|---|---|---|---|
| | I | II | III |
| Brazil [n = 4] | 0.98 mg Fe /L | 0.45 mg Fe /L (53.4% decrease *vs.* infusion I; p = 0.028) | 0.21 mg Fe /L (78.5% decrease *vs.* infusion I; p = 0.028) |
| Argentina [n = 6] | 0.82 mg Fe /L | 0.42 mg Fe /L (49.1% decrease *vs.* infusion I; p = 0.036) | 0.18 mg Fe /L (77.8% decrease *vs.* infusion I; p = 0.012) |
| Paraguay [n = 6] | 1.14 mg Fe /L | 0.36 mg Fe /L (68.6% decrease *vs.* infusion I; p = 0.386) | 0.15 mg Fe /L (87.2% decrease *vs.* infusion I; p = 0.005) |

**Table 2.** The influence of the number of infusions with 85°C water on Ca levels

| Origin of Yerba mate | The number of infusions with 85°C water | | |
|---|---|---|---|
| | I | II | III |
| Brazil [n = 4] | 109.13 mg Ca/L | 75.70 mg Ca/L (30.7% decrease *vs.* infusion I; p = 0.028) | 36.05 mg Ca/L (66.9% decrease *vs.* infusion I; p = 0.028) |
| Argentina [n = 6] | 82.66 mg Ca/L | 49.55 mg Ca/L (40.1% decrease *vs.* infusion I; p = 0.012) | 22.75 mg Ca/L (72.5 % decrease *vs.* infusion I; p = 0.012) |
| Paraguay [n = 6] | 98.95 mg Ca/L | 48.88 mg Ca/L (50.6% decrease *vs.* infusion I; p = 0.386) | 20.81 mg Ca/L (79.8% decrease *vs.* infusion I; p = 0.005) |

**Table 3.** The influence of the number of infusions with 85°C water on Mg levels

| Origin of Yerba mate | The number of infusions with 85°C water | | |
|---|---|---|---|
| | I | II | III |
| Brazil [n = 4] | 685.32 mg Mg /L | 369.49 mg Mg /L (46.1% decrease *vs.* infusion I; p = 0.028) | 205.39 mg Mg /L (70.0% decrease *vs.* infusion I; p = 0.028) |
| Argentina [n = 6] | 630.82 mg Mg /L | 346.65 mg Mg /L (45.0% decrease *vs.* infusion I; p = 0.012) | 176.89 mg Mg /L (71.9% decrease *vs.* infusion I; p = 0.012) |
| Paraguay [n = 6] | 673.80 mg Mg /L | 313.80 mg Mg /L (53.4% decrease *vs.* infusion I; p = 0.013) | 161.58 mg Mg /L (76.0% decrease *vs.* infusion I; p = 0.005) |

regarding the levels of elements in Yerba mate extracts, where hot extraction at 80°C resulted in much higher levels of elements than cold extraction at 8°C[36]. Apart from pure Yerba mate products, other mixtures of *Ilex dumosa* and *Ilex paraguariensis* (Yerba mate) 70:30 (v:v) are available. Research conducted by Maiocchi et al.[4] showed that Yerba mate extracts had higher levels of elements in comparison to *I. dumosa,* with the highest levels obtained for Ca, followed by Mg, and then Fe[4]. In turn, Bastos et al.[38] found the that the descending order in fresh leaf infusions of Yerba mate was Mg>Fe>Ca. Our results were identical to the analysis made by Garcia et al.[36], where the highest concentrations of Mg were observed in the infusions prepared from Yerba mate originating from Brazil, Argentina and Paraguay, followed by Ca and then Fe[37]. The discrepancy in the results obtained is most likely related to the type of material being tested: Yerba mate fresh leaves, mixture of dried Yerba mate and *Ilex dumosa,* and dried Yerba mate alone. It is well known that *Ilex dumosa* is characterized by relatively lower levels of micro- and macroelements compared to Yerba mate, and a high proportion (70%) in the leaf mixture can significantly affect the levels of extracted elements.

As in the case of antioxidant activity, the elemental composition of Yerba mate depends largely on the parts of the plant used to make the infusion and the elemental composition of the soils in which the plant grew. Research carried out by Baran et al.[39] on Yerba mate samples from Paraguay and Argentina showed similar mineral compositions for dried leaves and stems and infusions prepared from them with the elements arranged in the following descending orders: K>Ca->Mg>Mn>Fe>Zn>Na>Cu>Ni>Cr>Pb>Cd (leaves and stems) and K>Mg>Mn>Ca>Zn>Na>Fe>Cu->Ni>Cr>Pb>Cd (infusions). It was shown that in total 73% K, 69% Mn, 15% Ni and Cu, 9% Cr, 8% Zn, 7% Mg, 6% Pb, 3% Na, 2% Cd, 0.34% Ca and 0.13% Fe were extracted from the leaves and stems. Yerba mate infusions were also characterized by a very high antioxidant activity, with the Argentinean Yerba mate having slightly higher antioxidant activity than the Paraguayan variety. After converting the determined quantities of elements to standards, daily consumption of Yerba mate infusions provided essential minerals in amounts well below 1% of the recommended daily allowance (RDA) recommended in the US and Canada. The exception was the amount of Mn at 1.43% RDA for men and 1.82% RDA for women[39].

Our research indicated much higher levels of Mg, Ca and Fe in 1 liter of infusions prepared from this plant. Infusions from Brazilian Yerba mate provided 11% RDA for Ca for adults and as much as 163% RDA Mg and 5.5% RDA Fe for women and 12% RDA Fe for men. In the case of raw material originating in Argentina, the levels of elements were: 8% RDA for Ca, 150% RDA for Mg, and 4.5% RDA Fe for women and 10% RDA Fe for men. 1 liter of infusion prepared from a plant originating in Paraguay provided 10% RDA for Ca, 135% RDA for Mg and 6.7% RDA for Fe for women and 15% RDA for Fe for men according to recommendations in the USA and Canada. Based on the obtained results, it can be concluded that the plant originating from Brazil was the raw material with the highest levels of the studied mineral compounds.

### Statistical dependencies between the examined parameters

Regardless of the origin of the raw material, Mg levels correlated negative with the temperature of water used for infusions made (Table 4). A similar relationship was also observed for Fe content, but only for the samples from Paraguay and Argentina.This dependence in relation to Ca was only statistically significant for Yerba mate from Paraguay. The antioxidant activity was significantly affected by water temperature and the number of infusions. Relations between the antioxidant activity and the levels of Ca, Fe and Mg were negative, with varying statistical significance (Table 4).

Statistical analysis showed directly proportional correlations between the levels of the elements, with the exception of the Argentinean Yerba mate where the correlation between Ca and Fe levels was not significant (Table 5).

The correlations were also examined without taking into account the country of origin and the brewing method; there were significant positive correlations between the contents Ca *vs* Fe, Ca *vs* Mg and Mg *vs* Fe (Table 5).

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**Table 4.** Correlation coefficient (r) between antioxidant activity (DPPH), minerals content (Ca, Fe, Mg) and temperature of infusions

| Origin of Yerba mate | Correlation coefficient (r) between: | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ca and temperature | Fe and temperature | Mg and temperature | DPPH and temperature | DPPH and Ca | DPPH and Fe | DPPH and Mg |
| Brazil [n = 4] | –0.634 | –0.781* | –0.781* | 0.683 | –0.881* | –0.881* | –0.786* |
| Argentina [n = 6] | –0.552 | –0.820* | –0.777* | 0.497 | –0.697* | –0.364 | –0.483 |
| Paraguay [n = 6] | –0.820* | –0.863* | –0.777* | 0.561 | –0.461 | –0.440 | –0.468 |

* designates significance at p≤0.05

**Table 5.** Correlation coefficient (r) between minerals content (Ca, Fe, Mg) depending origin of Yerba and temperature of infusions

| Origin of Yerba mate | Correlation coefficient [r] between: | | |
|---|---|---|---|
| | Ca and Fe content | Ca and Mg content | Mg and Fe content |
| Brazil [n = 4] | 0.881* | 0.881* | 0.952* |
| Argentina [n = 6] | 0.539 | 0.648* | 0.979* |
| Paraguay [n = 6] | 0.979* | 0.895* | 0.881* |
| Without considering the country and how to prepare the infusion | 0.721* | 0.850* | 0.837* |

* designates significance at p≤0.05

## CONCLUSIONS

Yerba mate is a raw material with high antioxidant activity and rich in microelements and macroelements. Yerba mate infusions are characterized by high magnesium levels (from 135% to 163% RDA) and medium to high antioxidant activity depending on the brewing conditions (40.23%–86.47% DPPH inhibition). The extraction temperature affects both the antioxidant activity and the levels of calcium, magnesium and iron, and the literature data and our results show that the most efficient brewing method is hot extraction. The method of infusion influences the levels of elements and antioxidant activity, but we found no significant differences in the parameters depending on the country of origin of the plant material.

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