REVISTA Mundi
Meio Ambiente e Agrárias
INSTITUTO FEDERAL Paraná

CARBOIDRATOS, ÁCIDOS ORGÂNICOS E COMPOSTOS FENÓLICOS EM FOLHAS E INFUSÃO DE ERVA-MATE

CARBOHYDRATES, ORGANIC ACIDS AND PHENOLIC COMPOUNDS IN LEAVES AND INFUSION OF YERBA MATE

Marcela Moreira Terhaag[1]
Leonel Vinícius Constantino[2]
Lycio Shinji Watanabe[3]
Tiago Bervelieri Madeira[4]
Suzana Lucy Nixdorf[5]
Sandra Helena Prudencio3[6]

Resumo: A floresta de erva-mate (*Ilex paraguariensis*) pode ser nativa (NAT), composta por árvores nativas, de crescimento espontâneo, com espaçamentos e sombreamentos variáveis, ou composta por árvores nativas transplantadas e/ou melhoramento genético (PLANT), cultivadas de forma densa ou em consórcio silvicultural com eucalipto, pinus e outros. Alguns fatores como processamento industrial e forma de consumo interferem qualitativa e quantitativamente nos compostos bioativos da erva-mate. O objetivo deste trabalho foi avaliar o teor de compostos fenólicos totais (TPCs) e a atividade antioxidante (AA), identificar e quantificar os açúcares, ácidos orgânicos e minerais presentes nas folhas de erva-mate NAT e PLANT. Além disso, a partir das folhas com maior teor de TPCs e AA foi preparada uma infusão a fim de determinar a porcentagem de TPCs e minerais lixiviados e a atividade antioxidante. Ainda, foram identificados e quantificados os principais ácidos fenólicos, flavonóides e metilxantinas presentes na infusão. Nas folhas PLANT, foram determinados maiores teores de TPCs (14%) e AA (27, 22 e 13%, para DPPH, FRAP e ABTS, respectivamente) em relação às folhas de NAT, assim como diferentes perfis de açúcares e ácidos orgânicos. Dessa forma, as folhas da PLANT foram escolhidas para análise e preparo da infusão. Verificou-se que, embora parte do conteúdo mineral, TPCs e AA tenham sido retidos durante o preparo da infusão, compostos como cafeína, teobromina, ácido ascórbico, epicatequina e ácido clorogênico ainda estavam presentes, comprovando que a infusão preparada a partir da erva-mate plantada possui compostos com potenciais benefícios à saúde.

Palavras-chave: Ilex Paraguariensis. Chimarrão. Cafeina. Nativa. Plantio.

Abstract: O Yerba mate (*Ilex paraguariensis*) forest may be native (NAT), composed of native trees, spontaneously growing, with variable spacing and shading, or composed of native transplanted and/or plant breeding (PLANT), cultivated in dense form or as a silvicultural consortium with eucalyptus, pinus and others. Some factors such a industrial processing and form of consumption interfere qualitatively and quantitatively in bioactive compounds of yerba mate. The aim of this work was to evaluate the total phenolic compounds contend (TPCs) and antioxidant activity (AA), identify and quantify the sugars, organic acids and minerals present in

[1] Doutorado em Ciência de Alimentos, Instituto Federal do Paraná – Campus Umuarama, marcela.terhaag@ifpr.edu.br.
[2] Doutor em Agronomia e em Química, Universidade Estadual de Londrina, leonel@uel.br.
[3] Doutor em Química, Universidade Estadual de Londrina, shinjiwatanabe2003@hotmail.com.
[4] Doutor em Química, Universidade Estadual de Londrina, madeiratb@gmail.com.
[5] Doutora em Química, Universidade Estadual de Londrina, snixdorf@uel.br.
[6] Doutora em Ciência dos Alimentos, Universidade Estadual de Londrina, sandrah@uel.br.

NAT and PLANT leaves. Also, from the leaves with higher content of TPCs and AA was prepared an infusion to determine the percentage of TPCs and minerals leached, and antioxidant activity. There was identified and quantified the major phenolic acids, flavonoids and methylxanthines in the infusion. In PLANT leaves, higher content of TPCs (14%) and AA (27%, 22% and 13%, for DPPH, FRAP and ABTS, respectively) were determined in relation to NAT leaves, as well as different profiles of sugars and organic acids. That way, the PLANT leaves were chosen for analysis and preparation of infusion. It was found that, although part of the mineral content, TPCs and AA were retained during the preparation of the infusion, compounds such as caffeine, theobromine, ascorbic acid, epicatechin and chlorogenic acid were still present, proving that the infusion prepared from planted yerba mate has compounds with potential health benefits.

Keywords: Ilex paraguariensis. Chimarrão. Caffeine. Native. Intentional planting

1 INTRODUCTION

Yerba mate (*Ilex paraguariensis*), a native plant in South America, is consumed as chimarrao (or maté), tereré, hot or iced tea with fruit juice (ISOLABELLA et al., 2010; MEINHART et al., 2010).

The yerba mate has antioxidant properties and act in cardiovascular protection, as central nervous system stimulant, it has antimutagenic effect and in weight reduction (AZAM et al., 2003; HECK & DE MEJÍA, 2007; DARTORA et al., 2011; BARG et al., 2014). The yerba mate benefits occur because of its innumerous bioactive compounds such as saponins, phenolic acids, flavonoids and methylxanthines, whose contents vary according the edaphoclimatic conditions of mate tree cultivation (FRIZON et al., 2015).

Factors such as levels of radiation, progeny of plants, location and planting way interfere in the content of phenolic compounds (DONADUZZI et al., 2003; HECK et al., 2008) and micro and macro nutrients from mate leaves (GIULIAN et al., 2007; MARCELO et al., 2014). Frizon et al. (2015) evaluated the content of phenolic compounds in mate from different regions of the State of Paraná and observed that there is a greater content of phenolic compounds in herbal from central State, where cultivation was jointly with eucalyptus and different radiation levels.

The native mate cultivation is characterized by yerba mate trees from spontaneous growth and without spacing defined between them. There is shading or partial solar radiation level, due to the growth associated with other tree species such as Araucaria (*Araucaria angustifolia*) (EFING et al., 2009, BORGES et al., 2003).

The commercial mate cultivation consists of native plants transplanted and/or plants from genetic improvement, cultivated in thickened form or as forestry consortium with eucalyptus, pinus or others (FRIZON et al., 2015; PES et al., 1995). In consortium cultivation, the solar radiation level is variable and depends on the associated plant (PES et al., 1995).

Thus, it becomes important to check, among native mate cultivation and intentional planting, which one provides leaves with higher phenolic compounds content and antioxidant activity, besides determining the profile of minerals and bioactive compounds in mate infusions.

The bioactive compounds of mate may change qualitative and quantitative and depending on the industrial processing and consumption form (DARTORA et al., 2011; BOAVENTURA et al., 2013). Only part of the content of phenolic acids, flavonoids, methylxanthines and minerals can be carried into the aqueous fraction during the preparation of chimarrão, tereré, tea or infusion (JACQUES et al., 2007; BASTOS et al., 2014; BRAGHINI et al., 2014). Therefore, the determination of the content of bioactive compounds and minerals leached from the leaves to the infusion of yerba mate provide relevant information to the development of products with potential benefits to health, after proper adjustment of process and formulation.

This work aimed to evaluate the total phenolic content (TPCs) and antioxidant activity (AA), identifying and quantifying sugars, organic acids and minerals presents in leaves of native yerba mate (NAT) and yerba mate from intentional planting (PLANT). From the leaves with higher content of TPCs and AA an infusion was prepared to determine the percentage of TPC, minerals, antioxidant activity that was leaching, as well as identify and quantify the major phenolic acids, flavonoids and methylxanthines.

2 DEVELOPMENT

2.1 Material and Methods

2.1.1 Material

2.1.1.1 Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-S-triazine), ABTS (2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid), Folin-Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid), glucose, fructose, sucrose, stachyose, mannitol, citric acid, malic acid, tartaric acid, lactic acid, succinic acid, acetic acid, ascorbic acid, gallic acid, protocatechuic acid, theobromine, paraxanthine, epigallocatechin, catechin, epicatechin, quercetin, caffeic acid, caffeine, rutin, kaempferol, chlorogenic acid, p-coumaric acid, ferulic acid, sinapic acid, myricetin, theophylline and trigonelline were purchased from Sigma-Aldrich (purity ≥ 99%). Ethanol and the other reagents were obtained from Merck (Darmstadt, Germany). HPLC grade

acetonitrile and methanol was purchased from J.T. Baker (Xalostoc, Mexico). The ultrapure water used in the preparation of standard solutions and extraction was produced by the Milli-Q® System (Simplicity 185, Millipore, MA, USA).

2.1.1.2 Yerba mate: plant material, extracts and infusion

Plant material from native (NAT) and intentional cultivation (PLANT) of *Ilex paraguariensis* (Saint-Hilaire), with unknown age were obtained from Campina do Simão, Paraná, Brazil (25°4'8" S; 51°49'31"W; altitude: 963 m). The native cultivation was situated among Araucarias and livestock, random spacing and variable solar radiation level. The intentional cultivation was located nearby eucalyptus, pinus and others trees species, thickened form and getting a higher incidence of sunlight when compared to native cultivation.

Adult leaves were harvested in March 2015 and separated according to the origin as: native (NAT) or planted (PLANT). The leaves were washed into water and dried in an oven with forced circulation by 24 h at 45 °C. They were ground into knife mill to particle size less than 3.55 mm, and stored at -18 °C in polyethylene bags covered with foil (MURAKAMI et al 2011).

The infusion was prepared by adding water at 85 °C on dehydrated and ground leaves of mate in a proportion of 1:10 (leaves: water). The infusion was maintained for 15 minutes, filtered in paper filter Whatman n.1 and maintained at -15 °C until the tests which were carried out within 8 hours after the infusion.

2.1.2 Methods

2.1.2.1 Chemical Characterization of the Leaves and the Infusion

The moisture content, proteins, fibers and ashes were determined according to the methods described by AOAC (2005). Total carbohydrates were estimated by calculating the percentage difference of other components. The mineral analyses were performed in an atomic absorption spectrometer (AAS, Varian AA-140) and in mass spectrometer by inductively coupled plasma (ICP-MS, Varian 820-MS). The results were expressed in grams per 100 g (dry

weight basis (dw), except the moisture). All analyses were carried out in triplicate (n = 3).

2.1.2.2 Determination of Sugars and Organic Acids from Mate Leaves and Infusion

For identification and quantification of sugars and organic acids, 1 g of mate leaves or mate infusion was homogenized in 10 mL of water, centrifuged at 9056g for 15 min. The supernatant was collected and filtered in PVDF membrane (0.22 µm Millipore, Cork, Ireland).

The analyses were performed in a high performance liquid chromatograph (LC 20, Shimadzu Co., Kyoto, Japan) with a high pressure pump (LC-20AT), automatic injector (SIL-20AC HT), column oven (CTO-20A) and photodiode-array detector (SPD-M20A) and refractive index detector (RID-10A) coupled in series (HPLC-PDA-RID). Chromatographic analyses were in isocratic mode.

The chromatographic conditions for the determination of sugars and organic acids are described in Table 1. It was used the oven temperature of 85 °C, with ion exchange column Aminex HPX-87P (7.8 x 300 mm in ionic form Pb^{2+}, Biorad, CA, USA) previously balanced with 100% of ultrapure water as the mobile phase (MiliQ®). The flow rate was set 1.0 mL/min and the cell of RID was maintained at 40 °C. The wavelength of the PDA was set at 215 nm beyond scanning 200 to 400 nm (PAULI et al., 2011). The data acquisition and the integration of chromatographic peaks were performed in software LC Solutions (Shimadzu Co., Kyoto, Japan). The analyses were performed in duplicate (n=2) and the results were expressed in mg/g (dw).

Table 1 – Chromatographic conditions used for the determination of sugars and organic acids[1]

	Sugars	**Organic acids**
Mobile phase	100% ultrapure water	100% Phosphate buffer 25mM pH 2.4
Column	Aminex HPX-87P Ion	CapCell Pak C18 (250 x 4.6mm x

	exchange Pb^{2+} (7.8 x 300 mm) (Biorad, CA, USA)	5µm) (Shiseido Co., Ltd., Japan)
Oven temperature (°C)	85	40
Detector temperature (°C)	40	40
Flow rate (mL/min)	1.0	1.0
Injection volume (µL)	20	20

[1]Source: Pauli et al. (2011)

2.1.2.3 Determination of Phenolic Content and Antioxidant Activity

The extracts were obtained from the addition of 1 g of dehydrated and ground leaves to 10 mL 80% hydroethanolic solution (v/v). The mixture was stirred continuously for 20 minutes at room temperature, centrifuged to 699 g/5 min and the supernatant collected. The extraction procedure was repeated two more times. The supernatants were combined and completed with 80% ethanol up to achieve 30 mL. The extracts were obtained in triplicate for each yerba mate sample (n = 3). The extracts were stored at -80 °C until analysis of total phenolic content and antioxidant activity.

The total content of phenolic content (TPC) was determined as Singleton et al. (1999), with some modifications. The extract or infusion (0.5 mL) was added of Folin-Ciocalteau reagent 0.9 N and 0.5 mL of sodium carbonate solution 10% (m/v), kept in dark for 30 min and the absorbance was read at 760 nm in spectrophotometer (Biochrom Pound S22, United Kingdom). The water was used as white. The TPC content was obtained using gallic acid as standard (4 to 24 µg/mL) and the results were expressed in µg of gallic acid equivalents (GAE) per g of sample (dw).

For the determination of phenolics and methylxanthines composition in infusion, it was used ultra-high efficiency liquid chromatograph (UHPLC) (Waters® ACQUITY UPLC® I-Class System) with HSS C18 column 1.8 µm 2.1×100 mm (Waters), using two mobile phases: ultrapure water (A), and methanol (B), with 0.05% and 0.1% formic acid, respectively. The injection volume was 1.0 µL, partial loop mode with needle overfill, 0.4 mL/min of flow rate. The separation was performed with a gradient condition (0.00 – 10.00 min: 95% phase A; 10.00-10.10 min: 95% of phase B; 10.10 – 13.00 min: 95% phase A). The Diode Array Detector (DAD) was used to scan the wavelengths between 190 and 700 nm, and the reading in 270 and 320 nm. The analysis

was carried out in 13 min. The separation and quantification were performed in duplicate (n = 2). The results were expressed in µg/mL of infusion.

The antioxidant activity (AA) was evaluated by free radical reduction methods 2.2 '-diphenyl-1-picrylhydrazyl (DPPH), and of the $ABTS^{\bullet+}$ (2.2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and the ability of reduction from Fe^{3+} to Fe^{2+} according to Sánchez-González et al. (2005).

For the DPPH method, was added in a tube 1 mL of 100mM acetate buffer, pH 5.5; 1 mL of ethanol; 0.5 mL of ethanolic solution of DPPH 250µM and 50 µL of sample. After 15 min of incubation in the dark at room temperature the absorbance was read at 517 nm in a spectrophotometer. The positive control did not contain sample and blank was consisted of 1 mL of acetate buffer and 1.5 ml of ethanol.

The radical $ABTS^{\bullet+}$ was produced from the reaction of the solution of ABTS 7 mm with potassium persulfate 2.45 mM in dark environment for 16h. The $ABTS^{\bullet+}$ was diluted in 20mM phosphate buffer (pH 7.4) to the final absorbance of 0.700 at 730 nm. An aliquot of 10 µL of the sample was added in tube containing 4 mL of $ABTS^{\bullet+}$ and after 6 min of reaction the absorbance was read at 730 nm.

The FRAP reagent was obtained by the combination of 25 mL of acetate buffer (300mM, pH 3.6), 2.5 mL of TPTZ (10mM TPTZ in HCl 40mM) and 2.5 mL of iron chloride 20mM. The reagent was prepared at the time of analysis. After 30 µL reaction of sample with 70 µl of distilled water and 900 µl of reagent FRAP in dark environment at 37 °C, for 30 minutes, the absorbance was read at 595 nm spectrophotometer. Water was used as blank.

It was used standard curves of Trolox to DPPH (from 50 to 600µM), FRAP (from 50 to 600µM) and ABTS (aqueous solutions of Trolox with ethanol 80%, from 0.75 to 7.5µM). The results of the antioxidant activity were expressed in µmol of Trolox equivalents antioxidant activity (TEAC) per g of sample (dw).

2.1.2.4 Statistical Analysis

The data of the analyses of mate leaves in the two cropping systems (NAT and PLANT) were submitted to paired t-test. The Pearson test was

applied to determine the correlation among the content of sugars, organic acids, TPC and AA of PLANT and CULT leaves. All the statistics analysis was performed using Statistica 7.1 (2006), at a significance level of 5%.

2.2 Results and Discussion

2.2.1 Characterization of the Mate Leaves

NAT and PLANT leaves were similar in relation to moisture content, fiber and lipids (Table 2). The PLANT leaves had higher ashes content and total carbohydrates (~6% than NAT) and lower protein content (~22%). Edaphoclimatic factors such as fertilizing, luminosity, cultural practices, among others interfere on the chemical characteristics of the leaves of mate. Several authors (EFING et al., 2009; BRAGHINI et al., 2014; ESMELINDRO et al., 2002) identified similar centesimal composition in leaves of Brazilian yerba mate.

PLANT leaves had higher levels Ca, P and Fe, and NAT sample had a higher concentration of Mg. NAT and PLANT leaves were similar in boron, manganese (both with less than 0.02 µg/g, data not shown) and potassium content. In general, the order of mineral concentration decreased in the following order: P, K, Ca, Mn and Fe. Giulian et al. (2007) and Marcelo et al. (2014) detected the same minerals, but in higher concentrations in commercial mate.

The PLANT leaves presented a lower content of glucose and fructose than NAT leaves (51% and 53%, respectively, Table 2), and it was not detected sucrose, stachyose and mannitol in the samples.

The PLANT leaves presented smaller levels of malic, tartaric, succinic and lactic acids and highest of citric and acetic acids than NAT leaves (Table 2), possibly as a result of the genetic characteristics of this yerba mate variety and edaphoclimatic factors of cultivation. The glucose and fructose levels were correlated positively with citric and malic acid and negatively with succinic and lactic (Table 1 Supplementary).

Table 2 – Chemical composition and antioxidant activity of leaves of native (NAT) and planted (PLANT) mate

Parameters	Yerba mate	
	NAT	PLANT
Centesimal composition (g/100 g) [1]		
Moisture	6.74 (±1.54)	4.57 (±0.67)
Proteins	16.04 (±0.45)*	12.42 (±0.56)*
Total fiber	19.79 (±0.30)	19.53 (±0.38)
Ashes	5.82 (±0.03)*	6.18 (±0.07)*
Lipids	7.75 (±0.21)	8.13 (±0.16)
Total carbohydrates	50.60 (±0.49)*	53.74 (±0.19)*
Minerals (mg/100 g) [1]		
Potassium	667.25 (±54.02)	588.46 (±10.97)
Calcium	15.72 (±4.33)*	41.71 (±2.94)*
Phosphorus	7.13 (±0.31)*	12.64 (±0.25)*
Magnesium	2.87 (±0.19)*	2.35 (±0.15)*
Iron	0.019 (±0.0003)*	0.020 (±0.0002)*
Sugars (mg/g) [2]		
Glucose	2.12 (±0.01)*	1.03 (±0.00)*
Fructose	2.34 (±0.00)*	1.09 (±0.00)*
Organic acids (mg/g) [2]		
Citric acid	0.92 (±0.02)*	1.28 (±0.05)*
Malic acid	2.55 (±0.01)*	2.17 (±0.01)*
Tartaric acid	4.52 (±0.04)*	4.51 (±0.17)*
Lactic acid	0.75 (±0.01)*	0.34 (±0.03)*
Succinic acid	0.43 (±0.00)*	0.40 (±0.00)*
Acetic acid	0.39 (±0.00)*	0.59 (±0.00)*
Total phenolics [3] and antioxidant activity [4]		
TPC §	3.73 (±0.009)*	4.30 (±0.024)*
DPPH ¶	195.62 (±2.51)*	270.15 (±5.53)*
FRAP ¶	318.56 (±7.83)*	408.31 (±5.59)*
ABTS ¶	573.32 (±7.94)*	661.02 (±31.35)*

[1] Results are expressed in dry basis, except moisture, as average (n = 3) ± standard deviation.
[2] Results are expressed in dry basis, as average (n = 2) ± standard deviation.
[3]Results are expressed in dry basis, as g of gallic acid equivalent per 100 g of sample (g EAG/g).
[4]Results are expressed in dry basis, as µmol of Trolox equivalent antioxidant activity per g of sample (µmol TEAC/g). NAT: leaves of native mate; PLANT: leaves of intentional planting.
* Values followed by different letters in the same line do not differ by t test ($p < 0.05$).

These results can be explained by the participation of these sugars in the Embden-Meyerhof-Parnas pathway, since they are converted to citric acid during cellular metabolism. These characteristics are related to the genetic characteristics and physiology of the PLANT leaves before sample collection and, due to the biochemical nature of the cycle, are probably not related to

storage. Naturally, the organic acids occurring in leaves of mate as a result of physiological biochemical processes. Both the presence and the content of these compounds are related to secondary reactions of glycolytic way and the Krebs cycle. According to Rizzon and Sganzerla (2007), malic acid in grape vines is synthesized from secondary reactions of photosynthesis in mature leaves and can use citric acid as a precursor. Souza et al. (2015) found greater content of citric and lower of malic acid in leaves of mate.

Factors like progenies of plants, soil type, level of solar radiation, presence and type of fertilizing in mate culture interfere in mineral and sugars contents (DONADUZZI et al., 2003; JACQUES et al., 2007), and consequently in organic acids contents. Among these factors, the differences between NAT and PLANT leaves may be related to the level of solar radiation, since the PLANT cultivation received higher incidence of solar radiation than the NAT trees.

The TPC content (Table 2) was 14% higher in the PLANT than in the NAT leaves. These results were lower than related to the findings of Bravo et al. (2007) and Chandra and Mejia (2004) in aceto-methanolic extracts from mate. The efficiency of extraction of TPC depends on the polarity of solvent and the methodology employed (ANDREO & JORGE, 2006). Besides, it was observed higher antioxidant activity (AA) through the three methods in PLANT leaves than in NAT.

It was verified a positive correlation between the contents of TPC and AA (Table 1 Supplementary). These results confirm the ones indicated by Bravo et al. (2007) and Anesini et al. (2012) and show that most of AA comes from phenolic compounds. Also, it showed positive correlation among the results of AA assessed by the three methods used in this work.

Some authors (DARTORA et al., 2011; DONADUZZI et al., 2003; BRAVO et al., 2007) pointed a relationship between edaphoclimatic factors and TPC content and AA in mate leaves. In this way, the higher incidence of sunlight also provided higher TPC and AA to PLANT leaves.

2.2.2 Characteristics of infusion from mate PLANT leaves

Since the typical consumption of mate is in the form of mate or tereré, an infusion was prepared from the PLANT leaves, as they had higher TPC content and higher AA (Table 2).

Comparing the content of minerals, TPC and AA in 1 g of PLANT leaves and in the infusion prepared with the same mass of leaves, a reduction was observed in these parameters (Figure 1). Probably only a part of these compounds was dissolved in hot water and consequently was leached for the infusion. The minerals are part of numerous physiological processes in leaves of mate and your accessibility and solubility are related to the location and function of each of them in the plant tissue. Ways of processing and extraction from the leaves interfere in the minerals content spread during the preparation of the infusion of mate (Marcelo et al., 2014; Bastos et al., 2014). So, there was a difference in the percentage of each mineral leaching for the infusion. The elements Mg, Ca and P had a higher retention percentage in the mate leaves (95, 94 and 93%, respectively), while Fe and K showed a higher water solubility and subsequent leaching (about 28 and 16%, respectively).

It was detected in the infusion: 9.4; 2.51; 0.8; 1.2; and 0.005 mg/100 g, respectively for K, Ca, P, Mg and Fe. Although, only a fraction of the minerals was leached, this content should be considered for the consumption of drink or development of infusion products. Higher concentrations of minerals were identified by Heinrichs and Malavoltam (2001) in infusions of mate.

The TPC content observed in the infusion was of 252 mg/g (dw), i.e. almost 45% lower than the one found in PLANT leaves. This demonstrates that only the hot water was not efficient to leaching all TPCs. Pagliosa et al. (2010) indicate that the phenolic compounds may be linked to the polysaccharides in the cell wall, being found in suber cells and sclerenchyma in bark from residues from mate tree. This indicates that part of phenolic compounds is lignified and in regions with difficult extraction in the tissues of the leaves of yerba mate. Bravo et al. (2007) found lower contents of TPCs (7.76-8.12 g GAE/100 g) and higher AA by FRAP assay (803-846 µmol TEAC/g) in infusions from commercials yerba mate than the values observed in this study.

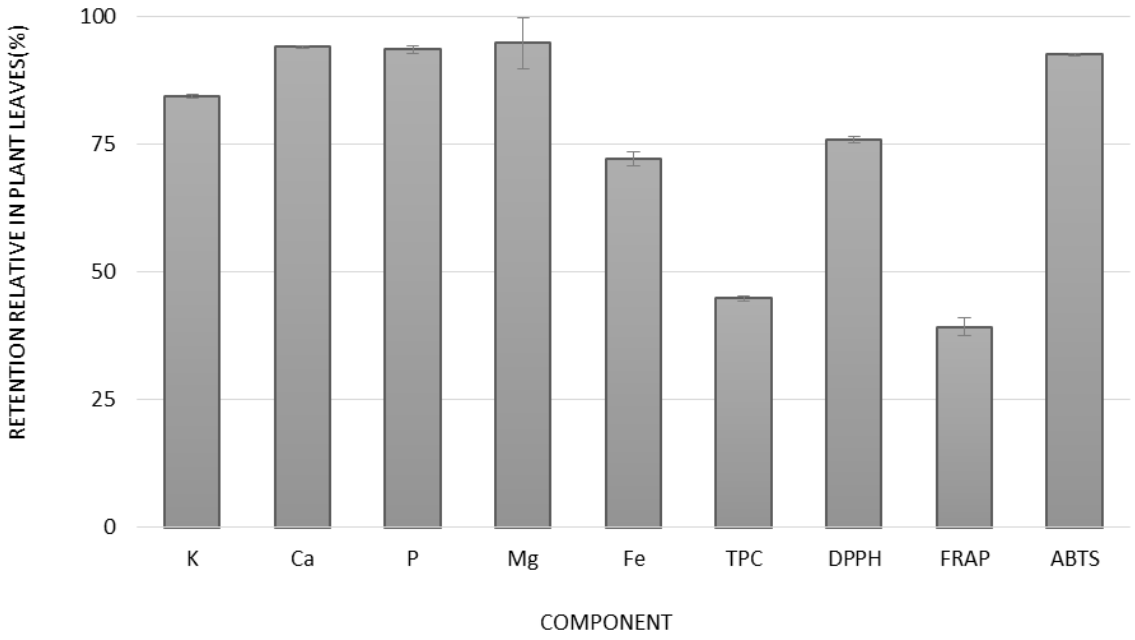

Figure 1 – Retention percentage in the mineral content, TPC and AA after the preparation of mate infusion from PLANT leaves[1]
[1] Average percent reduction calculated from the comparison between the results determined in 1 g of leaves of mate PLANT and infusion prepared from 1 g of leaves PLANT
PLANT: leaves of intentional planting

It was noted a decrease in AA of the infusion in relation to PLANT leaves, 39, 76 and 92% for the methods FRAP, DPPH, ABTS, respectively. As most of the AA of mate is related to the TPC (BRAVO et al., 2007), it can be inferred that the reduction on AA is related to a low leaching of TPC to infusion.

By chromatographic analysis and using 19 standards it was identified 17 compounds in PLANT infusion (Figure 2): six phenolic acids (gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid and ferulic acid), three methylxanthines (theobromine, paraxanthine and caffeine) and five flavonoids (catechin, epigallocatechin, epicatechin, rutin and kaempferol). Also, ascorbic acid and nicotinic acid were identified in the infusion, but trigonelline, quercetin, theophylline and sinapic acid were not detected. Among the compounds identified (Table 3), the highest relative concentration of bioactive compounds was found for caffeine (66%), theobromine (19%), ascorbic acid (8%), epicatechin (1.7%) and chlorogenic acid (1.5%). Bravo et al. (2007) identified a similar profile of phenolic compounds in mate and infusion.

The caffeine content was similar to the one observed by Anesini et al. (2012), Isolabella et al. (2010) and Meinhart et al. (2010) in leaves and infusion

of commercial yerba mate, and higher than those indicated by Pagliosa et al. (2010) in the waste processing of mate. Anesini et al. (2012) found higher contents of chlorogenic acid, caffeic acid and rutin while Isolabella et al. (2010) indicated highest content of rutin and theobromine. The p-coumaric acid and ferulic acid were identified in mate infusion. Pagliosa et al. (2010) also detected such compounds in leaves and in waste production of yerba mate, indicating that both are common constituents of cell walls. The same authors identified theophylline and 4.5 dicaffeoylquinic acid and lower contents of theobromine, chlorogenic acid and caffeic acid.

According to Dartora et al. (2011), during the drying of mate leaves occur plant cells disruption resulting in higher extraction of caffeine during the infusion preparation. Possibly drying and grinding of the leaves increase the hydroextraction and consequent leaching of methyl xanthines, phenolic acids and flavonoids during the infusion preparation.

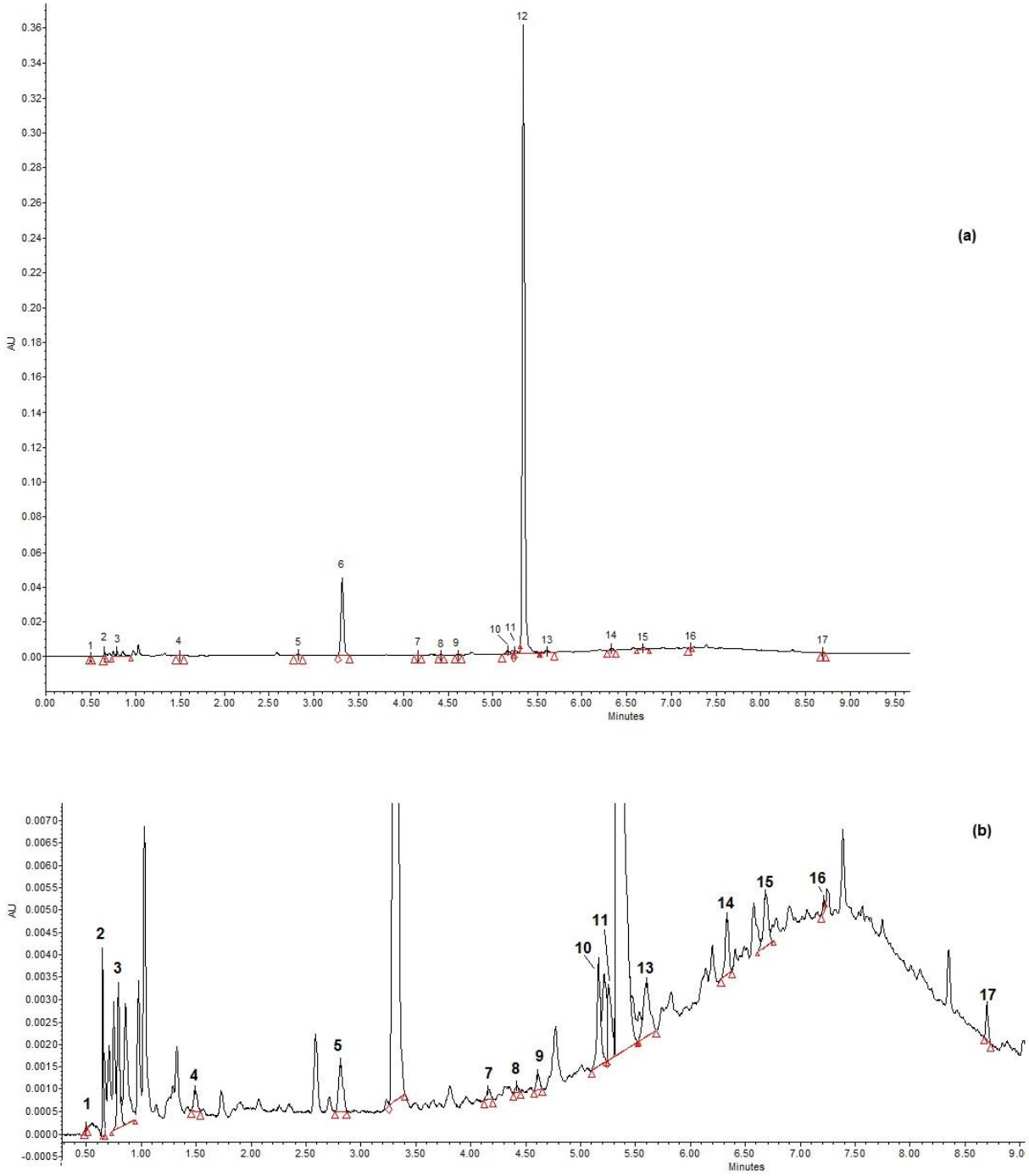

Figure 2 – UHPLC chromatogram of yerba mate infusion from PLANT leaves: (a) sign in 270 nm; (b) sign extended in 270 nm

PLANT: leaves of intentional planting

Table 3 – Concentration of bioactive compounds in infusion of yerba mate PLANT[1]

	Compound	RT (min)	µg/mL
1	Trigonelline	0.498	n.d.
2	Ascorbic acid	0.651	93.51 (±1.80)
3	Nicotinic acid	0.798	13.48 (±0.10)
4	Gallic acid	1.491	0.51 (±0.06)
5	Protocatechuic acid	2.819	3.20 (±0.10)
6	Theobromine	3.311	223.01 (±1.44)
7	Paraxanthine	4.162	0.89 (±0.05)
8	Epigallocatechin	4.415	0.055 (±0.07)
9	Catechin	4.612	6.45 (±0.50)
10	Chlorogenic acid	5.166	18.13 (±2.34)
11	Cafeic acid	5.25	7.90 (±2.87)
12	Caffeine	5.345	778.87 (±1.00)
13	Epicatechin	5.605	20.25 (±0.59)
14	P-coumaric acid	6.330	2.58 (±0.22)
15	Ferulic acid	6.678	5.23 (±0.00)
16	Rutin	7.208	0.001 (±0.01)
17	Kaempferol	8.694	5.08 (±0.27)

[1]Results are expressed as average (± standard deviation). Infusion at 10% (w/v), prepared from the leaves of mate PLANT. PLANT: leaves of intentional planting

3 CONSIDERAÇÕES FINAIS

The leaves of yerba mate from intentional planting system have higher contents of total phenolic compounds and antioxidant activity than native yerba mate. The leaves of yerba mate from intentional planting system present different profiles of sugars and organic acids when compared to those of native yerba mate.

Only a fraction of mineral (>72%) and phenolic contents (45%), and of the antioxidant activity (39, 76 and 92% by FRAP, DPPH, ABTS methods, respectively) are preserved in the mate infusion. Compounds such as caffeine, theobromine, ascorbic acid, chlorogenic acid and epicatechin are leached from the leaves to the yerba mate infusion. So an infusion of yerba mate has potential beneficial health effect, either in direct consumption or as an ingredient for the development of products.

REFERENCES

ANDREO, D.; JORGE, N. Antioxidantes naturais: técnicas de extração. **Boletim do Centro de Pesquisa de Processamento de Alimentos**, Curitiba, v. 24, n. 2, p. 319-326, 2006.

ANESINI, C.; TURNER, S.; COGOI, L.; FILIP, R. Study of the participation and polyphenols on the overall antioxidant activity of mate (Ilex paraguariensis). **LWT – Food Science and Technology**, London, v. 45, n. 2, p. 299-304, 2012.

AOAC. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis of Association of Official Analytical Chemists. 18 ed. Maryland: AOAC, 2005. 1094 p.

AZAM, S.; HADI, N.; KHAN, N. U.; HADI, S. M. Antioxidant and prooxidant properties of caffeine, theobromine and xanthine. **Medical Science Monitor**, Warsaw, v. 9, n. 9, BR 325-330, 2003.

BARG, M.; REZIN, G. T.; LEFFA, D. D.; BALBINOT, F.; GOMES, L. M.; CARVALHO-SILVA, M.; VUOLO, F.; PETRONILHO, F.; DAL-PIZZOL, F.; STRECK, E. L.; ANDRADE, V. M. Evaluation of the protective effect of Ilex paraguariensis and Camellia sinensis extracts on the prevention of oxidative damage caused by ultraviolet radiation. **Environmental Toxicology and Pharmacology**, Amsterdam v. 37, n. 1, p.195-201, 2014.

BASTOS, M. C.; REISSMANN, C. B.; KESEKERC J. F.; PAULETTI, V.; GAIAD, S.; STURION, J. A. Mineral content of young leaves of yerba mate. **Pesquisa Florestal Brasileira**, Colombo, v. 34, n. 77, p. 63-71, 2014.

BOAVENTURA, B. C. B.; MURAKAMI, A. N. N.; PRUDENCIO, E. S.; MARASCHIN, M.; MURAKAMI, F. S.; AMANTE, E. R.; AMBONI, R. D. M. C. Enhancement of bioactive compounds content and antioxidant activity of aqueous extract of mate (Ilex paraguariensis St. Hil.) through freeze concentration technology. **Food Research International**, Essex, v. 53, n. 2, p. 686-692, 2013.

BORGES, L.; LÁZZARI, S. M. N.; LÁZZARI, F. A. Comparação dos sistemas de cultivo nativo e adensado de erva mate, Ilex paraguariensis St. Hil., quanto à ocorrência e flutuação populacional de insetos. **Revista Brasileira de Entomologia**, Curitiba, v. 47, n. 4, p. 563-568, 2003.

BRAGHINI, F.; DE CARLI, C. G.; BONSAGLIA, B.; JUNIOR, J. F. S. S.; OLIVEIRA, D. F.; TRAMUIAS, J.; TONIAL, I. B. (2014). Composição físico-química de erva-mate, antes e após simulação do chimarrão. **Pesquisa Agropecuária Gaúcha**, Porto Alegre, v. 20, n. 1/2, p. 7-15, 2014.

BRAVO, L.; GOYA, L.; LECUMBERRI, E. LC/MS characterization of phenolic constituents of mate (Ilex paraguariensis, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. **Food Research International**, Essex, v. 40, n. 3, 393-405, 2007.

CHANDRA, S.; DE MEJIA, G. E. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of Ardisia

compressa in comparison to mate (Ilex paraguariensis) and green (Camellia sinensis) teas. **Journal of Agricultural and Food Chemistry**, Washington, v. 52, n. 11, p. 3583-3589, 2004.

DARTORA, N.; SOUZA, L. M.; SANTANA-FILHO, A. P.; IACOMINI, M.; VALDUGA, A. T.; GORIN, P. A. J.; SASSAKI, G. L. (2011). UPLC-PDA–MS evaluation of bioactive compounds from leaves of Ilex paraguariensis with different growth conditions, treatments and ageing. **Food Chemistry**, Barking, v. 129, n. 4, p. 1453-1461, 2011.

DONADUZZI, C. M.; JUNIOR, E. L.C.; DONADUZZI, E. M.; DA SILVA, M. M.; STURION, J. A.; CORREA, G. Variação nos teores de polifenóis totais e taninos em dezesseis progênies de Erva-Mate (Ilex paraguariensis St. Hill.) cultivadas em três municípios do Paraná. **Arquivo de Ciências da Saúde UNIPAR**, Umuarama, v. 7, n. 2, p. 129-133, 2003.

EFING, L. C.; CALIARI, T. K.; NAKASHIMA, T.; DE FREITAS, R. J. S. Caracterização química e capacidade antioxidante da erva-mate (Ilex paraguariensis St. Hil.). **Boletim do Centro de Pesquisa de Processamento de Alimentos**, Curitiba, v. 27, n. 2, p. 241-246, 2009.

ESMELINDRO, M. C.; TONIAZZO, G.; WACZUK, A.; DARIVA, C.; OLIVEIRA, D. Caracterização físico-química da erva-mate: influência das etapas do processamento industrial. **Ciência e Tecnologia de Alimentos**, Campinas, v. 22, n. 2, p. 193-204, 2002.

FRIZON, C. N. T.; OLIVEIRA, G. A.; PERUSSELLO, C. A.; PERALTA-ZAMORA, P. G.; CAMLOFSKI, A. M. O.; ROSSA, U. B.; HOFFMANN-RIBANI, R. Determination of total phenolic compounds in yerba mate (Ilex paraguariensis) combining near infrared spectroscopy (NIR) and multivariate analysis. **LWT - Food Science and Technology**, London, v. 60, p. 795-801, 2015.

GIULIAN, R.; DOS SANTOS, C. E. I.; SHUBEITA, S. M.; DA SILVA, L. M.; DIAS, J. F.; YONEAMA, M. L. Elemental Characterization of Commercial Mate Tea Leaves (Ilex paraguariensis St.-Hil.) before and after Hot Water Infusion Using Ion Beam Techniques. **Journal of Agricultural and Food Chemistry**, Washington, v. 55, n.3, p. 741−746, 2007.

HECK, C. I.; DE MEJIA, E. G. Yerba Mate Tea (Ilex paraguariensis): A Comprehensive Review on Chemistry, Health Implications, and Technological Considerations. **Journal of Food Science**, Chicago, v. 72, n.9, p. 138-151, 2007.

HECK, C. I.; SCHMALKO, M.; DE MEJÍA, G. Effect of growing and drying conditions on the phenolic composition of mate teas (Ilex paraguariensis). **Journal of Agricultural and Food Chemistry**, Washington, v. 56, n.18, p. 8394-8403, 2008.

HEINRICHS, R.; MALAVOLTA, E. Mineral composition of a commercial product from mate-herb (Ilex paraguariensis St. Hil.). **Ciência Rural**, Santa Maria, v. 31, n. 5, p. 781-785, 2001.

ISOLABELLA, S.; COGOI, L.; LÓPEZ, P.; ANESINI, C.; FERRARO, G.; FILIP, R. Study of the bioactive compounds variation during yerba mate (Ilex paraguariensis) processing. **Food Chemistry**, Barking, v. 122, n. 3, p. 695-699, 2010.

JACQUES, R.A.; ARRUDA, E.J.; OLIVEIRA, L.C.S.; OLIVEIRA, A.P.; DARIVA, C.; OLIVEIRA, J.V.; CARAMÃO, E.B. Influence of agronomic variables on the macronutrient and micronutrient contents and thermal behavior of mate tea leaves (Ilex paraguariensis). **Journal of Agricultural and Food Chemistry**, Washington, v. 55, n. 18, p. 7510-7516, 2007.

MARCELO, M. C. A.; MARTINS, C. A.; POZEBON, D.; DRESSLER, V. L.; FERRAO, M. F. Classification of yerba mate (Ilex paraguariensis) according to the country of origin based on element concentrations. **Microchemical Journal**, New York, v. 117, p. 164–171, 2014.

MEINHART, A. D.; BIZZOTTO, C. S.; BALLUS, C. A.; RYBKA, A. C. P.; SOBRINHO, M. R.; CERRO-QUINTANA, R. S.; TEIXEIRA, J.; GODOY, H. T. Methylxanthines and phenolics content extracted during the consumption of mate (Ilex paraguariensis St. Hil) Beverages. Journal of Agricultural and Food Chemistry, Washington, v. 58, n. 4, p. 2188-2193, 2010.

MURAKAMI, A. N. N.; AMBONI, R. D. M. C.; PRUDENCIO, E. S.; AMANTE, E. R.; ZANOTTA, L. M.; MARASCHIN, M.; PETRUS, J. C.; TEOFILO, R. F. Concentration of phenolic compounds in aqueous mate (Ilex paraguariensis A. St. Hil) extract through nanofiltration. **LWT - Food Science and Technology**, London, v. 44, p. 2211-2216, 2011.

PAGLIOSA, C. M.; VIEIRA, M. A.; PODESTÁ, R.; MARASCHIN, M.; ZENI, A. L. B.; AMANTE, E. R.; AMBONI, R. D. M. C. Methylxanthines, phenolic composition, and antioxidant activity of bark from residues from mate tree harvesting (Ilex paraguariensis A. St. Hil.). **Food Chemistry**, Barking, v. 122, n. 1, p. 173-178, 2010.

PAULI, E. D.; CRISTIANO, V.; NIXDORF, S. L. Método para determinação de carboidratos empregado na triagem de adulterações em café. **Química Nova**, São Paulo, v. 34, n. 4, p. 689-694, 2011.

PES, L.; HOPPE, J.M.; STORCK, L.; OLIVEIRA, O. S. Comportamento da erva-mate (Ilex paraguariensis St. Hil.) em consórcio silvicultural. **Ciência Florestal**, Santa Maria, v. 5, n. 1, p. 19-32, 1995.

RIZZON, L. A.; SGANZERLA, V. M. A. Ácidos tartárico e málico no mosto de uva em Bento Gonçalves-RS. **Ciência Rural**, Santa Maria, v. 37, n. 3, p. 911-914, 2007.

SÁNCHEZ-GONZÁLEZ, I.; JIMÉNEZ-ESCRIG, A.; SAURA-CALIXTO, F. In vitro antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter). **Food Chemistry**, Barking, v. 90, n. 1-2, p. 133-139, 2005.

SINGLETON, V. L.; ORTHOFER, R.; LAMUELA-RAVENTOS, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-

ciocalteu reagente. **Methods in enzymology**, New York, v. 299, p. 152-178, 1999.

SOUZA, A. H. P.; CORRÊA, R. C. G.; BARROS, L.; CALHELHA, R.; SANTOS-BUELGA, C.; PERALTA, R. M.; BRACHT, A.; MATSUSHITA, M.; FERREIRA, I. C. F. R. Phytochemicals and bioactive properties of Ilex paraguariensis: An in-vitro comparative study between the whole plant, leaves and stems. **Food Research International**, Essex, v. 78, p. 286–294, 2015.

STATSOFT. **STATISTICA for Windows: computer program manual**. Versão 7.1. [Tulsa]: Software Inc., 2006.

ACKNOWLEDGEMENTS

This work was supported by Coordination for the Improvement of Higher Level or Education Personnel - Ministry of Education (CAPES-MEC) and the National Council of Technological and Scientific Development (CNPQ). Thanks to Federal Institute of Paraná (IFPR) for releasing the first author to doctoral studies and the Multiuser Lab (LAPA-UEL/FINEP) through collaboration in the execution of the chromatographic and minerals analyses.

Enviado em: 12/01/2020

Aceito em: 30/06/2022

Editor Chefe: Prof. Dr. Everaldo dos Santos

Editor Adjunto: Dr. Wilian Demetrio