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A multivariate approach to differentiate *yerba mate* (*Ilex paraguariensis*) commercialized in the southern Brazil on the basis of phenolics, methylxanthines and *in vitro* antioxidant activity

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Abstract

The consumption of yerba mate beverages is related to several health benefits. These desirable properties are mainly due to the bioactive compounds. However, the levels of those compounds are directly affected by factors such as geographical origin. This study aimed to use chemometrics to evaluate the antioxidant compounds of yerba mate (consumed as *chimarrão*) marketed in southern Brazil. Neochlorogenic acid and caffeine were the main bioactive compounds found in this type of yerba mate beverage (*chimarrão*). The dataset was analyzed by chemometrics, and principal component analysis using the first three principal components explained 61.30% of the total variance. Hierarchical cluster analysis suggested three clusters, with cluster 1 containing the majority of the samples from the states of Rio Grande do Sul and Paraná, with higher levels of phenolics, methylxanthines, and antioxidant activity. The supervised methods, soft independent modeling of class analogy (SIMCA) and partial least squares-discriminant analysis (PLS-DA), had similar predictability (≥75% of accuracy) in terms of classifying yerba mate according to different Brazilian regions. Therefore, the chemometric tools used in the current study was suitable to monitor and assess a large variation of the antioxidant composition of yerba mate from different southern Brazilian states.

Keywords: bioactive compounds; geographical origin; *chimarrão*; HPLC; pattern recognition.

Practical Application: The use of chemometrics to discrimination and classification of commercial yerba mate.

1 Introduction

Yerba mate is the main product prepared from the dried and ground, leaves and twigs obtained from the ***Ilex paraguariensis*** **A. St.-Hil.** (Aquifoliaceae) tree. According to the Food and Agricultural Organization of the United Nations (2017), Brazil is the largest producer of the *yerba mate* worldwide, with around 602,000 tonnes per year, followed by Argentina (237,000 tonnes), and Paraguay (92,000 tonnes). Its natural occurrence is located in the latitude at 22°S to 30°S and longitude at 48°30'W to 56°10'W, with an altitude of 500-1500 m.a.s.l and a normal annual rainfall of 1500-2000 mm, and medium temperature of 15-18 °C (Empresa Brasileira de Pesquisa Agropecuária, 2017).

Traditionally *yerba mate* is consumed as a hot infusion, which is known as '*chimarrão*' in southern Brazil (Colpo et al., 2016). The beverage (*chimarrão*) is made in a typical artefact denominated '*cuia*' where the *yerba mate* is added and partially immersed in hot water (around 80 °C). Once the infusion is ready, this extract is drunk using a metal straw '*bomba*' (Silveira et al., 2016b). During consumption, water is poured over the *yerba mate* many times to make a partial infusion, and about 20-40 mL of fresh extract is drunk each time (Bracesco et al., 2011). Furthermore, it is important to emphasize that the drinking process is continuous all day long and throughout the year, usually accompanying daily activities, with an average consumption of 1-2 L per day per consumer.

Its proximate composition, expressed in dry matter, showed around 1.2% of protein, 10.4% of carbohydrates, and 0.8% of total fat, and has interesting contents of potassium (1100 mg/100 g), magnesium (296 mg/100 g), and manganese (54.4 mg/100 g) (Cardozo & Morand, 2016). The intake of *yerba mate* beverages, which is mainly by people from South America, is related to several health benefits that have been reported in the literature, including antioxidant, antimutagenic, anticancer, anti-inflammatory, and antimicrobial effects, as well as lipid reduction and vasodilation properties (Heck & Mejia, 2007; Bracesco et al., 2011; Silveira et al., 2016a; Gerke et al., 2017). These desirable properties are mainly due to the bioactive compounds (phenolic compounds and methylxanthines) in *yerba mate*, which have been attributed to antioxidant activity. The phenolic compounds in *yerba mate* beverages (*chimarrão* or *mate*) are mostly represented by chlorogenic acids (Silveira et al., 2016b). The main methylxanthines, on the other hand, are caffeine and theobromine (Bassani et al., 2007).

The levels of those bioactive compounds are directly affected by factors such as geographical origin, as well as variations in

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environmental conditions, processing and cultivation, harvest time, and soil composition (Pinto et al., 2015; Bastos et al., 2018). In southern Brazil there are a large number of brands of *yerba mate* that are commercialized from different regions, and the antioxidant potential of each of these is influenced by the factors mentioned above. Nowadays, the differences in foods that are mainly related to their geographical origin are investigated using the combination of analytical results and multivariate statistical tools (Herrera Alvarez et al., 2017; Bona et al., 2017). This association makes it possible to extract a maximum quantity of information from the dataset and it increases understanding about the data structure (Zielinski et al., 2014b).

Therefore, the aim of this study was to evaluate the chemical composition of *yerba mate* (to be used as *chimarrão*) marketed in southern Brazil in order to discriminate and classify the samples according to their geographical origin using multivariate statistical analysis.

2 Materials and methods

2.1 Materials

A total of 69 samples (around 10 kg each sample) of *yerba mate* (to be consumed as *chimarrão*) from three different Brazilian states (Paraná - PR; Santa Catarina - SC; and Rio Grande do Sul - RS) were purchased in the respective local markets from 2015 to 2016. Their geographical origin was stated by *yerba mate* companies. All the packages stated that the *yerba mate* was derived from the *I. paraguariensis* species. Folin-Ciocalteu reagent (2.0 N), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), TPTZ (2,4,6-tri (2-pyridyl)-s-triazine), trolox (6-hydroxy-2,5,7,8-tet ramethylchroman-2-carboxylic acid), gallic acid, chlorogenic acid, caffeine, theobromine and rutin were all purchased from Sigma-Aldrich (St. Louis, MO, USA), with purity ≥ 95% (HPLC-grade standards). The other reagents used in the experiments were of analytical grade and all the solutions were prepared using Milli-Q water (MILLI-Q, Millipore, Brazil).

2.2 Yerba mate infusion

Initially, each sample (10 kg, moisture content of 10%) was quartered, and 100 g was ground to obtain a homogeneous fine powder that passed through a 0.5 mm screen. Then, 2 g of each sample was extracted with 100 mL of distilled water at 80 °C under constant agitation (150 rpm in a magnetic stirrer) for eight min, in accordance with Zielinski et al. (2014a). All the infusions were centrifuged (8160 × *g*, 20 min at 4 °C), transferred to Falcon tubes, and immediately frozen at -20 °C until further analysis.

2.3 Phenolic and methylxantine composition

The total phenolic content (TPC) was determined in the extracts according to Singleton & Rossi (1965) using Folin-Ciocalteu reagent, with minor modifications. The analysis was performed in 96-well microplates, where 15 µL of diluted extracts, 240 µL of distilled water and 15 µL of Folin-Ciocalteu reagent (0.2 N) were added. After three min under agitation, 30 µL of sodium carbonate solution (1.0 N) was added into the microplates, followed by gentle shaking for five minutes in a vortex. The absorbance (725 nm) was measured after 30 minutes using a microplate reader (Epoch microplate spectrophotometer, Synergy-BIOTEK, Winooski, VT, USA). The absorbance values of the samples were compared with a calibration curve of gallic acid (GA) and the results were expressed as mg of gallic acid equivalents (GAE) per liter of infusion [mg GAE/L].

The total flavonols were determined in triplicate, as described by Kumaran & Karunakaran (2007), with minor modifications. Briefly, in microplates, 30 µL of each infusion was mixed with 60 µL of aluminum chloride (20 mg/mL) and 150 µL of sodium acetate (50 mg/mL). The solution was kept in the dark for 2.5 hours and the absorbance was measured at 440 nm using a microplate reader (Epoch microplate spectrophotometer, Synergy-BIOTEK, Winooski, VT, USA). The sample absorbance measurements were compared to a calibration curve of rutin and the results were expressed as milligrams of rutin equivalents (RE) per liter of infusion [mg RE/L].

The identification and quantification of individual phenolic compounds and methylxantines was performed by high performance liquid chromatography (HPLC) according to Mejía et al. (2010), with slight modifications. Prior to analysis, the samples were filtered through a 0.20 µm nylon syringe and 10 µL of the sample was injected into the chromatography system. The separation was performed using an Alliance HPLC system (model 2695, Waters, Milford, MA, USA), coupled with a photodiode array detector (model PDA 2998, Waters, Milford, MA, USA), a quaternary pump and an auto sampler. Separation was performed using a Gemini® C_{18} column with dimensions of 150 mm x 4.6 mm, 5 µm (Phenomenex, Torrance, CA, USA) at 25 °C with a flow rate of 0.9 mL/min. A linear gradient composed of mobile phase A (1.0% formic acid, v/v) and mobile phase B (acetonitrile) was applied from 3 to 45% B for 45 min, followed by the washing and reconditioning of the column. The peaks were compared to the retention time and the wavelengths of the standards, and the areas were compared against the regression equations of the standards ($R^2 > 0.99$, $p < 0.01$). The runs were monitored at 320 nm (chlorogenic acids), and at 350 nm (rutin). All the results were expressed as milligrams per liter of infusion [mg/L]. A typical chromatogram is presented in Figure 1.

2.4 In vitro antioxidant activity

The measurement of the free-radical scavenging activity by the DPPH assay of the *yerba mate* infusions was performed according to Brand-Williams et al. (1995). The samples were submitted to reading in a microplate reader (Epoch microplate spectrophotometer, Synergy-BioTek, Winooski, VT, USA) at 517 nm.

The measurement of the free-radical scavenging activity by the ABTS assay was performed as described by Re et al. (1999). The mixture reacted in the dark for 30 min and the measurement was performed using a microplate reader (Epoch microplate spectrophotometer, Synergy-BioTek, Winooski, VT, USA) at 734 nm.

The measurement of the total antioxidant potential by the FRAP method was performed according to Benzie & Strain

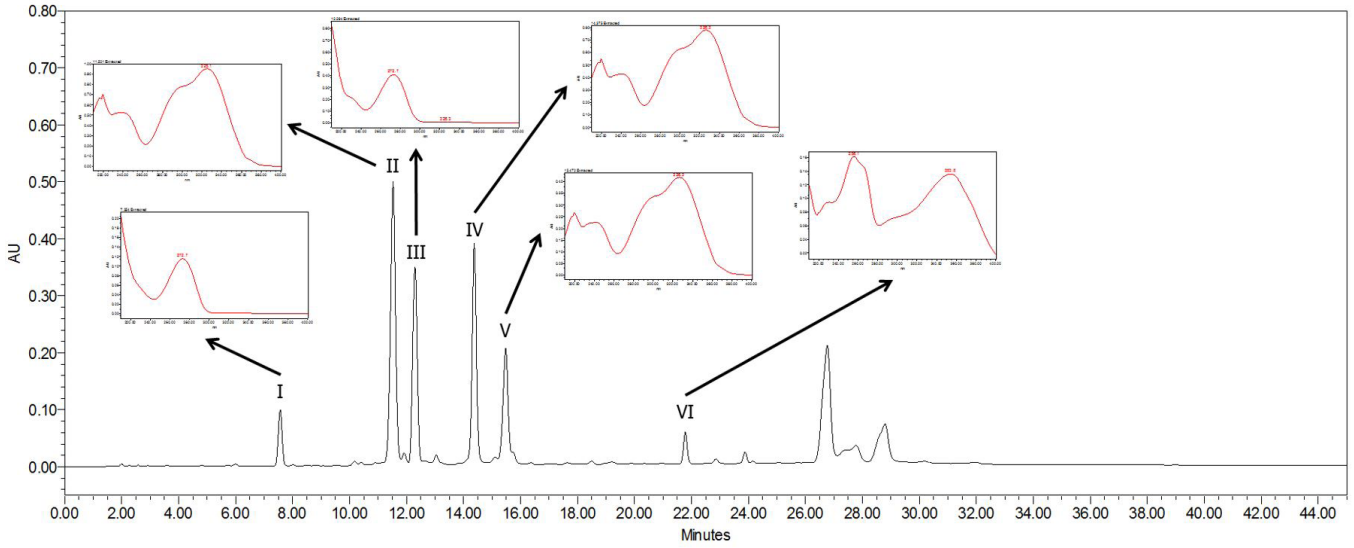

Figure 1. Typical chromatogram obtained from y*erba mate* extract ("*chimarrão*") for phenolic compounds and methylxanthines (280 nm). The UV spectra corresponding to the compounds are shown above the chromatogram. Peaks: (I) theobromine; (II) neochlorogenic acid; (III) caffeine; (IV) chlorogenic acid; (V) cryptochlorogenic acid; and (VI) rutin.

(1996). The reading was performed in a microplate reader (Biotec Instruments, Inc., Epoch, Winooski, USA) at 593 nm.

For all the *in vitro* antioxidant activity, a standard curve was plotted using different concentrations of Trolox (100-1000 µmol/L). The results were expressed in µmol Trolox equivalent per liter of infusion (µmol TE/L). All the determinations were performed in quadruplicate.

2.5 Data analysis

The data were presented as median and mean ± standard deviation (SD). All the data had their normality checked by the Shapiro-Wilk test, and their homogeneity of variance were verified by Levene's test. A significant difference between the samples from the Brazilian states was found by one-way ANOVA (parametric data) and Kruskal-Wallis ANOVA (non-parametric data). Furthermore, all the analytical parameters and samples were evaluated together by multivariate statistical tools. Firstly, all the variables were autoscaled (into z-score) and then principal component analysis (PCA) was used to separate the samples according to their phenolic and methylxanthine contents and *in vitro* antioxidant activity. Hierarchical cluster analysis (HCA) was applied to check the similarities between the samples based on the Euclidean distance. Ward's method was used to suggest clusters as the samples in the same group were considered to be statistically similar (Zielinski et al., 2014b).

An attempt to classify in accordance with geographical origin was also performed by soft independent modeling of class analogy (SIMCA), and partial least squares-discriminant analysis (PLS-DA). For classification, the dataset was divided into a training set (75%) and validation set (25%) by the Kennard-Stone algorithm. For SIMCA, each category was independently modeled using PCA. The number of PCs in each class ranged from 3 to 5. In addition, the class distance was determined by considering that each class was bounded by a region of space, which represents a confidence level of 95% (Herrera Alvarez et al., 2017). For the PLS-DA, the number of latent variables was defined using 10-fold cross-validation of calibration samples based on the mean of the areas under the receiver operating characteristic curve (ROC) for each class (Bona et al., 2017). The PLS-DA output was converted into *a posteriori* probabilities using Bayes' Theorem. Thereafter, a threshold value for the class separation was obtained and the performance of the classifier was evaluated using the figures of merit which had been previously defined (Marquetti et al., 2016).

The performance of the models was evaluated by means of the accuracy, sensitivity, specificity, and efficiency, Equations 1, 2, 3, and 4:

$$ACCY = 100 \text{ x} \frac{(TP + TN)}{(TP + TN + FP + FN)} \quad (1)$$

$$SENS = 100 \text{ x} \frac{TP}{(TP + FN)} \quad (2)$$

$$SPEC = 100 \text{ x} \frac{TN}{(TN + FP)} \quad (3)$$

$$EFFI = \frac{SENS + SPEC}{2} \quad (4)$$

where: ACCY is accuracy; SENS is sensitivity; SPEC is specificity; EFFI is efficiency; TP is the number of positive samples that are correctly identified as positive samples; FN is the number of positive samples that are misclassified as negative samples; FP is the number of negative samples that are incorrectly identified as positive samples; and TN is the number of negative samples that are correctly identified as negative samples.

The PCA and SIMCA were performed using Pirouette 4.1 software (Infometrix®, Bothell, WA, USA). The PLS-DA was performed using MATLAB R2008b (The MathWorks Inc., Natick, USA) and HCA was performed using Statistica v. 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

3 Results and discussion

The minimum, maximum and mean values of the *yerba mate* extracts are shown in Table 1. The total phenolics and total flavonols ranged from 500 to 1,531 mg GAE/L (median: 1,061 mg GAE/L), and from 230 to 541 mg RE/L (median: 349 mg/L), respectively. The methylxanthines, represented by caffeine and theobromine, varied from 66 to 201 mg/L (median: 140 mg/L), and from 334 to 904 mg/L (median: 542 mg/L), respectively. In terms of the individual phenolics, neochlorogenic acid ranged from 259 to 635 mg/L (median: 487 mg/L), chlorogenic acid from 204 to 359 mg/L (median: 275 mg/L), cryptochlorogenic acid from 132 to 257 mg/L (median: 179 mg/L), and rutin from 36 to 105 mg/L (median: 69 mg/L). Statistically significant differences were not observed ($p > 0.05$) between the geographical origin of the samples of Brazilian *yerba mate* (Table 1), except for neochlorogenic acid ($p = 0.01$), which was present at a higher concentration in the samples from Rio Grande do Sul (n = 31).

According to Peres et al. (2013), caffeoylquinic acids like neochlorogenic (183-263 mg/L), chlorogenic (153-242 mg/L), and cryptochlorogenic (123-188 mg/L) acids are the major phenolic compounds found in *yerba mate* beverages (*chimarrão* and *tererê*); neochlorogenic acid stands out as having higher levels in these beverages. Butiuk et al. (2016) observed that the leaves and stems from the early harvesting season have the highest chlorogenic acid content; and the roasting step during *yerba mate* processing is responsible for a substantial loss of this phenolic acid. In the present study, significant correlation coefficients ($p < 0.01$) were observed between neochlorogenic and chlorogenic acids ($r = 0.40$) and with TPC ($r = 0.42$, $r = 0.38$, respectively). Furthermore, the consumption of caffeoylquinic acids from *yerba mate* beverages can contribute to the prevention of chronic and cardiovascular diseases (Cardozo & Morand, 2016). Theobromine, caffeine, and rutin have also been found in *I. paraguariensis* (Souza et al., 2015; Baeza et al., 2018). Methylxanthines presents good hydroxyl radical scavenging ability which is attributed their anticarcinogenic properties (Zielinski et al., 2016). As reported by Silveira et al. (2014), *yerba mate* beverages also are good sources of rutin (flavonol), confirming the significant correlation ($p < 0.05$) in our study between flavonols and TPC ($r = 0.44$, $r = 0.24$) for the analyzed samples.

Chemical methods are the main and easiest techniques to measure the *in vitro* antioxidant activity of foods and beverages. However, these methods differ in their mechanisms and some problems have been reported. Therefore, the i*n vitro* antioxidant activity was estimated by using three different methods (ABTS, DPPH and FRAP) (Table 1). The radical scavenging determined by ABTS ranged from 9,840 to 16,209 µmol TE/L (median: 12,988 µmol/L) and by DPPH from 8,869 to 24,793 µmol TE/L (median: 16,248 µmol/L). The total antioxidant potential measured by FRAP varied from 6,265 to 17,252 µmol TE/L (median: 11,829 µmol/L). In our study, the ABTS assay was the most suitable method to measure antioxidant activity. Its results showed correlation with those of the other spectrophotometric methods that were used ($r > 0.32$, $p < 0.03$), and the individual phenolics and methylxanthines that were evaluated ($r > 0.26$,

Table 1. Phenolics, methylxanthines and *in vitro* antioxidant actitivity of Brazilian *Yerba Mate* according to the geographical origin.

Analytical parameters	Paraná (n = 21)	Santa Catarina (n = 17)	Rio Grande do Sul (n = 31)	p-value*
TPC (mg GAE/L)	1019.4 ± 276.6 (500.0-1520.8)	1033.3 ± 276.6 (611.1-1493.9)	1067.8 ± 288.4 (624.1-1531.3)	0.77
Flavonols (mg RE/L)	366.9 ± 72.4 (274.5-540.8)	332.2 ± 58.7 (230.4-429.2)	364.9 ± 61.9 (262.6-477.8)	0.19
ABTS (µmol TE/L)	12887.0 ± 1358.6 (9839.5-15304.8)	12434.0 ± 1409.0 (10271.2-15081.8)	13144.7 ± 1477.9 (10014.1-16209.2)	0.26
DPPH (µmol TE/L)	16120.1 ± 2685.6 (12131.2-21955.4)	15382.0 ± 3712.2 (8869.3-23450.9)	16829.6 ± 2860.9 (10329.9-24793.3)	0.29
FRAP (µmol TE/L)	11831.3 ± 2392.6 (6264.5-16464.3)	11397.7 ± 2210.1 (8122.2-15780.8)	12329.9 ± 1966.8 (8560.3-17251.9)	0.35
Theobromine (mg/L)	127.6 ± 29.6 (65.8-193.9)	144.0 ± 36.2 (72.7-200.6)	143.6 ± 31.1 (69.8-196.3)	0.17
Neochlorogenic acid (mg/L)	464.4 [b] ± 65.5 (356.0-592.1)	440.0 [b] ± 75.5 (259.0-569.2)	505.5 [a] ± 63.5 (376.9-635.4)	0.01
Caffeine (mg/L)	543.5 ± 143.8 (333.54-839.3)	546.5 ± 111.2 (356.7-754.3)	599.2 ± 121.6 (423.2-904.4)	0.21
Chlorogenic acid (mg/L)	284.2 ± 34.5 (206.4-359.2)	269.0 ± 36.4 (204.4-318.7)	271.4 ± 27.1 (222.3-332.3)	0.26
Cryptochlorogenic acid (mg/L)	189.6 ± 29.0 (131.7-257.1)	174.8 ± 17.3 (142.8-205.7)	176.6 ± 19.5 (142.6-231.2)	0.07
Rutin (mg/L)	68.8 ± 16.5 (37.3-105.1)	62.0 ± 10.8 (39.3-77.6)	72.5 ± 16.2 (35.6-104.9)	0.08

*Probability values obtained by *one-way* ANOVA. TPC: total phenolic compounds, and *in vitro* antioxidant activity by ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power.

$p < 0.03$) except for rutin ($r = 0.21$, $p = 0.08$). The same was observed by Deetae et al. (2012), who evaluated the antioxidant properties of Thai herbal teas and conventional teas. The ABTS assay has advantages over the other assays because it is able to react with both hydrophilic and hydrophobic antioxidants, therefore covering a broader range of antioxidants in a sample (Gorjanović et al., 2012).

As observed in our study, a high variation regarding the phenolic composition and *in vitro* antioxidant activity of *yerba mate* can be due to the manufacturing conditions. Briefly, the following processing steps are involved in the production of commercial *yerba mate*: harvesting, roasting, drying, milling, aging, and packing. Furthermore, the companies that process *yerba mate* employ different conditions, which can modify the qualitative and quantitative phenolic composition. Isolabella et al. (2010) observed that *zapecado* (roasting or pre-drying), drying and aging steps changed the biologically active principles of the samples. In addition, each company uses different leaf-to-stem ratios, and *I. paraguariensis* trees grow in many different climatic and geographical locations (Butiuk et al., 2016; Souza et al., 2015; Marquez et al., 2013). The leaves of *Ilex paraguariensis* show higher levels of phenolic compounds and higher antioxidant capacity than stems (Souza et al., 2015), and the harvest season influences the composition of both leaves and stems (Butiuk et al., 2016). Therefore, the wide range that was observed in the raw materials explains the low significant ($p < 0.05$) correlation coefficients that were found between all the parameters.

As well as the previously mentioned factors that influence the levels of antioxidant compounds, it is important to emphasize that *yerba mate* beverages (*chimarrão* or *mate* and *tererê*) are consumed with successive infusions in southern Brazil, Argentina and Uruguay. According to Colpo et al. (2016), antioxidant compounds can be extracted in different amounts during the consumption of *chimarrão*.

In order to explore the differences between the samples from the distinct Brazilian regions that were studied, principal component analysis (PCA) was performed. Principal component 1 (PC1) explained up to 37.64% of total variance, PC2 explained 13.20%, and PC3 explained 10.47%, totaling 61.30%. Using a 3D-scatter plot, it was possible to observe an overlap between the samples from Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR) (Figure 2A). However, the majority of the samples from RS remained associated, while the samples from SC and PR showed a higher dispersion. The clearest separation was observed when PCA was conducted again using the SC and RS samples (53.74% of total variance explained by the two first PCs); there was a higher number of RS samples on the right side (Figure 2B). On the other hand, the PCA applied between the samples from PR and RS, and PR and SC, continued to overlap (Figures 2C, 2D). In contrast to our study, Marcelo et al. (2014) used PCA and reported a clear separation between *yerba mate* samples from different countries (Brazil, Argentina, Uruguay, and Paraguay); they considered mineral analysis to discriminate the country of origin.

In addition to PCA, the similarity between the samples was also evaluated using HCA and three clusters were suggested (Figure 3). Cluster 1 mainly grouped samples from RS (50%, 16 samples) and PR (31%, ten samples), and showed significant difference using one-way ANOVA ($p < 0.01$) between clusters 2 and 3 (Table 2), with higher levels in all the analyzed parameters (phenolics, methylxanthines, and antioxidant activity). Cluster 2 was also mainly formed by samples from RS (53%, ten samples), and cluster 3 by samples from SC (39%, seven samples).

A tentative classification of the *yerba mate* samples according to geographical origin was also performed by SIMCA and PLS-DA. The SIMCA model was developed with the selection of four principal components for each Brazilian state class (Paraná, Santa Catarina, and Rio Grande do Sul), which was able to explain more than 79% of the total variance in each class. Furthermore, the model had an overall accuracy of 84% (external validation of 75%). The PLS-DA model, with seven latent variables, explained 99.99% of variance in X and 17.49% in Y. The low variance of Y indicates that the variables of X did not show a satisfactory correlation with the classes represented in Y. The overall accuracy that was determined was 75% (external validation of 81%). The classification achieved by both proposed models to differentiate *yerba mate* from different Brazilian states (PR, SC and RS) showed similar performance, with a consistency in the efficiency, sensitivity and specificity values (Table 3).

Table 2. Chemical composition of yerba mate samples clustered using hierarchical cluster analysis.

Variables	Cluster 1 (n = 32)	Cluster 2 (n = 19)	Cluster 3 (n = 18)	p-value*	p-value**
TPC (mg GAE/L)	1166 ± 214	1074 ± 176.49	796.81 ± 180.96	0.10	<0.01
Flavonols (mg RE/L)	401.97 ± 58.92	329.57 ± 42.59	307.75 ± 39.47	0.99	<0.01
ABTS (µmol TE/L)	13910 ± 972	12510 ± 1114	11483 ± 984	0.57	<0.01
DPPH (µmol TE/L)	17371 ± 3038	15934 ± 2900	14618 ± 2500	0.20	<0.01
FRAP (µmol TE/L)	12572 ± 1644	12265 ± 2286	10507 ± 2288	0.40	<0.01
Theobromine (mg/L)	153.61 ± 27.09	119.86 ± 32.32	132.56 ± 29.97	0.33	<0.01
Neochlorogenic acid (mg/L)	528.15 ± 45.96	455.74 ± 57.15	407.93 ± 52.79	0.37	<0.01
Caffeine (mg/L)	638.04 ± 116.56	523.11 ± 97.80	495.62 ± 114.14	0.62	<0.01
Chlorogenic acid (mg/L)	291.69 ± 26.61	267.29 ± 13.57	252.32 ± 38.53	0.01	<0.01
Cryptochlorogenic acid (mg/L)	193.43 ± 23.35	171.49 ± 10.70	165.59 ± 18.65	0.07	<0.01
Rutin (mg/L)	76.24 ± 13.87	69.38 ± 12.46	54.93 ± 11.90	0.73	<0.01

Results expressed as mean ± standard deviation. TPC: total phenolic compounds, and in vitro antioxidant activity by ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power. *Probability values obtained by the Levene test for homogeneity of variances; **Probability values obtained by one-way ANOVA or Kruskal-Wallis test. Different letters in the same line represent statistically different results (P < 0.05).

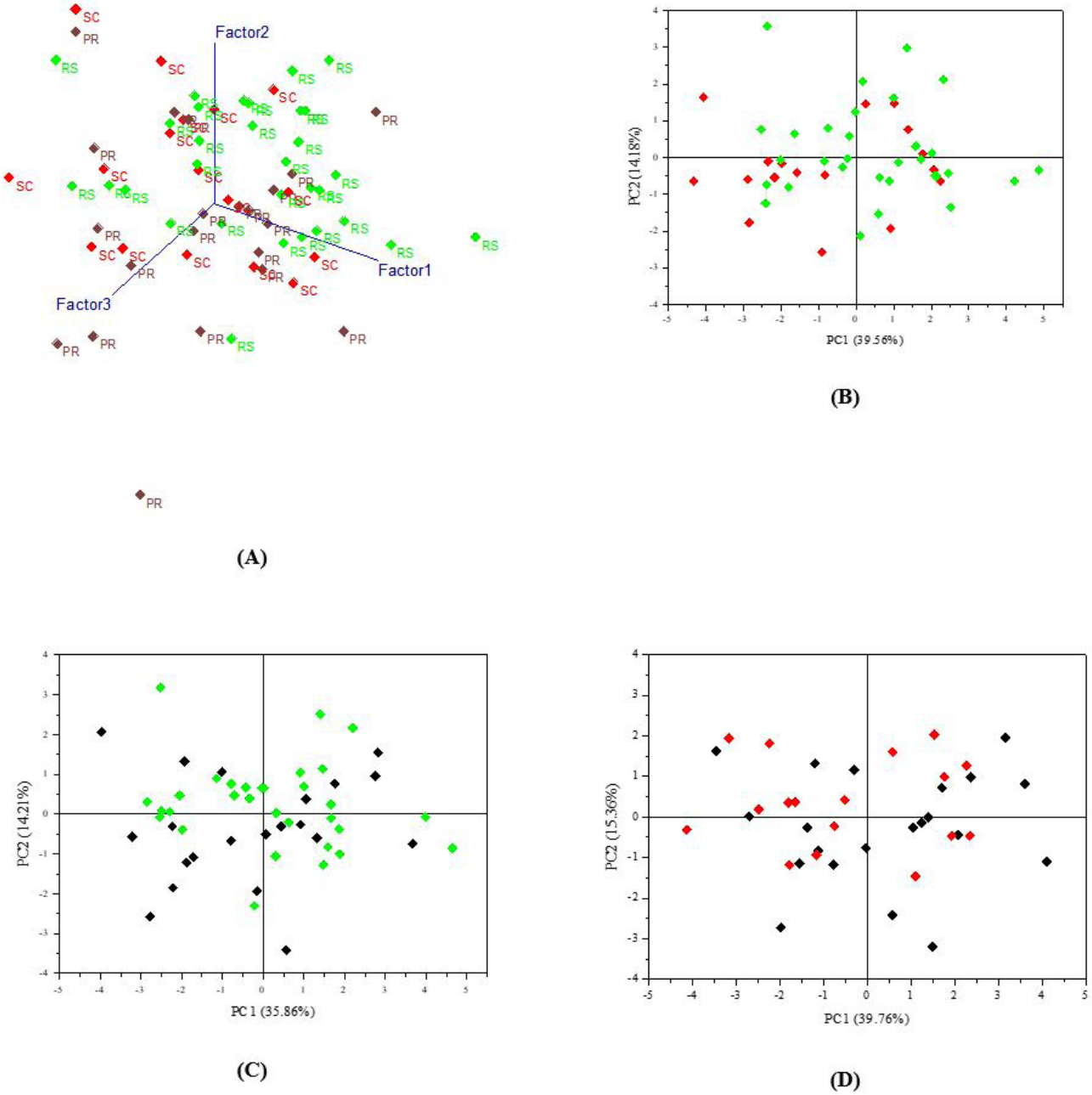

Figure 2. PCA scatter plots obtained for all samples (A), SC vs. RS (B), PR vs. RS (C), PR vs. SC (D). Note: red (Santa Catarina), black (Paraná), green (Rio Grande do Sul). PC1: Principal component 1; PC2: Principal component 2; RS: Rio Grande do Sul; SC: Santa Catarina; PR: Parana.

Table 3. Predictive abilities of classification models (SIMCA and PLS-DA) using the chemical dataset for *Yerba Mate* samples produced in Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS).

Predictive abilities		Calibration			Validation		
		PR	SC	RS	PR	SC	RS
SIMCA (%)	**Accuracy**	86.00	84.00	82.00	78.95	73.68	73.68
	Sensitivity	80.00	83.33	69.57	66.67	80.00	50.00
	Specificity	88.57	84.21	92.59	84.62	71.43	90.91
	Efficiency	84.29	83.77	81.08	75.64	75.71	70.45
PLS-DA (%)	**Accuracy**	80.00	76.00	68.00	68.42	84.21	89.47
	Sensitivity	46.67	33.33	89.47	33.33	40.00	100.00
	Specificity	94.29	89.47	66.67	84.62	100.00	81.82
	Efficiency	70.48	61.40	78.07	58.98	70.00	90.91

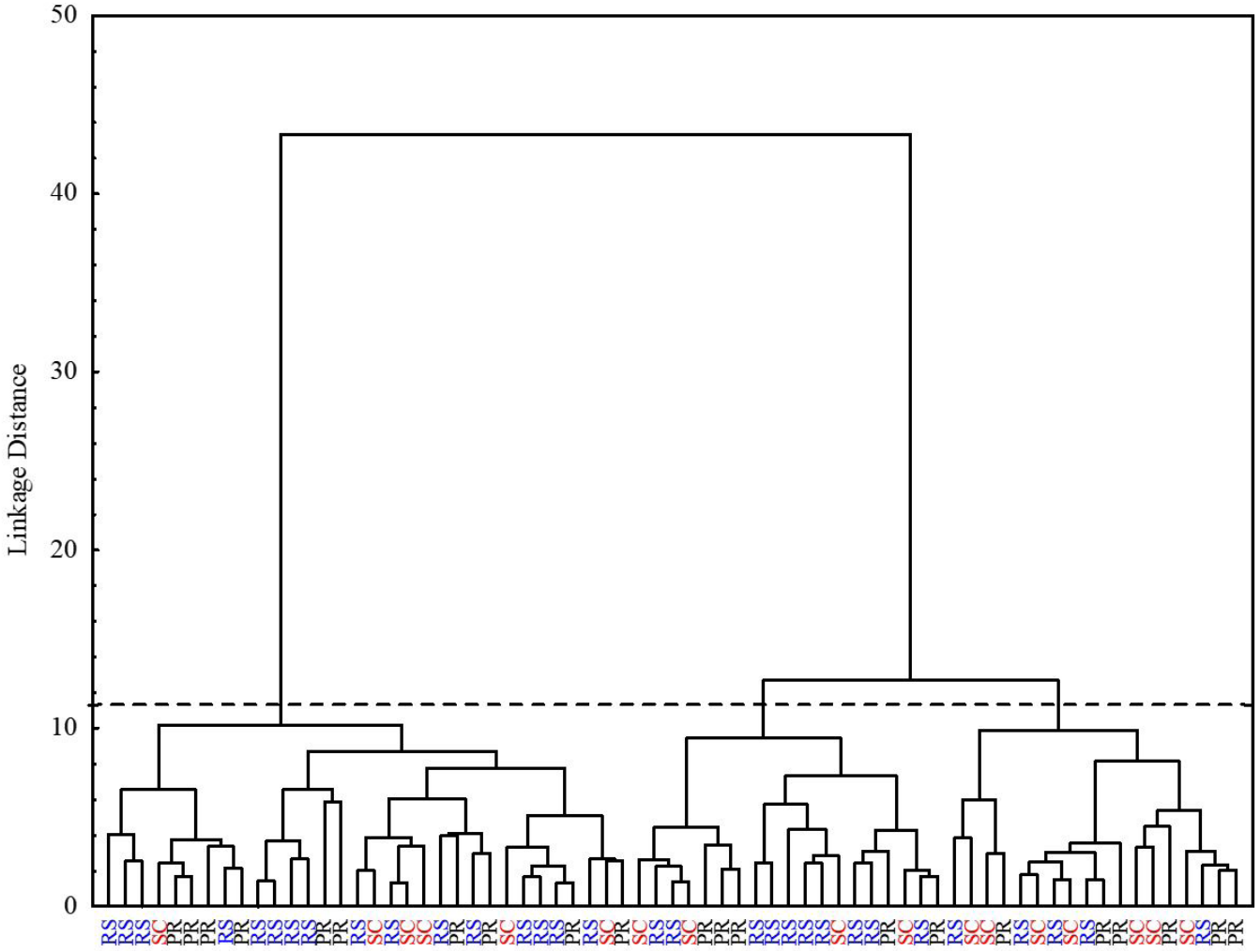

Figure 3. Dendrogram obtained by HCA for y*erba mate* samples produced in the southern Brazil. RS: Rio Grande do Sul; SC: Santa Catarina; PR: Parana.

Although, our results had intermediate levels (< 80% of accuracy) of classification, as far as we know this is the first study that has used antioxidant composition together with chemometrics to differentiate and classify *yerba mate* according to geographical origin (Brazilian states). Therefore, the methods that were employed can be useful for the quality control of *yerba mate* and its correct classification according to geographical origin.

4 Conclusion

A large variation in the level of antioxidant compounds was verified between the *yerba mate* samples from different southern Brazilian states. Using PCA, the first three principal components explained 61.30% of total variance, with the majority of the samples from RS staying associated; while the samples from SC and PR overlapped. Furthermore, HCA suggested three clusters, with cluster 1 (mainly samples from RS and PR) showing the highest levels of phenolics, methylxanthines, and antioxidant activity. A tentative classification of *yerba mate* according to geographical origin was also performed and both models that were employed (SIMCA and PLS-DA) had a similar predictability (≥ 75% of accuracy). Therefore, the chemometric tools used in the current study can be suitable to monitor and assess the antioxidant composition of *yerba mate* and also to suggest its correct classification according to its geographical origin.

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