# Discrimination of yerba mate (*Ilex paraguayensis* Saint-Hilaire 1838) samples according to their geographic origin by means of near infrared spectroscopy and chemometrics

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## Introduction

In recent years, the search for antioxidants from natural sources has received much attention, and efforts have been made to identify new natural resources for active antioxidant compounds in plants and natural products.[1,2] These naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage from occurring in the body. Mate tea, often known as "yerba mate", is one of the most commonly consumed beverages in several South American countries, including Uruguay, Paraguay, Argentina and Brazil.[1] It is manufactured from the caffeine rich leaves of *Ilex paraguayensis* Saint-Hilaire 1838 (Aquifoliaceae) by a method similar to that used to produce green tea. The material plant *Ilex paraguayensis* is a perennial tree growing in the jungle of the Parana basin.[1] This plant material has a very characteristic odour, used in stimulants, bitter tonics, and diaphoretics; and has a characteristic mature flavour which is somewhat sweet, sour, withered leaf like, and cigarette-like.[1,2] Leaves from this plant contain between 1–1.5% caffeine and 7–11% tannin similar to other beverages like tea or coffee.[1,2] In spite of its popularity, the composition of its flavor constituents was not reported until recently.[1]

Many reports have been found on the classification, discrimination or authentication of natural products by means of near infrared (NIR) spectroscopy and chemometrics.[3] Analytical control, either qualitative or quantitative, is essential in order to assess raw materials, products and by-products as well as to optimise the manufacturing process.[3] In addition to chemical composition, important aspects of product description might be related to the process history (e.g. fresh as opposed to frozen food) or geographic origin (e.g. as Italian olive oil produced only with olives grown in Italy).[3] Foods or raw ingredients that are most likely to be targets for adulteration include those which are of high value, or are subject to the effects of environment conditions (e.g. rain, sun exposure) during their growth or harvesting.[3] Visual examination of the near infrared (NIR) spectra may not discriminate between authentic and adulterated products, or determine geographic origins.[3] Therefore, the application of multivariate techniques like principal component analysis (PCA) or discriminant analysis (e.g. discriminant partial least squares, linear discriminant analysis) is crucial to unravel and interpret the spectral properties of the sample and to allow classification without using chemical information.[4] The objective of this study was to explore the usefulness of NIR reflectance spectroscopy combined with chemometrics to discriminate the geographic origin of yerba mate (*Ilex paraguayensis* Saint-Hilaire 1838) samples obtained from commercial suppliers.

## Materials and methods

### Plant materials

Five different trademarks of yerba mate *Ilex paraguayensis* Saint-Hilaire 1838 (Aquifoliaceae) produced in industries located in Brazil (BR) (n = 2), Argentina (ARG) (n = 2) and a commercial blend of mate tea from Brazil with herbal tea from Uruguay (URU) (n = 1) were used. Additionally, two mixtures were made by weighing equal quantities of BR and ARG yerba mate samples (w/w). Five bags (1 kg w/w) of each trademark were purchased in the local market, yielding a total of 25 samples. Before NIR analysis, samples were dried in an oven at 100°C for 24 h and ground in a Wiley forage mill to pass a 1 mm screen (Arthur H. Thomas, Philadelphia, PA, USA).

### Spectra collection

Samples were analysed in a scanning spectrophotometer NIRSystems 6500 (NIRSystems, Silver Springs, MD, USA) in reflectance mode (1100–2500 nm) using the sample transport module. Dry samples were scanned in a small circular quartz cup (50 mm diameter) back sealed with disposal paper. Two pairs of lead sulphide detectors collected the reflectance spectra and the readings were referenced using a ceramic disk. Reflectance data were stored as log (1/R) (R = reflectance) at 2 nm intervals (700 data points). Samples were

scanned once (no repeated spectral measurements were made) and were not rotated during spectral collection. Computer operation and spectral data collection were performed with ISI 3.1 (InfraSoft International, Port Matilda, PA, USA).

## Data manipulation and analysis

Spectra were exported from ISI 3.1 in ASCII format into The Unscrambler 7.5 (CAMO ASA, Oslo, Norway) for chemometric analysis. Principal component analysis was performed on raw and preprocessed spectra. Spectra were preprocessed by calculating the second derivative (to reduce baseline variation and enhance spectral features), and by standard normal variate (SNV) transformation.[5] Discrimination models were developed using partial least squares (PLS2) discriminant regression (PLS-DA) and linear discriminant analysis (LDA).[4] In PLS-DA, each sample in the calibration set is assigned a dummy variable as a reference value. The discrimination model is then developed by regression of the spectral data against the assigned reference value (dummy variable).[4] The PLS-DA models were developed using a non-metric dummy variable (set to 1 = URU; 2 = BR; 3 = ARG and 4 = mixtures). The classification of the samples according to geographic origin was based on a 0.5 cut-off value. LDA was performed with JMP 5.01 (SAS Institute Inc., Cary, NC, USA) on the scores of principal components (PCs) 1 to 3, which gave the highest level of separation (high variance) in the PCA models developed. Full cross validation (CV) (leave-one-out) was used when PCA and discriminant models were developed.[4]

## Results and Discussion

Figure 1 shows the score plot of the first two principal components derived from the raw NIR spectra. Samples were separated by geographic origin. The highest eigenvectors in PC1 (79% of variance explained) were at 1300 nm (C-H first overtone), at 1500 nm, 1700 nm and 1932 nm (O-H stretch first overtone associated with water content), and at 2104 nm and 2282 nm (C-H combination tones associated with aromatic compounds and essential oils).[6] The highest negative eigenvectors observed in PC2 (20% of variance explained) were at 1726 nm (C-H overtones associated with essential oils), where the highest positive eigenvectors were at 1418 nm and 1932 nm (O-H overtones associated with water content).[6] The highest eigenvectors in PC3 (1% of the variance explained) were at 1400 nm and 1900 nm (associated with water). Wavelengths at both 1750 nm and around 2300 nm have also been reported to be associated with phenolic compounds, mainly tannins.[6, 7] The overall classification rates obtained were 76% and 100% using PLS-DA and LDA to classify yerba mate samples according to their geographic origin.
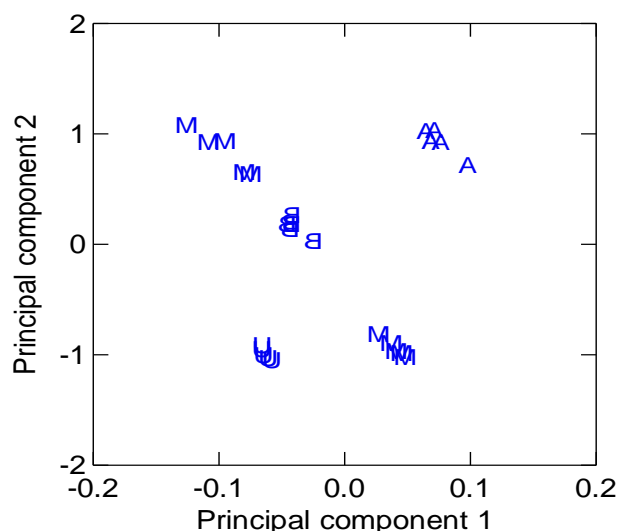


**Figure 1.** Score plot of the first two principal components (PC) of the NIR spectra of yerba mate samples (*Ilex paraguayensis*) (A= Argentina, M= mixtures, B= Brazil, U= Uruguay).

It was observed that some of the yerba mate samples from Argentina and Brazil were incorrectly classified using PLS-DA. The ability of the NIR model to classify yerba mate samples is based on the vibrational responses of chemical bonds in the NIR region (O-H, N-H and C-H). Hence, sample sets with larger variations in the chemical entities which respond in these spectral regions will produced more accurate

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models. The results also suggested that other characteristics (e.g. essential oils, tannins, aromatic compounds) would explain the discrimination among samples. As suggested by other authors, NIR spectroscopy might be used for initial testing of accidental contamination (e.g. cross contamination) or fraudulent practices, or for screening the raw material. The inclusion of NIR spectroscopic methods might provide initial screening in the food chain and enable costly methods to be used more productively on suspicious specimens; NIR could easily be implemented in the trade and quality control of yerba mate. In addition, to be able to control and optimise the processing of the yerba mate it is important to measure and analyse the chemical composition of the raw material used to manufacture the final product.

## Conclusions

The results from this study showed that qualitative analysis of yerba mate might be possible with NIR spectroscopy. All samples were classified correctly accordingly to their geographic origin using the LDA technique. These results also suggested that NIR spectroscopy combined with multivariate data analysis methods might be used by the yerba mate industry for the identification and authentication of the product. The work reported here constitutes a preliminary study and requires further development.

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